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ISSN: 0973-4945; CODEN ECJHAO
E-Journal of Chemistry
2011, **8(4),** 1734-1746

InVitro Antioxidant Activities of New 4,5-Dihydro-1*H*-1,2,4-triazol-5-ones having Thiophene Ring with their Acidic Properties

H. YUKSEK, F. ISLAMOGLU[*], O. GURSOY KOL,
S. BAHCECI[§], M. BEKAR[§] and M. AKSOY[§]

Department of Chemistry, Faculty of Science and Arts
Kafkas University, 36100 Kars, Turkey

[*]Department of Chemistry, Faculty of Science and Arts
Rize University, 53100 Rize, Turkey

[§]Fatih Education Faculty, Karadeniz Technical University
61355 Trabzon, Turkey

fatihislamoglu53@hotmail.com

Received 19 February 2011; Accepted 16 April 2011

Abstract: Seven new 3-alkyl(aryl)-4-(2-thienymethylenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**2**) were synthesized by the reactions of 3-alkyl(aryl)-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**1**) with thiophene-2-carbaldehyde. In addition, *N*-acetyl derivatives of compounds **2d-2g** were also prepared. The structures of eleven new compounds synthesized were determined by elemental analysis as well as IR, NMR and UV spectral data. In addition, compounds **2a-g** and **3a, 3b, 3d-f** were also screened for their antioxidant activities and **2a-g** were potentiometrically titrated with tetrabutylammonium hydroxide (TBAH) in four nonaqueous solvents (isopropyl alcohol, *t*-butyl alcohol, acetonitrile and *N,N*-dimethyl formamide). Also half-neutralization potential values and the corresponding pKa values were determined in all cases.

Keywords: 4,5-Dihydro-1*H*-1,2,4-triazol-5-ones, Synthesis, Schiff bases, Acetylation, Acidity, Potentiometric titrations

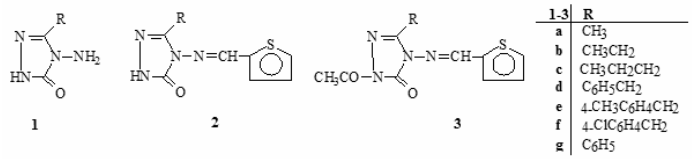
Introduction

Several articles, involving the synthesis of some *N*-arylidenamino-4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives, have been published[1-9]. Also, the reactions of some 4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives with acetic anhydride were investigated[5-10]. In addition, 1,2,4-triazole and 4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives are reported to show a broad

spectrum of biological activities[4,11-15]. On the other hand, there have been a number of systematic studies of the acidity in different media using different techniques[6-30], but unfortunately very few have dealt with triazoles. It is well known that two major factors influence the acidity of a molecule[31-34], namely, structural and solvent effects. In most molecules there are two or more structural effects and it is usually very difficult to assess how much each effect contributes to the acidity of a molecule. Moreover, it is sometimes extremely difficult to differentiate between structural and solvent effects.

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, that could provide active components to prevent or reduce the impact of oxidative stress on cells[35]. 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 ROS (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[36].

This paper describes the synthesis of a series of 3-alkyl(aryl)-4-(2-thienymethylenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**2**) from the reactions of 3-alkyl(aryl)-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones **(1)** with thiophene-2-carbaldehyde. Besides, the reactions of compounds **2a, 2b, 2d-g** with acetic anhydride affording the corresponding compounds **3a, 3b, 3d-g** were investigated (Scheme 1). Furthermore, the antioxidant activities of 13 new compounds was determined.



Scheme 1. Synthesis route of compounds **1** - **3**

Experimental

Melting points were taken on a electrothermal digital melting point apparatus and are uncorrected. IR spectra (wavenumbers in cm^{-1}) were registered on a Perkin-Elmer 1600 FTIR spectrometer. ^1H NMR spectra were recorded in deuterated dimethyl sulfoxide on a Varian 60A spectrometer with TMS as internal standard. Chemical shifts are given in ppm (δ-scale). UV absorption spectra (λ_{max} in nm) were measured in 10 mm quartz cells between 200 and 400 nm using a Shimadzu UV-1201 spectrophotometer. Elemental analyses were performed using a Carlo-Erba 1106 analyzer. The starting compounds **1a-1g** were prepared from the reactions of the corresponding ester ethoxycarbonylhydrazones with hydrazine hydrate according to the literature[10,37].

General procedure for preparation of 3-alkyl(aryl)-4-(2-thienymethylenamino)-4,5-dihydro-1H-1,2,4-triazol-5-ones (2)

3-Alkyl(aryl)-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-one (**1**) (0.01 mol) was heated with thiophene-2-carbaldehyde (1.12 g, 0.01 mol) at 160-165 °C (170-175 °C for compounds **2d-2g**) for 1.5 h and cooled. Several recrystallizations of the residue from an appropriate solvent gave pure compounds **2**.

*3-Methyl-4-(2-thienymethylenamino)-4,5-dihydro-1H-1,2,4-triazol-5-ones (**2a**)*

Colourless crystals, yield 99%. M.p. 174 °C (EtOH). For $C_8H_8N_4OS$ (208.2) calculated: 46.14% C, 3.87% H, 26.91% N; found: 45.65% C, 3.63% H, 27.70% N. ^{1}H NMR (DMSO-d_6): 2.30 (s, 3H, CH_3); 7.10-7.34 (m, 1H, Ar-H); 7.60-7.90 (m, 2H, Ar-H); 9.82 (s, 1H, N=CH); 11.70 (s, 1H, NH). IR (KBr): 3140 (NH), 1698 (C=O), 1598, 1585 (C=N) cm^{-1}. UV (ethanol), λ_{max} (ε): 313 (11020), 264 (7200), 207 (5940) nm. MS: *m/z* 231 (M+23), 185, 169, 142, 115, 96 (100), 69, 60.

*3-Ethyl-4-(2-thienymethylenamino)-4,5-dihydro-1H-1,2,4-triazol-5-ones (**2b**)*

Colourless crystals, yield 98%. M.p. 157 °C (EtOH-H_2O, 1 : 3). For $C_9H_{10}N_4OS$ (222.3) calculated: 48.64% C, 4.54% H, 25.22% N; found: 48.49% C, 4.45% H, 25.39% N. ^{1}H NMR (DMSO-d_6): 1.34 (t, 3H, CH_3); 2.76 (q, 2H, CH_2); 7.10-7.34 (m, 1H, Ar-H); 7.60-7.98 (m, 2H, Ar-H); 9.82 (s, 1H, N=CH); 11.70 (s, 1H, NH). IR (KBr): 3150 (NH), 1708 (C=O), 1600, 1590 (C=N) cm^{-1}. UV (ethanol), λ_{max} (ε): 332 (10490), 319 (11560), 280 (8850), 222 (8260) nm. MS: *m/z* 245 (M+23), 239, 231, 185, 169, 142, 96 (100), 69, 60.

*3-n-Propyl-4-(2-thienymethylenamino)-4,5-dihydro-1H-1,2,4-triazol-5-ones (**2c**)*

Colourless crystals, yield 98%. M.p. 158 °C (EtOH-H_2O, 1 : 3). For $C_{10}H_{12}N_4OS$ (236.3) calculated: 50.83% C, 5.12% H, 23.71% N; found: 49.29% C, 4.76% H, 23.88% N. ^{1}H NMR (DMSO-d_6): 0.98 (t, 3H, CH_3); 1.68 (sext, 2H, CH_2); 2.64 (t, 2H, CH_2); 7.08-7.28 (m, 1H, Ar-H); 7.60-7.86 (m, 2H, Ar-H); 9.76 (s, 1 H, N=CH); 11.80 (s, 1H, NH). IR (KBr): 3192 (NH), 1718 (C=O), 1602, 1590 (C=N) cm^{-1}. UV (ethanol), λ_{max} (ε): 312 (11310, 264 (6910), 203 (6510) nm. MS: *m/z* 259 (M+23), 237 (M+1), 236 (M^+), 234, 185, 169, 142, 96 (100), 93, 69.

*3-Benzyl-4-(2-thienymethylenamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (**2d**)*

Colourless crystals, yield 97%. M.p. 188 °C (EtOH-H_2O, 1 : 3). For $C_{14}H_{12}N_4OS$ (284.3) calculated: 59.14% C, 4.26% H, 19.72% N; found: 59.00% C, 4.15% H, 19.62% N. ^{1}H NMR (DMSO-d_6): 4.14 (s, 2H, CH_2); 7.14-7.44 (m, 6H, Ar-H); 7.60-7.90 (m, 2H, Ar-H); 9.80 (s, 1H, N=CH); 11.90 (s, 1H, NH). IR (KBr): 3192 (NH), 1715 (C=O), 1605, 1590 (C=N), 755, 700 (monosubstituted benzenoid ring) cm^{-1}. UV (ethanol), λ_{max} (ε): 319 (10560), 305 (12900), 264 (9650), 210 (11690) nm. MS: *m/z* 307 (M+23), 285 (M+1), 284 (M^+), 265, 234, 198, 185, 161, 142, 96 (100), 93, 69.

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

Colourless crystals, yield 89%. M.p. 151 °C (EtOH-H_2O, 1 : 3). For $C_{15}H_{14}N_4OS$ (298.4) calculated: 60.38% C, 4.73% H, 18.79% N; found: 59.89% C, 4.53% H, 18.07% N. ^{1}H NMR (DMSO-d_6): 2.30 (s, 3H, CH_3); 3.96 (s, 2H, CH_2); 6.90-7.26 (m, 5H, Ar-H); 7.56-7.80 (m, 2H, Ar-H); 9.76 (s, 1H, N=CH); 11.80 (s, 1H, NH). IR (KBr): 3195 (NH), 1720 (C=O), 1605, 1595 (C=N), 820 (1,4-disubstituted benzenoid ring) cm^{-1}. UV (ethanol), λ_{max} (ε): 320 (8640), 281 (7060), 223 (10890) nm. MS: *m/z* 321 (M+23), 299 (M+1), 265, 234, 231, 198, 185, 169, 161, 142, 96 (100), 93, 69, 60.

*3-(4-Chlorobenzyl)-4-(2-thienymethylenamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (**2f**)*

Colourless crystals, yield 94%. M.p. 192 °C (EtOH). For $C_{14}H_{11}N_4OSCl$ (318.8) calculated: 52.82% C, 3.49% H, 17.61% N; found: 52.93% C, 3.31% H, 17.48% N. ^{1}H NMR (DMSO-d_6): 4.04 (s, 2H, CH_2); 7.08-7.28 (m, 1H, Ar-H); 7.34 (s, 4H, Ar-H); 7.56-7.86 (m, 2H, Ar-H); 9.76 (s, 1H, N=CH); 11.84 (s, 1H, NH). IR (KBr): 3195 (NH), 1715 (C=O), 1600, 1585 (C=N), 820 (1,4-disubstituted benzenoid ring) cm^{-1}. UV (ethanol), λ_{max} (ε): 311 (13090), 266 (10670), 220 (14420) nm. MS: *m/z* 321, 320 (M+2), 319 (100), 309, 268, 265, 254, 234, 142, 110, 93, 79, 60.

*3-Phenyl-4-(2-thienymethylenamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (**2g**)*

Colourless crystals, yield 69%. M.p. 164 °C (benzene). For $C_{13}H_{10}N_4OS$ (270.3) calculated: 57.76% C, 3.73% H, 20.73% N; found: 57.64% C, 3.60% H, 20.55% N. ^{1}H NMR (DMSO-d_6): 7.10-8.00 (m, 8H, Ar-H); 9.74 (s, 1H, N=CH); 12.16 (s, 1H, NH). IR (KBr): 3195 (NH), 1720 (C=O), 1595, 1570 (C=N), 762, 710 (monosubstituted benzenoid ring) cm^{-1}. UV (ethanol), λ_{max} (ε): 325 (8450), 319 (10780), 305 (12990), 264 (17220), 225 (8450), 210 (11720) nm. MS: *m/z* 293 (M+23), 271 (M+1), 234, 198, 185, 169, 142, 96 (100), 93, 60.

*General procedure for preparation of 1-Acetyl-3-alkyl(aryl)-4-(2-thienymethylenamino)-4,5-dihydro-1H-1,2,4-triazol-5-ones (**3**)*

The corresponding compound **2** (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. Evaporation of the resulting solution at 40-45 °C *in vacuo* and several recrystallizations of the residue from an appropriate solvent gave pure compounds **3**.

*1-Acetyl-3-methyl-4-(2-thienymethylenamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (**3a**)*

Colourless crystals, yield 91%. M.p. 178 °C (ethanol). ^{1}H NMR (DMSO-d_6): 2.25 (s, 3H, CH_3); 2.44 (s, 3H, $COCH_3$); 7.19 (t, 1H, Ar-H, *J*=4.76 Hz); 7.71 (d, 1H, Ar-H, *J*=3.66 Hz); 7.82 (d, 1H, Ar-H, *J*=5.12 Hz); 9.67 (s, 1H, N=CH). ^{13}C NMR (DMSO-d_6): 11.81 (CH_3); 24.08 ($CO\underline{C}H_3$); 129.06, 132.33, 135.45, 138.11 (ar-C); 147.02 (triazole-C_3); 148.57 (N=CH); 151.52 (triazole-C_5); 166.65 (C=O). IR (KBr): 1763 (C=O), 1613, 1587 (C=N) cm^{-1}. UV (ethanol), λ_{max} (ε): 312 (16056), 260 (11280), 218 (17140) nm. MS: *m/z* 273 (M+23), 251 (M+1), 234, 231, 209, 147, 142, 120, 93, 74 (100), 69.

*1-Acetyl-3-ethyl-4-(2-thienymethylenamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (**3b**)*

Colourless crystals, yield 79%. M.p. 179 °C (ethanol). ^{1}H NMR (DMSO-d_6): 1.20 (t, 3H, CH_3, *J*=7.32 Hz); 2.46 (s, 3H, $COCH_3$); 2.65 (q, 2H, CH_2, *J*=7.32 Hz); 7.20 (t, 1H, Ar-H, *J*=4.39 Hz); 7.73 (d, 1H, Ar-H, *J*=3.66 Hz); 7.83 (d, 1H, Ar-H, *J*=5.12 Hz); 9.68 (s, 1H, N=CH). ^{13}C NMR (DMSO-d_6): 10.03 (CH_3); 19.29 (CH_3); 24.12 ($CO\underline{C}H_3$); 129.10, 132.34, 135.43, 138.20 (ar-C); 148.83 (triazole-C_3); 150.53 (N=CH); 151.59 (triazole-C_5); 166.63 (C=O). IR (KBr): 1769, 1687 (C=O), 1613, 1587 (C=N) cm^{-1}. UV (ethanol), λ_{max} (ε): 312 (17412), 260 (12684), 218 (7625) nm. MS: *m/z* 291, 223 (100), 217, 200, 120.

*1-Acetyl-3-benzyl-4-(2-thienymethylenamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (**3d**)*

Colourless crystals, yield 95%. M.p. 167 °C (benzene-petroleum ether, 1 : 3). For $C_{16}H_{14}N_4O_2S$ (326.4) calculated: 58.88% C, 4.32% H, 17.17% N; found: 59.07% C, 4.27% H, 16.65% N. ^{1}H NMR (DMSO-d_6): 2.58 (s, 3H, $COCH_3$); 4.10 (s, 2H, CH_2); 7.00-7.50 (m, 6H, Ar-H); 7.60-7.90 (m, 2H, Ar-H); 9.80 (s, 1H, N=CH). IR (KBr): 1753 (C=O), 1625, 1572 (C=N), 756, 696 (monosubstituted benzenoid ring) cm^{-1}. UV (ethanol), λ_{max} (ε): 314 (11730), 260 (9660), 209 (14790) nm. MS: *m/z* 327 (M+1), 308, 307, 285, 265, 231, 207, 185, 169, 147, 142, 96, 93, 74 (100), 69, 60.

*1-Acetyl-3-(p-methylbenzyl)-4-(2-thienymethylenamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (**3e**)*

Colourless crystals, yield 95%. M.p. 188 °C (EtOH). For $C_{17}H_{16}N_4O_2S$ (340.4) calculated: 59.98% C, 4.74% H, 16.46% N; found: 59.50% C, 4.68% H, 16.02% N. ^{1}H NMR (DMSO-d_6): 2.34 (s, 3H, CH_3); 2.62 (s, 3H, $COCH_3$); 4.10 (s, 2H, CH_2); 7.00-7.40 (m, 5H, Ar-H); 7.60-7.90 (m, 2H, Ar-H); 9.80 (s, 1H, N=CH). IR (KBr): 1734 (C=O), 1607, 1560 (C=N),

827 (1,4-disubstituted benzenoid ring) cm^{-1}. UV (ethanol), λ_{max} (ε): 312 (12018), 261 (9790), 207 (16210) nm. MS: *m/z* 340 (M+1), 321, 301, 299, 296, 282, 255, 239, 231, 209, 195, 185, 173, 169, 147, 145, 142 (100), 139, 120, 110.

*1-Acetyl-3-(4-chlorobenzyl)-4-(2-thienymethylenamino)-4,5-dihydro-1H-1,2,4-triazol -5-one (**3f**)*

Colourless crystals, yield 95%. M.p. 200 °C (benzene-petroleum ether, 1 : 3). For $C_{16}H_{13}N_4O_2SCl$ (360.8) calculated: 53.26% C, 3.63% H, 15.53% N; found: 53.67% C, 3.60% H, 14.98% N. ^{1}H NMR (DMSO-d_6): 2.60 (s, 3H, $COCH_3$); 4.16 (s, 2H, CH_2); 7.08-7.28 (m, 1H, Ar-H); 7.36 (s, 4H, Ar-H); 7.66-7.94 (m, 2H, Ar-H); 9.68 (s, 1H, N=CH). IR (KBr): 1753 (C=O), 1625, 1565 (C=N), 820 (1,4-disubstituted benzenoid ring) cm^{-1}. UV (ethanol), λ_{max} (ε): 312 (14320), 261 (11010), 207 (17740) nm. MS: *m/z* 352, 341, 321, 319, 279, 255, 226, 209, 195, 176, 147, 139, 125, 120 (100), 115.

*1-Acetyl-3-phenyl-4-(2-thienymethylenamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (**3g**)*

Colourless crystals, yield 86%. M.p. 160 °C (EtOH). For $C_{15}H_{12}N_4O_2S$ (312.4) calculated: 57.68% C, 3.87% H, 17.94% N; found: 57.90% C, 3.85% H, 17.55% N. ^{1}H NMR (DMSO-d_6): 2.60 (s, 3H, $COCH_3$); 7.06-7.28 (m, 1H, Ar-H); 7.36-8.00 (m, 7H, Ar-H); 9.64 (s, 1H, N=CH). IR (KBr): 1727 (C=O), 1600, 1570 (C=N), 762, 700 (monosubstituted benzenoid ring) cm^{-1}. UV (ethanol), λ_{max} (ε): 313 (13 920), 262 (19 650), 206 (18 440) nm.

Antioxidant activity

Chemicals

Butylated hydroxytoluene (BHT) was purchased from E. Merck. Ferrous chloride, α-tocopherol, 1,1-diphenyl-2-picryl-hydrazyl (DPPH·), 3-(2-pyridyl)-5,6-bis(phenylsulfonic acid)-1,2,4-triazine (ferrozine), butylated hydroxyanisole (BHA) 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[38]. 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 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·, using the method of Blois[39]. 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 spectrophometer. 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):

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

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

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

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.*[40] 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

Potentiometric titrations an Orion 720A model pH-ionmeter equipped with a combined pH electrode (Ingold) and indicator electrode were used. A magnetic stirrer, a semi-micro burette and a 25 mL beaker were also used in titrations. Before potentiometric titrations, the pH meter was calibrated according to the instructions supplied by the manufactures of the pH meter. During the titrations, the titrant was added in increments of 0.05 mL after each stable reading and mV values were recorded. The necessary chemicals were supplied from Fluka and Merck. After purifications, isopropyl alcohol was used to prepare 0.05 N tetra-butylammonium hydroxide. For all potentiometric titrations, 0.05 N tetra-butylammonium hydroxide in isopropyl alcohol, which was prepared from 0.1 N tetra-butylammonium hydroxide (TBAH) by dilution, was used. The 0.05 M solution of TBAH in isopropyl alcohol, which is widely used in the titration of acids, was used as titrant. The half-neutralization potentials and the corresponding pKa values for all compounds, obtained from the potentiometric titrations with 0.05 M tetrabutylammonium hydroxide in isopropyl alcohol, *t*-butyl alcohol, *N,N*-dimethylformamide and acetonitrile. The mV values, that were obtained in pH-meter, were recorded. The half-neutralization potential (HNP) values and the corresponding p*K*a values of all compounds, obtained from the potentiometric titrations with 0.05 M TBAH in isopropyl alcohol, *t*-butyl alcohol, acetonitrile and *N,N*-dimethylformamide, are presented in Table 1. Finally, the half-neutralization potential (HNP) values were determined by drawing the mL (TBAH)-mV graphic. From the titration curves, the HNP values were measured and the corresponding pKa values were calculated.

Results and Discussion

In this study, the structures of seven new 3-alkyl(aryl)-4-(2-thienymethylenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**2**) and four new 1-acetyl-3-alkyl(aryl)-4-(2-thieny-methylenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**3**) were identified by using elemental analysis and IR, ^{1}H NMR and UV spectral data, and the obtained spectral values were seen as compatible with literature[4-6,15].

Antioxidant activity

The compounds **2a-g** and **3a, 3b, 3d-f** 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.

Table 1. Half-neutralization potential (HNP) values and the corresponding pKa values of all triazole derivatives in isopropyl alcohol, *N,N*-dimethyl formamide, *t*-butyl alcohol and acetonitrile

Compd. No	Isopropyl alcohol		*N,N*-Dimethyl-formamide		*t*-Butyl alcohol		Acetonitrile	
	pKa	HNP, mV	pKa	HNP, mV	pKa	HNP, mV	pKa	HNP, mV
a	13.03±0.13	-356.8±1.3	14.43±0.19	-439.9±2.3	14.15±0.23	-422.7±1.8	14.97±0.19	-471.1±2.2
b	13.30±0.17	-372.7±1.9	14.64±0.15	-452.1±1.9	14.60±0.16	-448.8±2.2	14.96±0.15	-471.7±1.7
c	13.22±0.14	-368.0±1.8	15.59±0.17	-507.3±2.1	14.92±0.11	-468.8±1.5	15.53±0.12	-504.4±1.9
d	12.84±0.16	-345.4±1.5	14.37±0.11	-436.2±1.8	14.24±0.15	-428.4±1.7	14.67±0.20	-453.7±1.4
e	12.88±0.21	-347.9±1.6	14.59±0.19	-448.4±1.7	15.13±0.18	-481.1±2.3	14.91±0.13	-468.3±1.6
f	12.59±0.18	-330.6±1.4	14.13±0.13	-420.9±1.4	14.08±0.20	-418.8±1.6	14.72±0.11	-456.3±1.7
g	12.57±0.15	-329.5±1.8	14.04±0.16	-416.5±2.0	14.28±0.14	-431.3±1.4	14.27±0.18	-430.1±1.5

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. 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 activity[41]. 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[42]. 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 take part in any electron transfer reaction. In other words, compounds did not show the reductive activities.

DPPH$^{\bullet}$ 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 ability[43]. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule[44]. The reduction capability of DPPH radicals was determined by decrease in its absorbance at 517 nm induced by antioxidants. The absorption maximum of a stable DPPH radical in ethanol was at 517 nm.

The decrease in absorbance of DPPH radical was caused by antioxidants, because of reaction between antioxidant molecules and radical, progresses, which 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[45]. BHA and α-tocopherol were used as a reference to antioxidant compounds. Scavenging effect values of compounds **2g**, **3e,** BHA and α-tocopherol at different concentrations are given Figure 1. The newly synthesized compounds showed no activity as a radical scavenger.

Ferrous ion chelating activity

The chelating effect towards ferrous ions by the compounds and standards was determined according to the method of Dinis[40]. 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[46]. Transition metals have pivotal role in the generation oxygen free radicals in living organism.

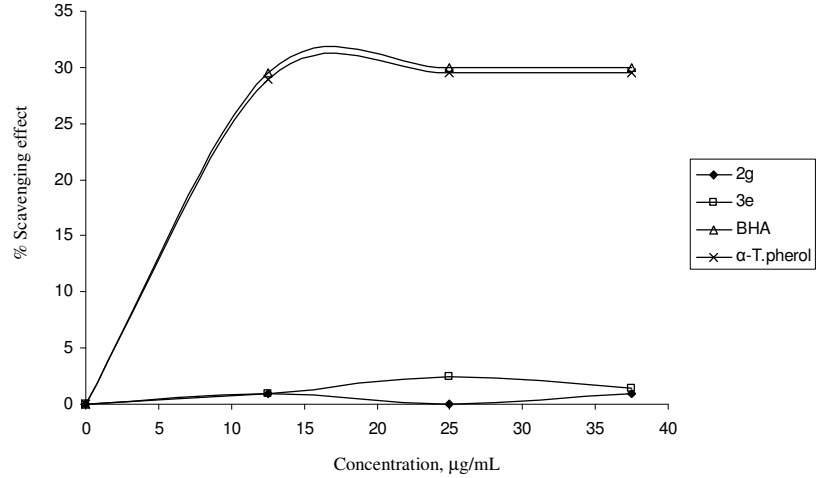


Figure 1. Scavenging effect of compounds **2g**, **3e,** BHA and α-tocopherol at different concentrations (12.5-25-37.5 µg/mL)

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[47] pH 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[48]. 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:

$$O^{\cdot}_2 + H_2O_2 \rightarrow O_2 + OH^- + OH^{\cdot} \quad (1)$$

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:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^{\cdot} \quad (2)$$

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[49]. Ferrous ion chelating activities of the compounds, BHT, BHA and α-tocopherol are shown in Figures 2 and 3. 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 σ-bonds with a metal are effective as secondary antioxidants because they reduce the redox potential thereby stabilizing the oxidized form of metal ion[50].

The data obtained from Figures 2 and 3 reveal that the compounds, except **2e**, 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.

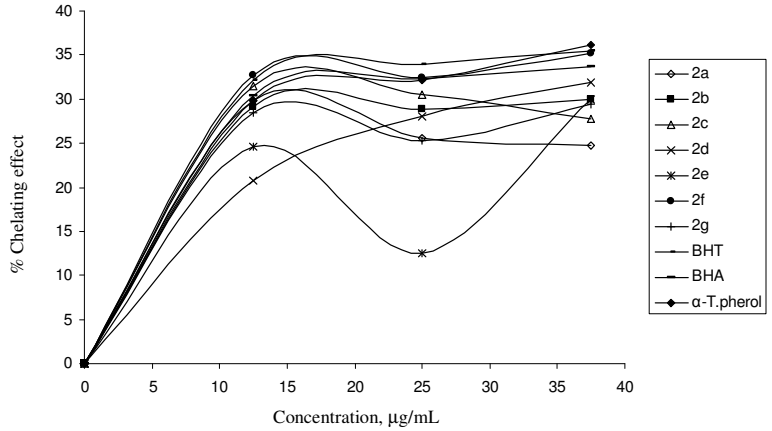


Figure 2. Metal chelating effect of different amount of the compounds **2a-g**, BHT, BHA and α-tocopherol on ferrous ions

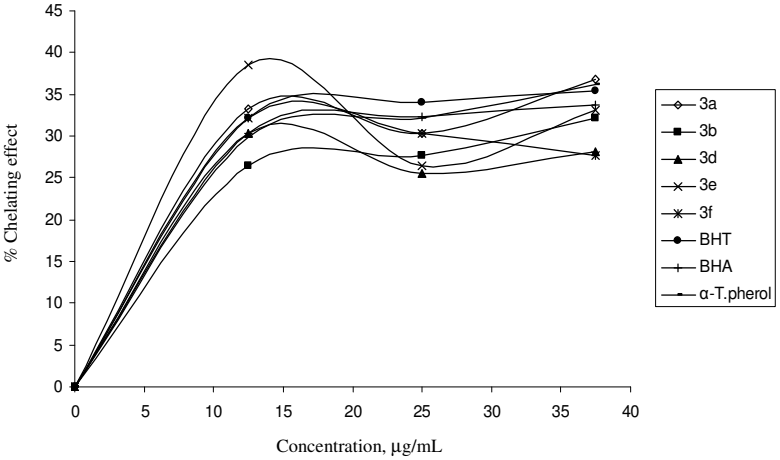


Figure 3. Metal chelating effect of different amount of the compounds **3a**, **3b, 3d-f,** BHT, BHA and α-tocopherol on ferrous ions

Potentiometric titrations

In this study, compounds 2**a-2g** were titrated potentiometrically with TBAH in isopropyl alcohol, *t*-butyl alcohol, acetonitrile and *N,N*-dimethyl formamide. The mV values read in each titration were drawn against TBAH volumes (mL) added and potentiometric titration curves were formed for all the cases. From the titration curves, the HNP values were measured and the corresponding pKa values were calculated. The half-neutralization potential (HNP) values and the corresponding pKa values of all triazole derivatives, obtained from the potentiometric titrations with 0.05 M TBAH in isopropyl alcohol, *t*-butyl alcohol, acetonitrile and *N,N*-dimethyl formamide, are presented in Table 1. Also the derived potentiometric titration curves were plotted (Figures 4 – 8 for **2a**). The pH of the weak acids are given by the following equation:

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

pH = pKa occurs when $[A^-]$ is equal to [HA] at the half-neutralization point. Therefore, the pH values can be regarded as pKa at the half-neutralization points. When the dielectric

permittivity of solvents is taken into consideration, the acidic arrangement can be expected as follows: *N,N*-dimethyl formamide (ε=36.7) > acetonitrile (ε=36.0) > isopropyl alcohol (ε=19.4) > *t*-butyl alcohol (ε=12.0). The acidity of a compound depends on several factors. The two most important factors are the solvent effect and molecular structure. Table 1 shows that the half neutralization potential (HNP) values and the corresponding pKa values obtained from potentiometric titrations depend on the type of non-aqueous solvents used and molecular structure of the compound.

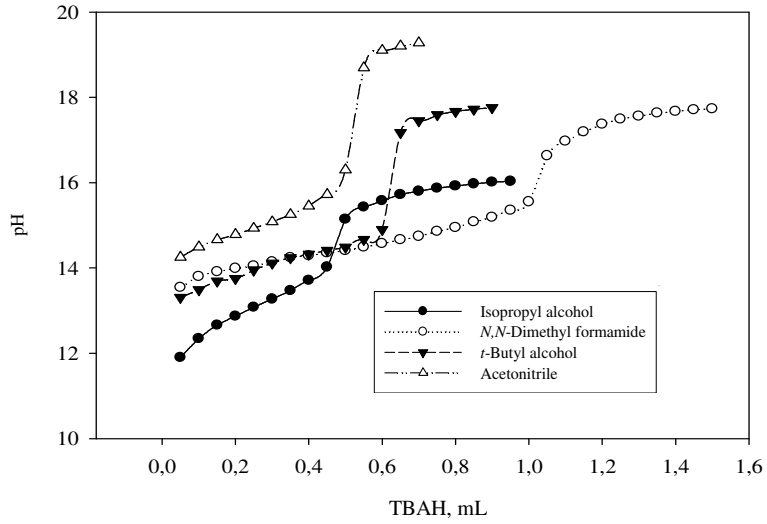


Figure 4. pH - mL (TBAH) potentiometric titration curves of 0.001 M solutions of compound **2a** titrated with 0.05 M TBAH in isopropyl alcohol, *t*-butyl alcohol, acetonitrile and *N,N*-dimethyl formamide at 25 °C

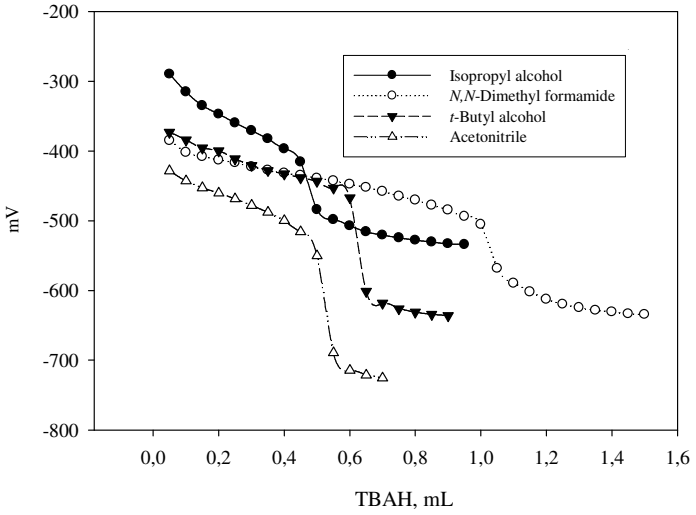


Figure 5. mV – mL (TBAH) potentiometric titration curves of 0.001 M solutions of compound **2a** titrated with 0.05 M TBAH in isopropyl alcohol, *t*-butyl alcohol, acetonitrile and *N,N*-dimethyl formamide at 25 °C

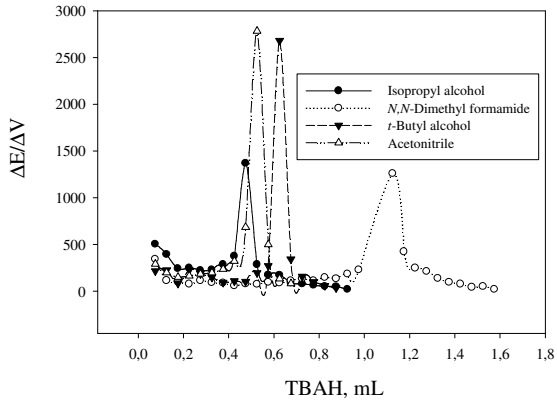


Figure 6. ΔE/ΔV - mL (TBAH) potentiometric titration curves of 0.001 M solutions of compound **2a** titrated with 0.05 M TBAH in isopropyl alcohol, *t*-butyl alcohol, acetonitrile and *N,N*-dimethyl formamide at 25 °C

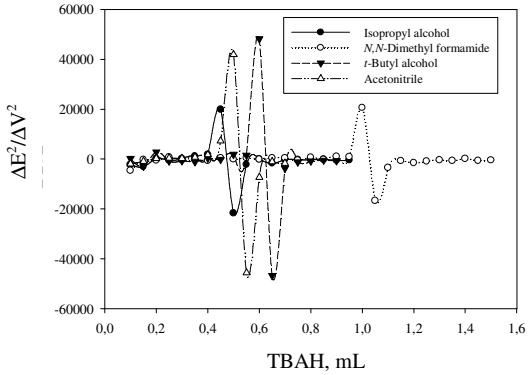


Figure 7. $\Delta^2E/\Delta V^2$ - mL (TBAH) potentiometric titration curves of 0.001 M solutions of compound **2a** titrated with 0.05 M TBAH in isopropyl alcohol, *t*-butyl alcohol, acetonitrile and *N,N*-dimethyl formamide at 25 °C

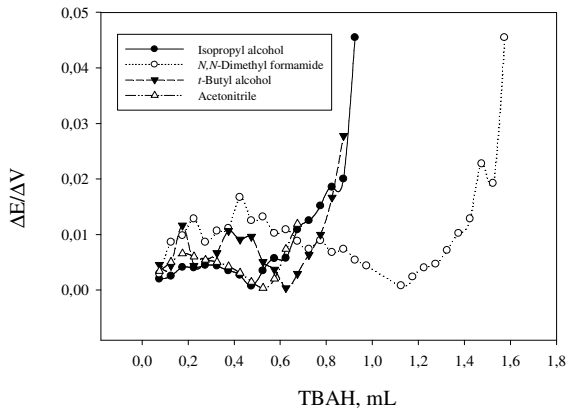


Figure 8. ΔV/ΔE - mL (TBAH) potentiometric titration curves of 0.001 M solutions of compound **2a** titrated with 0.05 M TBAH in isopropyl alcohol, *t*-butyl alcohol, acetonitrile and *N,N*-dimethyl formamide at 25 °C

As seen in Table 1, the acidic order for compounds **a**, **b**, **c, d** and **f** is: isopropyl alcohol > *t*-butyl alcohol > *N,N*-dimethyl formamide > acetonitrile, for compounds **e** and **g** is: isopropyl alcohol > *N,N*-dimethyl formamide > acetonitrile > *t*-butyl alcohol. In isopropyl alcohol, **a**, **b**, **c**, **d, e, f** and **g** compounds show the strongest acidic properties. **a**, **b**, **c**, **d** and **f** compounds show the weakest acidic properties in acetonitrile, **e** and **g** compounds show the weakest acidic properties in *t*-butyl alcohol. This situation may be attributed to the hydrogen bonding between the negative ions formed and the solvent molecules in the amphiprotic neutral solvents. Autoprotolysis is an acid-base reaction between identical solvent molecules is which some act as an acid and others as a base.

Consequently, the extent of an autoprotolysis reaction depends both on the intrinsic acidity and the instrinsic basicity of the solvent. The importance of the autoprotolysis constant in titrations lies in its effect on the completeness of a titration reaction.

Half-neutralization potential (HNP) values and corresponding pKa values obtained from the potentiometric titrations rely on the non-aqueous solvents used and the substituents at C-3, in triazole ring.

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. All of the compounds demonstrate a marked capacity for iron binding. Acidity of a compound depends on mainly two factors, *i.e.* solvent effect and molecular structure.

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