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Synthesis and Antioxidant Activities of Some New 4-(4-Hydroxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one Derivatives with Their Acidic Properties

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Fourteen new compounds having 4,5-dihydro-1*H*-1,2,4-triazol-5-one ring, namely six new 3-alkyl(aryl)-4-(4-hydroxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**3**), four new 1-acetyl-3-alkyl(aryl)-4-(4-acetyloxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**4**), two new 1-methyl-3-alkyl-4-(4-methoxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**5**) and two new 3-alkyl(aryl)-4-[4-(*p*-tolylsulfonyl)-oxybenzylidenamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**6**), were synthesized. The structures of the newly synthesized compounds were determined by elemental analysis as well as IR, ^{1}H NMR, ^{13}C NMR and UV spectral data and their antioxidant activities, except compounds **3e** and **4f**, were investigated. In addition, compounds **3a–f** were titrated potentiometrically with tetrabutylammonium hydroxide in three non-aqueous solvents such as isopropyl alcohol, *t*-butyl alcohol and N,N-dimethyl formamide. The half-neutralization potential values and the corresponding pK_a values were determined for all the cases.

Key Words: Synthesis, 4,5-Dihydro-1*H*-1,2,4-triazol-5-one, Schiff base, Methylation, Acetylation, Acidity, Potentiometric titrations, pK_a, Antioxidant,

INTRODUCTION

Compounds of the 1,2,4-triazole and 4,5-dihydro-1*H*-1,2,4-triazol-5-one type are reported to show a broad spectrum of biological activities such as antifungal, antimicrobial, hypoglycemic, antihypertensive, analgesic, antiparasitic, hypocholesteremic, antiviral, anti-inflammatory, antitumor, antioxidant, antiradical and anti-HIV properties[1–12] and several articles deal with the synthesis of N-arylidenamino-1,2,4-triazole and N-arylidenamino-4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives[4,13–19].

In addition, one of the important research fields of the recent years has been based on antioxidants, which play an important role in health by scavenging the free radicals that cause many diseases and ageing[20]. Scientists in many different disciplines became more interested in new compounds, either synthesized or obtained from natural sources, that could provide active components to prevent or reduce the impacts of oxidative stress on cells[21, 22].

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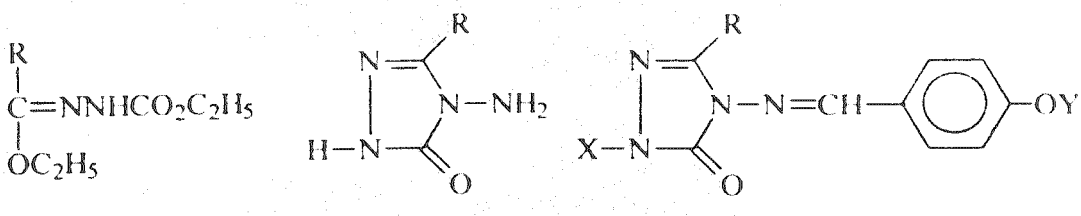
Many widely-used methods for measuring antioxidant activity involve the generation of radical species and the radical concentration is monitored as the present antioxidants scavenge them. The scavenging of radicals and monitoring the absorbance caused by them are applied in 2,2-diphenyl-1-picrilhydrazyl (DPPH) scavenging activity measurement method. Lower sample concentration required to scavenge oxidants in this assay means higher antioxidant activity[23, 24].

Several articles, involving the acetylation and methylation of 1,2,4-triazole and 4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives, have also been published up to date[5, 6, 15, 25]. On the other hand, some 1,2,4-triazole and 4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives were titrated potentiometrically with tetrabutylammonium hydroxide in non-aqueous solvents and the pK_a values of the compounds were determined[14–19, 26, 27]. Determination of pK_a values of active constituent of certain pharmaceutical preparations is important, because their distribution, transport behaviour, bonding to receptors and contributions to metabolic behaviour of the active constituent molecules depend on the ionization constant[28].

In this study, a series of 3-alkyl(aryl)-4-(4-hydroxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**3**) were synthesized from the reactions of 3-alkyl(aryl)-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**2**) with 4-hydroxybenzaldehyde. Besides, the reactions of compounds **3** with acetic anhydride, NaOH/dimethyl sulphate and toluene-4-sulfonyl chloride gave compounds **4**, **5** and **6**, respectively (**Scheme-1**). Next, part of the current study was aimed at the determination of *in-vitro* antioxidant activities of these new compounds synthesized in the study to evaluate their nutraceutical and medicinal values. Furthermore, in order to determine the pK_a values of the compounds **3a–f** they were titrated potentiometrically with tetrabutylammonium hydroxide (TBAH) in three non-aqueous solvents, including isopropyl alcohol, *t*-butyl alcohol and N,N-dimethyl formamide. For each new compound **3a–f**, the half-neutralization potential (HNP) and the corresponding pK_a value were determined in three dif-



1 **2** **3-6**

a) R=CH_3; **b)** R=CH_2CH_3; **c)** R=$CH_2C_6H_5$;
d) R=$CH_2C_6H_4.CH_3$ (*p*-); **e)** R=$CH_2C_6H_4.Cl$ (*p*-); **f)** R=C_6H_5

	X	Y
3	H	H
4	$COCH_3$	$COCH_3$
5	CH_3	CH_3
6	H	$SO_2C_6H_4.CH_3$ (*p*-)

Scheme-1

ferent non-aqueous solvents. The data obtained from the potentiometric titrations were interpreted, and substituent effects attached to C-3 position in 4,5-dihydro-1*H*-1,2,4-triazol-5-one ring and solvent effects were studied[14–19, 26–29].

EXPERIMENTAL

Butylated hydroxytoluene (BHT) was obtained from Applichem. Trolox. (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), 1,1-diphenyl-2-picryl hydrazil (DPPH), dimethyl sulfoxide (DMSO) and L-ascorbic acid were purchased from Sigma Chemical Co. Potassium ferricyanide ($K_3Fe(CN)_6$) and ferric chloride ($FeCl_3$) were purchased from Merck Co. All the reagents used were of analytical grade. All absorbance measurements were obtained with an ATI-Unicam UV-2 UV-Vis spectrophotometer.

Melting points were taken on an Electrothermal digital melting point apparatus and are uncorrected. IR spectra were registered using KBr disks on a Perkin-Elmer 1600 FTIR 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 and 50 MHz, respectively. UV absorption spectra were measured for ethanol solutions in 10 mm quartz cells between 200 and 400 nm using a Shimadzu UV-1201 spectrophotometer.

The starting compounds **2a–f** were prepared from the reactions of the corresponding ester ethoxycarbonyl hydrazones (**1a–f**) with hydrazine hydrate according to literature[25, 30].

General method for the preparation of 3-alkyl(aryl)-4-(4-hydroxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (3): 3-Alkyl(aryl)-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-one (**2**) (0.01 mol) was dissolved in acetic acid (15 mL) and treated with 4-hydroxy benzaldehyde (1.22 g, 0.01 mol). The mixture was refluxed for 1 h and then evaporated at 50-55°C *in vacuo*. Several recrystallizations of the residue from an appropriate solvent gave pure compounds **3**.

3-Methyl-4-(4-hydroxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (3a): Yield 91%; m.p. 284–285°C (AcOH-H_2O, 1 : 2). IR (KBr, cm^{-1}): 3150 ν(OH, NH), 1715 ν(C=O), 1610, 1588 ν(C=N), 820 ν(1,4-disubstituted benzenoid ring). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 2.27 (s, 3H, CH_3), 6.91 (d, 2H, Ar-H, J = 8.24 Hz), 7.69 (d, 2H, Ar-H, J = 8.24 Hz), 9.57 (s, 1H, N=CH), 10.20 (s, 1H, OH), 11.79 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 50 MHz): δ 11.11 (aliphatic carbon), 115.80 (2C), 124.38, 129.61 (2C), 160.50 (aromatic carbons), 146.16 (triazole C_3), 151.35 (N=CH), 154.34 (triazole C_5). UV (ethanol) λ_{max} (ε, L mol^{-1} cm^{-1}): 306 (20130), 223 (11600), 206 (12580) nm. Anal. Calcd. for $C_{10}H_{10}N_4O_2$: C, 55.0; H, 4.6; N, 25.7; Found: C, 55.7; H, 4.5; N, 24.1%.

3-Ethyl-4-(4-hydroxybenzylidenamino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (3b): Yield 93%; m.p. 249–250°C (AcOH-H_2O, 1 : 2). IR (KBr, cm^{-1}): 3150 ν(OH, NH), 1710 ν(C=O), 1600, 1580 ν(C=N), 825 ν(1,4-disubstituted benzenoid ring). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 1.24 (t, 3H, CH_3), 2.68 (q, 2H, CH_2), 6.95 (m, 2H, Ar-H), 7.69–7.71 (m, 2H, Ar-H), 9.59 (s, 1H, N=CH), 10.21 (s, 1H, OH), 11.83 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 50 MHz): δ 10.08, 18.62 (aliphatic carbons), 115.92 (2C), 124.60, 129.66 (2C), 160.70 (aromatic carbons), 148.10

(triazole C_3), 151.40 (N=CH), 154.20 (triazole C_5). UV (ethanol) λ_{max} (ε, L mol^{-1} cm^{-1}): 307 (18510), 223 (11540), 207 (11690) nm. Anal. Calcd. for $C_{11}H_{12}N_4O_2$: C, 56.9; H, 5.2; N, 24.1;. Found: C, 57.5; H, 5.1; N, 24.1%.

3-Benzyl-4-(4-hydroxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (3c): Yield 90%; m.p. 275–276°C (AcOH-H_2O, 1 : 2). IR (KBr, cm^{-1}): 3150 ν(OH, NH), 1710 ν(C=O), 1610, 1595 ν(C=N), 835 ν(1,4-disubstituted benzenoid ring), 765, 700 ν(monosubstit. benzenoid ring). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 4.04 (s, 2H, CH_2), 6.90 (d, 2H, Ar-H, J = 7.63 Hz), 7.32 (s, 5H, Ar-H), 7.66 (d, 2H, Ar-H, J = 7.63 Hz), 9.54 (s, 1H, N=CH), 10.21 (s, 1H, OH), 11.94 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 50 MHz): δ 31.25 (aliphatic carbon), 116.00 (2C), 124.53, 126.81, 128.55 (2C), 128.95 (2C), 129.81 (2C), 136.02, 160.81 (aromatic carbons), 146.30 (triazole C_3), 151.52 (N=CH), 154.25 (triazole C_5). UV (ethanol) λ_{max} (ε, L mol^{-1} cm^{-1}): 309 (15060), 210 (13340) nm. Anal. Calcd. for $C_{16}H_{14}N_4O_2$: C, 65.3; H, 4.8; N, 19.0; Found: C, 65.4; H, 4.8; N, 18.4%.

3-*p*-Methylbenzyl-4-(4-hydroxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (3d): Yield 95%; m.p. 280–281°C (AcOH-H_2O, 1 : 2). IR (KBr, cm^{-1}): 3200 ν(OH, NH), 1720 ν(C=O), 1600, 1580 ν(C=N), 840, 813 ν(1,4-disubstituted benzenoid ring). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 2.24 (s, 3H, CH_3), 3.99 (s, 2H, CH_2), 6.93 (d, 2H, Ar-H, J = 7.63 Hz), 7.09–7.24 (m, 4H, Ar-H), 7.68 (d, 2H, Ar-H, J = 7.63 Hz), 9.56 (s, 1H, N=CH), 10.23 (s, 1H, OH), 11.96 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 50 MHz): δ 20.62, 30.80 (aliphatic carbons), 115.92 (2C), 124.50, 128.71 (2C), 129.01 (2C), 129.70 (2C), 132.70, 135.90, 160.71 (aromatic carbons), 146.30 (triazole C_3), 151.50 (N=CH), 154.10 (triazole C_5). UV (ethanol) λ_{max} (ε, L mol^{-1} cm^{-1}): 292 (7610), 256 (7970), 210 (13490) nm. Anal. Calcd. for $C_{17}H_{16}N_4O_2$: C, 66.2; H, 5.2; N, 18.2; Found: C, 66.5; H, 5.2; N, 17.8%.

3-*p*-Chlorobenzyl-4-(4-hydroxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (3e): Yield 90%; m.p. 265–267°C (AcOH-H_2O, 1 : 2). IR (KBr, cm^{-1}): 3200 ν(OH, NH), 1715 ν(C=O), 1595, 1580 ν(C=N), 840, 810 ν(1,4-disubstituted benzenoid ring). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 4.05 (s, 2H, CH_2), 6.92 (d, 2H, Ar-H, J = 8.24 Hz), 7.37 (s, 4H, Ar-H), 7.66 (d, 2H, Ar-H, J = 8.55 Hz), 9.53 (s, 1H, N=CH), 10.35 (s, 1H, OH), 12.03 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 50 MHz): δ 30.70 (aliphatic carbon), 115.87 (2C), 124.20, 128.33 (2C), 129.70 (2C), 130.70 (2C), 131.40, 134.80, 160.60 (aromatic carbons), 145.90 (triazole C_3), 151.40 (N=CH), 154.20 (triazole C_5). UV (ethanol) λ_{max} (ε, L mol^{-1} cm^{-1}): 309 (13110), 223 (14200) nm. Anal. Calcd. for $C_{16}H_{14}N_4O_2Cl$: C, 58.5; H, 4.0; N, 17.0; Found: C, 58.7; H, 3.9; N, 17.1%.

3-Phenyl-4-(4-hydroxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (3f): Yield 88%; m.p. 256-257°C (AcOH-H_2O, 1 : 2). IR (KBr, cm^{-1}): 3200 ν(OH, NH), 1715 ν(C=O), 1613, 1580 ν(C=N), 815 ν(1,4-disubstituted benzenoid ring), 760, 690 (monosubstituted benzenoid ring). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 6.95 (d, 2H, Ar-H, J = 7.63 Hz), 7.53 (m, 3H, Ar-H), 7.71 (d, 2H, Ar-H, J = 7.63 Hz), 7.94 (m, 2H, Ar-H), 9.49 (s, 1H, N=CH), 10.29 (s, 1H, OH), 12.38 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 50 MHz): δ 115.94 (2C), 124.10, 127.10, 127.78 (2C), 128.44 (2C), 129.92 (3C), 160.90 (aromatic carbons), 144.60 (triazole C_3), 151.50 (N=CH), 157.70 (triazole C_5). UV (ethanol) λ_{max} (ε, L mol^{-1} cm^{-1}): 310 (9010), 225 (8640), 205 (10520) nm. Anal. Calcd. for $C_{15}H_{12}N_4O_2$: C, 64.3; H, 4.3; N, 20.0; Found: C, 63.9; H, 4.8; N, 19.5%.

General method for the preparation of 1-acetyl-3-alkyl(aryl)-4-(4-acetyloxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (4): The corresponding compound **3** (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 **4**.

1-Acetyl-3-methyl-4-(4-acetyloxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (4a): Yield 95%; m.p. 210–211°C (EtOH-toluene, 1 : 2). IR (KBr, cm^{-1}): 1780, 1750, 1705 ν(C=O), 1630, 1605 ν(C=N), 810 ν(1,4-disubstituted benzenoid ring) cm^{-1}. ^1H NMR (DMSO-d_6, 200 MHz): δ 2.32 (s, 6H, $2CH_3$), 2.47 (s, 3H, CH_3), 7.28 (d, 2H, Ar-H, $J = 7.33$ Hz), 7.88 (d, 2H, Ar-H, $J = 7.33$ Hz), 9.56 (s, 1H, N=CH). ^{13}C NMR (DMSO-d_6, 50 MHz): δ 11.44, 21.15, 23.60 (aliphatic carbons), 122.85 (2C), 129.59 (2C), 130.90, 148.50 (aromatic carbons), 144.20 (triazole C_3), 151.60 (N=CH), 154.50 (triazole C_5), 165.90, 168.80 (2C=O). UV (ethanol) λ_{max} (ε, L mol^{-1} cm^{-1}): 291 (8760), 255 (8810), 208 (10040) nm. Anal. Calcd. for $C_{14}H_{14}N_4O_4$: C, 55.6; H, 5.2; N, 24.1; Found: C, 55.3; H, 5.2; N, 23.4%.

1-Acetyl-3-benzyl-4-(4-acetyloxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (4c): Yield 93%; m.p. 165-166°C (EtOH-toluene, 1 : 2). IR (KBr, cm^{-1}): 1780, 1760, 1703 ν(C=O), 1620, 1605 ν(C=N), 810 ν(1,4-disubstituted benzenoid ring), 760, 705 ν(monosubstituted benzenoid ring). ^1H NMR (DMSO-d_6, 200 MHz): δ 2.30 (s, 3H, CH_3), 2.50 (s, 3H, CH_3), 4.15 (s, 2H, CH_2), 7.27-7.37 (m, 7H, Ar-H), 7.87 (d, 2H, Ar-H, $J = 8.24$ Hz), 9.57 (s, 1H, N=CH). ^{13}C NMR (DMSO-d_6, 50 MHz): δ 20.80, 23.47, 30.92 (aliphatic carbons), 122.57 (2C), 126.91, 128.45 (2C), 128.93 (2C), 129.22 (2C), 130.53, 134.60, 148.23 (aromatic carbons), 147.95 (triazole C_3), 153.03 (N=CH), 154.34 (triazole C_5), 166.00, 168.80 (2C=O). UV (ethanol) λ_{max} (ε, L mol^{-1} cm^{-1}): 292 (9360), 256 (9500) nm. Anal. Calcd. for $C_{20}H_{18}N_4O_4$: C, 63.5; H, 4.8; N, 14.8; Found: C, 63.5; H, 4.9; N, 14.3%.

1-Acetyl-3-*p*-methylbenzyl-4-(4-acetyloxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (4d): Yield 95%; m.p. 172–173°C (EtOH-toluene, 1 : 2). IR (KBr, cm^{-1}): 1780, 1750, 1702 ν(C=O), 1620, 1608 ν(C=N), 810 ν(1,4-disubstituted benzenoid ring). ^1H NMR (DMSO-d_6, 200 MHz): δ 2.25 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 2.50 (s, 3H, CH_3), 4.08 (s, 2H, CH_2), 7.12 (d, 2H, Ar-H, $J = 7.63$ Hz), 7.24–7.31 (m, 4H, Ar-H), 7.88 (d, 2H, Ar-H, $J = 8.24$ Hz), 9.56 (s, 1H, N=CH). ^{13}C NMR (DMSO-d_6, 50 MHz): δ 20.80, 20.90, 23.80 (aliphatic carbons), 122.74 (2C), 128.95 (2C), 129.16 (2C), 129.38 (2C), 130.80, 131.90, 136.40, 148.80 (aromatic carbons), 148.00 (triazole C_3), 153.30 (N=CH), 154.60 (triazole C_5), 166.10, 169.10 (2C=O). UV (ethanol) λ_{max} (ε, L mol^{-1} cm^{-1}): 291 (9620), 256 (11740), 210 (9840) nm. Anal. Calcd. for $C_{21}H_{20}N_4O_4$: C, 64.3; H, 5.1; N, 14.3; Found: C, 64.1; H, 5.2; N, 14.4%.

1-Acetyl-3-phenyl-4-(4-acetyloxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (4f): Yield 92%; m.p. 134–135°C (EtOH). IR (KBr, cm^{-1}): 1770, 1710 ν(C=O), 1610, 1595 ν(C=N), 820 ν(1,4-disubstituted benzenoid ring), 760, 695 (monosubstituted benzenoid ring). ^1H NMR (DMSO-d_6, 200 MHz): δ 2.30 (s, 3H, CH_3), 2.30 (s, 3H, CH_3), 7.29 (d, 2H, Ar-H, $J = 8.24$ Hz), 7.51–7.61

(m, 3H, Ar-H), 7.84–7.95 (m, 4H, Ar-H), 9.52 (s, 1H, N=CH). ^{13}C NMR (DMSO-d_6, 50 MHz): δ 21.34, 24.03 (aliphatic carbons), 123.13, 123.24, 125.73, 128.39, 129.00, 129.11 (2C), 129.15 (2C), 129.63, 129.96, 130.88, 131.76, 146.50 (aromatic carbons), 148.64 (triazole C_3), 153.77 (N=CH), 156.27 (triazole C_5), 166.70, 169.42 (2C=O). Anal. Calcd. for $C_{19}H_{16}N_4O_4$: C, 63.6; H, 4.4; N, 15.4; Found: C, 63.4; H, 4.9; N, 14.8%.

General method for the preparation of 1-methyl-3-alkyl-4-(4-methoxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (5): The corresponding compound **3** (0.01 mol) was dissolved in 2 N NaOH (10 mL) and treated with dimethyl sulphate (3.2 mL). After stirring the mixture at room temperature for 1 h, the solid formed was filtered, washed with cold water and dried *in vacuo*. Several recrystallizations of crude product from a proper solvent gave pure compound **5**.

1,3-Dimethyl-4-(4-methoxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (5a): Yield 63%; m.p. 167–168°C (EtOH-H_2O, 1 : 3). IR (KBr, cm^{-1}): 1710 ν(C=O), 1610, 1595 ν(C=N), 835 (1,4-disubstituted benzenoid ring). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 2.25 (s, 3H, CH_3), 3.31 (s, 3H, NCH_3), 3.83 (s, 3H, OCH_3), 7.04 (m, 2H, Ar-H), 7.75 (m, 2H, Ar-H), 9.58 (s, 1H, N=CH). ^{13}C NMR (DMSO-d_6, 50 MHz): δ 11.00, 31.90, 55.60 (aliphatic carbons), 114.46 (2C), 126.00, 129.53 (2C), 162.00 (aromatic carbons), 143.00 (triazole C_3), 150.00 (N=CH), 154.00 (triazole C_5). UV (ethanol) λ_{max} (ε, L mol^{-1} cm^{-1}): 307 (16980), 222 (11470) nm. Anal. Calcd. for $C_{12}H_{14}N_4O_2$: C, 58.5; H, 5.7; N, 22.8; Found: C, 58.1; H, 5.6; N, 22.9%.

1-Methyl-3-benzyl-4-(4-methoxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (5c): Yield 72%; m.p. 112–113°C (EtOH-H_2O, 1 : 3). IR (KBr, cm^{-1}): 1715 ν(C=O), 1617, 1598 ν(C=N), 822 ν(1,4-disubstituted benzenoid ring), 760, 697 ν(monosubstituted benzenoid ring). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 3.28 (s, 3H, NCH_3), 3.85 (s, 3H, OCH_3), 4.12 (s, 2H, CH_2), 7.31–7.42 (m, 7H, Ar-H), 7.86 (m, 2H, Ar-H), 9.54 (s, 1H, N=CH). ^{13}C NMR (DMSO-d_6, 50 MHz): δ 30.78, 31.75, 55.80 (aliphatic carbons), 114.46, 116.14, 126.00, 129.57, 128.82, 129.22 (2C), 129.53 (2C), 134.55, 144.14, 162.00 (aromatic carbons), 146.14 (triazole C_3), 151.64 (N=CH), 154.82 (triazole C_5). Anal. Calcd. for $C_{18}H_{18}N_4O_2$: C, 67.1; H, 5.6; N, 17.4; Found: C, 66.6; H, 5.3; N, 17.8%.

General method for the preparation of 3-alkyl(aryl)-4-[4-(*p*-tolylsulfonyl)-oxybenzylidenamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (6): The corresponding compound **3** (0.01 mol) was refluxed with a solution of toluene-4-sulfonyl chloride (0.01 mol, 1.91 g) in pyridine (30 mL) for 3 h and then allowed to cool. The solid formed was collected, washed with cold water and dried *in vacuo*. Several recrystallizations of crude product from a proper solvent gave pure compounds **6**.

3-Methyl-4-[4-(*p*-tolylsulfonyl)oxybenzylidenamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-one (6a): Yield 40%; m.p. 130–131°C (EtOH-toluene, 1: 2). IR (KBr, cm^{-1}): 3200 ν(NH), 1705 ν(C=O), 1610, 1580 ν(C=N), 1185, 1155 ν(S=O), 820 ν(1,4-disubstituted benzenoid ring). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 2.09 (s, 3H, CH_3), 2.22 (s, 3H, CH_3), 6.77–7.62 (m, 8H, Ar-H), 9.55 (s, 1H, N=CH), 11.72 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 50 MHz): δ 11.33, 21.50 (aliphatic

carbons), 116.08 (2C), 122.86, 124.32, 128.46 (2C), 129.83 (2C), 130.44 (2C), 146.20, 160.80 (aromatic carbons), 144.49 (triazole C_3), 152.00 (N=CH), 154.50 (triazole C_5). UV (ethanol) λ_{max} (ε, L mol^{-1} cm^{-1}): 306 (9860), 223 (9460), 204 (12110) nm. Anal. Calcd. for $C_{17}H_{16}N_4O_2S$: C, 60.0; H, 4.7; N, 16.5; Found: C, 60.5; H, 4.9; N, 16.4%.

3-Ethyl-4-[4-(*p*-tolylsulfonyl)-oxybenzylidenamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-one (6b): Yield 46%; m.p. 180-181°C (EtOH-toluene, 1 : 2). IR (KBr, cm^{-1}) 3200 ν(NH), 1703 ν(C=O), 1600, 1590 ν(C=N), 1180, 1155 ν(S=O), 820 ν(1,4-disubstituted benzenoid ring). ^1H NMR (DMSO-d_6, 200 MHz): δ 1.21 (t, 3H, CH_3), 2.43 (s, 3H, CH_3), 2.67 (q, 2H, CH_2), 7.18–7.82 (m, 8H, Ar-H), 9.74 (s, 1H, N=CH), 11.81 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 50 MHz): δ 9.97, 18.47, 21.17 (aliphatic carbons), 122.70 (2C), 128.27 (2C), 129.28 (2C), 130.28 (2C), 131.20, 132.74, 147.99, 150.85 (aromatic carbons), 145.98 (triazole C_3), 151.32 (N=CH), 151.84 (triazole C_5). UV (ethanol) λ_{max} (ε, L mol^{-1} cm^{-1}): 298 (14700), 259 (16170), 209 (19760) nm. Anal. Calcd. for $C_{18}H_{18}N_4O_2S$: C, 61.0; H, 5.1; N, 15.8; Found: C, 61.7; H, 5.0; N, 15.8%.

Pharmacology

Ferric reducing/antioxidant power (FRAP) assay: The reducing power of the synthesized compounds in DMSO solutions was determined according to a modified version of ferric reducing/antioxidant power (FRAP) assay of Oyaizu[31]. Different concentrations of the samples (0.500–0.125 mg/mL) and ascorbic acid for comparison were mixed with phosphate buffer (0.2 M, 2.5 mL, pH = 6.6) and 1% potassium ferricyanide (2.5 mL). The mixture was incubated at 20°C for 20 min. After incubation period, the mixture was vortexed, and the absorbance was measured at 700 nm. Higher absorbance value means higher reducing power of a sample.

Free radical scavenging activity: The free radical scavenging activity of compounds **3–6** was measured by utilizing DPPH in the method of Cuendet *et al.*[32] Briefly, 0.1 mM solution of DPPH in ethanol (0.75 mL) was added to the sample solution in DMSO (0.75 mL) at different concentrations (1.000–0.0312 mg/mL). After 30 min, absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. 50% inhibitory concentration (IC_{50}) of the compounds, which was calculated from the curves drawn by plotting absorbance values for corresponding sample concentration, was used to evaluate radical scavenging activities of the compounds. Higher IC_{50} values pointed to higher compound concentrations required to scavenge half of the radicals present and, thus, to lower radical scavenging activities.

Potentiometric titrations: For potentiometric titrations, a Jenway 3040 ion analyzer pH-meter, calibrated according to the instructions of the manufacturers and equipped with an Ingold pH electrode, was used. During the titrations, the titrant was added in increments of 0.05 mL after each stable reading and the corresponding mV values were recorded. After purification, isopropyl alcohol was used as solvent to prepare 0.05 M tetrabutylammonium hydroxide (TBAH), which was used for all potentiometric titrations.

RESULTS AND DISCUSSION

In this study, the structures of six new 3-alkyl(aryl)-4-(4-hydroxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**3**), four new 1-acetyl-3-alkyl(aryl)-4-(4-acetyloxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**4**), two new 1-methyl-3-alkyl-4-(4-methoxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**5**) and two new 3-alkyl(aryl)-4-[4-(p-tolylsulfonyl)-oxybenzylidenamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**6**) were identified using elemental analysis and IR, ^{1}H NMR, ^{13}C NMR and UV spectral data.

In addition, the newly synthesized compounds **3**–**6**, except compounds **3e** and **4f**, were screened for their *in-vitro* antioxidant activities. Several methods are used to determine total antioxidant capacity (TAC)[33]. In this study, ferric reducing/antioxidant power (FRAP) method was used to determine TAC of the compounds. Any substance that can donate electrons and has a half-reaction reduction potential lower than that of the Fe^{3+}/Fe^{2+} redox couple will be reactive in the FRAP method[34].

The absorbance values obtained with FRAP method for three different concentrations are presented in Table-1.

TABLE-1
REDUCING POWER AND FREE RADICAL SCAVENGING ACTIVITY OF THE NEW COMPOUNDS

Compound	FRAP[a] activity (mg/mL)			DPPH[b] scavenging activity
	$A_{700\,nm}$[c]			IC_{50}[d] (mg/mL)
	0.5	0.25	0.125	
BHT[e]	x	x	x	0.43
Ascorbic acid	0.042	0.010	0	x
3a	—	—	—	15.73
3b	—	—	—	—
3c	0.360	0.031	0	—
3d	0.229	0.149	0	—
3f	—	—	—	Oxidant
4a	—	—	—	—
4c	0.262	0	0	—
4d	0.275	0.155	0.099	—
5a	—	—	—	—
5c	0.287	0	0	3.20
6a	0.036	0	0	13.0
6b	0.344	0.149	0	—

[a] FRAP: ferric reducing antioxidant power
[b] DPPH: 1,1-diphenyl-2-picryl-hydrazil, free radical
[c] A_{700nm}: Absorbance at 700 nm, larger values represent higher antioxidant power
[d] IC_{50}: The concentration of the sample compound providing 50 per cent scavenging of DPPH free radical
[e] BHT: Butylated hydroxytoluene; BHT and ascorbic acid are used as reference standards
— : No activity was observed
x: Not tested

Higher absorbance values in this method mean higher antioxidant capacity. Compounds **3c, 3d, 4c, 4d, 5c, 6a** and **6b** showed antioxidant activity, generally higher than the antioxidant activity of ascorbic acid, which was used as reference antioxidant compound. Only compound **4d** showed antioxidant activity at 0.125 mg/mL concentration in this method, again exceeding the antioxidant activity of ascorbic acid. Compounds **3c, 3d, 4d** and **6b** had concentration dependent activity at the concentrations utilized in the test method (Fig. 1).

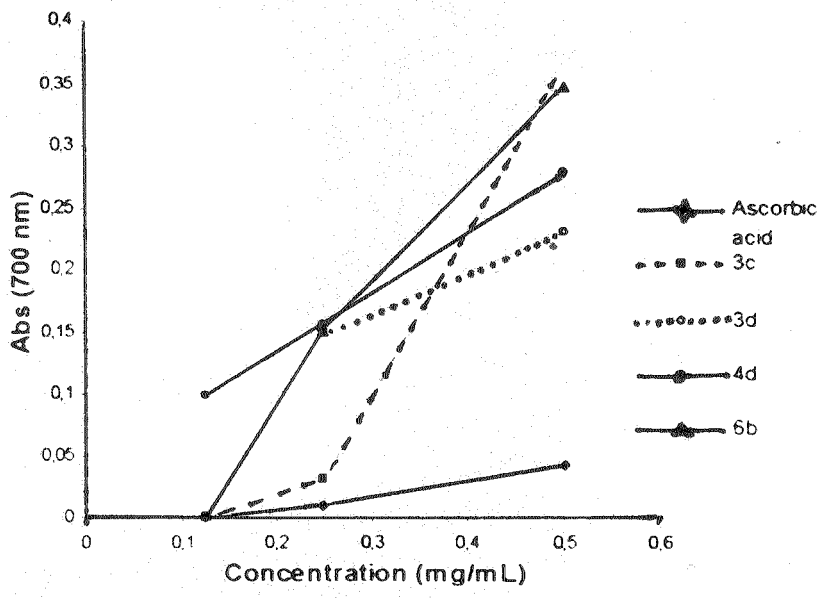


Fig. 1. Concentration dependence of the ferric reducing/antioxidant power (FRAP) of compounds **3c, 3d, 4d** and **6b**. Higher absorbance values represent higher antioxidant activity

DPPH can generate stable free radicals in solutions. Free radicals are well-known to be able to induce lipid peroxidation. In the DPPH test, sample solutions were able to scavenge DPPH stable radical to yellow coloured diphenylpicrylhydrazine. The method is based on the reduction of DPPH in the solution in the presence of a hydrogen-donating antioxidant resulting in the formation of the non-radical form DPPH-H. The compounds synthesized were tested in this method at five different concentrations and the graphs obtained by plotting compound concentration against absorbance at 517 nm were used to calculate the compound concentrations providing 50% scavenging of the DPPH radicals present in the reaction medium (IC_{50}). The activities of the compounds compared with that of the reference antioxidant compound BHT are given in Table-1.

Only three of the 12 compounds tested with this method showed radical scavenging and one showed prooxidant character, *i.e.*, increased radical formation. The remaining eight compounds did not show any effect on radicals. All three compounds possessing scavenging activity exhibited scavenging capacities lower than the standard antioxidant BHT. Compound **5c**, among the three, showed the highest activity, with the lowest IC_{50} value.

Compounds **5c** and **6a** showed antioxidant capacity in the two methods, suggesting better antioxidant behaviour. Compound **5c** was especially active. Despite the limitation from the insufficient variation and number of the compounds, the

antioxidant activity results suggest structure-activity relationship, since compounds of close similarity exhibited quite a high degree of variation in antioxidant capacity.

On the other hand, the newly synthesized **3** type compounds were titrated potentiometrically with tetrabutylammonium hydroxide (TBAH) in the non-aqueous solvents such as isopropyl alcohol, *t*-butyl alcohol and N,N-dimethyl formamide. The non-aqueous solvents N,N-dimethyl formamide from aprotic solvent and isopropyl alcohol and *t*-butyl alcohol from amphiprotic neutral solvents, were used in potentiometric titrations.

The mV values read in each titration were drawn against TBAH volumes (mL), and potentiometric titration curves were obtained for all the cases.

As an example, the potentiometric titration curves of 0.001 M 3-phenyl-4-(4-hydroxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (**3f**) solutions titrated with 0.05 M TBAH in isopropyl alcohol, *t*-butyl alcohol, N,N-dimethyl formamide are presented in Fig. 2.

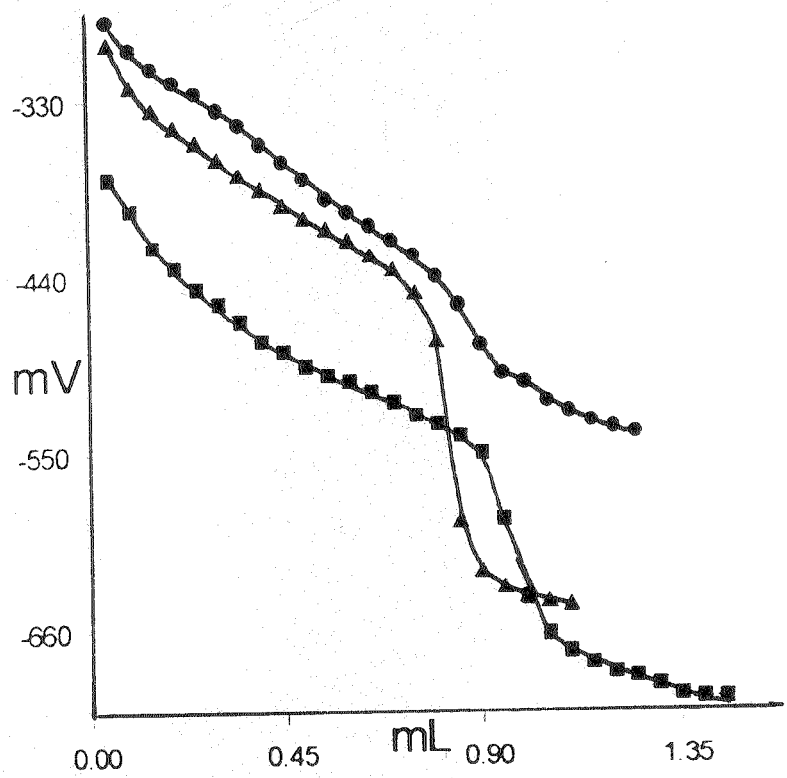


Fig. 2. Potentiometric titration curves of 10^{-3} M 3-phenyl-4-(4-hydroxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (**3f**) solutions titrated with 0.05 M TBAH in isopropyl alcohol (●), *t*-butyl alcohol (▲) and N,N-dimethyl formamide (■) at 25°C

The half-neutralization potential (HNP) values and the corresponding pKa values for compounds **3a–f**, obtained from the potentiometric titrations with 0.05 M TBAH in isopropyl alcohol, *t*-butyl alcohol and N,N-dimethyl formamide, are given in Table-2.

The pH of the weak acids can be found by the following equality:

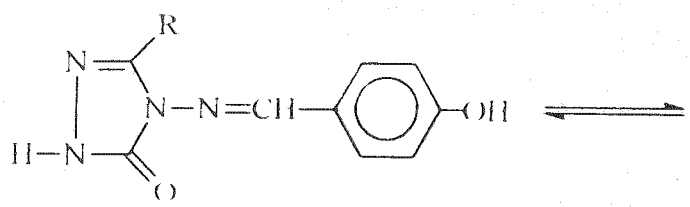
$$pH = pK_a + \log [A^-]/[HA]$$

$pH = pK_a$ occurs in $[A^-]$ due to the fact that it is equal to [HA] at the half-neutralization points. Therefore, the pH values were regarded as pK_a at the half-neutralization points.

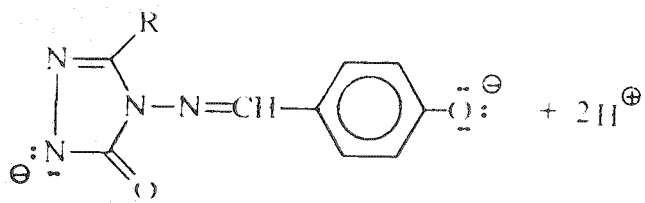
TABLE-2
THE HALF-NEUTRALIZATION POTENTIAL (HNP) VALUES AND THE CORRESPONDING pK_a VALUES OF COMPOUNDS **3a–f** IN ISOPROPYL ALCOHOL, *t*-BUTYL ALCOHOL AND N,N-DIMETHYLFORMAMIDE

Compd. No.	N,N-Dimethyl formamide		Isopropyl alcohol		*t*-butyl alcohol	
	HNP (mV)	pK_a	HNP (mV)	pKa	HNP (mV)	pKa
3a	–494	15.31	–316	12.08	–432	14.14
3b	–533	15.85	–293	12.84	–411	13.86
3c	–500	15.46	–396	13.60	–382	13.78
3d	–418	14.45	–339	12.58	–357	12.76
3e	–503	15.76	–387	13.87	–414	14.26
3f	–496	15.35	–369	13.00	–392	13.45

When the dielectric permittivity of solvents is taken into consideration, the acidic arrangement can be expected as follows: N,N-dimethyl formamide ($\varepsilon = 37$) > isopropyl alcohol ($\varepsilon = 19.4$) > *t*-butyl alcohol ($\varepsilon = 12$). As seen in Table-2, the arrangement for compounds **3a–f** is: isopropyl alcohol > *t*-butyl alcohol > N,N-dimethyl formamide. These results are in accordance with the theoretical arrangement, except N,N-dimethyl formamide. In N,N-dimethyl formamide, these compounds show the weakest acidic properties. This situation can be attributed to the hydrogen bonding between the negatively formed ions (B) and the solvent molecules in the amphiprotic neutral solvents **(Scheme-2).**



3A



3B

Scheme-2

As it is well known, the acidity of a compound depends on some factors. The two most important factors are the solvent effect and molecular struc- ture[14–19, 26–29]. Table-2 and Fig. 2 show that the HNP values and the corresponding pK_a values obtained from potentiometric titrations rely on the type of non-aqueous solvents used and molecular structure of the compound tested.

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