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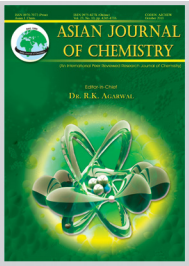

Study of Antioxidant Properties and DNA Interaction of Some Novel 4,5-Dihydro-1*H*-1,2,4-triazol-5-one Derivatives

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3-Alkyl(aryl)-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**2**) reacted with 4-benzenesulfonyloxybenzaldehyde (**3**) to afford the corresponding seven novel 3-alkyl(aryl)-4-(4-benzenesulfonyloxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**4**). The acetylation reactions of compounds **4** were investigated and **5** type compounds were obtained. The 14 new compounds synthesized were characterized by using IR, ^{1}H NMR, ^{13}C NMR and UV spectral data together with elemental analysis. The synthesized compounds were analyzed for their *in vitro* potential antioxidant activities in three different methods. Those antioxidant activities were compared to standard antioxidants such as BHA, BHT and α-tocopherol. In addition, the interaction of the synthesized compounds **4** with cat DNA was investigated by using electrophoresis measurements. Results suggest that the compounds do not interact with the DNA. Furthermore, to investigate the effects of solvents and molecular structure upon acidity, compounds **4** were titrated potentiometrically with tetrabutylammonium hydroxide in five non-aqueous solvents (isopropyl alcohol, *tert*-butyl alcohol, *N,N*-dimethylformamide, acetone and dimethyl sulphoxide). The half-neutralization potential values and the corresponding pK_a values were determined for all cases.

Key Words: 1,2,4-Triazol-5-one, Schiff base, Synthesis, Antioxidant activity, Acetylation, Acidity, DNA interaction.

INTRODUCTION

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, antiinflammatory, antitumor and anti-HIV properties[1-7]. In addition, several research papers reporting the synthesis of some *N*-arylidenamino-4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives[6,8-14]. The acetylation and methylation of 4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives have also been reported[9-15].

Global studies of antioxidants concern both natural and synthetic antioxidants. Any molecule that can retard or prevent the action of oxidants could be considered to be an antioxidant. Antioxidants are in the first line of antioxidative defense and are therefore of high importance in cellular response to oxidative stress. Many studies suggest that a plethora of synthetic antioxidant compounds can be potentially useful in therapy. Researchers in many different disciplines has become more concerned the search for new natural antioxidants, synthesis of new antioxidant compounds and evaluation and elucidation of mechanisms of action of both natural and synthetic antioxidants[16]. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are formed under physiological conditions in human body or in food system and are removed by cellular antioxidant defense system. During oxidative stress their increased formation leads to several diseases including atherosclerosis, neurodegenerative diseases, such as Alzheimer's and Parkinson's disease, cancer, diabetes mellitus, inflammatory diseases, as well as psychological diseases or aging processes[17]. Recent studies pointed out the necessity to maintain the natural oxidative homeostasis of the organism and the importance to help the organism to keep this homeastasis both health and disease. Therefore the efforts raised to synthesize multi-functional antioxidant which could be considered as "biological response modifiers" maintaining oxidative homeostasis.

In the present work, the antioxidant potential of sythesized compounds has been exploited using three different assays: DPPH (2,2-diphenylpicryl hydrazyl) scavenging activity, metal chelating activity and reducing power assay. On the other hand, recently DNA binding studies with small molecules over molecular levels get more attention[18,19]. Especially,

new small compounds get considerable attention because of their importance in the development of new therapeutic materials[20,21].

Although water is an extraordinarily versatile solvent in which to carry out acid-base titrations, there are occasions when a nonaqueous solvent may be necessary or preferred, such as when the reagent is not water soluble and the neutralization reactions is not sufficiently complete in water. The completeness of a neutralization reaction depends, in part, on acid or base strength of the analyte. But Bronsted and Lowry have made it clear that the observed acidity or basicity depends on the solvent because it is participates in the ionization[22]. It is well known that there are two major factors influencing the acidity or basicity of a molecule, namely, solvent and structural effects. An acid or base too weak to titrate in water some times can be titrated in a nonaqueous solvent, where its observed acidity is greater[23,24]. Thus, 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 tetrabutylammonium hydroxide (TBAH) in non-aqueous solvents and the pK_a values of the compounds were determined[2,5,6,8-14,25,26].

EXPERIMENTAL

Chemical reagents and all the solvents used in this study were purchased from Merck AG, Aldrich and Fluka. The starting materials **2a-g** were prepared from the reactions of the corresponding ester ethoxycarbonylhydrazones **1a-g** with an aqueous solution of hydrazine hydrate as described in the literature[15,27]. Melting points were determined in open glass capillaries using an Electrothermal 9100 digital melting point apparatus and are uncorrected. The IR spectra were obtained on a Perkin-Elmer Instruments Spectrum One FT-IR spectrometer. ^{1}H and ^{13}C NMR spectra were recorded in deuterated dimethyl sulfoxide with TMS as internal standard using 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-1201 UV/Vis spectrometer. Extinction coefficients (ε) are expressed in L mol^{-1} cm^{-1}. Elemental analyses were carried out on an ECS 4010 Costech Instruments Elemental Combustion System (CHNS-O) for C, H and N.

Synthesis of compounds 4: 4-Hydroxybenzaldehyde (0.01 mol) dissolved in ethyl acetate (15 mL) was treated with benzenesulfonyl chloride (0.01 mol) and to this solution was added triethylamine (0.01 mol) slowly with stirring at 0-5 ºC. Stirring was continued for 2 h and the mixture was refluxed for 4 h and filtered. The filtrate was evaporated *in vacuo* and the crude product was washed with water and recrystallized from ethanol to afford compound **3**, m.p. 85 ºC; yield 2.25 g (85.9 %). IR (KBr, ν_{max}, cm^{-1}): CHO 2854, 2746; C=O 1701; SO_2 1374 and 1175; 1,4-disubstituted benzenoid ring 852; monosubstituted benzenoid ring 761 and 694. The corresponding compound **2** (0.01 mol) was dissolved in acetic acid (15 mL) and treated with 4-benzenesulfonyloxybenzaldehyde (**3**) (0.01 mol). The mixture was refluxed for 1.5 h and then evaporated at 50-55 ºC *in vacuo*. Several recrystallizations of the residue from ethanol gave pure compounds **4** as colourless crystals.

3-Methyl-4-(4-benzenesulfonyloxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (4a): Yield 3.24 g (90.5 %). m.p. 218 ºC. IR (KBr, ν_{max}, cm^{-1}): 3190 (NH); 1700 (C=O); 1600 (C=N); 1375 and 1176 (SO_2); 845 (1,4-disubstituted benzenoid ring); 750 and 694 (monosubstituted benzenoid ring). ^{1}H NMR (DMSO-d_6): δ 2.25 (s, 3H, CH_3), 7.15 (d, 2H, ArH, *J* = 8.7 Hz), 7.67-7.70 (m, 2H, ArH), 7.82-7.90 (m, 5H, ArH), 9.70 (s, 1H, N=CH), 11.84 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 11.52 (CH_3), 123.16 (2C), 128.72 (2C), 129.22 (2C), 130.36 (2C), 133.25, 134.53, 135.68, 144.75 (arom-C), 151.23 (triazole C_3), 151.60 (N=CH), 152.67 (triazole C_5). UV λ_{max} (ε): 296 (21826), 256 (24664), 224 (17672) nm.

3-Ethyl-4-(4-benzenesulfonyloxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (4b): Yield 3.32 g (89.3 %). m.p. 194 ºC. IR (KBr, ν_{max}, cm^{-1}): 3190 (NH); 1700 (C=O); 1600 (C=N); 1375 and 1176 (SO_2); 845 (1,4-disubstituted benzenoid ring); 750 and 694 (monosubstituted benzenoid ring). ^{1}H NMR (DMSO-d_6): δ 1.18 (t, 3H, CH_3, *J* = 7.5 Hz), 2.65 (q, 2H, CH_2, *J* = 7.5 Hz), 7.15 (d, 2H, ArH, *J* = 8.6 Hz), 7.68 (t, 2H, ArH, *J* = 7.5 Hz), 7.81-7.85 (m, 3H, ArH), 7.89 (d, 2H, ArH, *J* = 7.6 Hz), 9.70 (s, 1H, N=CH), 11.87 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 10.48 (CH_3), 18.92 (CH_2), 123.18 (2C), 128.71 (2C), 129.78 (2C), 130.36 (2C), 133.27, 134.56, 135.67, 148.49 (arom-C), 151.23 (triazole C_3), 151.74 (N=CH), 152.40 (triazole C_5). UV λ_{max} (ε): 296 (13860), 260 (17005), 224 (13488) nm.

3-Benzyl-4-(4-benzenesulfonyloxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (4c): Yield 4.25 g (97.9 %). m.p. 199 ºC. IR (KBr, ν_{max}, cm^{-1}): 3212 (NH); 1704 (C=O); 1600, 1589 (C=N); 1371 and 1181 (SO_2); 820 (1,4-disubstituted benzenoid ring); 753 and 702, 750 and 683 (monosubstituted benzenoid ring). ^{1}H NMR (DMSO-d_6): δ 3.99 (s, 2H, CH_2), 7.15 (d, 2H, ArH, *J* = 8.7 Hz), 7.20-7.23 (m, 1H, ArH), 7.27-7.33 (m, 4H, ArH), 7.66-7.70 (m, 2H, ArH), 7.79-7.85 (m, 3H, ArH), 7.89-7.91 (m, 2H, ArH), 9.68 (s, 1H, N-CH), 12.02 (s,1H, NH). ^{13}C NMR (DMSO-d_6): δ 31.47 (CH_2), 123.17 (2C), 127.19, 128.71 (2C), 128.91 (2C), 129.26 (2C), 129.82 (2C), 130.34 (2C), 133.22, 134.58, 135.65, 136.19, 146.68 (arom-C), 151.24 (triazol C-3), 151.60 (N=CH), 152.20 (triazol C-5). UV λ_{max} (ε): 300 (12896), 260 (15920), 226 (14552) nm. Anal. calcd. for $C_{22}H_{18}N_4O_4S$ (434.47): C, 60.82; H, 4.17; N, 12.89; S, 7.38. Found: C, 59.86; H, 4.29; N, 12.43; S, 7.96.

3-*p*-Methylbenzyl-4-(4-benzenesulfonyloxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (4d): Yield 4.35 g (97.1 %). m.p. 152 ºC. IR (KBr, ν_{max}, cm^{-1}): 3191 (NH); 1710 (C=O); 1591 (C=N); 1376 and 1176 (SO_2); 844, 820 (1,4-disubstituted benzenoid ring); 744 and 686 (monosubstituted benzenoid ring). ^{1}H NMR (DMSO-d_6): δ 2.24 (s, 3H, CH_3), 3.98 (s, 2H, CH_2), 7.10 (d, 2H, ArH, *J* = 7.9 Hz), 7.15-7.20 (m, 4H, ArH), 7.67-7.71 (m, 2H, ArH), 7.80-7.85 (m, 3H, ArH), 7.89-7.91 (m, 2H, ArH), 9.66 (s, 1H, N-CH), 11.98 (s,1H, NH). ^{13}C NMR (DMSO-d_6): δ 21.07 (CH_3), 31.06 (CH_2), 123.19 (2C), 128.73 (2C), 129.12 (2C), 129.48 (2C), 129.85 (2C), 130.37 (2C), 133.08, 133.23, 134.56, 135.69, 136.25, 146.83 (arom-C), 151.23 (triazol C-3), 151.58 (N=CH), 152.21 (triazol C-5). UV λ_{max} (ε): 300 (11558), 260 (14340), 224 (16267) nm.

3-*p*-Chlorobenzyl-4-(4-benzenesulfonyloxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (4e): Yield 4.55 g (97.0 %). m.p. 151 ºC. IR (KBr, ν_{max}, cm^{-1}): 3187 (NH); 1716 (C=O); 1595, 1590 (C=N); 1376 and 1177 (SO_2); 847, 820 (1,4-disubstituted benzenoid ring); 760 and 691 (monosubstituted benzenoid ring). ^{1}H NMR (DMSO-d_6): δ 4.06 (s, 2H, CH_2), 7.16 (d, 2H, ArH, *J* = 8.7 Hz), 7.32-7.38 (m, 4H, ArH), 7.69 (t, 2H, ArH, *J* = 7.8 Hz), 7.80-7.89 (m, 3H, ArH), 7.90 (d, 2H, ArH, *J* = 7.3 Hz), 9.67 (s, 1H, N-CH), 12.02 (s,1H, NH). ^{13}C NMR (DMSO-d_6): δ 30.78 (CH_2), 123.19 (2C), 128.73 (2C), 128.85 (2C), 129.98 (2C), 130.37 (2C), 131.21 (2C), 131.90, 133.17, 134.54, 135.17, 135.69, 146.36 (arom-C), 151.26 (triazol C-3), 151.56 (N=CH), 152.33 (triazol C-5). UV λ_{max} (ε): 300 (12567), 260 (15076), 226 (19414) nm. Anal. calcd. for $C_{22}H_{17}N_4O_4SCl$ (468.91): C, 56.35; H, 3.65; N, 11.95; S, 6.84. Found: C, 56.07; H, 3.76; N, 11.71; S, 6.74.

3-Phenyl-4-(4-benzenesulfonyloxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (4f): Yield 4.10 g (97.6 %). m.p. 184 ºC. IR (KBr, ν_{max}, cm^{-1}): 3181 (NH); 1720 (C=O); 1599 (C=N); 1381 and 1180 (SO_2); 809 (1,4-disubstituted benzenoid ring); 745 and 688 (monosubstituted benzenoid ring). ^{1}H NMR (DMSO-d_6): δ 7.18 (d, 2H, ArH, *J* = 8.7 Hz), 7.51-7.54 (m, 3H, ArH), 7.67-7.71 (m, 2H, ArH), 7.81-7.84 (m, 3H, ArH), 7.85-7.92 (m, 4H, ArH), 9.67 (s, 1H, N-CH), 12.41 (s,1H, NH). ^{13}C NMR (DMSO-d_6): δ 123.29 (2C), 127.00, 128.45 (2C), 128.70 (2C), 129.02 (2C), 130.07 (2C), 130.39 (2C), 130.60, 133.04, 134.57, 135.70, 145.07 (arom-C), 151.40 (triazol C-3), 151.74 (N=CH), 155.26 (triazol C-5). UV λ_{max} (ε): 266 (17424), 224 (13430) nm. Anal. calcd. for $C_{21}H_{16}N_4O_4S$ (420.44): C, 59.99; H, 3.84; N, 13.33; S, 7.63. Found: C, 59.25; H, 3.97; N, 12.91; S, 8.28.

3-Cyclopropyl-4-(4-benzenesulfonyloxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (4g): Yield 2.60 g (67.7 %). m.p. 182 ºC. IR (KBr, ν_{max}, cm^{-1}): 3176 (NH); 1701 (C=O); 1627, 1588 (C=N); 1366 and 1195 (SO_2); 831 (1,4-disubstituted benzenoid ring); 760 and 697 (monosubstituted benzenoid ring). ^{1}H NMR (DMSO-d_6): δ 0.87-0.95 (m, 4H, CH_2CH_2), 2.08-2.15 (m, 1H, CH), 7.69 (t, 2H, ArH, *J* = 7.8 Hz), 7.82-7.91 (m, 5H, ArH), 9.70 (s, 1H, N-CH), 11.81 (s,1H, NH). ^{13}C NMR (DMSO-d_6): δ 5.97 (CH), 6.96 (CH_2CH_2), 123.14, 123.18, 128.72, 129.85, 130.36 (2C), 130.52, 133.27, 134.54, 134.56, 135.68, 148.68 (arom-C), 151.25 (triazol C-3), 151.71 (N=CH), 152.78 (triazol C-5). UV λ_{max} (ε): 300 (11480), 260 (15437), 226 (12683) nm.

Synthesis of compound 5: The corresponding compound **4** (0.01 mol) was refluxed with acetic anhydride (20 mL) for 0.5 h. After the addition of absolute ethanol (100 mL), the mixture was refluxed for 1 h more. Evaporation of the resulting solution at 40-45 ºC *in vacuo* and several recrystallizations of the residue from EtOH gave pure compounds **5** as colourless needles.

1-Acetyl-3-methyl-4-(4-benzenesulfonyloxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (5a): Yield 3.55 g (88.8 %). m.p. 191 ºC. IR (KBr, ν_{max}, cm^{-1}): 1778, 1702 (C=O); 1625, 1606 (C=N); 1366 and 1179 (SO_2); 978, 872 and 790 (S-O-C); 846 (1,4-disubstituted benzenoid ring); 758 and 682 (monosubstituted benzenoid ring). ^{1}H NMR (DMSO-d_6): δ 2.30 (s, 3H, CH_3), 2.45 (s, 3H, $COCH_3$), 7.15 (d, 2H, ArH, *J* = 8.6 Hz), 7.68 (d, 2H, ArH, *J* = 7.8 Hz), 7.79-7.89 (m, 5H, ArH), 9.55 (s, 1H, N=CH). ^{13}C NMR (DMSO-d_6): δ 11.87 (CH_3), 24.16 ($COCH_3$), 123.51 (2C), 128.96 (2C), 130.42 (2C), 130.61 (2C), 132.94, 134.61, 135.97, 154.64 (arom-C), 147.39 (triazole C_3), 148.45 (N=CH), 151.77 (triazole C_5), 166.73 ($COCH_3$). UV λ_{max} (ε): 292 (17480), 254 (20850), 226 (15620) nm. Anal. calcd. for $C_{18}H_{16}N_4O_5S$ (400.41): C, 53.99; H, 4.03; N, 13.99; S, 8.00. Found: C, 54.01; H, 4.21; N, 13.36; S, 8.83.

1-Acetyl-3-ethyl-4-(4-benzenesulfonyloxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (5b): Yield 3.65 g (88.2 %). m.p. 186 ºC. IR (KBr, ν_{max}, cm^{-1}): 1776, 1699 (C=O); 1619, 1604 (C=N); 1372 and 1179 (SO_2); 978, 873 and 793 (S-O-C); 843 (1,4-disubstituted benzenoid ring); 756 and 700 (monosubstituted benzenoid ring). ^{1}H NMR (DMSO-d_6): δ 1.19 (t, 3H, CH_3, *J* = 7.4 Hz), 2.45 (s, 3H, $COCH_3$), 2.70 (q, 2H, CH_2, *J* = 7.4 Hz), 7.15 (d, 2H, ArH, *J* = 8.6 Hz), 7.68 (t, 2H, ArH, *J* = 7.8 Hz), 7.78-7.88 (m, 5H, ArH), 9.54 (s, 1H, N=CH). UV λ_{max} (ε): 292 (16300), 254 (20200), 224 (18460) nm.

1-Acetyl-3-benzyl-4-(4-benzenesulfonyloxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (5c): Yield 4.50 g (94.5 %). m.p. 162 ºC. IR (KBr, ν_{max}, cm^{-1}): 1779, 1705 (C=O); 1610, 1599 (C=N); 1367 and 1177 (SO2); 979 and 876 (S-O-C); 825 (1,4-disubstituted benzenoid ring); 762 and 699 (monosubstituted benzenoid ring). ^{1}H NMR (DMSO-d_6): δ 2.46 (s, 3H, $COCH_3$), 4.10 (s, 2H, CH_2), 7.14 (d, 2H, ArH, *J* = 8.6 Hz), 7.24-7.31 (m, 5H, ArH), 7.68 (d, 2H, ArH, *J* = 7.8 Hz), 7.82-7.89 (m, 5H, ArH), 9.51 (s, 1H, N-CH). ^{13}C NMR (DMSO-d_6): δ 24.23 ($COCH_3$), 31.60 (CH_2), 123.49 (2C), 127.67, 128.94 (2C), 129.21 (2C), 129.67 (2C), 130.41 (2C), 130.61 (2C), 132.91, 134.65, 135.28, 135.96, 154.30 (arom-C), 148.61 (triazol C-3), 148.94 (N=CH), 151.74 (triazol C-5), 166.66 ($COCH_3$). UV λ_{max} (ε): 294 (18400), 256 (21760), 222 (26200) nm.

1-Acetyl-3-*p*-methylbenzyl-4-(4-benzenesulfonyloxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (5d): Yield 3.73 g (76.1 %). m.p. 182 ºC. IR (KBr, ν_{max}, cm^{-1}): 1779, 1702 (C=O); 1617, 1602 (C=N); 1368 and 1177 (SO_2); 980, 875 and 798 (S-O-C); 853, 820 (1,4-disubstituted benzenoid ring); 765 and 689 (monosubstituted benzenoid ring). ^{1}H NMR (DMSO-d_6): δ 2.22 (s, 3H, CH_3), 2.46 (s, 3H, $COCH_3$), 4.05 (s, 2H, CH_2), 7.05-7.25 (m, 6H, ArH), 7.60-7.70 (m, 2H, ArH), 7.77-7.90 (m, 5H, ArH), 9.55 (s, 1H, N-CH). ^{13}C NMR (DMSO-d_6): δ 21.33 (CH_3), 24.26 ($COCH_3$), 31.23 (CH_2), 123.52 (2C), 128.97 (2C), 129.57 (2C), 129.78 (2C), 130.44 (2C), 130.64 (2C), 132.15, 132.94, 134.65, 135.98, 136.79, 154.35 (arom-C), 148.62 (triazol C-3), 149.12 (N=CH), 151.75 (triazol C-5), 166.69 ($COCH_3$). UV λ_{max} (ε): 292 (16120), 256 (19044), 220 (19926), 212 (17345) nm. Anal. calcd. for $C_{25}H_{22}N_4O_5S$ (490.53): C, 61.21; H, 4.52; N, 11.42; S, 6.53. Found: C, 60.60; H, 4.60; N, 11.22; S, 7.07.

1-Acetyl-3-*p*-chlorobenzyl-4-(4-benzenesulfonyloxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (5e): Yield 4.55 g (93.0 %). m.p. 153 ºC. IR (KBr, ν_{max}, cm^{-1}): 1778, 1699 (C=O); 1618, 1603 (C=N); 1370 and 1177 (SO_2); 980 and 872 (S-O-C); 849, 803 (1,4-disubstituted benzenoid ring); 766 and 689 (monosubstituted benzenoid ring). ^{1}H NMR (DMSO-d_6): δ 2.46 (s, 3H, $COCH_3$), 4.11 (s, 2H, CH_2), 7.14 (d, 2H, ArH, *J* = 8.6 Hz), 7.36 (m, 4H, ArH), 7.68 (d, 2H, ArH, *J* = 7.8 Hz), 7.79-7.89 (m, 5H, ArH), 9.52 (s, 1H, N-

CH). ^{13}C NMR (DMSO-d_6): δ 24.24 ($COCH_3$), 30.95 (CH_2), 123.52 (2C), 128.97 (2C), 129.13 (2C), 130.46 (2C), 130.63 (2C), 131.65 (2C), 132.38, 132.90, 134.32, 134.64, 135.98, 154.35 (arom-C), 148.69 (triazol C-3), 151.77 (N=CH), 151.77 (triazol C-5), 166.66 ($COCH_3$). UV λ_{max} (ε): 292 (20460), 256 (23410), 228 (21600) nm.

1-Acetyl-3-phenyl-4-(4-benzenesulfonyloxybenzylidenamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (5f): Yield 4.35 g (94.2 %). m.p. 144 ºC. IR (KBr, ν_{max}, cm^{-1}): 1733 (C=O); 1604, 1587 (C=N); 1370 and 1180 (SO_2); 971, 867 and 778 (S-O-C); 841 (1,4-disubstituted benzenoid ring); 750 and 698 (monosubstituted benzenoid ring). ^{1}H NMR (DMSO-d_6): δ 2.48 (s, 3H, $COCH_3$), 7.16 (d, 2H, ArH, *J* = 7.8 Hz), 7.56 (m, 3H, ArH), 7.64 (d, 2H, ArH, *J* = 7.8 Hz), 7.69-7.84 (m, 7H, ArH), 9.50 (s, 1H, N-CH). ^{13}C NMR (DMSO-d_6): δ 24.29 ($COCH_3$), 123.36, 123.64, 125.87, 128.44, 128.94 (2C), 129.38 (2C), 129.42 (2C), 129.66, 130.64 (2C), 132.03, 132.73, 134.67, 136.00, 157.51 (arom-C), 146.75 (triazol C-3), 148.80 (N=CH), 151.92 (triazol C-5), 166.97 ($COCH_3$). UV λ_{max} (ε): 258 (27960), 230 (20980) nm.

1-Acetyl-3-cyclopropyl-4-(4-benzenesulfonyloxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (5g): Yield 3.45 g (81.0 %). m.p. 144 ºC. IR (KBr, ν_{max}, cm^{-1}): 1767, 1706 (C=O); 1605 (C=N); 1373 and 1178 (SO_2); 978 and 869 (S-O-C); 840 (1,4-disubstituted benzenoid ring); 753 and 695 (monosubstituted benzenoid ring). ^{1}H NMR (DMSO-d_6): δ 0.85-1.06 (m, 4H, CH_2CH_2), 2.10-2.20 (m, 1H, CH), 2.43 (s, 3H, $COCH_3$), 7.16 (d, 2H, ArH, *J* = 8.2 Hz), 7.68 (d, 2H, ArH, *J* = 7.4 Hz), 7.78-7.93 (m, 5H, ArH), 9.55 (s, 1H, N-CH). ^{13}C NMR (DMSO-d_6): δ 6.07 (CH), 8.08 (CH_2CH_2), 24.14 ($COCH_3$), 123.20, 123.52, 128.96 (2C), 130.07, 130.44, 130.61, 132.18, 132.95, 134.64, 135.97, 155.01 (arom-C), 148.72 (triazol C-3), 151.20 (N=CH), 152.98 (triazol C-5), 166.53 ($COCH_3$). UV λ_{max} (ε): 290 (12400), 260 (16500), 224 (11480) nm.

Antioxidant activity: Butylated hydroxytoluene (BHT) was the product of E. Merck, Darmstadt, Germany. 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 purchased from Sigma Chemical Company, Saint Louis, Missouri, USA.

Reducing power: The synthesized compounds (50-250 μg/mL) in DMSO (1 mL) were mixed with 2.5 mL of 0.2 mol/L of phosphate buffer, pH = 6.5 and 2.5 mL of 1 g/100 mL of potassium ferricyanide and than, incubated at 50 ºC for 20 min. 2.5 mL trichloroacetic acid (10 %) was added to the mixture and centrifuged at 3000 g for 10 min at room temperature. The resulting supernatant was taken and mixed with 2.5 mL of H_2O and 0.5 mL of ferric chloride (% 1) and than, incubated 37 ºC for 10 min. the absorbance at 700 nm was measured. Increased absorbance indicated increased reducing power[28].

Free radical scavenging activity: Free radical scavenging activity of compounds was measured by DPPH$^•$, using the method of Blois[29]. Briefly, reactions were performed in 1 mL of ethanol containing 0.1 mmol/L freshly made DPPH and 3 mL of 50-250 μg/mL of synthesized compounds. Reaction mixtures were incubated at 37 ºC for 0.5 h and the absorbance at 517 nm was measured. The DPPH$^•$ concentration (mM) in the reaction medium was calculated from the following calibration curve and determined by linear regression (R: 0.997): Absorbance = 0.0003 × DPPH$^•$ - 0.0174.

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

$$\text{DPPH}^• \text{ scavenging effect (\%)} = \frac{(A_0 - A_1)}{A_0} \times 100$$

where A_0 is the absorbance of the control reaction and A_1 is the absorbance in the presence of the samples or standards.

Metal chelating activity: Fe^{2+}-chelating activity was measured by the method of Dinis *et al.*[30]. The reaction mixture contained synthesized compounds (50-250 μg/mL), 2 mM $FeCl_2$ and 5 mM ferrozine at the ratio of 8:1:4. The absorbance of the resulting ferrous ion-ferrozine complex was noted at 562 nm after the mixture was left at room temperature for 10 min. A lower absorbance indicates a higher chelating effect and *vice versa*. The percentage of inhibition of ferrozine-Fe^{2+} complex formation was given by the formula:

$$\text{Inhibition (\%)} = \frac{(A_0 - A_1)}{A_0} \times 100$$

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.

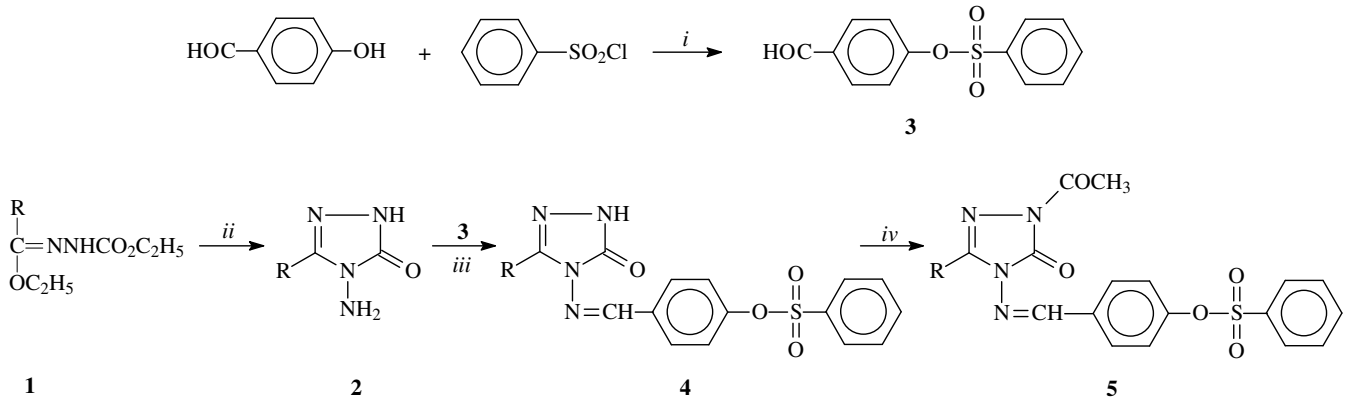
Gel elecrophoresis study of novel 4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives with cat genomic DNA: The interaction of the synthesized compounds with cat DNA was carried out using gel electrophoresis using 10 μM of the DNA in 10 mM *tris*-HCl buffer and and 1 mM of compounds **4** at pH 7.1 was treated with varying concentration of compouns. The mixtures of reaction were incubated at 37 ºC for 3 h. Then, the reaction mixtures were cooled down by adding the loading buffer. 15 μL of sample mixtures were loaded on a 1.5 % agarose gel that contains of ethidium bromide in TBE buffer. The agarose gel was run at 100 V for 3 h and photographed under UV light[31,32].

Potentiometric titrations: A Jenway 3040-model ion analyzer was used for potentiometric titrations. An Ingold pH electrode was preferred because of the advantage. All chemicals used were of analytical reagent grade or similar. Tetra-*n*-butylammonium hydroxide (TBAH), isopropyl alcohol, *tert*-butyl alcohol, acetone, *N,N*-dimethylformamide and dimethyl sulfoxide (DMSO) (Merck Darmstadt, Germany)) were used throughout the work without further purification.

Procedure: 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 half-neutralization potential values and the corresponding pK_a values were determined by drawing the mL (TBAH)-mV graphic.

RESULTS AND DISCUSSION

The 3-alkyl(aryl)-4-(4-benzenesulfonyloxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (**4a-g**) were prepared. The starting compounds 3-alkyl(aryl)-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**2a-g**) were prepared from



i) Et_3N, AcOEt, 0-5 ºC; ii) N_2H_4, H_2O, reflux, 6 h; iii) AcOH, reflux, 1 h; iv) Ac_2O, reflux, 1 h

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, g) R = cyclopropyl

Scheme-I: Synthesis route of compounds **2**, **4**, **5**

the reactions of the corresponding ester ethoxycarbonylhydrazones (**1a-g**) with an aqueous solution of hydrazine hydrate as described in the literature[15,27]. Compounds **4** were obtained from the reactions of compounds **2** with 4-benzenesulfonyloxybenzaldehyde (**3**) which were synthesized by the reactions of 4-hydroxybenzaldehyde with benzenesulfonyl chloride by using triethylamine. Then the reactions of compounds **4a-g** with acetic anhydride were investigated and compounds **5a-g** were prepared (**Scheme-I**).

The structures of seven new 3-alkyl(aryl)-4-(4-benzenesulfonyloxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (**4a-g**) and seven new 1-acetyl-3-alkyl(aryl)-4-(4-benzenesulfonyloxibenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (**5a-g**) were identified using IR, ^{1}H NMR, ^{13}C NMR, UV and elemental analyses data.

Antioxidant activity: All the 14 new compounds **4a-g** and **5a-g** were screened for their *in vitro* antioxidant activities. Several methods are used to determine antioxidant activities. The methods used in this study are discussed below:

Total reductive capability using the potassium ferricyanide reduction method: The 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 BHT and BHA. The reducing capacity of a compound may serve as a significant indicator for its potential antioxidant activity[33]. 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[34]. In this study, all of the amounts of the compounds showed lower absorbance then blank except compound **4f** for 250 µg/mL (Table-1). 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[35]. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule[36]. 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.

TABLE-1
TOTAL REDUCTIVE CAPABILITY OF THE NEW COMPOUNDS (BLANK: 0.356)

Compound	100	250	500
BHT	0.362	0.586	0.698
BHA	0.417	0.704	1.243
4a	0.278	0.311	0.320
4b	0.269	0.304	0.282
4c	0.303	0.353	0.330
4d	0.285	0.266	0.279
4e	0.249	0.251	0.282
4f	0.305	0.358	0.347
4g	0.165	0.270	0.237
5a	0.261	0.257	0.347
5b	0.284	0.264	0.251
5c	0.259	0.273	0.227
5d	0.274	0.289	0.256
5e	0.349	0.195	0.215
5f	0.302	0.254	0.244
5g	0.293	0.292	0.247

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 discolouration from purple to yellow. Hence, DPPH is usually used as a substrate to evaluate antioxidative activity of antioxidants[37]. BHT and BHA were used as a reference to antioxidant compounds. All the compounds tested with this method exhibited marked DPPH free radical scavenging activity in a concentration-dependent manner. Figs. 1 and 2 illustrate a decrease in the concentration of DPPH radical due to the scavenging ability of these compounds. These results indicate that the newly synthesized **4** and **5** type compounds showed moderate activities as a radical scavenger, indicating that it has good activities as hydrogen donors.

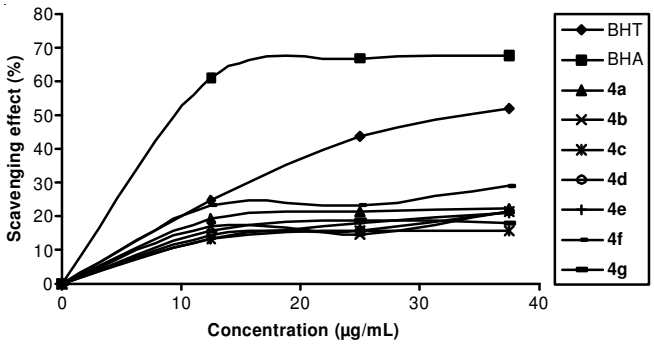


Fig. 1. Scavenging effect of compounds **4a-g**, BHT and BHA at different concentrations (12.5-25-37.5 µg/mL)

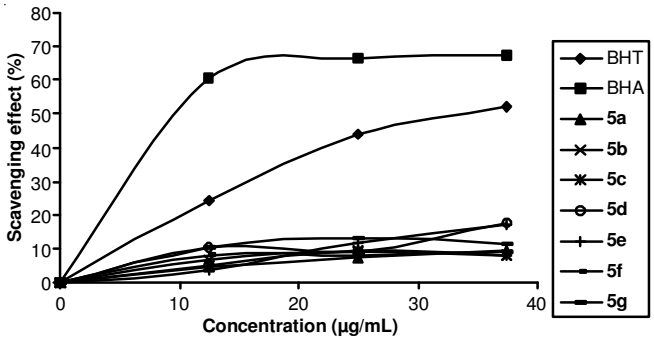


Fig. 2. Scavenging effect of compounds **5a-g**, BHT and BHA at different concentrations (12.5-25-37.5 µg/mL)

Ferrous ion chelating activity: The chelating effect towards ferrous ions by the compounds and standards was determined according to the method of Dinis *et al.*[30]. Ferrozine can quantitatively form complexes with Fe^{2+}. In 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[38]. 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[39] 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[40]. Also, the production of highly active ROS such as $O_2^{\bullet -}$, H_2O_2 and $OH^{\bullet}$ is also catalyzed by free iron though Haber-Weiss reactions:

$$O_2^{\bullet} + H_2O_2 \longrightarrow O_2 + OH^- + OH^{\bullet}$$

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:

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

Fe^{3+} ion also produces radicals from peroxides, although the rate is ten-fold less than that of Fe^{2+} ion, which is the most powerful pro-oxidant among the various types of metal ions[41]. Ferrous ion chelating activities of the compounds except **5b-g**, BHT and α-tocopherol are shown in Fig. 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[42].

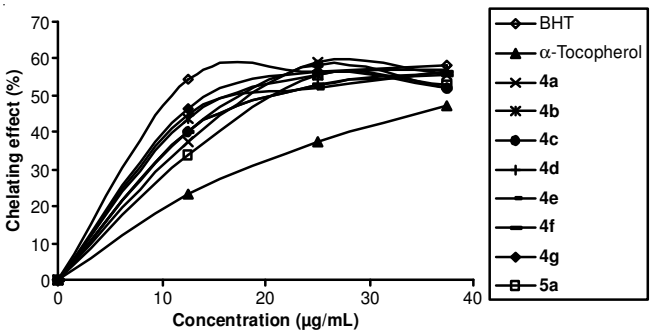


Fig. 3. Metal chelating effect of different amount of the compounds **4a-g**, **5a**, BHT and α-tocopherol on ferrous ions

The data obtained from Fig. 3 reveal that the compounds **4b**, **4d-f** 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 iron complex may also be active, since it can participate in iron-catalyzed reactions.

DNA interaction: Firstly, in this work genomic DNA was used to perform the gel electrophoresis for the novel 4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives. When the concentration of 4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives was increased, the interaction between the DNA and 4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives was not observed. Mobility of the bands (1) was not significantly changed and visibility of band was a little faint compared to that of the untreated DNA (Fig. 4). Therefore, it can be said that the observed unchanges in the DNA bands in the presence of 4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives is an evidence that DNA does not interact with the synthesized compounds. It can be concluded that the compound does not interact with genomic DNA.

Potentiometric titrations: In order to determine the pK_a values of the new compounds **4a-g** they were titrated potentiometrically with tetrabutyl ammonium hydroxide (TBAH) in the non-aqueous solvents such as acetone, dimethyl sulfoxide, isopropyl alcohol, *tert*-butyl alcohol and *N,N*-dimethylformamide. The mV values obtained from pH meter were plotted *versus* tetrabutyl ammonium hydroxide volumes (mL) added and thus potentiometric titration curves were formed for all cases. From these curves, the half neutralization potential values were determined and the corresponding pK_a values were calculated.

As an example, the potentiometric titration curves of 0.001 M compound **4d** solutions titrated with 0.05 N TBAH in acetone, dimethyl sulfoxide, isopropyl alcohol, *tert*-butyl alcohol and *N,N*-dimethylformamide are presented in Fig. 5.

TABLE-2
HALF NEUTRALIZATION POTENTIAL (HNP) VALUES AND THE CORRESPONDING pK_a VALUES OF COMPOUNDS **4a-g** IN ACETONE, DIMETHYL SULFOXIDE, ISOPROPYL ALCOHOL, *tert*-BUTYL ALCOHOL AND *N,N*-DIMETHYLFORMAMIDE AT 25 ºC

Compd.	DMF		Acetone		*tert*-Butyl alcohol		*iso*-Propyl alcohol		DMSO	
	pK_a	HNP (mV)	pK_a	HNP (mV)	pK_a	HNP (mV)	pK_a	HNP (mV)	pK_a	HNP (mV)
4a	15.56	-454	15.71	-463	15.17	-458	12.80	-277	14.14	-367
4b	14.76	-402	16.20	-476	14.51	-631	12.65	-293	12.93	-314
4c	15.08	-428	14.57	-486	14.76	-279	13.00	-318	13.47	-341
4d	13.95	-332	11.93	-235	9.50	-118	9.34	-113	13.63	-316
4e	14.27	-378	-	-	-	-	12.34	-275	12.80	-276
4f	14.35	-389	14.65	-431	14.00	-373	-	-	-	-
4g	13.63	-323	14.93	-388	11.49	-212	12.05	-257	12.65	-274

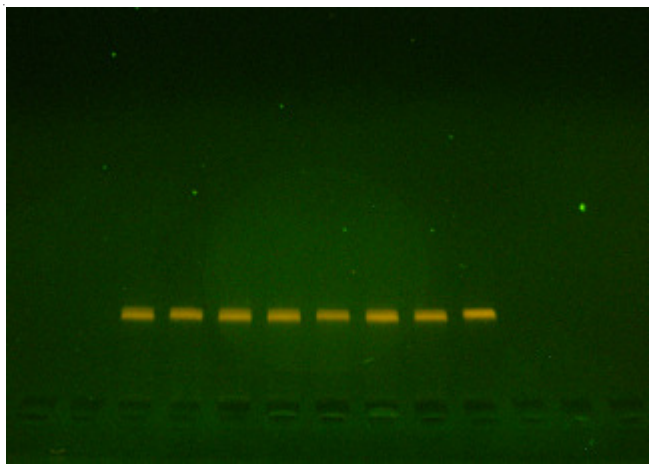


Fig. 4. DNA interaction by various concentration of the synthesized compounds. Lane C is control DNA in absence of the compounds; lane **4a**, lane **4b**, lane **4c**, lane **4d**, lane **4e**, lane **4f** and lane **4g** are DNA + [compounds]

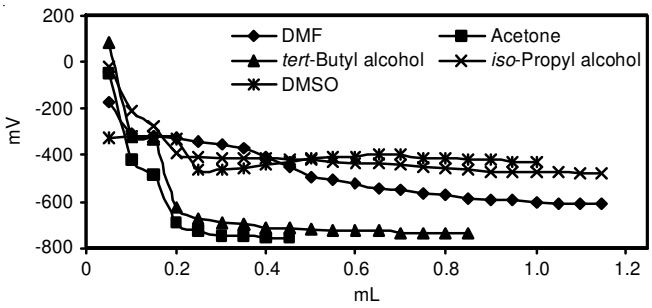


Fig. 5. Potentiometric titration curves of 10^{-3} M compound **4d** solutions titrated with 0.05 N tetrabutyl ammonium hydroxide at 25 ºC

The half neutralization potential (HNP) values and the corresponding pK_a values for compounds **4a-g**, which were obtained from the potentiometric titrations with 0.05 M tetrabutyl ammonium hydroxide in non-aqueous solvents such as isopropyl alcohol, *tert*-butyl alcohol, acetone, dimethyl sulfoxide and *N,N*-dimethyl-formamide, are presented in Table-2.

When the dielectric permittivity of solvents is taken into consideration, the acidic arrangement may be expected as follows: dimethyl sulfoxide ($\varepsilon = 46$) > *N,N*-dimethylformamide ($\varepsilon = 37$) > acetone ($\varepsilon = 20.7$) > *iso*-propyl alcohol ($\varepsilon = 19.4$) > *tert*-butyl alcohol ($\varepsilon = 12$). The experimental and theoretical acidic arrangement, along with the error for each compound, are presented in Table-2. All these compounds show the weakest acidic properties in five solvents.

The exchange of the pK_a values with autoprotolysis constant and dielectric constant are given in Fig. 6.

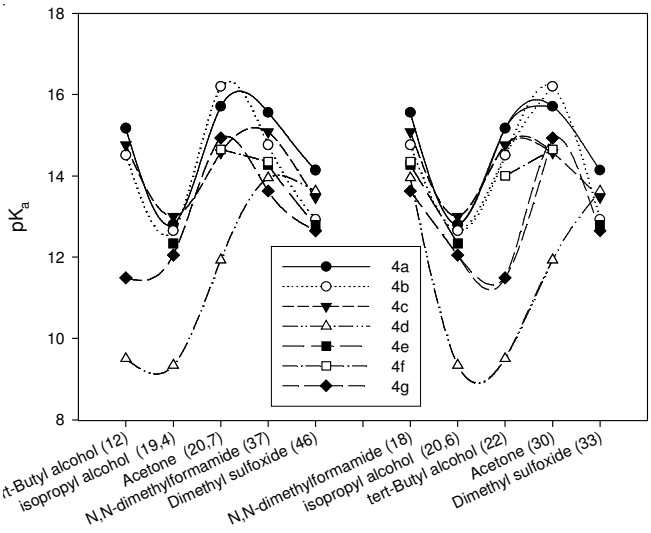


Fig. 6. Variation of the pK_a values for synthesized componds **4a-g** with autoprotolysis constant and dielectric constant

From all the titrations in *N,N*-dimethylformamide, dimethyl sulfoxide, acetone *iso*-propyl alcohol and *tert*-butyl alcohol typical S-shaped titration curves were obtained. For this reason, these solvents for the compounds mentioned above are suitable titration solvents. But, typical S-shaped curves were not obtained for compound **4f** in isopropyl alcohol, dimethyl sulfoxide and **4e** in *tert*-butyl alcohol, acetone. Therefore, the half neutralization potential values and the pK_a values were not determined clearly.

It is seen from the Table-2 and Fig. 5 that the molecular structure of the compounds titrated alters the half neutralization potential values and the corresponding pK_a values: that is, in the same type compounds, the half neutralization potential values and related pK_a values are connected to the substituents linked to C-3.

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