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Synthesis and Antioxidant Properties of Some 3-Alkyl(Aryl)-4-[3-ethoxy-2-(4-toluenesulfonyloxy)-benzylidenamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-ones

[1]Faruk Kardaş, [2]Sevda Manap, [2]Özlem Gürsoy-Kol[*], [2]Murat Beytur and [2]Haydar Yüksek

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

ABSTRACT

*A series of novel 3-alkyl(aryl)-4-[3-ethoxy-2-(4-toluenesulfonyloxy)-benzyliden-amino]-4,5-dihydro-1H-1,2,4-triazol-5-ones (**4a-i**) were synthesized from the reactions of 3-alkyl(aryl)-4-amino-4,5-dihydro-1H-1,2,4-triazol-5-ones (**2a-i**) with 3-ethoxy-2-(4-toluenesulfonyloxy)-benzaldehyde (**3**). The new compounds were characterized using by IR, ^{1}H-NMR and ^{13}C-NMR 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. These antioxidant activities were compared to those from standard antioxidants, such as EDTA, BHA, BHT and α-tocopherol.*

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

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 properties [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 [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 cells [10]. 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 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 [11].

In the present study, due to a wide range of applications to find their possible radical scavenging and antioxidant activity, the newly synthesized compounds were investigated by using different antioxidant methodologies: 1,1-diphenyl-2-picryl-hydrazyl ($DPPH^{\cdot}$) free radical scavenging, reducing power and metal chelating activities.

MATERIALS AND METHODS

Chemicals and Apparatus

Chemical reagents used in this paper were bought from Merck AG, Aldrich and Fluka. Melting points were taken using an Electrothermal Melting-point Apparatus in an open capillary tube and were not corrected. The infrared spectra were recorded on a Perkin Elmer Instruments Spectrum One FT-IR spectrophotometer. ^{1}H and ^{13}C NMR spectra were determined in deuterated dimethyl sulfoxide with TMS as internal standard using a Bruker spectrophotometer at 200 MHz and 50 MHz, respectively.

Synthesis of 3-alkyl(aryl)-4-[3-ethoxy-2-(4-toluenesulfonyloxy)- benzylidenamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (4a-i)

3-Ethoxy-2-hydroxybenzaldehyde (0.01 mol) dissolved in ethyl acetate (100 mL) was reacted with p-toluenesulfonyl chloride (0.01 mol), and to this solution was slowly mixed triethylamine (0.01 mol) by stirring at 0-5 °C. Stirring was continued for 2 h, and after that the mixture was refluxed for 3 hours and filtered. The filtrate was evaporated *in vacuo*, and the crude product was washed with water and recrystallized from ethanol to afford compound **3.** Yield 92%, mp 116-117 °C; IR (υ, cm^{-1}): 2887 and 2765 (CHO), 1687 (C=O), 1363 and 1153 (SO_2), 819 (1,4-disubstituted benzenoid ring). Then the corresponding compound **2** (0.01 mol) was dissolved in acetic acid (20 mL) and by treated 3-ethoxy-2-(4-toluenesulfonyloxy)-benzaldehyde (**3**) (0.01 mol). The mixture was refluxed for 1.5 hours and then evaporated at 50-55 °C *in vacuo*. A few recrystallizations of the residue from ethanol gave pure compounds **4a-i** as uncolored crystals.

3-Methyl-4-[3-ethoxy-2-(4-toluenesulfonyloxy)-benzylidenamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-one (4a)

Yield 92%, mp. 245-246 ºC. IR (KBr) cm^{-1}: 3171 (NH); 1696 (C=O); 1596 (C=N); 1341 and 1152 (SO_2); 856 (1,4-disubstituted benzene ring). ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.15 (3H, t, J = 6.80 Hz, OCH_2CH_3), 2.25 (3H, s, CH_3), 2.35 (3H, s, $PhCH_3$), 3.92 (2H, q, J = 6.80 Hz, OCH_2CH_3), 7.27 (1H, d, J = 8.40 Hz, Ar-H), 7.38-7.42 (3H, m, Ar-H), 7.49 (1H, d, J = 8.00 Hz, Ar-H), 7.71 (2H, d, J = 8.40 Hz, Ar-H), 9.62 (1H, s, N=CH), 11.83 (1H, s, NH). ^{13}C NMR (50Mz, DMSO-d_6): δ 151.73 (triazole C_5), 147.60 (N=CH), 145.63 (triazole C_3), [150.73, 144.05, 137.39, 132.21, 129.81 (2C), 128.75, 128.22, 128.14 (2C), 117.27, 116.48] (arom-C), 64.23 ($O\underline{C}H_2CH_3$), 20.94 ($PhCH_3$), 13.99 ($OCH_2\underline{C}H_3$), 10.95 (CH_3).

3-Ethyl-4-[3-ethoxy-2-(4-toluenesulfonyloxy)-benzylidenamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-one (4b)

Yield 93%, mp. 220-221 ºC. IR (KBr) cm^{-1}: 3175 (NH); 1694 (C=O); 1591 (C=N); 1353 and 1152 (SO_2); 854 (1,4-disubstituted benzene ring). ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.17 (3H, t, J = 7.60 Hz, CH_2CH_3), 1.21 (3H, t, J = 7.20 Hz, OCH_2CH_3), 2.35 (3H, s, $PhCH_3$), 2.62 (2H, q, J =7.60 Hz, CH_2CH_3), 3.93 (2H, q, J = 7.20 Hz, OCH_2CH_3), 7.27 (1H, d, J = 8.40 Hz, Ar-H), 7.36-7.42 (3H, m, Ar-H), 7.48 (1H, d, J = 8.00 Hz, Ar-H), 7.71 (2H, d, J = 8.40 Hz, Ar-H), 9.61 (1H, s, N=CH), 11.86 (1H, s, NH). ^{13}C NMR (50Mz, DMSO-d_6): δ 151.77 (triazole C_5), 147.80 (N=CH), 147.56 (triazole C_3), [150.90, 145.61, 137.43, 132.22, 129.82 (2C), 128.79, 128.25, 128.15 (2C), 117.18, 116.48] (arom-C), 64.26 ($O\underline{C}H_2CH_3$), 20.95 ($PhCH_3$), 18.39 ($\underline{C}H_2CH_3$), 14.00 ($OCH_2\underline{C}H_3$), 9.97 ($CH_2\underline{C}H_3$).

3-(*n*-Propyl)-4-[3-ethoxy-2-(4-toluenesulfonyloxy)-benzylidenamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-one (4c)

Yield 83%, mp. 217-218 ºC. IR (KBr) cm^{-1}: 3169 (NH); 1691 (C=O); 1590 (C=N); 1335 and 1150 (SO_2); 851 (1,4-disubstituted benzene ring). ^{1}H NMR (200 MHz, DMSO-d_6): δ 0.97 (3H, t, J = 7.20 Hz, $CH_2CH_2CH_3$), 1.15 (3H, t, J = 7.20 Hz, OCH_2CH_3), 1.68 (2H, sext, J = 7.20 Hz, $CH_2\underline{C}H_2CH_3$), 2.35 (3H, s, $PhCH_3$), 2.60 (2H, t, J = 7.20 Hz, $\underline{C}H_2CH_2CH_3$), 3.92 (2H, q, J = 6.80 Hz, OCH_2CH_3), 7.27 (1H, d, J = 8.40 Hz, Ar-H), 7.37-7.42 (3H, m, Ar-H), 7.48 (1H, d, J = 8.00 Hz, Ar-H), 7.72 (2H, d, J = 8.40 Hz, Ar-H), 9.62 (1H, s, N=CH), 11.86 (1H, s, NH). ^{13}C NMR (50Mz, DMSO-d_6): δ 151.83 (triazole C_5), 147.62 (N=CH), 146.75 (triazole C_3), [150.90, 145.66, 137.51, 132.30, 129.89 (2C), 128.86, 128.34, 128.20 (2C), 117.20, 116.55] (arom-C), 64.32 ($O\underline{C}H_2CH_3$), 26.63 ($\underline{C}H_2CH_2CH_3$), 21.03 ($PhCH_3$), 18.86 ($CH_2\underline{C}H_2CH_3$), 14.05 ($OCH_2\underline{C}H_3$), 13.50 ($CH_2CH_2\underline{C}H_3$).

3-Benzyl-4-[3-ethoxy-2-(4-toluenesulfonyloxy)-benzylidenamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-one (4d)

Yield 90%, mp. 204-205 ºC. IR (KBr) cm^{-1}: 3173 (NH); 1696 (C=O); 1591 (C=N); 1350 and 1149 (SO_2); 833 (1,4-disubstituted benzene ring); 748 and 715 (monosubstituted benzene ring). δ 1.15 (3H, t, J = 6.80 Hz, OCH_2CH_3), 2.29 (3H, s, $PhCH_3$), 3.93 (2H, q, J = 6.80 Hz, OCH_2CH_3), 4.02 (2H, s, CH_2Ph), 7.25-7.40 (9H, m, Ar-H), 7.44 (1H, d, J = 8.00 Hz, Ar-H), 7.63-7.65 (2H, m, Ar-H), 9.51 (1H, s, N=CH), 11.97 (1H, s, NH). ^{13}C NMR (50Mz, DMSO-d_6): δ 151.86 (triazole C_5), 147.40 (N=CH), 146.10 (triazole C_3), [150.74, 145.60, 137.62, 135.71, 132.28, 129.86

(2C), 129.86 (2C), 128.91 (2C), 128.72, 128.45, 128.32 (2C), 128.09 (2C), 126.81, 117.17, 116.59] (arom-C), 64.33 ($O\underline{CH_2}CH_3$), 30.99 (CH_2Ph), 20.98 ($PhCH_3$), 14.08 ($OCH_2\underline{CH_3}$).

3-(*p*-Methylbenzyl)-4-[3-ethoxy-2-(4-toluenesulfonyloxy)-benzylidenamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-one (4e)

Yield 93%, mp. 177-178 ºC. IR (KBr) cm^{-1}: 3174 (NH); 1698 (C=O); 1591 (C=N); 1353 and 1149 (SO_2); 853 (1,4-disubstituted benzene ring). ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.15 (3H, t, *J* = 6.80 Hz, OCH_2CH_3), 2.27 (3H, s, $PhCH_3$), 2.29 (3H, s, $PhCH_3$), 3.93 (2H, q, *J* = 7.20 Hz, OCH_2CH_3), 3.96 (2H, s, CH_2Ph), 7.14 (2H, d, *J* = 8.00 Hz, Ar-H), 7.20-7.28 (6H, m, Ar-H), 7.46 (1H, d, *J* = 8.00 Hz, Ar-H), 7.64-7.66 (2H, m, Ar-H), 9.52 (1H, s, N=CH), 11.94 (1H, s, NH). ^{13}C NMR (50Mz, DMSO-d_6): δ 151.77 (triazole C_5), 147.29 (N=CH), 146.17 (triazole C_3), [150.68, 145.54, 137.52, 135.81, 132.51, 132.21, 129.78 (2C), 128.91 (2C), 128.69 (3C), 128.24, 128.02 (2C), 117.09, 116.50] (arom-C), 64.25 ($O\underline{CH_2}CH_3$), 30.49 (CH_2Ph), 20.91 ($PhCH_3$), 20.52 ($PhCH_3$), 13.99 ($OCH_2\underline{CH_3}$).

3-(*p*-Methoxybenzyl)-4-[3-ethoxy-2-(4-toluenesulfonyloxy)-benzylidenamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-one (4f)

Yield 81%, mp. 152-153 ºC. IR (KBr) cm^{-1}: 3181 (NH); 1700 (C=O); 1617, 1591 (C=N); 1351 and 1149 (SO_2); 854 (1,4-disubstituted benzene ring). ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.16 (3H, t, *J* = 7.20 Hz, OCH_2CH_3), 2.29 (3H, s, $PhCH_3$), 3.72 (3H, s, OCH_3), 3.93 (2H, q, *J* = 7.20 Hz, OCH_2CH_3), 3.95 (2H, s, CH_2Ph), 6.91 (2H, d, *J* = 8.80 Hz, Ar-H), 7.24-7.29 (5H, m, Ar-H), 7.39 (1H, t, *J* = 8.00 Hz, Ar-H), 7.48 (1H, d, *J* = 8.00 Hz, Ar-H), 7.66 (2H, d, *J* = 8.00 Hz, Ar-H), 9.54 (1H, s, N=CH), 11.95 (1H, s, NH). ^{13}C NMR (50Mz, DMSO-d_6): δ 151.80 (triazole C_5), 147.33 (N=CH), 146.35 (triazole C_3), [158.12, 150.72, 145.55, 137.56, 132.25, 129.91 (2C), 129.80 (2C), 128.72, 128.26, 128.04 (2C), 127.40, 117.13, 116.49, 113.81 (2C)] (arom-C), 64.27 ($O\underline{CH_2}CH_3$), 54.98 (OCH_3), 30.07 (CH_2Ph), 20.92 ($PhCH_3$), 14.01 ($OCH_2\underline{CH_3}$).

3-(*p*-Chlorobenzyl)-4-[3-ethoxy-2-(4-toluenesulfonyloxy)-benzylidenamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-one (4g)

Yield 89%, mp. 203-204 ºC. IR (KBr) cm^{-1}: 3171 (NH); 1693 (C=O); 1592 (C=N); 1349 and 1150 (SO_2); 838, 812 (1,4-disubstituted benzene ring). ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.14 (3H, t, *J* = 7.20 Hz, OCH_2CH_3), 2.31 (3H, s, $PhCH_3$), 3.92 (2H, q, *J* = 7.20 Hz, OCH_2CH_3), 4.04 (2H, s, CH_2Ph), 7.26 (1H, d, *J* = 8.00 Hz, Ar-H), 7.32-7.45 (8H, m, Ar-H), 7.67-7.69 (2H, m, Ar-H), 9.58 (1H, s, N=CH), 12.00 (1H, s, NH). ^{13}C NMR (50Mz, DMSO-d_6): δ 151.82 (triazole C_5), 147.53 (N=CH), 145.77 (triazole C_3), [150.77, 145.63, 137.59, 134.65, 132.32, 131.52, 130.83 (2C), 129.87 (2C), 128.76, 128.39, 128.33 (2C), 128.15 (2C), 117.20, 116.60] (arom-C), 64.32 ($O\underline{CH_2}CH_3$), 30.30 (CH_2Ph), 21.01 ($PhCH_3$), 14.06 ($OCH_2\underline{CH_3}$).

3-(*p*-Chlorobenzyl)-4-[3-ethoxy-2-(4-toluenesulfonyloxy)-benzylidenamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-one (4h)

Yield 82%, mp. 212-214 ºC. IR (KBr) cm^{-1}: 3175 (NH); 1696 (C=O); 1593 (C=N); 1349 and 1150 (SO_2); 831 (1,4-disubstituted benzene ring); 793 and 701 (*m*-disubstituted benzene ring). ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.15 (3H, t, *J* = 7.20 Hz, OCH_2CH_3), 2.30 (3H, s, $PhCH_3$), 3.93 (2H, q, *J* = 7.20 Hz, OCH_2CH_3), 4.06 (2H, s, CH_2Ph), 7.25-7.46 (9H, m, Ar-H), 7.65-7.67 (2H, m, Ar-H), 9.53 (1H, s, N=CH), 12.01 (1H, s, NH). ^{13}C NMR (50Mz, DMSO-d_6): δ 151.78 (triazole C_5), 147.37 (N=CH), 145.52 (triazole C_3), [150.65, 145.52, 138.00, 137.55, 132.88, 132.22, 130.22, 129.77 (2C), 128.99, 128.63, 128.20, 128.04 (2C), 127.63, 126.75, 117.10, 116.55] (arom-C), 64.25 ($O\underline{CH_2}CH_3$), 30.53 (CH_2Ph), 20.90 ($PhCH_3$), 13.99 ($OCH_2\underline{CH_3}$).

3-Phenyl-4-[3-ethoxy-2-(4-toluenesulfonyloxy)-benzylidenamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-one (4i)

Yield 90%, mp. 210-211 ºC. IR (KBr) cm^{-1}: 3164 (NH); 1699 (C=O); 1591 (C=N); 1362 and 1152 (SO_2); 858 (1,4-disubstituted benzene ring); 750 and 696 (monosubstituted benzene ring). ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.17 (3H, t, *J* = 6.80 Hz, OCH_2CH_3), 2.30 (3H, s, $PhCH_3$), 3.95 (2H, q, *J* = 7.20 Hz, OCH_2CH_3), 7.29 (1H, d, *J* = 8.00 Hz, Ar-H), 7.36-7.41 (4H, m, Ar-H), 7.54-7.57 (3H, m, Ar-H), 7.72-7.75 (2H, m, Ar-H), 7.86-7.88 (2H, m, Ar-H), 9.58 (1H, s, N=CH), 12.41 (1H, s, NH). ^{13}C NMR (50Mz, DMSO-d_6): δ 151.83 (triazole C_5), 149.50 (N=CH), 145.66 (triazole C_3), [150.88, 144.32, 137.62, 132.18, 130.09, 129.87 (2C), 128.47 (2C), 128.40, 128.17 (2C), 127.90 (3C), 126.32, 117.17, 116.79] (arom-C), 64.31 ($O\underline{CH_2}CH_3$), 20.94 ($PhCH_3$), 14.00 ($OCH_2\underline{CH_3}$).

Antioxidant Activity

Chemicals

Butylated hydroxytoluene (BHT), ferrous chloride, $DPPH^{\cdot}$, α-tocopherol, 3- butylated hydroxyanisole (BHA), (2-pyridyl)-5,6-bis(phenylsulfonic acid)-1,2,4-triazine (ferrozine) and trichloroacetic acid (TCA) were obtained from E. Merck or Sigma.

Reducing power

The reducing power of the synthesized compounds was determined according to the method of Oyaizu [12]. 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 and afterwards a portion (2.5 mL) of trichloroacetic acid (10%) was added to the mixture, which was centrifuged for 10 min at 1000 x g. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%), and then the absorbance at 700 nm was measured in a spectrophometer. Higher absorbance of the reaction mixture indicated greater reducing power.

Free radical scavenging activity

Free radical scavenging effect of the compounds was measured via $DPPH^{\cdot}$, by using the method of Blois [13]. 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 µg/mL). The mixture was shaken vigorously and allowed to remain at the room temperature for 30 min. Then, the absorbance was measured at 517 nm in a spectrophometer. The 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): Absorbance = $(0.0003 \times DPPH^{\cdot}) - 0.0174$

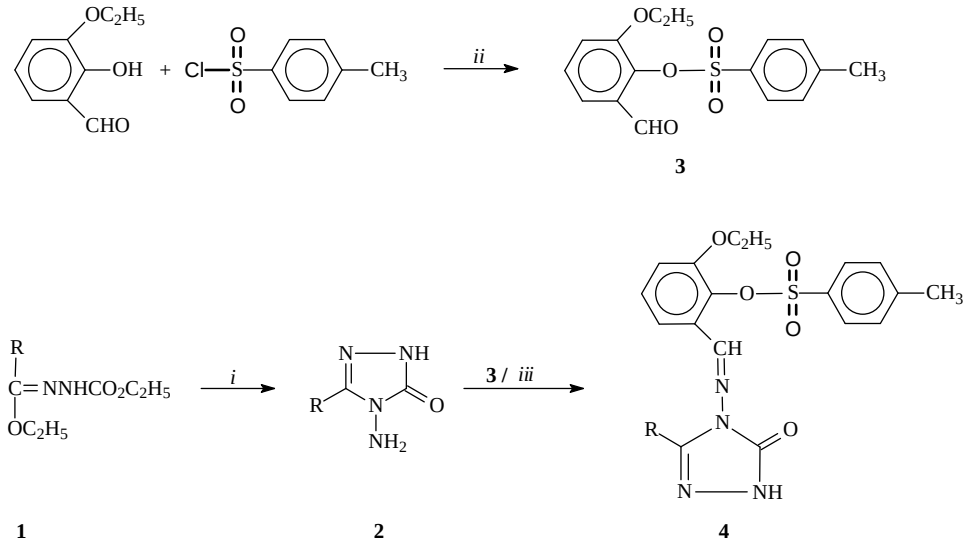
The capability to scavenge the DPPH radical was calculated by using the following equation: $DPPH^{\cdot}$ scavenging effect (%) = $(A_0 - A_1/A_0) \times 100$, where A_0 is the absorbance of the control reaction, and A_1 is the absorbance in the presence of the samples or standards.

Metal chelating activity

The chelating of ferrous ions by the synthesized compounds and estimated by the method of Dinis et al. [14]. In the study, all of the compounds and the standard antioxidants were dissolved in ethanol. Briefly, the synthesized compounds (30–90 µg/mL) were added to a 2 mM solution of $FeCl_2.4H_2O$ (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 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.

RESULTS AND DISCUSSION

In the study, 3-alkyl(aryl)-4-[3-ethoxy-2-(4-toluenesulfonyloxy)-benzyliden-amino]-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**4a-i**) were synthesized. The starting compounds **2a-i** were prepared as explained in the literature [15,16]. Compounds **4a-i** were prepared from the reactions of compounds **2a-i** with 3-ethoxy-2-(4-toluenesulfonyloxy)benzaldehyde **3**, which were obtained through the reactions of 3-ethoxy-2-hydroxybenzaldehyde including 4-toluenesulfonyl chloride with triethylamine (**Scheme 1**). Nine novel 3-alkyl(aryl)-4-[3-ethoxy-2-(4-toluenesulfonyloxy)-benzylidenamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**4a-i**) were characterized with IR, ^{1}H-NMR and ^{13}C-NMR spectral data.



***i*) $N_2H_4.H_2O$, reflux, 6 h; *ii*) Et_3N; *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 = $CH_2C_6H_4OCH3$ (p-), g) R = $CH_2C_6H_4Cl$ (p-), h) R = $CH_2C_6H_4Cl$ (m-), i) R = C_6H_5

Scheme 1. Synthetic route of compounds 4a-i

Antioxidant activity

The antioxidant capacities of ten newly synthesized compounds **4a-i** were determined. Different processes have been used to identify antioxidant capacities. The processes used in the paper are clarified below:

Reducing power

The compounds **4a-i** were screened for their in-vitro reducing activities by Fe^{3+}-Fe^{2+} transformation in the presence compounds samples. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant capacity. The presence of reductants like antioxidants substances in the antioxidant examples causes the reduction of the Fe^{3+} / ferricyanide complex to the ferrous form. Therefore, the Fe^{2+} may be monitored by measuring the formation of Perl's Prussian blue at 700 nm [17]. 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 [18]. In the present study, all of the concentrations of the compounds had a lower absorbance than the reference antioxidants. 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.

$DPPH^{\cdot}$ 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 $DPPH^{\cdot}$ scavenging was thought to be due to their hydrogen donating ability [19]. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [20]. The reduction capacity of DPPH radicals was defined of a decrease in the absorbance at 517 nm induced by antioxidants, resulting a color change from purple to yellow. In the study, antiradical capacities of compounds **4a-i** and reference antioxidants such as α-tocopherol, BHA and BHT were detected by using $DPPH^{\cdot}$ method. The newly synthesized compounds did not show significant ability like a radical scavenger as seen in the Figure 1. Compounds **4b** and **4i** showed very low activity in a concentration-dependent manner. The radical scavenging effect of the compounds and standards decreased in the order of α-tocopherol = BHA > BHT > **4i** > **4b** which were 73.8, 73.8, 50.8, 10.2, 5.3 (%), at the highest concentration, respectively.

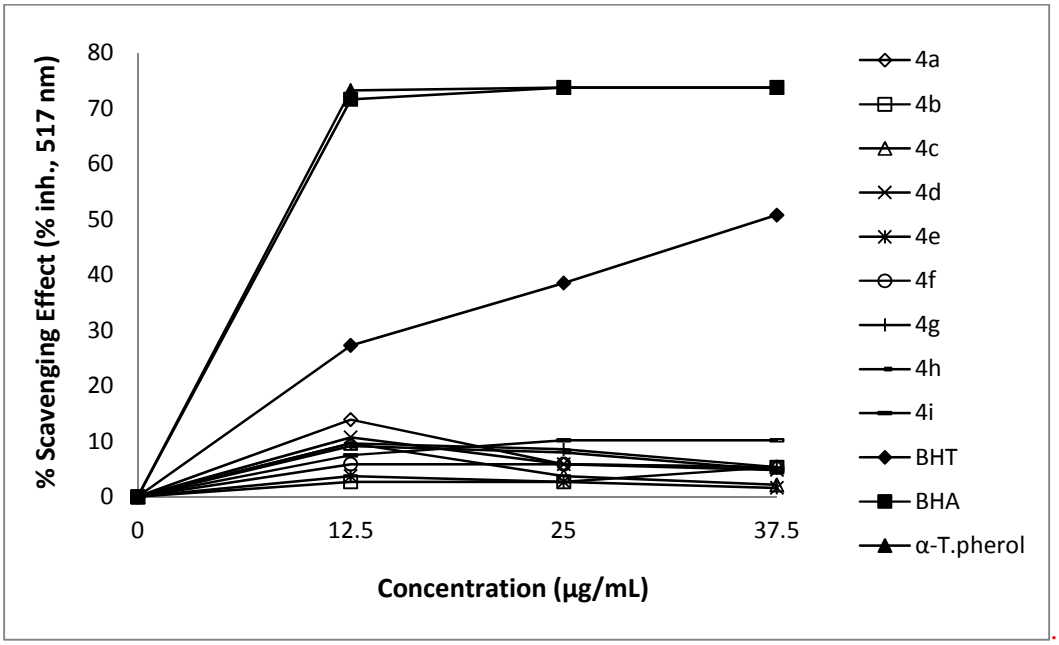


Figure 1. Scavenging effect of compounds 4, BHT, BHA and α-tocopherol at different concentrations (12,5-25-37.5 µg/mL).

Iron binding capacity

The chelating effect towards ferrous ions by the compounds and standards was determined. 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 [21]. 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 [22] 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 [23]. 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 [24].

Iron binding activities of the compounds **4**, EDTA and α-tocopherol are shown in Figure **2**. In the present paper, high iron binding capacity of synthesized compounds would be beneficial in retarding metal-chelating oxidation. The data acquired from Figure **2** disclose that the metal chelating effects of the compounds were not concentration-dependent.

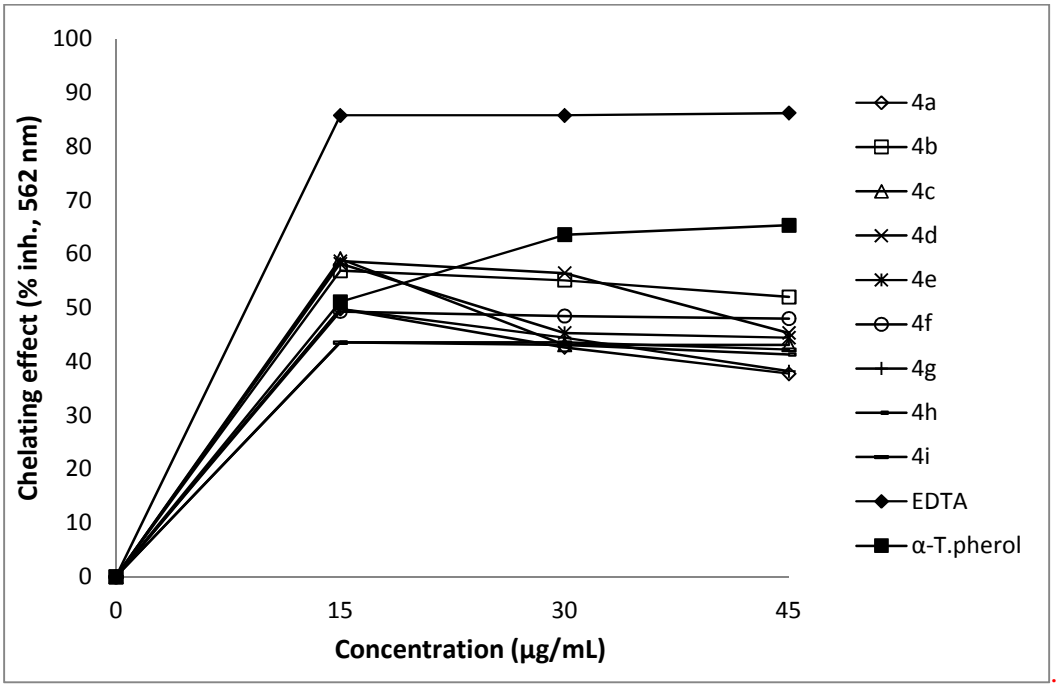


Figure 2. Iron binding effect of diverse amount of the compounds 4, and reference antioxidants

CONCLUSION

1,2,4-Triazoles have broad spectrum of biological activities. The synthesis and *in vitro* antioxidant evaluation of new 4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives are described.

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