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Research Article

INVESTIGATION OF ANTIOXIDANT OF NOVEL 4-[2-(2-THIENYL-CARBONYLOXY)-3-METHOXYBENZYLIDENEAMINO]-4,5-DIHYDRO-1H-1,2,4-TRIAZOL-5-ONE DERIVATIVES

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ABSTRACT

A series of novel 3-alkyl(aryl)-4-[2-(2-thienylcarbonyloxy)-3-methoxybenzylideneamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**4**) were synthesized from the reactions of 3-alkyl(aryl)-4-amino-4,5-dihydro-1H-1,2,4-triazol-5-ones (**2**) with 2-(2-thienylcarbonyloxy)-3-methoxy-benzaldehyde (**3**). Then, the acetylation reactions of compounds **3** were investigated and 1-acetyl-3-alkyl(aryl)-4-[2-(2-thienylcarbonyloxy)-3-methoxybenzylideneamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**5**) were obtained. The structures of thirteen new compounds were characterized from IR, ^{1}H NMR, ^{13}C NMR and UV spectral data. In addition, the synthesized new **4** and **5** type compounds were analyzed for their *in vitro* potential antioxidant activities in three different methods; including 1,1-diphhenlyl-2-picryl-hydrazyl free radical (DPPH.) scavenging, reducing activity by Fe^{+3} – Fe^{+2} transformation and ferrous metal (Fe^{+2}) chelating activities.

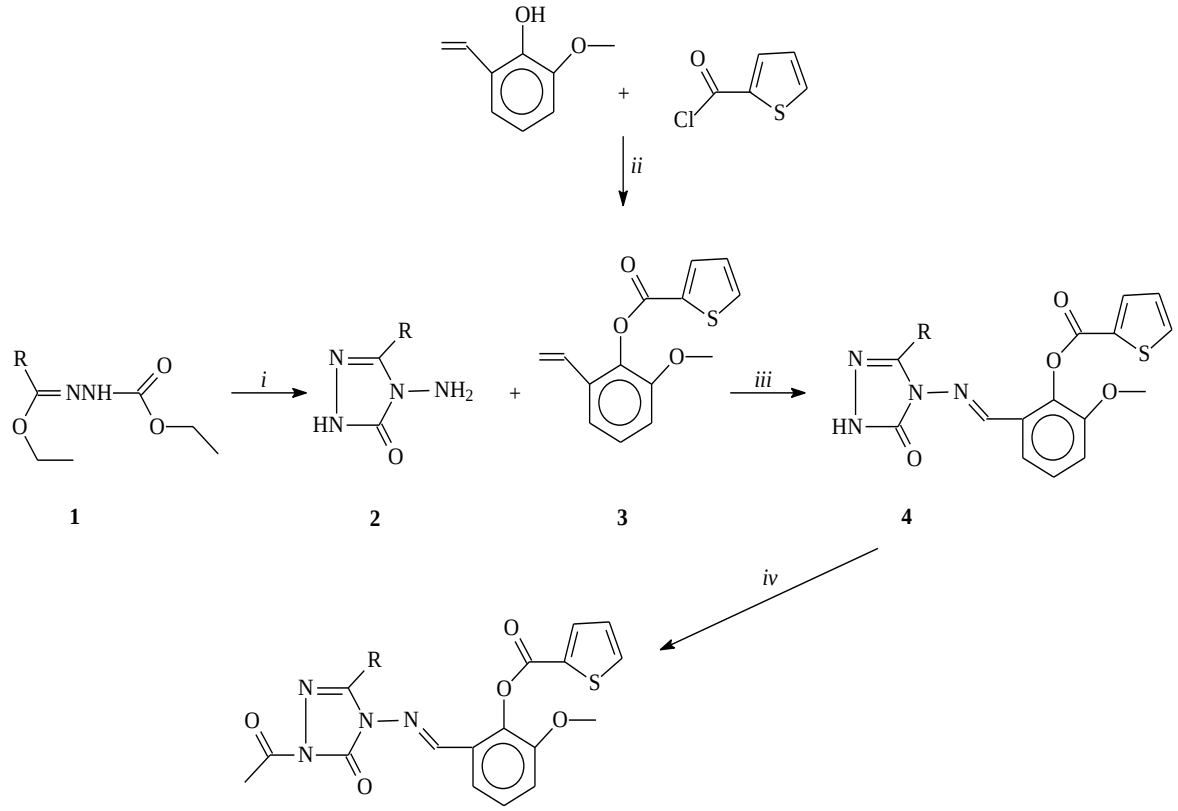
Keywords: 1,2,4-triazol-5-one, Schiff base, Acetylation and Antioxidant activity.

INTRODUCTION

Schiff bases having the azomethine group or CH=N imine bonds are prepared by the condensation between amines and active carbonyl compounds[1,2]. Schiff-bases have been extensively studied due to their applicability in various areas such as biological, chemical, industrial and pharmaceutical applications[3-6]. Schiff bases derivatives have recently increased studies related to corrosion inhibitors, optical sensors, highly selective polymer membrane electrodes, semiconducting, therapeutic properties, highly thermal stability, modern technology (nonlinear optical materials), various coordination, homogenous catalysis and biological probes[7-13]. As a result of well-synthesized structures, all properties make them and derivatives useful in organic structure in electronic and opto-electronic devices, pharmaceutical products or thermo-durable materials[14,15]. They are widely used in pharmaceutical industry because of their valuable clinical and pharmacological properties[16]. The azomethine moiety plays a very important role in biological active systems. It has also been shown to exhibit a wide range of biological activities including anti-bacterial, anti-tumor, anti-proliferative, anti-malarial, anti-inflammatory and antioxidant[3,4,17-23].

In the present study, we present the synthesis of a series of 3-alkyl(aryl)-4-[2-(2-thienylcarbonyloxy)-3-methoxybenzylide neamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**4**) and 1-acetyl-3-alkyl(aryl)-4-[2-(2-thienylcarbonyloxy)-3-methoxy benzylidene amino]-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**5**). The starting compounds 3-alkyl/aryl-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones **(2)** were prepared from the reactions of the corresponding ester ethoxycarbonyl hydrazones **(1)** with an aqueous solution of hydrazine hydrate as described in the literature[24,25]. The

Compounds **4** were obtained from the reactions of compounds **2** with 2-(2-thienylcarbonyloxy)-3-methoxybenzaldehyde (**3**), which was synthesized by the reactions of 2-hydroxy-3-methoxybenzaldehyde with 2-thienyl chloride by using triethylamine. In addition, the reactions of compounds **4** with acetic anhydride were investigated and compounds **5** were prepared (Figure **1**). The structures of new compounds were identified by using IR, ^{1}H NMR, ^{13}C NMR, UV spectral data.The compounds**4** and **5** were analyzed for their antioxidant activities in three different methods (reducing power, free radical scavenging and metal chelating activity), were drawn their graphs and their results were interpreted.



i) $N_2H_4.H_2O$, reflux; *ii*) Et_3N; *iii*)AcOH, reflux, 1h; *iv*) Ac_2O, reflux
a) R = CH_3, b) R = CH_2CH_3, c) R = $CH_2CH_2CH_3$, d) R = $CH_2C_6H_5$,
e) R = $CH_2C_6H_4CH_3$ (*p*-), f) R = $CH_2C_6H_4Cl$ (*p*-), g) R = C_6H_5

Fig. 1: Synthetic route of compounds 2-5

EXPERIMENTAL

Chemistry

Chemical reagents used in the study were supplied from Sigma (Sigma-Aldrich GmbH, Germany), Fluka (Switzerland) and Merck AG, (Germany). Melting points were identified using a Stuart SMP30 melting point apparatus with open glass capillaries (United Kingdom). ^{1}H and ^{13}C-NMR spectra were recorded in deuterated dimethyl sulfoxide (DMSO-d_6) using a Bruker spectrometer (Germany).

General procedure for the synthesis of 3-alkyl (aryl)-4-[2-(2-thienylcarbonyloxy)-3-methoxybenzylideneamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (4)

2-Hydroxy-3-methoxybenzaldehyde (0.01 mol) dissolved in ethyl acetate (50 mL) was treated with 2-thienyl chloride (0.01 mol), and to this solution was slowly added triethylamine (0.01 mol) in 10 mL ethyl acetate 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 ethanol to afford compound **3**.Yield: 83.7%; m.p. 94°C; IR (cm^{-1}) υ_{max}:2848 and 2765 (CHO),1723, 1692 (C=O),1249 (COO). ^{1}H-NMR (400 MHz, DMSO-d_6) (ppm) δ H: 3.86 (s, 3H, OCH_3), 7.34 (dd, 1H, ArH, *J* = 5.2, 4.0 Hz), 7.52-7.57 (m, 3H, ArH), 8.08 (dd, 1H, ArH, *J* = 3.6, 1.2 Hz), 8.13 (dd, 1H, ArH, *J* = 5.2, 1.2 Hz), 10.13 (s, 1H, CHO); ^{13}C-NMR (100 MHz, DMSO-d_6) (ppm) δ C: 56.41 (OCH_3), [118.74, 121.72, 127.49, 128.18, 128.76, 129.10, 130.93, 135.59, 139.61, 151.66] (ArC), 159.35 (COO), 189.68 (CHO). Then, the corresponding compound **2** (0.01 mol) was dissolved in acetic acid (20 mL) and treated with 2-(2-thienylcarbonyloxy)-3-methoxybenzaldehyde (**3**)(0.01 mol). The mixture was refluxed for 2 h and then evaporated at 50-55 °C in vacuo. Several recrystallizations of the residue from ethanol gave pure compounds **4**as colorless crystals.

3-Methyl-4-[2-(2-thienylcarbonyloxy)-3-methoxybenzylideneamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-one(4a)

White solid; yield: 96.4%; m.p. 212°C; IR (cm^{-1})υ_{max}: 3207 (NH), 1733, 1698 (C=O), 1607 (C=N), 1249 (COO). ^{1}H-NMR (200 MHz, DMSO-d_6) (ppm) δ H:2.05 (s, 3H, CH_3), 3.75 (s, 3H, OCH_3), 7.27-7.52 (m, 4H, ArH), 8.00-8.10 (m, 2H, ArH), 9.81 (s, 1H, N=CH), 11.74 (s, 1H, NH);UV [Etanol, λ_{max}, nm (ε, $L.mol^{-1}.cm^{-1}$)]: 284 (28.410), 256 (29.870), 214 (21.310).

3-Ethyl-4-[2-(2-thienylcarbonyloxy)-3-methoxybenzylideneamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-one (4b)

White solid; yield: 96.8%; m.p. 208°C; IR (cm^{-1}) υ_{max}: 3222 (NH), 1708, 1698 (C=O), 1607, 1591 (C=N), 1265 (COO). ^{1}H-NMR (200 MHz, DMSO-d_6) (ppm) δ H: 1.08 (t, *J* 7.42, 3H, CH_2CH_3), 2.40 (q, *J* 7.42, 2H, CH_2CH_3), 3.75 (s, 3H, OCH_3), 7.26-7.50 (m, 4H, ArH), 8.00-8.08 (m, 2H, ArH), 9.82 (s, 1H, N=CH), 11.78 (s, 1H, NH); UV [Etanol, λ_{max}, nm (ε, $L.mol^{-1}.cm^{-1}$)]: 254 (29.010), 232 (24.740), 214 (20.980).

3-(*n*-Propyl)-4-[2-(2-thienylcarbonyloxy)-3-methoxybenzylideneamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-one(4c)

White solid; yield: 95.9%; m.p. 203°C; IR (cm^{-1}) υ_{max}: 3205 (NH), 1716, 1700 (C=O), 1606, 1590 (C=N), 1247 (COO). ^{1}H-NMR (200 MHz, DMSO-d_6) (ppm) δ H: 0.80 (t, *J* 7.42, 3H, $CH_2CH_2CH_3$), 1.51 (sext, *J* 7.42, 3H, $CH_2\underline{CH_2}CH_3$), 2.43 (q, *J* 7.42, 2H, $\underline{CH_2}CH_2CH_3$), 3.75 (s, 3H, OCH_3), 7.25-7.48 (m, 4H, ArH), 7.98-8.07 (m, 2H, ArH), 9.80 (s, 1H, N=CH), 11.77 (s, 1H, NH); UV [Etanol, λ_{max}, nm (ε, $L.mol^{-1}.cm^{-1}$)]: 252 (18.370), 222 (15.180).

3-Benzyl-4-[2-(2-thienylcarbonyloxy)-3-methoxybenzylideneamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-one (4d)

White solid; yield: 96.8%; m.p. 218°C; IR (cm^{-1}) υ_{max}: 3203 (NH), 1727, 1710 (C=O), 1585 (C=N), 1263 (COO), 767 and 701 (monosubstituted benzenoid ring). ^{1}H-NMR (200 MHz, DMSO-d_6) (ppm) δ H: 3.68 (s, 3H, OCH_3), 3.81 (s, 2H, CH_2Ph), 7.15-7.38 (m, 9H, ArH), 7.92-7.99 (m, 2H, ArH), 9.72 (s, 1H, N=CH), 11.80 (s, 1H, NH); UV [Etanol, λ_{max}, nm (ε, $L.mol^{-1}.cm^{-1}$)]: 254 (27.410), 232 (24.180), 214 (21.070).

3-(*p*-Methylbenzyl)-4-[2-(2-thienyl carbonyloxy)-3-methoxy benzylidene amino]-4,5-dihydro-1*H*-1,2,4-triazol-5-one (4e)

White solid; yield: 89.3%; m.p. 224°C; IR (cm^{-1}) υ_{max}: 3174 (NH), 1739, 1702 (C=O), 1609, 1590 (C=N), 1263 (COO), 811 (1,4-disubstituted benzenoid ring). ^{1}H-NMR (200 MHz, DMSO-d_6) (ppm) δ H: 2.21 (s, 3H, $PhCH_3$), 3.77 (s, 3H, OCH_3), 3.84 (s, 2H, CH_2Ph), 7.08-7.51 (m, 8H, ArH), 8.00-8.09 (m, 2H, ArH), 9.81 (s, 1H, N=CH), 11.89 (s, 1H, NH); UV [Etanol, λ_{max}, nm (ε, $L.mol^{-1}.cm^{-1}$)]: 254 (27.360), 232 (24.290), 214 (21.290).

3-(*p*-Chlorobenzyl)-4-[2-(2-thienyl carbonyloxy)-3-methoxybenzylideneamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-one(4f)

White solid; yield: 96.2%; m.p. 215°C; IR (cm^{-1}) υ_{max}: 3177 (NH), 1746, 1700 (C=O), 1588 (C=N), 1262 (COO), 820 (1,4-disubstituted benzenoid ring). ^{1}H-NMR (200 MHz, DMSO-d_6) (ppm) δ H: 3.75 (s, 3H, OCH_3), 3.89 (s, 2H, CH_2Ph), 7.21-7.58 (m, 8H, ArH), 7.99-8.08 (m, 2H, ArH), 9.80 (s, 1H, N=CH), 11.93 (s, 1H, NH); UV [Etanol, λ_{max}, nm (ε, $L.mol^{-1}.cm^{-1}$)]: 254 (24.630), 228 (24.010), 214 (20.900).

3-Phenyl-4-[2-(2-thienylcarbonyloxy)-3-methoxybenzylideneamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-one(4g)

White solid; yield: 96.2%; m.p. 212°C; IR (cm^{-1}) υ_{max}: 3180 (NH), 1720, 1709 (C=O), 1610 (C=N), 1265 (COO), 769 and 696 (monosubstituted benzenoid ring). ^{1}H-NMR (200 MHz, DMSO-d_6) (ppm) δ H: 3.74 (s, 3H, OCH_3), 7.24-8.03 (m, 11H, ArH), 7.92-7.99 (m, 2H, ArH), 9.76 (s, 1H, N=CH), 12.26 (s, 1H, NH); UV [Etanol, λ_{max}, nm (ε, $L.mol^{-1}.cm^{-1}$)]: 254 (26.740), 212 (18.570).

General procedure for the synthesis of 1-acetyl-3-alkyl(aryl)-4-[2-(2-thienyl carbonyl oxy)-3-methoxy benzylideneamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (5)

The corresponding compound **4**(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 **5**.

1-Acetyl-3-methyl-4-[2-(2-thienyl carbonyloxy)-3-methoxybenzylideneamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-one (5a)

White solid; yield: 81.1%; m.p. 178°C; IR (cm^{-1}) υ_{max}: 1745, 1713 (C=O), 1623 (C=N), 1252 (COO). ^{1}H-NMR (400 MHz, DMSO-d_6) (ppm) δ H: 2.22 (s, 3H, CH_3), 2.43 (s, 3H, $COCH_3$), 3.84 (s, 3H, OCH_3), 7.36 (dd, *J* 5.20, 4.00, 1H, ArH), 7.39-7.41 (m, 1H, ArH), 7.46 (t, *J* 8.00, 1H, ArH,), 7.61 (dd, *J* 8.00, 1.60, 1H, ArH), 8.08 (dd, *J* 4.00, 1.60, 1H, ArH), 8.16 (dd, *J* 4.80, 1.20, 1H, ArH), 9.75 (s, 1H, N=CH); ^{13}C-NMR (100 MHz, DMSO-d_6) (ppm) δ C: 10.95 (CH_3), 23.39 ($CO\underline{C}H_3$), 56.29 (OCH_3), [115.88, 118.40, 126.74, 127.43, 128.93, 130.71, 135.73, 135.86, 138.67, 149.91] (ArC), 144.46 (triazole-C_3), 147.78 (N=CH), 151.60 (triazole-C_5), 159.16 (COO), 166.06 ($\underline{C}OCH_3$); UV [Etanol, λ_{max}, nm (ε, $L.mol^{-1}.cm^{-1}$)]: 288 (22.915), 250 (18.655), 218 (17.900).

1-Acetyl-3-ethyl-4-[2-(2-thienyl carbonyloxy)-3-methoxybenzylideneamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-one (5b)

White solid; yield: 77.6%; m.p. 164°C; IR (cm^{-1}) υ_{max}: 1766, 1725 (C=O), 1613 (C=N), 1265 (COO).^{1}H-NMR (400 MHz, DMSO-d_6) (ppm) δ H: 1.16 (t, *J* 7.60, 3H, CH_2CH_3), 2.44 (s, 3H, $COCH_3$), 2.59 (q, *J* 7.60, 2H, CH_2CH_3), 3.83 (s, 3H, OCH_3), 7.35-7.41 (m, 2H, ArH), 7.46 (t, *J* 8.00, 1H, ArH), 7.58 (dd, *J* 8.00, 1.60, 1H, ArH), 8.09 (dd, *J* 3.60, 1.20, 1H, ArH), 8.16 (dd, *J* 4.80, 1.20, 1H, ArH), 9.75 (s, 1H, N=CH);^{13}C-NMR (100 MHz, DMSO-d_6) (ppm) δ C: 9.35 ($CH_2\underline{C}H_3$), 18.38 ($\underline{C}H_2CH_3$), 23.39 ($CO\underline{C}H_3$), 56.28 (OCH_3), [115.85, 118.60, 126.75, 127.46, 128.91, 130.78, 135.71, 135.83, 138.60, 150.10] (ArC), 148.00 (triazole-C_3), 149.93 (N=CH), 151.62 (triazole-C_5), 159.17 (COO), 166.00 ($\underline{C}OCH_3$);UV [Etanol, λ_{max}, nm (ε, $L.mol^{-1}.cm^{-1}$)]: 288 (13.530), 252 (17.990), 232 (15.940).

1-Acetyl-3-(*n*-propyl)-4-[2-(2-thienyl carbonyloxy)-3-methoxybenzylideneamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-one (5c)

White solid; yield: 74.5%; m.p. 149°C; IR (cm^{-1}) υ_{max}: 1772, 1738 (C=O), 1590 (C=N), 1246 (COO).^{1}H-NMR (400 MHz, DMSO-d_6) (ppm) δ H: 0.93 (t, *J* 7.60, 3H, $CH_2CH_2CH_3$), 1.65 (sext, *J* 7.20, 3H, $CH_2\underline{CH_2}CH_3$), 2.44 (s, 3H, $COCH_3$), 2.56 (q, *J* 7.20, 2H, $\underline{CH_2}CH_2CH_3$), 3.84 (s, 3H, OCH_3),7.35-7.38 (m, 1H, ArH), 7.39-7.42 (m, 1H, ArH), 7.47 (t, *J* 8.00, 1H, ArH,), 7.58 (dd, *J* 8.00, 1.60, 1H, ArH), 8.09 (dd, *J* 4.00, 1.60, 1H, ArH), 8.16 (dd, *J* 4.80, 1.20, 1H, ArH), 9.74 (s, 1H, N=CH);^{13}C-NMR (100 MHz, DMSO-d_6) (ppm) δ C: 13.35 ($CH_2CH_2\underline{C}H_3$), 18.29 ($CH_2\underline{C}H_2CH_3$), 23.42 ($CO\underline{C}H_3$), 26.22 ($\underline{C}H_2CH_2CH_3$), 56.50 (OCH_3), [115.90, 118.64, 126.74, 127.51, 128.92, 130.75, 135.71, 135.84, 138.59, 150.35] (ArC), 147.97 (triazole-C_3), 148.80 (N=CH), 151.63 (triazole-C_5), 159.16 (COO), 166.03 ($\underline{C}OCH_3$).

1-Acetyl-3-benzyl-4-[2-(2-thienyl carbonyloxy)-3-methoxybenzylideneamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-one (5d)

White solid; yield: 93.4%; m.p. 150°C; IR (cm^{-1}) υ_{max}: 1750, 1725 (C=O), 1607, 1593 (C=N), 1247 (COO), 770 and 706 (monosubstituted benzenoid ring). ^{1}H-NMR (400 MHz, DMSO-d_6) (ppm) δ H: 2.44 (s, 3H, $COCH_3$), 3.83 (s, 3H, OCH_3), 4.06 (s, 2H, CH_2Ph), 7.26-7.29 (m, 1H, ArH), 7.32-7.36 (m, 5H, ArH), 7.38-7.40 (m, 1H, ArH), 7.45 (t, *J* 8.00, 1H, ArH,), 7.55 (dd, *J* 8.00, 1.60, 1H, ArH), 8.08 (dd, *J* 4.00, 1.20, 1H, ArH), 8.15 (dd, *J* 4.80, 1.20, 1H, ArH), 9.74 (s, 1H, N=CH);^{13}C-NMR (100 MHz, DMSO-d_6) (ppm) δ C: 23.47 ($CO\underline{C}H_3$), 30.80 (CH_2Ph), 56.29 (OCH_3), [115.90, 117.86, 126.75, 127.47, 128.95, 130.57, 135.78, 135.93, 138.88, 149.42] (ArC), [126.96, 127.47 (2C), 128.48 (2C), 134.53] (ArC linked C-3), 147.97 (triazole-C_3), 148.05 (N=CH), 151.54 (triazole-C_5), 159.17 (COO), 166.01 ($\underline{C}OCH_3$); UV [Etanol, λ_{max}, nm (ε, $L.mol^{-1}.cm^{-1}$)]: 278 (16.225), 252 (20.470), 230 (20.665), 216 (19.890).

1-Acetyl-3-(*p*-methylbenzyl)-4-[2-(2-thienyl carbonyloxy)-3-methoxybenzylidene-amino]-4,5-dihydro-1*H*-1,2,4-triazol-5-one (5e)

White solid; yield: 81.5%; m.p. 166°C; IR (cm^{-1}) υ_{max}: 1740, 1722 (C=O), 1609 (C=N), 1282 (COO), 824 (1,4-disubstituted benzenoid ring). ^{1}H-NMR (400 MHz, DMSO-d_6) (ppm) δ H: 2.27 (s, 3H, $PhCH_3$), 2.43 (s, 3H, $COCH_3$), 3.83 (s, 3H, OCH_3), 4.00 (s, 2H, CH_2Ph), 7.14 (d, *J* 8.60, 2H, ArH), 7.22 (d, *J* 8.00, 2H, ArH),7.34-7.36 (m, 1H, ArH), 7.38-7.40 (m, 1H, ArH), 7.45 (t, *J* 8.00, 1H, ArH,), 7.56 (dd, *J* 8.00, 1.60, 1H, ArH), 8.08 (dd, *J* 4.00, 1.20, 1H, ArH), 8.15 (dd, *J* 4.80, 1.20, 1H, ArH), 9.73 (s, 1H, N=CH);^{13}C-NMR (100 MHz, DMSO-d_6) (ppm) δ C: 20.62 ($PhCH_3$), 23.46 ($CO\underline{C}H_3$), 30.42 (CH_2Ph), 56.29 (OCH_3), [115.89, 117.90, 126.76, 127.47, 128.94, 130.59, 135.77, 135.92, 138.87, 149.43] (ArC), [128.82 (2C), 129.04 (2C), 131.37, 136.07] (ArC linked C-3), 147.96 (triazole-C_3), 148.20 (N=CH), 151.55 (triazole-C_5), 159.17 (COO), 166.00 ($\underline{C}OCH_3$).

1-Acetyl-3-(*p*-chlorobenzyl)-4-[2-(2-thienyl carbonyloxy)-3-methoxybenzylidene-amino] -4,5-dihydro-1*H*-1,2,4-triazol-5-one (5f)
White solid; yield: 87.7%; m.p. 191°C; IR (cm^{-1}) υ_{max}: 1750, 1713 (C=O), 1608 (C=N), 1261 (COO), 802 (1,4-disubstituted benzenoid ring).^{1}H-NMR (400 MHz, DMSO-d_6) (ppm) δ H: 2.43 (s, 3H, $COCH_3$), 3.83 (s, 3H, OCH_3), 4.07 (s, 2H, CH_2Ph), 7.34-7.42 (m, 6H, ArH), 7.45 (t, *J* 8.00, 1H, ArH,), 7.54 (dd, *J* 7.60, 1.60, 1H, ArH), 8.08 (dd, *J* 4.00, 1.20, 1H, ArH), 8.15 (dd, *J* 5.20, 1.60, 1H, ArH), 9.75 (s, 1H, N=CH);^{13}C-NMR (100 MHz, DMSO-d_6) (ppm) δ C: 23.45 ($CO\underline{C}H_3$), 30.12 (CH_2Ph), 56.29 (OCH_3), [115.93, 117.91, 126.72, 127.48, 128.95, 130.89, 135.78, 135.94, 138.87, 149.48] (ArC), [128.40 (2C), 130.58 (2C), 131.68, 133.52] (ArC linked C-3), 147.75 (triazole-C_3), 147.95 (N=CH), 151.55 (triazole-C_5), 159.17 (COO), 165.98 ($\underline{C}OCH_3$); UV [Etanol, λ_{max}, nm (ε, $L.mol^{-1}.cm^{-1}$)]: 272 (16.190), 250 (19.710), 226 (24.020), 220 (23.425).

Antioxidant Activity
Reducing power
The reducing power of the synthesized compounds was determined according to the method of Oyaizu[26]. Different concentrations of the samples (50-250 µg/mL) in DMSO (1 mL) were mixed with phosphate buffer (2.5 mL, 0.2 M, pH = 6.6) and potassium ferricyanide (2.5 mL, 1%). The mixture was incubated at 50°C for 20 min and afterwards a portion (2.5 mL) of trichloroacetic acid (10%) was added to the mixture, which was centrifuged for 10 min at 1000 x g. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%), and then the absorbance at 700 nm was measured in a spectrophometer. Higher absorbance of the reaction mixture indicated greater reducing power.

Free radical scavenging activity
Free radical scavenging activity of compounds was measured by DPPH., using the method of Blois[27]. 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 remain at the room temperature for 30 min. Then, the absorbance was measured at 517 nm in a spectrophometer. The 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 × DPPH·) – 0.0174

The capability to scavenge the DPPH radical was calculated by using the following equation: DPPH· scavenging effect (%) = $(A_0 - A_1/A_0) \times 100$, 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 of Dinis et al.[28]. Shortly, the synthesized compounds (50-250 µgxmL^{-1}) were added to a 2 mM solution of $FeCl_2$ (0.05 mL). The reaction was initiated by the addition of 5 mM ferrozine (0.2 mL), and then the mixture was shaken vigorously and left remaining at the 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 carried out 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.

RESULTS AND DISCUSSION
Chemistry
In the present work, 3-alkyl(aryl)-4-[2-(2-thienylcarbonyloxy)-3-methoxy benzylidene amino]-4,5-dihydro-1*H*-1,2,4-triazol -5-ones (**4**) were synthesized from the reactions of 3-alkyl(aryl)-4-amino-4,5-dihydro-1H-1,2,4-triazol-5-ones (**2**) with 2-(2-thienylcarbonyloxy)-3-methoxy-benzaldehyde (**3**). Then, the acetylation reactions of compounds **3** were investigated and 1-acetyl-3-alkyl(aryl)-4-[2-(2-thienylcarbonyloxy)-3-methoxybenzylideneamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**5**) were obtained. The structures of compounds 3-5 were identified by using IR, ^{1}H-NMR,^{13}C-NMR and UV spectral data.

Antioxidant activity
The antioxidant activities of thirteen new compounds **4** and**5**were determined. Several methods have been used to determine antioxidant activities and the methods used in the study are given below:

Total reductive capability using the potassium ferricyanide reduction method
In the present study, all the concentrations of the compounds had a lower absorbance than the

reference antioxidants. Hereby, no activity was observed for reducing metal ion complexes to their lower oxidation state or for any electron transfer reaction. Therefore, the compounds did not exhibit any reductive activity.

DPPH· radical scavenging activity

The reduction capability of DPPH radicals was determined by decrease in its absorbance at 517 nm induced by antioxidants. In the study, antiradical activities of compounds and standard antioxidants such as BHT, BHA and α-tocopherol were determined. The newly synthesized compounds showed no activity as a radical scavenger.

Ferrous ion chelating activity

In this study, Ferrous ion chelating activities of the compounds **4**, **5**, EDTA and α-tocopherol are respectively shown in Figure **2**.The 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 a metal ion[29]. The metal chelatingeffect of the newly synthesized compounds and standard antioxidants are shown as% inhibition in the graph of Figure **2**. It is observed that the synthesized compounds have a low degree of chelator at the lowest concentration. The results are not promising.

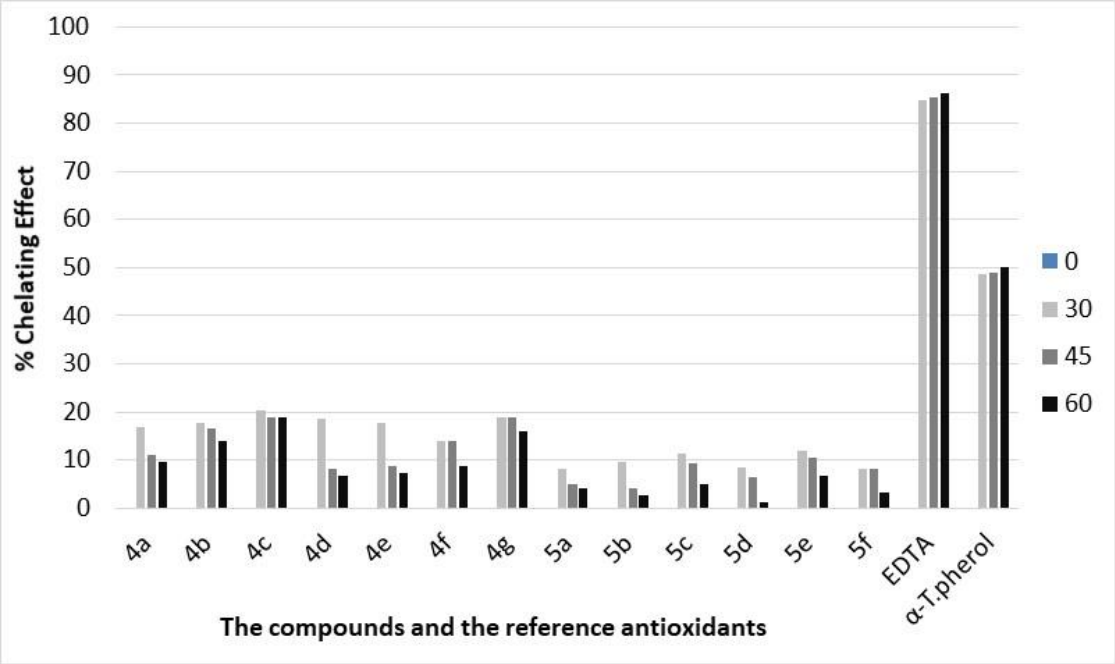


Fig. 2: Iron binding effect of diverse amount of the compounds 4, 5 and the reference antioxidants

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