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A study on 4-(3/4-carboxyphenyl) -4,5-dihydro-1*H*-1,2,4-triazol-5-ones

O. GURSOY KOL and H. YUKSEK*

Department of Chemistry, Faculty of Science and Education, Kafkas University, Kars (Turkey).

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ABSTRACT

A series of 3-alkyl(aryl)-4-(3/4-carboxyphenyl)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (2, 3) were obtained from the reactions of ester ethoxycarbonylhydrazones (1) with 3-aminobenzoic acid and 4-aminobenzoic acid, respectively. The chemical structures of the compounds were elucidated by IR, ^{1}H NMR, ^{13}C-NMR and UV spectral data. The synthesized compounds were analyzed for their *in vitro* antioxidant activities in three different methods including reducing power, free radical scavenging and metal chelating activity. In addition, the compounds 2 and 3 were titrated potentiometrically with tetrabutylammonium hydroxide in four non-aqueous solvents. The half-neutralization potential values and the corresponding pK_a values were determined for all cases.

Key words: 4,5-Dihydro-1*H*-1,2,4-triazol-5-one, Schiff base, antioxidant activity, potentiometric titrations.

INTRODUCTION

Several articles on the synthesis and some reactions of various ester ethoxycarbony lhydrazones have been reported[1-5]. In addition, 1,2,4-triazole and 4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives are reported to possess a broad spectrum of biological activities such as antifungal, antimicrobial, hypoglycemic, antihypertensive, analgesic, antiparasitic, hypocholesteremic, antiviral, anti-inflammatory, antitumor and anti-HIV properties[6-14].

Besides, antioxidants have become one of the major areas of scientific research. Antioxidants are extensively studied for their capacity to protect organism and cell from damage that are induced by oxidative stress. Scientists in many different disciplines become more interested in new compounds, either synthesized or obtained from natural sources that could provide active components to prevent or reduce the impact of oxidative stress on 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 play a significantly 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 and which exceeds the antioxidant capacity of the organism leads to variety of pathophysiological processes such as inflammation, diabetes, genotoxicity and cancer[16]. In the present study, due to a wide range applications and 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.

On the other hand, it is known that 1,2,4-triazole and 4,5-dihydro-1*H*-1,2,4-triazol-5-one rings have weak acidic properties, so some 1,2,4-triazole and 4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives were titrated potentiometrically with tetrabutyl ammonium hydroxide in non-aqueous solvents, and the corresponding p*K*a values of the compounds were determined[14,17-23].

In this paper, we present the synthesis of a series of 3-alkyl(aryl)-4-(3-carboxyphenyl)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (2b-f) and 3-alkyl(aryl)-4-(4-carboxyphenyl)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (3a-f). The starting compounds (1a-f) were prepared according to literature (5, 24). Compounds 2 and 3 were obtained from the reactions of compounds 1 with 3-aminobenzoic acid and 4-aminobenzoic acid (Scheme 1). Next part of the study, the antioxidant activities of 11 new compounds was determined. Furthermore, we also examined the potentiometric titrations of the synthesized compounds 2 and 3 with tetrabutylammonium hydroxide (TBAH) in four non-aqueous solvents (isopropyl alcohol, *tert*-butyl alcohol, *N,N*-dimethylformamide and acetone) to determine the corresponding half-neutralization potentials (HNP) and the corresponding p*K*a values. 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 were studied[14,17-23].

EXPERIMENTAL

Chemical reagents and all solvents used in this study were purchased from Merck AG, Aldrich and Fluka. Melting points were taken on an Electrothermal 9100 digital melting point apparatus and are uncorrected. IR spectra were registered on a Perkin-Elmer Instruments Spectrum One FT-IR spectrometer. ^{1}H-NMR and ^{13}C-NMR spectra were recorded in deuterated dimethyl sulfoxide with TMS as internal standard on a Varian Mercury spectrometer at 200 MHz and 50 MHz, respectively. UV absorption spectra were measured in 10 mm quartz cells between 200 and 400 nm using a Shimadzu UV-1201 spectrophotometer. Combustion analyses were performed on a ECS 4010 Costech Elemental Elemental Analyzer. Butylated hydroxytoluene (BHT) was purchased from E. Merck. Ferrous chloride, á-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) and trichloracetic acid (TCA) were bought from Sigma. In this study, a Jenway 3040 ion analyser pH meter equipped with an Ingold pH electrode was used for potentiometric titrations.

General method for the synthesis of 3-alkyl(aryl)-4-(3/4-carboxyphenyl)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (2b-f / 3a-f)

The corresponding compound 1 (0.01 mol) was heated with 3/4-aminobenzoic acid (1.37 g, 0.01 mol) at 110-185 ºC for 1.5 h and cooled. Several recrystallizations of the crude product from EtOH-H_2O (1:3) gave pure compounds 2b-f and 3a-f as colourless crystals.

3-Benzyl-4-(3-carboxyphenyl)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (2b)

This compound was obtained as white needles (yield: 2.16 g, 73%); M.P. 254 °C; IR (KBr) (ν, cm^{-1}): 3351-2508 (COOH), 3198 (NH), 1736, 1682 (C=O), 1606, 1588 (C=N), 819 and 705 (1,3-disubstitue benzenoid ring), 755 and 683 (monosubstitue benzenoid ring); ^{1}H-NMR (DMSO-d_6, δ ppm): 3.82 (s, 2H, CH_2), 6.91-6.95 (m, 2H, ar-H), 7.13-7.15 (m, 3H, ar-H), 7.51-7.55 (m, 2H, ar-H), 7.69 (s, 1H, ar-H), 7.92-7.95 (m, 1H, ar-H), 11.85 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6, δ ppm): 31.96 (CH_2), ar-C: [126.66, 128.17, 128.25 (2C), 128.50 (2C), 129.22, 129.61, 131.69, 131.85, 133.02, 134.80], 145.79 (triazole-C_3), 154.32 (triazole-C_5), 166.33 (C=O); UV λ_{max} (e): 214 (31451) nm. *Anal.* Calcd. (%) for $C_{16}H_{13}N_3O_3$: C, 65.08; H, 4.44; N, 14.23. Found; C, 65.08; H, 4.42; N, 14.21.

3-*p*-Methylbenzyl-4-(3-carboxyphenyl)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (2c)

This compound was obtained as white needles (yield: 2.48 g, 80%); M.P. 238 °C; IR (KBr) (ν, cm^{-1}): 3420-2532 (COOH), 3194 (NH), 1716, 1700 (C=O), 1595, 1573 (C=N), 760 and 681 (1,3-disubstitue benzenoid ring); ^{1}H-NMR (DMSO-d_6, δ ppm): 2.17 (s, 3H, CH_3), 3.75 (s, 2H, CH_2), 6.80 (d,

2H, ar-H, *J*=7.72 Hz), 6.95 (d, 2H, ar-H, *J*=7.72 Hz), 7.52-7.55 (m, 2H, ar-H), 7.66 (s, 1H, ar-H), 7.92-7.96 (m, 1H, ar-H), 11.82 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6, δ ppm): 21.24 (CH_3), 32.28 (CH_2), ar-C: [128.91, 129.06 (2C), 129.54 (2C), 129.91, 130.30, 132.44 (2C), 132.56, 133.76, 136.43], 146.64 (triazole-C_3), 155.04 (triazole-C_5), 167.05 (C=O); UV λ_{max} (e): 213 (21407) nm.

3-*p*-Chlorobenzyl-4-(3-carboxyphenyl)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (2d)

This compound was obtained as white needles (yield: 2.25 g, 68%); M.P. 225 °C; IR (KBr) (ν, cm^{-1}): 3300-2519 (COOH), 3269 (NH), 1703, 1653 (C=O), 1609, 1587 (C=N), 825 (1,4-disubstitue benzenoid ring), 799 and 684 (1,3-disubstitue benzenoid ring); ^{1}H-NMR (DMSO-d_6, δ ppm): 3.82 (s, 2H, CH_2), 6.99 (d, 2H, ar-H, *J*=8.39 Hz), 7.22 (d, 2H, ar-H, *J*=8.39 Hz), 7.54-7.57 (m, 2H, ar-H), 7.70 (s, 1H, ar-H), 7.93 (m, 1H, ar-H), 11.84 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6, δ ppm): 31.24 (CH_2), ar-C: [128.11 (2C), 129.21, 129.60, 130.46 (2C), 130.98, 131.30, 131.64, 131.87, 132.91, 133.77], 145.43 (triazole-C_3), 154.23 (triazole-C_5), 166.25 (C=O); UV λ_{max} (e): 213 (21429) nm. *Anal.* Calcd. (%) for $C_{16}H_{12}ClN_3O_3$: C, 58.28; H, 3.67; N, 12.74. Found; C, 58.27; H, 3.69; N, 12.77.

3-Phenyl-4-(3-carboxyphenyl)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (2e)

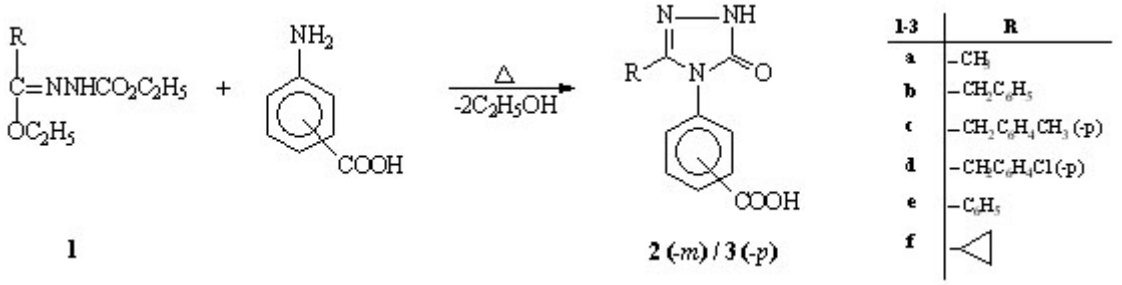
This compound was obtained as white needles (yield: 1.92 g, 68%); M.P. 293 °C; IR (KBr) (ν, cm^{-1}): 3500-2501 (COOH), 3233 (NH), 1699, 1653 (C=O), 1608, 1588 (C=N), 802 and 715 (1,3-disubstitue benzenoid ring), 761 and 694 (monosubstitue benzenoid ring); ^{1}H-NMR (DMSO-d_6, δ ppm): 7.25-7.59 (m, 7H, ar-H), 7.82 (s, 1H, ar-H), 7.94-7.98 (m, 1H, ar-H), 12.24 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6, δ ppm): ar-C: [126.75, 127.64 (2C), 128.09, 128.51 (2C), 129.05, 129.55, 129.84, 131.81 (2C), 133.85], 145.12 (triazole-C_3), 154.29 (triazole-C_5), 166.29 (C=O); UV λ_{max} (e): 256 (5473), 214 (18382) nm. *Anal.* Calcd. (%) for $C_{15}H_{11}N_3O_3$: C, 64.05; H, 3.94; N, 14.94. Found; C, 63.98; H, 3.99; N, 14.98.

3-Cyclopropyl-4-(3-carboxyphenyl)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (2f)

This compound was obtained as white needles (yield: 1.59 g, 65%); M.P. 226 °C; IR (KBr) (ν, cm^{-1}): 3300-2493 (COOH), 3282 (NH), 1707, 1654 (C=O), 1579 (C=N), 791 and 718 (1,3-disubstitue benzenoid ring); ^{1}H-NMR (DMSO-d_6, δ ppm): 0.77-0.86 (m, 4H, CH_2CH_2), 1.45-1.60 (m, 1H, CH), 7.65-7.72 (m, 2H, ar-H), 7.98-8.01 (m, 2H, ar-H), 11.66 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6, δ ppm): aliph-C: [6.15 (2C), 6.61 (C)], ar-C: [127.56, 128.88, 129.65, 131.23, 131.91, 133.29], 147.72 (triazole-C_3), 154.13 (triazole-C_5), 166.43 (C=O); UV λ_{max} (e): 219 (19850) nm.

3-Methyl-4-(4-carboxyphenyl)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (3a)

This compound was obtained as white needles (yield: 1.50 g, 68%); M.P. 294 °C; IR (KBr) (ν, cm^{-1}): 3200-2615 (COOH), 3181 (NH), 1713 (C=O), 1596 (C=N), 816 (1,4-disubstitue benzenoid ring); ^{1}H-NMR (DMSO-d_6, δ ppm): 2.09 (s, 3H, CH_3), 7.54 (d, 2H, ar-H, *J*=8.39 Hz), 8.05 (d, 2H, ar-H, *J*=8.39 Hz), 11.73 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6, δ ppm): 12.44 (CH_3), ar-C: [112.53, 126.82 (2C), 130,30 (2C), 136.78], 143.52 (triazole-C_3), 153.91 (triazole-C_5), 166.61 (C=O); UV λ_{max} (e): 256 (3274), 214 (9022) nm. *Anal.* Calcd. (%) for $C_{10}H_9N_3O_3$: C, 54.79; H, 4.14; N, 19.17. Found; C, 54.79; H, 4.13; N, 19.14.



Scheme 1.

3-Benzyl-4-(4-carboxyphenyl)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (3b)

This compound was obtained as white needles (yield: 2.05 g, 69%); M.P. 266 °C; IR (KBr) (ν, cm^{-1}): 3300-2520 (COOH), 3176 (NH), 1721, 1699 (C=O), 1606, 1575 (C=N), 824 (1,4-disubstitue benzenoid ring), 769 and 699 (monosubstitue benzenoid ring); ^{1}H-NMR (DMSO-d_6, δ ppm): 3.86 (s, 2H, CH_2), 6.93-6.96 (m, 2H, ar-H), 7.12-7.16 (m, 3H, ar-H), 7.38 (d, 2H, ar-H, *J*=8.39 Hz), 7.96 (d, 2H, ar-H, *J*=8.39 Hz), 11.89 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6, δ ppm): 31.97 (CH_2), ar-C: [126.69, 127.27 (2C), 128.27 (2C), 128.52 (2C), 130.18 (2C), 130.44, 136.57, 137.79], 145.65 (triazole-C_3), 154.10 (triazole-C_5), 166.58 (C=O); UV λ_{max} (e): 255 (5662), 216 (14662) nm. *Anal.* Calcd. (%) for $C_{16}H_{13}N_3O_3$: C, 65.08; H, 4.44; N, 14.23. Found; C, 65.06; H, 4.41; N, 14.24.

3-*p*-Methylbenzyl-4-(4-carboxyphenyl)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (3c)

This compound was obtained as white needles (yield: 1.95 g, 63%); M.P. 245 °C; IR (KBr) (ν, cm^{-1}): 3520-2500 (COOH), 3215 (NH), 1712, 1698 (C=O), 1595, 1574 (C=N), 806 (1,4-disubstitue benzenoid ring); ^{1}H-NMR (DMSO-d_6, δ ppm): 2.17 (s, 3H, CH_3), 3.80 (s, 2H, CH_2), 6.82 (d, 2H, ar-H, *J*=8.06 Hz), 6.96 (d, 2H, ar-H, *J*=8.06 Hz), 7.38 (d, 2H, ar-H, *J*=8.39 Hz), 7.97 (d, 1H, ar-H, *J*=8.39 Hz), 11.88 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6, δ ppm): 21.24 (CH_3), 32.25 (CH_2), ar-C: [127.95 (2C), 129.06 (2C), 129.57 (2C), 129.67, 130.91 (2C), 131.15, 132.41, 136.44], 146.47 (triazole-C_3), 154.80 (triazole-C_5), 167.30 (C=O); UV λ_{max} (e): 256 (4059), 213 (25426) nm.

3-*p*-Chlorobenzyl-4-(4-carboxyphenyl)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (3d)

This compound was obtained as white needles (yield: 2.17 g, 66%); M.P. 253 °C; IR (KBr) (ν, cm^{-1}): 3520-2505 (COOH), 3222 (NH), 1711, 1700 (C=O), 1573 (C=N), 830, 801 (1,4-disubstitue benzenoid ring); ^{1}H-NMR (DMSO-d_6, δ ppm): 3.87 (s, 2H, CH_2), 7.00 (d, 2H, ar-H, *J*=8.39 Hz), 7.22 (d, 2H, ar-H, *J*=8.06 Hz), 7.41 (d, 2H, ar-H, *J*=8.39 Hz), 7.97 (d, 2H, ar-H, *J*=8.39 Hz), 11.89 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6, δ ppm): 31.99 (CH_2), ar-C: [127.96 (2C), 128.91 (2C), 130.93 (2C), 131.24 (3C), 132.07, 134.51, 137.20], 146.05 (triazole-C_3), 154.77 (triazole-C_5), 167.27 (C=O); UV λ_{max} (e): 254 (7907), 214 (35926) nm.

3-Phenyl-4-(4-carboxyphenyl)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (3e)

This compound was obtained as white needles (yield: 2.73 g, 97%); M.P. 280 °C; IR (KBr) (ν, cm^{-1}): 3461-2520 (COOH), 3283 (NH), 1701 (C=O), 1607 (C=N), 808 (1,4-disubstitue benzenoid ring), 771 and 698 (monosubstitue benzenoid ring); ^{1}H-NMR (DMSO-d_6, δ ppm): 7.24-7.38 (m, 7H, ar-H), 7.97 (d, 2H, ar-H), 12.29 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6, δ ppm): ar-C: [126.72, 127.49 (2C), 127.64 (2C), 128.56 (2C), 129.90, 130.15 (2C), 130.36, 137.31], 145.09 (triazole-C_3), 154.09 (triazole-C_5), 166.52 (C=O); UV λ_{max} (e): 253 (6254), 212 (17727) nm. *Anal.* Calcd. (%) for $C_{15}H_{11}N_3O_3$: C, 64.05; H, 3.94; N, 14.94. Found; C, 64.07; H, 3.94; N, 14.92.

3-Cyclopropyl-4-(4-carboxyphenyl)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (3f)

This compound was obtained as white needles (yield: 1.62 g, 66%); M.P. 234 °C; IR (KBr) (ν, cm^{-1}): 3480-2511 (COOH), 3237 (NH), 1711, 1694 (C=O), 1608, 1590 (C=N), 810 (1,4-disubstitue benzenoid ring); ^{1}H-NMR (DMSO-d_6, δ ppm): 0.74-0.85 (m, 4H, CH_2CH_2), 1.58 (pentet, 1H, CH), 7.60 (d, 2H, ar-H, *J*=8.39 Hz), 8.07 (d, 2H, ar-H, *J*=8.39 Hz), 11.70 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6, δ ppm): aliph-C: [6.30, 6.71, 7.55], ar-C: [118.14, 126.82 (2C), 130.26 (2C), 136.81], 147.67 (triazole-C_3), 153.97 (triazole-C_5), 166.61 (C=O); UV λ_{max} (e): 261 (4425), 212 (15625) nm. *Anal.* Calcd. (%) for $C_{12}H_{11}N_3O_3$: C, 58.77; H, 4.52; N, 17.13. Found; C, 58.73; H, 4.54; N, 17.12.

Reducing power

The reducing power of the synthesized compounds (except 5f) was determined according to the method of Oyaizu (25). Different concentrations of the samples (50-250 ìg/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 oC 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 spectrophometer. Higher

absorbance of the reaction mixture indicated greater reducing power.

Free radical scavenging activity

Free radical scavenging activity of compounds was measured by $DPPH^{\cdot}$, using the method of Blois[26]. 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 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 $DPPH^{\cdot}$ concentration (mM) in the reaction medium was calculated from the following calibration curve and determined by linear regression (R: 0.997): Absorbance = 0.0003x$DPPH^{\cdot}$ – 0.0174. The capability to scavenge the DPPH radical was calculated using the following equation:

$$DPPH^{\cdot} \text{ scavenging effect (\%)} = (A_0 - A_1/A_0) \times 100 \quad ...(1)$$

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

Potentiometric titrations

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

RESULTS AND DISCUSSION

In this study, the structures of five new 3-alkyl(aryl)-4-(3-carboxyphenyl)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (2b-f) and six new 3-alkyl(aryl)-4-(4-carboxyphenyl)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (3a-f) were identified using elemental analysis and IR, ^{1}H-, ^{13}C-NMR and UV spectral data, and the observed spectral values were seen to be compatible with literature values[12-14,17-21].

Antioxidant Activity

The compounds 2 and 3 were screened for their *in vitro* antioxidant activities. Several methods are used to determine antioxidant activities. The methods used in the study are given below:

Total reductive capability using the potassium ferricyanide reduction method: The reductive capabilities of compounds are assessed by the extent of conversion of the Fe^{3+} / ferricyanide complex to the Fe^{2+} / ferrous form using the method of Oyaizu[25]. The reducing powers of the compounds were observed at different concentrations, and results were compared with BHA, BHT and á-tocopherol. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity[28]. 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[29]. In this study, all of the amounts of the compounds showed lower absorbance then blank. Hence, no activities were observed to reduce metal ions complexes to their lower oxidation state or to

Table 1: The HNP and the corresponding p*K*a values of compounds 2 in isopropyl alcohol, *tert*-butyl alcohol, DMF and acetone at 25 °C

Comp.	Isopropyl alcohol				*tert*-Butyl alcohol				DMF				Acetone			
	HNP_1 (mV)	pKa_1	HNP_2 (mV)	pKa_2	HNP_1 (mV)	pKa_1	HNP_2 (mV)	pKa_2	HNP_1 (mV)	pKa_1	HNP_2 (mV)	pKa_2	HNP_1	pKa_1 (mV)	HNP_2	pKa_2 (mV)
2b	-105	7,55	-	-	-181	9,60	-525	16,23	-303	11,61	-569	17,12	-342	12,44	-608	17,97
2c	-108	7,60	-	-	-241	10,03	-579	17,35	-322	12,02	-582	17,44	-370	13,04	-632	18,49
2d	-159	8,62	-	-	-264	10,69	-	-	-477	15,19	-	-	-375	13,05	-619	18,08
2e	-109	7,64	-	-	-158	7,48	-468	15,02	-452	14,61	-	-	-340	12,38	-561	16,94
2f	-104	7,50	-	-	-171	8,71	-494	15,54	-287	11,26	-	-	-351	12,59	-	-

Table 2: The HNP and the corresponding pKa values of compounds 3 in isopropyl alcohol, tert-butyl alcohol, DMF and acetone at 25°C

Comp.	Isopropyl alcohol				*tert*-Butyl alcohol				DMF				Acetone			
	HNP_1 (mV)	pKa_1	HNP_2 (mV)	pKa_2	HNP_1 (mV)	pKa_1	HNP_2 (mV)	pKa_2	HNP_1 (mV)	pKa_1	HNP_2 (mV)	pKa_2	HNP_1	pKa_1 (mV)	HNP_2	pKa_2 (mV)
3a	-100	7,59	-	-	-173	8,71	-540	13,93	-313	11,78	-415	13,73	-314	11,79	-624	17,70
3b	-136	8,22	-	-	-228	10,04	-531	15,82	-319	11,83	-568	16,82	-351	12,49	-619	17,83
3c	-124	7,97	-	-	-216	9,79	-540	16,32	-357	12,65	-592	17,39	-376	13,01	-603	17,59
3d	-113	7,76	-	-	-189	9,35	-496	15,44	-313	11,78	-563	16,85	-370	12,92	-561	16,86
3e	-147	8,38	-453	14,45	-225	9,95	-638	17,88	-297	11,30	-534	15,89	-329	11,91	-570	16,58
3f	-154	8,74	-	-	-164	8,82	-531	15,08	-356	12,59	-594	17,12	-482	14,98	-	-

take part in any electron transfer reaction. In other words, compounds did not show the reductive activities.

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[30]. 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 caused by antioxidants, because of reaction between antioxidant molecules and radical, progresses, which result in the scavenging of the radical by hydrogen donation. It is visually noticeable as a discoloration from purple to yellow. Hence, DPPH· is usually used as a substrate to evaluate antioxidative activity of antioxidants[31]. In the study, antiradical activities of compounds and standard antioxidants such as BHA and α-tocopherol were determined by using DPPH· method. Scavenging effect values of compounds 2, 3, BHA and á-tocopherol at different concentrations are given Figures 1, 2 respectively. All the compounds tested with this method showed lower absorbance than absorbance of the control reaction and higher absorbance than that of the standard antioxidant reactions. The data obtained in the study indicate that the newly synthesized compounds showed mild activities as a radical scavenger, indicating that it has moderate activities as hydrogen donors.

Ferrous ion chelating activity

The chelating effect towards ferrous ions by the compounds and standards was determined according to the method of Dinis[32]. 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[33]. 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 [34] 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[35]. 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:

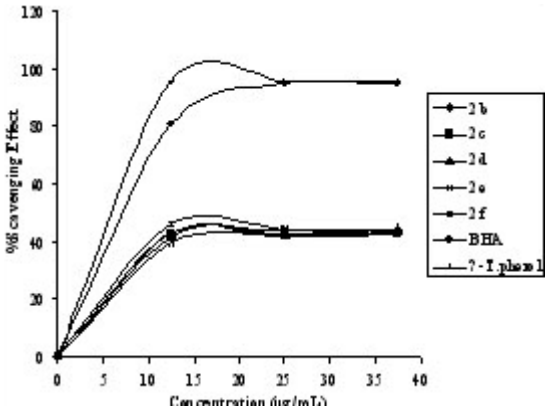


Fig. 1: Scavenging effect of compounds 2b-f, BHA and α-tocopherol at different concentrations (12.5-25-37.5 µg/mL)

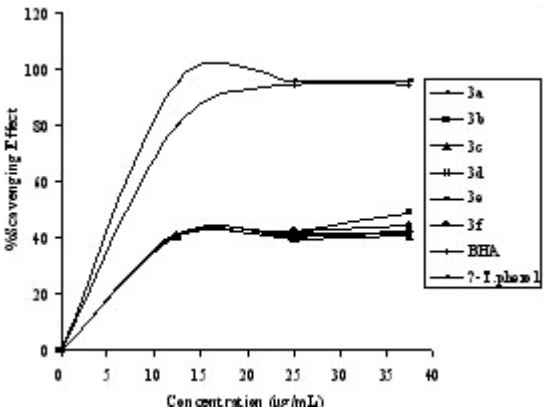


Fig. 3: Scavenging effect of compounds 3a-f, BHA and α-tocopherol at different concentrations (12.5-25-37.5 µg/mL)

$$O^{\cdot}_2 + H_2O_2 \rightarrow O_2 + OH^- + OH^-$$

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:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^-$$

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[36]. Ferrous ion chelating activities of the compounds 2, 3, BHT and á-tocopherol are shown in Figures 3, and 4, 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 ó-bonds with a metal are effective as secondary antioxidants because they reduce the redox potential thereby stabilizing the oxidized form of metal ion[37]. The data obtained from Figures 3 and 4 reveal that the compounds, demonstrate a marked capacity for iron binding, suggesting that their action as peroxidation protectors may be related to their iron binding capacity. On the other hand, free iron is known to have low solubility and a chelated iron complex has greater solubility in solution, which can be contributed solely by the ligand. Furthermore, the compound-iron complex may also be active, since it can participate in iron-catalyzed reactions.

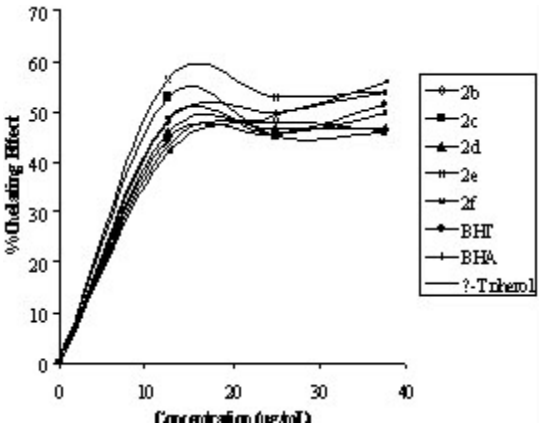


Fig. 3: Metal chelating effect of different amount of the compounds 2b-f, BHT and α-tocopherol on ferrous ions

Potentiometric titrations

In order to determine the pK_a values of the compounds 2b-f and 3a-f were titrated potentiometrically with TBAH in four non-aqueous solvents: isopropyl alcohol, *tert*-butyl alcohol, acetone and DMF. After the potentiometric titrations

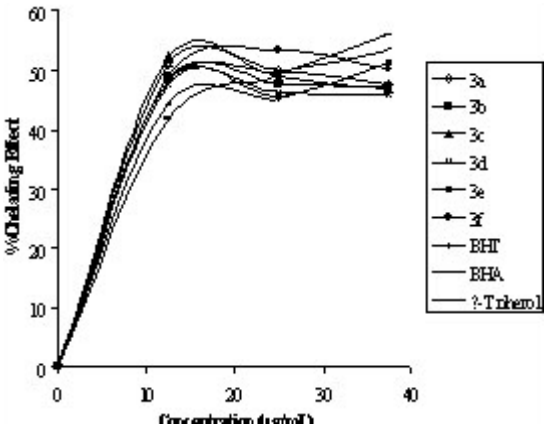


Fig. 4: Metal chelating effect of different amount of the compounds 3a-f, BHT and α-tocopherol on ferrous ions

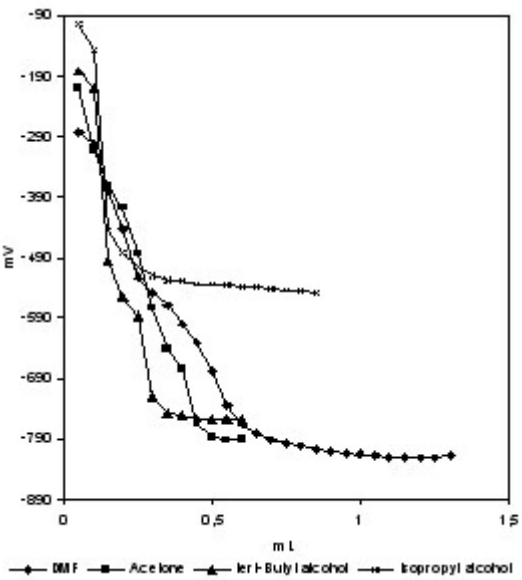


Fig. 5: Potentiometric titration curves of 0.001 M solutions of compound 2b titrated with 0.05 M TBAH in four non-aqueous solvents at 25 °C

of compounds 2 and 3 with TBAH in non-aqueous solvents, the mV values from each titration were plotted against TBAH volumes used (mL) and the potentiometric titration curves were obtained for all the cases. From the titration curves, the HNP values and the corresponding p*K*a values were obtained. As an example, the potentiometric titration curves for 0.001 M solutions of 3-benzyl-4-(3-carboxyphenyl)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (2b) titrated with 0.05 N TBAH in isopropyl alcohol, *tert*-butyl alcohol, *N,N*-dimethylformamide and acetone are presented in Fig. 5.

The half-neutralization potential (HNP) values and the corresponding p*K*a values of compounds 2 and 3, obtained from the potentiometric titrations with 0.05 M TBAH in isopropyl alcohol, *tert*-butyl alcohol, *N,N*-dimethylformamide and acetone are presented in Tables 1 and 2, respecticely.

The *p*H of weak acids can be calculated using the following equation:

$$pH = pKa + \log[A^-] / [HA]$$

where *p*H = *pK*a when [A-] is equal to [HA] at the half-neutralization points. Therefore, the *p*H values at the half-neutralization points were taken as *pK*a. Taking into consideration the dielectric permittivity of the solvents, the acidity ranking might be expected to be as follows: *N,N*-dimethylformamide (å=37) > acetone (20,6) > isopropyl alcohol (å=19.4) > *tert*-butyl alcohol (å=12).

As seen in Scheme 1, there is one weak acidic N-H group in 4,5-dihydro-1*H*-1,2,4-triazol-5-one ring and one carboxyl group in compounds 2 and 3. Thus, these compounds give two end points as well as two half neutralization potential values. The potentiometric titration curves of these compounds titrated with TBAH in isopropyl alcohol, *tert*-butyl alcohol, DMF and acetone resemble the titration curves of diprotic acids.

As seen in Table 1, for compounds 2b-f in isopropyl alcohol, compounds 2d-f in DMF, compound 2d in *tert*-butyl alcohol and compound 2f in acetone, the second p*K*a values have not been obtained. According to pKa_1 values the acidity order for compounds 2b, 2c and 2f is: isopropyl alcohol > *tert*-butyl alcohol > DMF > acetone, for compound 2d is isopropyl alcohol > *tert*-butyl alcohol > acetone > DMF and for compound 2e is *tert*-butyl alcohol > isopropyl alcohol > acetone > DMF. In addition, for compounds 3a-d and 3f in isopropyl alcohol and compound 3f in acetone, the second p*K*a values have not been obtained. According to pKa_1 values the acidity order for all the compounds 3 is: isopropyl alcohol > *tert*-butyl alcohol > DMF > acetone.

As it is well known, the acidity of a compound depends on several factors. The two most important ones are the solvent effect and molecular structure (14,17-23). Tables 1-2 and Figure 5 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.

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