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In-vitro biological activity of some new 1,2,4-triazole derivatives with their potentiometric titrations

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In this study, 3-alkyl(aryl)-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones **2** have been reacted with 3-(4-methoxybenzenesulfonyloxy)-4-methoxybenzaldehyde **1** to afford the corresponding nine new 3-alkyl(aryl)-4-[3-(4-methoxybenzenesulfonyloxy)-4-methoxybenzylideneamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-ones **3**. The structures of nine new compounds have been characterized by IR, ^{1}H and ^{13}C NMR spectral data. The synthesized compounds have been analyzed for their *in vitro* antioxidant and antimicrobial activities. Furthermore, the half-neutralization potential values and the corresponding pK_a values were determined for all the newly synthesized compounds.

Keywords: Synhesis, Antioxidant activity, Antimicrobial activity, pK_a, Acidity

INTRODUCTION

The compounds containing the 1,2,4-triazole moiety have been reported to possess different biological activities such as anti-inflammatory, antibacterial, antioxidant, antifungal, anticancer, analgesic, anticonvulsant, antiparasitic, antiviral, anti-HIV, antihypertensive and diuretic properties [1-16]. In addition, during the last 50 years, depending on developments in medicine, antimicrobial studies in the field of chemistry have been increased. Especially, many compounds in organic chemistry have been used in drug industry. In particularly, 1,2,4-triazoles and their derivatives are often used in antimicrobial studies. However, many studies including antimicrobial effects of triazole derivatives have also been studied [2, 6, 11, 15]. On the other hand, it is known that 1,2,4-triazole and 4,5-dihydro-1*H*-1,2,4-triazol-5-one ring have weak acidic properties, so that some 1,2,4-triazole and 4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives were titrated potentio-metrically with TBAH in non-aqueous solvents, and the pK_a values of the compounds were determined [4, 5, 14-18]. In the present study, 3-alkyl(aryl)-4-[3-(4-methoxybenzenesulfonyloxy)-4-methoxybenzylideneamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**3a-i**) were synthesized from the reactions of 3-alkyl(aryl)-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**2a-i**) with 3-(4-methoxybenzenesulfonyloxy)-4-methoxy-benzaldehyde (**1**), which was synthesized by the reaction of 3-hydroxy-4-methoxybenzaldehyde with 4-methoxy-benzenesulfonyl chloride by using triethylamine. In addition, due to a wide range of applications to find their possible radical scavenging and antioxidant activity, the newly synthesized compounds were investigated by using different antioxidant methodologies: 1,1-diphenyl-2-picryl-hydrazyl (DPPH.) free-radical scavenging, reducing power and metal chelating activities. And, the antimicrobial activities of new compounds were investigated by used agar well diffusion method. Furthermore, the newly synthesized compounds were titrated potentiometrically with tetrabutyl-ammonium hydroxide (TBAH) in isopropyl alcohol, tert-butyl alcohol, acetone and N,N-dimethylformamide (DMF) and the half-neutralization potential values and the corresponding pK_a values were determined for all cases.

EXPERIMENTAL

Materials and chemicals

Chemical reagents and all solvents used in this study were purchased from Merck, Aldrich and Fluka. The necessary solvents are obtained from domestic or foreign sources. Melting points were determined in using an electrothermal melting point apparatus which is Stuart SMP30. The IR spectra were taken in ALPHA-P BRUKER FT-IR spectrometer. ^{1}H and ^{13}C NMR spectra were recorded in deuterated dimethyl sulfoxide with TMS as an internal standard using a Bruker AVANCE III spectrometer at 400 MHz. The starting compounds **2a-i** were prepared as described in the literature [19-20].

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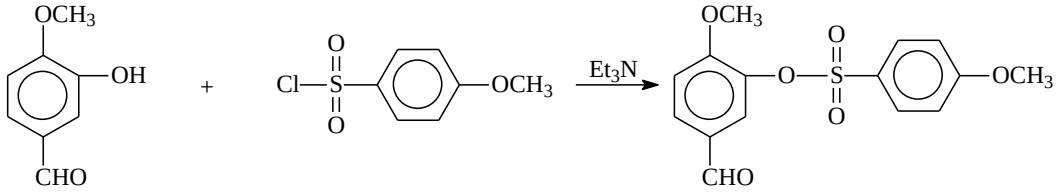

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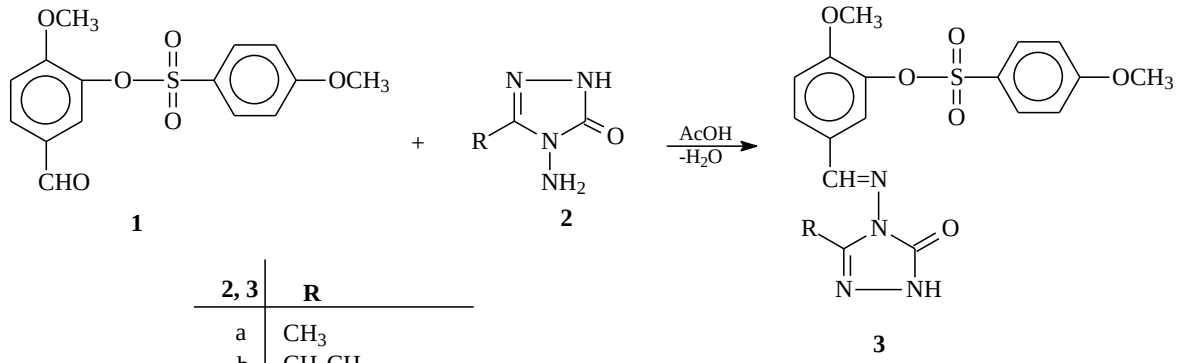

2, 3	R
a	CH_3
b	CH_2CH_3
c	$CH_2CH_2CH_3$
d	$CH_2C_6H_5$
e	$CH_2C_6H_4CH_3$(*p*-)
f	$CH_2C_6H_4OCH_3$ (*p*-)
g	$CH_2C_6H_4Cl$(*p*-)
h	$CH_2C_6H_4Cl$(*m*-)
i	C_6H_5

Scheme1. Synthetic pathway of compounds **3**

Procedure for the synthesis of 3-(4-methoxybenzenesulfonyloxy)-4-methoxybenzaldehyde (**1**)

3-Hydroxy-4-methoxy-benzaldehyde (0.01 mol) dissolved in ethyl acetate (100 mL) was treated with 4-methoxybenzenesulfonyl chloride (0.01 mol), and to this solution was slowly added triethylamine (0.01 mol) with stirring at 0-5 °C. Stirring was continued for 2 h, and then the mixture was refluxed for 3 h and filtered. The filtrate was evaporated *in vacuo*, and the crude product was washed with water and recrystallized from the DMSO-water (1:3) mixture to afford compound **1**, yield (2.87 g, 89.3 %), m.p. 134 °C. IR (KBr) υ_{max} 2830 and 2732 (CHO), 1682 (C=O), 1360 and 1170 (SO_2) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 3.64 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 7.16 (d, 2H, ArH; *J*=8.80 Hz), 7.27 (d, 1H, ArH; *J*=8.40 Hz), 7.63 (d, 1H, ArH; *J*=1.60 Hz), 7.76 (d, 2H, ArH; *J*=8.80 Hz), 7.88 (d, 1H, ArH; *J*=8.40, 1.60 Hz), 9.86 (s, 1H, CHO). ^{13}C NMR (100 MHz, DMSO-d_6): δ 56.43 (OCH_3), 56.75 (OCH_3), 114.02 (CH), 115.10 (2CH), 123.89 (CH), 126.28 (C), 129.85 (C), 131.20 (2CH), 131.75 (CH), 138.37 (C), 156.79 (C), 164.60 (C), 191.23 (CHO).

*General method for the preparation of 3-alkyl(aryl)-4-[3-(4-methoxy-benzenesulfonyloxy)-4-methoxy-benzylideneamino]-4,5-dihydro-1H-1,2,4-triazol-5-ones (**3a-i**)*

The 3-(4-methoxybenzene-sulfonyloxy)-4-methoxybenzaldehyde (**1**) (0.01 mol) was dissolved in acetic acid (15 mL) and treated with the corresponding compound **2** (0.01 mol). The mixture was refluxed for 1.5 h and then evaporated at 50-55 °C *in vacuo*. Several recrystallizations of the residue from appropriate solvent gave pure compounds **3a-i** as colorless crystals.

3-Methyl-4-[3-(4-methoxybenzene-sulfonyloxy)-4-methoxybenzylidene-amino]-4,5-dihydro-1*H*-1,2,4-triazol-5-one (3a). Yield (3.86 g, 92.6 %), m.p. 212 °C, IR (KBr) υ_{max} 3167 (NH), 1694 (C=O), 1593 (C=N), 1565 and 1171 (SO_2) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 2.21 (s, 3H, CH_3), 3.64 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 7.15 (d, 1H, ArH; *J*=7.20 Hz), 7.19 (d, 2H, ArH; *J*=8.80 Hz), 7.50 (d, 1H, ArH; *J*=2.0 Hz), 7.71 (dd, 1H, ArH; *J*=8.40, 2.0 Hz), 7.78 (d, 2H, ArH; *J*=8.80 Hz), 9.60 (s, 1H, N=CH), 11.80 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 11.44 (CH_3), 56.11 (2OCH_3), 114.03

(CH), 115.13 (2CH), 121.42 (CH), 126.53 (C), 126.70 (C), 129.88 (CH), 131.21 (2CH), 138.53 (C), 154.40 (C), 164.53 (C) (ArC), 144.62 (triazole C_3), 151.67 (triazole C_5), 152.80 (N=CH).

3-Ethyl-4-[3-(4-methoxybenzene-sulfonyloxy)-4-methoxybenzylidene-amino]-4,5-dihydro-1*H*-1,2,4-triazol-5-one (3b). Yield (4.13 g, 95.9 %), m.p. 219 °C, IR (KBr) υ_{max} 3179 (NH), 1703 (C=O), 1590 (C=N), 1367 and 1168 (SO_2) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 1.18 (t, 3H, CH_2CH_3; *J*=7.20 Hz), 2.57 (q, 2H, CH_2CH_3; *J*=7.20 Hz), 3.65 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), (7.15 (d, 1H, ArH; *J*=7.20 Hz), 7.19 (d, 2H, ArH; *J*=8.0 Hz), 7.47 (d, 1H, ArH; *J*=1.60 Hz), 7.70 (dd, 1H, ArH; *J*=8.80, 1.60 Hz), 7.76 (d, 2H, ArH; *J*=8.00 Hz), 9.60 (s, 1H, N=CH), 11.83 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 10.38 ($CH_2\underline{CH_3}$), 18.92 ($\underline{CH_2}CH_3$), 56.40 (OCH_3), 56.52 (OCH_3), 114.04 (CH), 115.12 (2CH), 121.26 (CH), 126.56 (C), 126.73 (C), 129.90 (CH), 131.18 (2CH), 138.57 (C), 154.43 (C), 164.53 (C) (ArC), 148.38 (triazole C_3), 151.82 (triazole C_5), 152.74 (N=CH).

3-*n*-Propyl-4-[3-(4-methoxybenzene-sulfonyloxy)-4-methoxybenzylidene-amino]-4,5-dihydro-1*H*-1,2,4-triazol-5-one (3c). Yield (4.19 g, 94.3 %), m.p. 186 °C, IR (KBr) υ_{max} 3171 (NH), 1697 (C=O), 1590 (C=N), 1565 and 1169 (SO_2) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 0.94 (t, 3H, $CH_2CH_2\underline{CH_3}$; *J*=7.20 Hz), 1.65 (sext, 2H, $CH_2\underline{CH_2}CH_3$; *J*=7.20 Hz), 2.57 (t, 2H, $\underline{CH_2}CH_2CH_3$; *J*=7.20 Hz), 3.64 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 7.17 (d, 2H, ArH; *J*=8.80 Hz), 7.19 (d, 1H, ArH; *J*=8.40 Hz), 7.51 (d, 1H, ArH; *J*=1.60 Hz), 7.71 (dd, 1H, ArH; *J*=8.40, 1.20 Hz), 7.78 (d, 2H, ArH; *J*=8.80 Hz), 9.60 (s, 1H, N=CH), 11.83 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 13.89 ($CH_2CH_2\underline{CH_3}$), 19.34 ($CH_2\underline{CH_2}CH_3$), 27.09 ($\underline{CH_2}CH_2CH_3$), (56.41 (OCH_3), 56.51 (OCH_3), 114.07 (CH), 115.11 (2CH), , 121.42 (CH), 126.53 (C), 126.75 (C), 126.79 (CH), 131.18 (2CH), 138.54 (C), 154.40 (C), 164.52 (C) (ArC), 147.25 (triazole C_3), 151.75 (triazole C_5), 152.80 (N=CH).

3-Benzyl-4-[3-(4-methoxybenzene-sulfonyloxy)-4-methoxybenzylidene-amino]-4,5-dihydro-1*H*-1,2,4-triazol-5-one (3d). Yield (4.76 g, 96.6 %), m.p. 165 °C, IR (KBr) υ_{max} 3167 (NH), 1702 (C=O), 1594 (C=N), 1374 and 1190 (SO_2), 758 and 700 (monosubstituted benzenoid ring) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 3.60 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 3.99 (s, 2H, CH_2ph), 7.17 (d, 2H, ArH; *J*=8.80 Hz), 7.24-7.32 (m, 6H, ArH), 7.60 (d, 1H, ArH; *J*=2.00 Hz), 7.67 (dd, 1H, ArH; *J*=8.40, 1.60 Hz), 7.78 (d, 2H, ArH; *J*=8.80 Hz), 9.57 (s, 1H, N=CH), 11.96 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 31.64 (CH_2Ph), 56.38 (OCH_3), 56.47 (OCH_3), 113.99 (CH), 115.09 (2CH), 121.63 (CH), 126.53 (C), 126.72 (C), 127.24 (CH), 128.92 (2CH), 129.33 (2CH), 129.86 (CH), 131.18 (2CH), 136.15 (C), 138.49 (C), 154.31 (C), 164.51 (C) (ArC), 146.63 (triazole C_3), 151.65 (triazole C_5), 152.56 (N=CH).

3-*p*-Methylbenzyl-4-[3-(4-methoxy-benzenesulfonyloxy)-4-methoxy-benzylideneamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-one (3e). Yield (4.88 g, 96.4 %), m.p. 198 °C, IR (KBr) υ_{max} 3167 (NH), 1703 (C=O), 1590 (C=N), 1369 and 1188 (SO_2), 833 (1,4-disubstituted benzenoid ring) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 2.25 (s, 3H, $PhCH_3$), 3.60 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 3.93 (s, 2H, CH_2Ph), 7.10-7.18 (m, 7H, ArH), 7.60 (s, 1H, ArH), 7.67 (d, 1H, ArH; *J*=8.40 Hz), 7.78 (d, 2H, ArH; *J*=8.80 Hz), 9.56 (s, 1H, N=CH), 11.94 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 21.09 ($PhCH_3$), 31.24 (CH_2Ph), 56.38 (OCH_3), 56.47 (OCH_3), 113.99 (CH), 115.09 (2CH), , 121.60 (CH), 126.53 (C), 126.73 (C), 129.19 (2CH), 129.49 (2CH), 129.87 (CH), 131.18 (2CH), 133.04 (C), 136.29 (C), 138.50 (C), 154.30 (C), 164.52 (C) (ArC), 146.78 (triazole C_3), 151.65 (triazole C_5), 152.51 (N=CH).

3-*p*-Methoxybenzyl-4-[3-(4-methoxy-benzenesulfonyloxy)-4-methoxy-benzylideneamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-one (3f). Yield (5.0 g, 95.7 %), m.p. 230 °C, IR (KBr) υ_{max} 3168 (NH), 1704 (C=O), 1589 (C=N), 1366 and 1186 (SO_2), 834 (1,4-disubstituted benzenoid ring) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 3.61 (s, 3H, OCH_3), 3.71 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 3.91 (s, 2H, $PhCH_2$), 6.87 (d, 2H, ArH; *J*=8.40 Hz), 7.16 (d, 2H, ArH; *J*=8.80 Hz), 7.18 (d, 1H, ArH; *J*=8.40 Hz), 7.21 (d, 2H, ArH; *J*=8.40 Hz), 7.60 (d, 1H, ArH; *J*=1.60 Hz), 7.68 (d, 1H, ArH; *J*=8.80 Hz), 7.78 (d, 2H, ArH; *J*=8.80 Hz), 9.57 (s, 1H, N=CH), 11.92 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 30.79 (CH_2Ph), 55.46 (OCH_3), 56.38 (OCH_3), 56.48 (OCH_3), 114.00 (CH), 114.33 (2CH), 115.09 (2CH), 121.60 (CH), 126.53 (C), 126.75 (C), 127.92 (C), 129.91 (CH), 130.39 (2CH), 131.19 (2CH), 138.51 (C), 154.31 (C), 158.56 (C), 164.53 (C) (ArC), 146.93 (triazole C_3), 151.67 (triazole C_5), 152.53 (N=CH).

3-*p*-Chlorobenzyl-4-[3-(4-methoxy-benzenesulfonyloxy)-4-methoxy-benzylideneamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-one (3g). Yield (5.13 g, 97.4 %), m.p. 228 °C, IR (KBr) υ_{max} 3168 (NH), 1703 (C=O), 1591 (C=N), 1368 and 1188 (SO_2), 834 (1,4-disubstituted benzenoid ring) cm^{-1}. ^1H NMR (400 MHz, DMSO-

d_6): δ 3.60 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 4.00 (s, 2H, CH_2Ph), 7.16 (d, 2H, ArH; *J*=8.80 Hz), 7.17 (d, 1H, ArH; *J*=8.80 Hz), 7.33 (d, 2H, ArH; *J*=8.40 Hz), 7.38 (d, 2H, ArH; *J*=8.40 Hz), 7.59 (d, 1H, ArH; *J*=2.00 Hz), 7.67 (dd, 1H, ArH; *J*=8.40,1.60 Hz), 7.78 (d, 2H, ArH; *J*=8.80 Hz), 9.57 (s, 1H, N=CH), 11.98 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 30.79 (CH_2Ph), 56.39 (OCH_3), 56.48 (OCH_3), 114.00 (CH), 115.08 (2CH), 121.68 (CH), 126.51 (C), 126.66 (C), 128.85 (2CH), 129.88 (CH), 131.19 (2CH), 131.26 (2CH), 131.92 (C), 135.15 (C), 138.48 (C), 154.34 (C), 164.52 (C) (ArC), 146.30 (triazole C_3), 151.64 (triazole C_5), 152.69 (N=CH).

3-*m*-Chlorobenzyl-4-[3-(4-methoxy-benzenesulfonyloxy)-4-methoxy-benzylideneamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-one (3h). Yield (5.09 g, 96.6 %), m.p. 200 °C, IR (KBr) υ_{max} 3175 (NH), 1703 (C=O), 1594 (C=N), 1371 and 1168 (SO_2), 832 and 706 (1,3-disubstituted benzenoid ring) cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.61 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 4.02 (s, 2H, CH_2Ph), 7.15 (d, 2H, ArH; *J*=8.80 Hz), 7.17 (d, 1H, ArH; *J*=8.40 Hz), 7.27-7.35 (m, 4H, ArH), 7.57 (d, 1H, ArH; *J*=1.20 Hz), 7.68 (d, 1H, ArH; *J*=8.40 Hz), 7.77 (d, 2H, ArH; *J*=8.80 Hz), 9.58 (s, 1H, N=CH), 11.99 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 31.15 (CH_2Ph), 56.38 (OCH_3), 54.49 (OCH_3), 114.01 (CH), 115.08 (2CH), 121.77 (CH), 126.55 (C), 126.63 (C), 127.30 (CH), 128.22 (CH), 129.27 (CH), 129.79 (CH), 130.73 (CH), 131.19 (2CH), 133.43 (C), 138.51 (2C), 154.39 (C), 164.51 (C) (ArC), 146.13 (triazole C_3), 151.62 (triazole C_5), 152.82 (N=CH).

3-Phenyl-4-[3-(4-methoxybenzene-sulfonyloxy)-4-methoxybenzylidene-amino]-4,5-dihydro-1*H*-1,2,4-triazol-5-one (3i). Yield (4.56 g, 95.2 %), m.p. 202 °C, IR (KBr) υ_{max} 3160 (NH), 1697 (C=O), 1594 (C=N), 1359 and 1170 (SO_2), 788 and 714 (monosubstituted benzenoid ring) cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.61 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 7.12 (d, 2H, ArH; *J*=8.80 Hz), 7.19 (d, 1H, ArH; *J*=8.40 Hz), 7.51-7.54 (m, 3H, ArH), 7.62 (d, 1H, ArH; *J*=1.60 Hz), 7.73 (dd, 1H, ArH; *J*=8.40, 1.60 Hz), 7.67 (dd, 1H, ArH; *J*=8.40,1.60 Hz), 7.76 (d, 2H, ArH; *J*=8.80 Hz), 7.87-7.89 (m, 2H, ArH; *J*=8.80 Hz), 9.56 (s, 1H, N=CH), 12.37 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 56.38 (OCH_3), 56.50 (OCH_3), 114.14 (CH), 115.05 (2CH), 122.16 (CH), 126.41 (C), 126.57 (C), 127.08 (C), 128.32 (2CH), 128.96 (2CH), 129,72 (CH), 130.60 (CH), 131.14 (2CH), 138.46 (C), 155.60 (C), 164.50 (C) (ArC), 144.90 (triazole C_3), 151.80 (triazole C_5), 154.47 (N=CH).

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.

Reducing power. The reducing power of the synthesized compounds was determined according to the method [21]. 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 50ºC 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 [22]. 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):

$$Absorbance = 0.0003\ x\ DPPH^{\cdot} - 0.0174$$

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

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

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

Metal chelating activity. The chelation of ferrous ions by the synthesized compounds and standards were estimated by the method [23]. Briefly, the synthesized compounds (15–45 μg/mL) were added to a 2 mM solution of $FeCl_2 \cdot 4H_2O$ (0.05 mL). The reaction was initiated by the addition of 5 mM ferrozine (0.2 mL), and then the mixture was shaken vigorously and left standing at room temperature for

10 min. After the mixture had reached equilibrium, the absorbance of the solution was measured at 562 nm in a spectrophotometer. All tests and analyses were run in triplicate and averaged. The percentage of inhibition of ferrozine–Fe^{2+} complex formation was given by the formula: % inhibition = $(A_0 - A_1 / A_0) \times 100$, where A_0 is the absorbance of the control, and A_1 is the absorbance in the presence of the samples or standards. The control did not contain compound or standard.

Antimicrobial activity

Chemicals. All test microorganisms were obtained from the Microbiologics Environmental Protection Laboratories Company in France and are follows: (E. *coli* ATCC 259222, *P. aeruginosa* ATCC 27853, *K. pneumonia* ATCC 4352, *S. aureus* ATCC 6538, *B. subtilis* ATCC 11774, *B. cereus* ATCC 11778.

Agar well diffusion method. Simple susceptibility screening test using agar well-diffusion method was used [24, 25]. Each microorganism was suspended in Mueller–Hinton (MH) broth and diluted approximately 10^6 colony forming unit (cfu)/mL. They were "flood-inoculated" onto the surface of MH agar and Sabouraud Dextrose Agar (SDA) and then dried. Five-millimeter diameter wells were cut from the agar using a sterile cork-borer, and 250-5000 μL/50 of the chemical substances were delivered into the wells. The plates were incubated for 18 h at 35 °C. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism. Dimethylsulphoxide was used as solved control.

Potentiometric titrations

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

RESULTS AND DISCUSSION

In this study, the structures of nine new compounds of 3-alkyl(aryl)-4-[3-(4-methoxybenzenesulfonyloxy)-4-methoxy-benzylideneamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-ones were identified using IR, ^{1}H NMR and ^{13}C NMR spectral data.

Antioxidant activity

Total reductive capability using the potassium ferricyanide reduction method. The reductive capabilities of compounds were 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. It has been observed that the reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity [26]. The antioxidant activity of putative antioxidant has been attributed to various mechanisms, among which are prevention chain initiation, binding of transition metal ion catalyst, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging [27]. In this study, all of the concentrations of the compounds showed lower absorbance than blank. Hence, no reductive activities were observed.

DPPH· radical scavenging activity. The model of scavenging the stable DPPH radical model is a widely used method to evaluate antioxidant activities in a relatively short time compared with other methods. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability [28]. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [29]. The reduction capability of DPPH radicals was determined by the 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 the reaction between antioxidant molecules and radical, progresses, which resulted 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 [30]. In the study, antiradical activities of compounds and standard antioxidants such as BHA, BHT and α-tocopherol were determined by using DPPH· method. Fig. 1 illustrates that the radical scavenging effects of the compounds **3c**, **3g** and **3h** were concentration-dependent, the other compounds were not. Nevertheless, these newly synthesized compounds showed poor radical scavenging activity.

Ferrous ion chelating activity. The chelating effect towards ferrous ions by the compounds and standards was determined. Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of chelating agents, the complex formation

is disrupted with the result that the red colour of the complex is decreased. Measurement of colour reduction therefore allows estimation of the chelating activity of the coexisting chelator [31]. 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 [32] 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 [33]. 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:

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

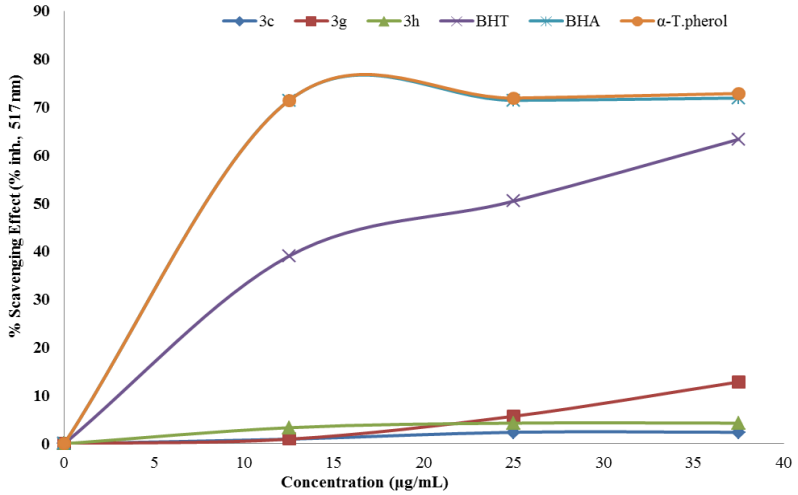

Fig. 1. Scavenging effect of compounds **3**, BHT, BHA and α-tocopherol at different concentrations (12,5-25-37.5 µg/mL)

Among the transition metals, iron is known as the most important lipid oxidation pro-oxidant due to its high reactivity. The ferrous state of iron accelerates lipid oxidation by breaking down the hydrogen and lipid peroxides to reactive free radicals via the Fenton reactions:

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

Fe^{3+} ion also produces radicals from peroxides, even though 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 [34]. 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 [35].

Low absorbance at 562 nm indicates high metal chelating activity. In the study, chelating activities of compounds and standard antioxidants such as BHA, BHT and α-tocopherol were determined. The data obtained from Fig. 2 reveal that the metal chelating effects of the compounds **3,** except **3i,** were not concentration-dependent. The chelating effect of the compounds, except **3i**, is high at low concentrations.

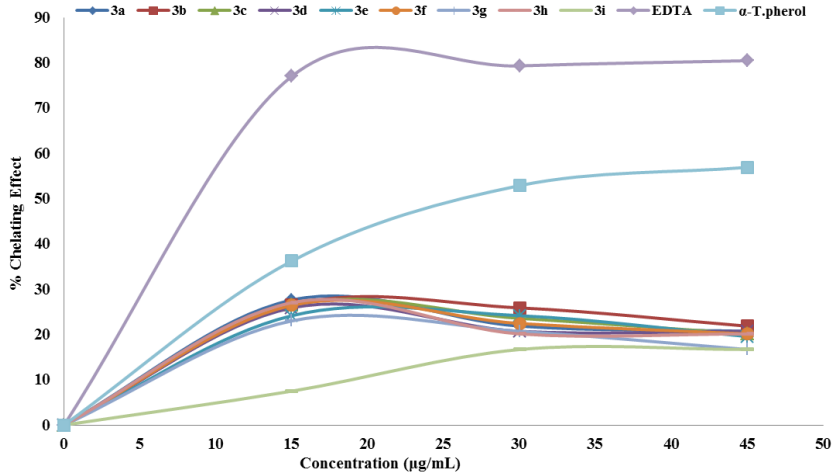

Fig. 2. Metal chelating effect of different amount of the compounds **3**, BHT, BHA and α-tocopherol on ferrous ions

Antimicrobial activity

Results were interpreted in terms of the diameter of inhibition zone:
(-): < 5.5 mm (No activity)
(+): 5.5-10 mm (Low level of activity)
(++): 11-16 mm (Moderate activity)
(+++): ≥ 17 mm (High level of activity)

Ampicillin, neomycin and streptomycin were standard antibacterial and antifungal agents. DMSO was used as solvent control. The observed data for the antimicrobial activity of **3** type compounds were given in Table 1. All of the compounds did not display any antimicrobial activity against Bacillus subtilis. The data reveal that, the highest zone diameter was obtained from compound **3d** against Bacillus cereus and **3h** against Pseudomonas aeruginosa.

Table 1. Antimicrobial activity of the compounds **3**

Comp.	Microorganisms and zone of inhibition (mm)					
	Bs	Bc	Pa	Kp	Sa	Ec
3a	-	8	8	8	9	-
3b	-	**14**	8	**12**	8	-
3c	-	9	12	7	7	8
3d	-	**16**	10	8	8	-
3e	-	8	**13**	8	7	9
3f	-	7	**13**	8	7	10
3g	-	8	10	9	9	**12**
3h	-	-	**16**	8	8	8
3i	-	9	**11**	**11**	**14**	-
Amp.	33	36	36	35	37	34
Neom.	17	17	17	16	13	16
Strep.	12	12	12	11	21	10

Bs: *Bacillus subtilis* (ATCC-11774), Bc: *Bacillus cereus* (ATCC-11778), Pa: *Pseudomonas aeruginosa* (ATCC-27853), Kp: *Klebsiella pneumoniae* (ATCC-4352), Sa: *Staphylococcus aureus* (ATCC-6538), Ec: *Escherichia coli* (ATCC-25922), Amp.: Ampicillin (3261), Neo.: Neomycin 3360, Str.: Streptomycin 3385 (-): no activite.

Table 2. The half-neutralization potential (HNP) and the corresponding pK_a values of new compounds **3a-i** in four non-aqueous solvents at 25°C.

Compd.	Isopropyl alcohol		*tert*-Butyl alcohol		DMF		Acetone	
No	**HNP (mV)**	**pK_a**	**HNP (mV)**	**pK_a**	**HNP (mV)**	**pK_a**	**HNP(mV)**	**pK_a**
3a	-	-	-	-	-	-	-166	10.51
3b	-	-	-	-	-	-	-451	15.49
3c	-	-	-	-	-	-	-460	12.50
3d	-	-	-60	8.59	-124	9.72	-297	12.79
3e	-	-	-	-	-	-	-442	15.38
3f	-	-	-	-	-240	11.69	-274	12.22
3g	-	-	-	-	-59	8.37	-407	14.64
3h	-	-	-	-	-309	12.85	-	-
3i	-	-	-138	9.98	-	-	-239	11.80

Potentiometric titrations

Newly synthesized **3** type compounds were titrated potentiometrically with TBAH in non-aqueous solvents, the mV values from each titration were plotted against TBAH volumes used (mL) and the potentiometric titration curves were obtained for all the cases. From the titration curves, the HNP values and the corresponding pK_a values were calculated. As an example, the potentiometric titrations curves for 0.001 M solutions of 3-ethyl-4-[3-(4-methoxy-benzenesulfonyloxy)-4-methoxybenzylideneamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-one (**3b**) titrated with 0.05 M TBAH in DMF, acetone, *tert*- butyl alcohol, isopropyl alcohol are shown in Fig. 3.

The half-neutralization potential (HNP) values and the corresponding pK_a values for new compounds **3a-i**, obtained from the potentiometric titrations with 0.05 M TBAH in isopropyl alcohol, *tert*-butyl alcohol, acetone and *N*,*N*-dimethyl formamide are presented in Table 2.

When the dielectric permittivity of solvents is taken into consideration, the acidity order can be given as follows: *N*,*N*-dimethylformamide (ε=37.0)

> acetone (ε=20.7)> isopropyl alcohol (ε=19.4) > *tert*-butyl alcohol (ε=12.0). As seen in Table 1, the acidity order for compound **3d** is *tert*-butyl alcohol > DMF > acetone, for compounds **3f** and **3g** it is: DMF > acetone, for compound **3i** it is: *tert*-butyl alcohol > acetone > DMF. Moreover, as seen in Table 1, for compounds **3a-i** in isopropyl alcohol, for compounds **3a-c**, **3e-h** in tert-butyl alcohol, for compounds **3a-c**, **3e**, **3i** in DMF and for compound **3h** in acetone, the HNP values and the corresponding pK_a values were not obtained.

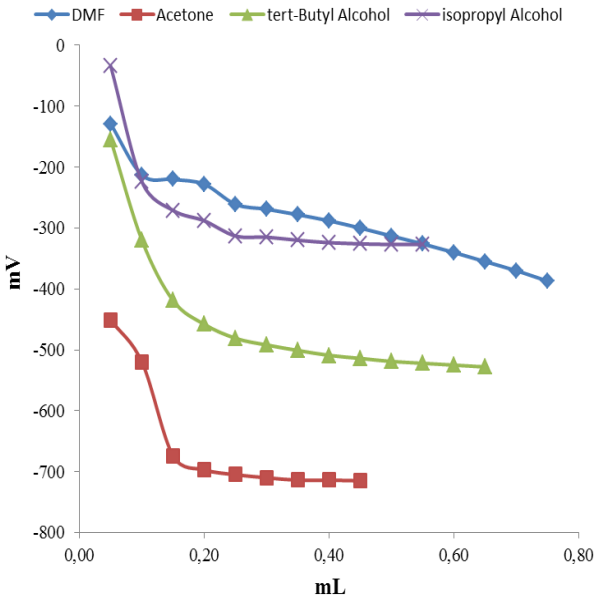

Fig. 3. Potentiometric titration curves of 0.001 M solutions of compound **3b** titrated with 0.05 M TBAH in isopropyl alcohol, tertbutyl alcohol, DMF, acetone at 25°C.

As it is well known, the acitidy of a compound depends on some factors. The two most important factors are the solvent effect and moleculer structure [36]. Table 2 shows that the HNP values and corresponding pK_a values obtained from the potentiometric titrations rely on the non-aqueous solvents used and the substituens at C-3 in 4,5-dihydro-1*H*-1,2,4-triazole-5-one ring.

CONCLUSIONS

The synthesis and in vitro antioxidant and antimicrobial evaluation also acidic properties of new 4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives are described in the study. Design and synthesis of novel small molecules can play specifically a protective role in biological systems and in modern medicinal chemistry. The results on the investigation of their biological activities might be helpful in the future drug development process.

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