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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and biological evaluation of new 1,2,4-triazole derivatives with their potentiometric titrations

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Abstract In the present study, 3-alkyl(aryl)-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**1a**, **b**, **d**–**g**) reacted with 3-phenoxybenzaldehyde to afford 3-alkyl(aryl)-4-(3-phenoxy-benzylidenamino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**2a**, **b**, **d**–**g**). Then, the acetylation reactions of compounds **2a** and **2d**–**g** were investigated. The structures of 11 new compounds were established from the elemental analysis, IR, ^{1}H NMR, ^{13}C NMR, MS, and UV spectral data. The synthesized compounds were analyzed for their in vitro potential antioxidant activities in three different methods. Compounds **2f** and **3d** showed best activity for the iron binding. Moreover, the compounds **2** were titrated potentiometrically with tetrabutylammonium hydroxide in four non-aqueous solvents (isopropyl alcohol, *tert*-butyl alcohol, acetonitrile, and *N*,*N*-dimethyl formamide). Thus, the half-neutralization potential values and the corresponding pK_a values were determined in all cases. Furthermore, these 11 new compounds and 13 recently reported 3-alkyl(aryl)-4-(2-thienylmethyleneamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones **(4a–g)** and 1-acetyl-3-alkyl (aryl)-4-(2-thienylmethyleneamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones **(5a**, **b**, **d–g)** were screened for their antimicrobial activities.

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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, hypolipidemic, antihypertensive, analgesic, hypocholesteremic, antiviral, anti-inflammatory, and antioxidant properties (Ikizler *et al*., 1998; Iqbal *et al*., 2012; Li *et al*., 2013; Sahoo *et al*., 2010; Siddiqui *et al*., 2011; Sujith *et al*., 2012; Yuksek *et al*., 2013). In addition, several articles, reporting the synthesis of some *N*-arylidenamino-4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives, have been published so far (Bahceci *et al*., 2002; Gursoy-Kol *et al*., 2012; Yuksek *et al*., 2011). The acetylation of 4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives has also been reported (Bahceci *et al*., 2002; Gursoy-Kol *et al*., 2012; Yuksek *et al*., 2011).

On the other hand, 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 that 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 (Bahceci *et al*., 2002; Gursoy-Kol *et al*., 2012; Yuksek *et al*., 2011; Yuksek and Gursoy-Kol, 2008). Determination of pK_a values of the active constituent of certain pharmaceutical preparations is important because the distribution, transport behavior, bonding to receptors, and contributions to the metabolic behavior of the active constituent molecules depend on the ionization constant (Frey *et al*., 1971; Putun *et al*., 1995; Demirbas *et al*., 1998).

Furthermore, antioxidants are extensively studied for their capacity to protect organisms and cells from damage induced by oxidative stress. Scientists in various disciplines have become more interested in new compounds, either synthesized or obtained from natural sources that could provide active components to prevent or reduce the impact of oxidative stress on cells (Hussain *et al*., 2003). Exogenous chemicals and endogenous metabolic processes in human body or in food system might produce highly reactive-free radicals, especially oxygen-derived radicals, which are capable of oxidizing biomolecules, resulting in cell death and tissue damage. Oxidative damages play a significant pathological role in human diseases. For example, cancer, emphysema, cirrhosis, atherosclerosis, and arthritis have all been correlated with oxidative damage. Also, excessive generation of reactive oxygen species (ROS) induced by various stimuli and which exceeds the antioxidant capacity of the organism leads to a variety of pathophysiological processes such as inflammation, diabetes, genotoxicity, and cancer (McClements and Decker, 2000).

In addition, in the past 25 years, the incidence of microbial infection has increased on alarming levels all over the world as a result of antimicrobial resistance. A growing number of immuno-compromised patients are as a result of cancer chemotherapy, organ transplantation, and HIV infection which are the major factors contributing to this increase. The health problem demands to search and synthesize a new class of antimicrobial compounds effective against pathogenic microorganisms that developed resistance to the antibiotics used in the current regiment (Bayrak *et al*., 2009; Bonde and Gaikwad, 2004; Koca *et al*., 2005; Yu and Huiyuan, 2002).

In this study, six new 3-alkyl(aryl)-4-(3-phenoxybenzylidenamino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones **(2a**, **b**, **d–g)** were synthesized from the reactions of 3-alkyl(aryl)-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones **(1a**, **b**, **d–g)** with 3-phenoxybenzaldehyde. Besides, the reactions of compounds **(2a**, **b**, **d–g)** with acetic anhydride gave compounds **(3a**, **d–g)** (Fig. 1). In addition, due to a wide range of applications to find their possible radical scavenging and antioxidant activity, the newly synthesized compounds were investigated using different antioxidant methodologies: 1,1-diphenyl-2-picryl-hydrazyl ($DPPH^{\cdot}$) free radical scavenging, reducing power, and metal chelating activities. The newly synthesized type **2** compounds were titrated potentiometrically with tetrabutylammonium hydroxide in four non-aqueous solvents such as acetonitrile, isopropyl alcohol, *tert*-butyl alcohol, and *N*,*N*-dimethylformamide, and the half-neutralization potential values and the corresponding pK_a values were determined for all cases. Thus, the effects of solvents and molecular structure upon acidity were investigated. Furthermore, the antimicrobial activity of six new 3-alkyl(aryl)-4-(3-phenoxybenzylidenamino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones **(2a**, **b**, **d–g)** and five new 1-acetyl-3-alkyl(aryl)-4-(3-phenoxybenzylidenamino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones **(3a**, **d–g)** and seven recently reported 3-alkyl(aryl)-4-(2-thienylmethyleneamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones **(4a–g)**, six recently reported 1-acetyl-3-alkyl(aryl)-4-(2-thienylmethyleneamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones **(5a**, **b**, **d–g)** which was synthesized according to reference (Yuksek *et al*., 2011) was determined.

Experimental

Instrumentation

Melting points were taken on an electrothermal digital melting point apparatus and are uncorrected. IR spectra were registered on a Perkin-Elmer Spectrum One 1600 FT-IR spectrometer. ^{1}H and ^{13}C NMR spectra were recorded in deuterated DMSO-d_6 on a Bruker Spectrospin Adance DPX 400 Ultrashield spectrometer at 400 and 100 MHz, respectively. UV absorption spectra were measured in 10-mm quartz cells between 200 and 400 nm using a Schimadzu-160 UV/VIS/N spectrometer. Extinction coefficients (ε) are expressed in L/mol/cm. Electrospray ionization mass spectrometry (ESI–MS) was performed on a TSQ Quantum Access Max Triple Stage Quadrupole Mass Spectrometer. Elemental analyses were carried out on a LECO, CHNS-932 for C, H, and N. The starting compounds **1a–g** were prepared according to references (Ikizler and Un, 1979; Ikizler and Yuksek, 1993). Compounds **4** and **5** were obtained through recently reported methods (Yuksek *et al*., 2011).

Synthesis

*General method for the preparation of 3-alkyl(aryl)-4-(3-phenoxy-benzylidenamino-4,5-dihydro-1H-1,2,4-triazol-5-ones (**2**)*

The corresponding compound 1 (0.01 mol) was dissolved in acetic acid (15 mL) and treated with 3-phenoxybenzaldehyde (1.98 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 **2a**, **2b**, **2d–g** as colorless crystals.

Spectral data of each compound are given below:

*3-Methyl-4-(3-phenoxy-benzylidenamino-4,5-dihydro-1H-1,2,4-triazol-5-one (**2a**)* Yield 88.5; mp 151 °C; UV (EtOH) λ_{max} (log ε) 294 (4.11), 225 (4.32), 259 (3.22) nm; IR (KBr) υ_{max} 3,172, 1,713, 1,613, 1,575, 785 and 695 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz,): $\delta = 11.83$

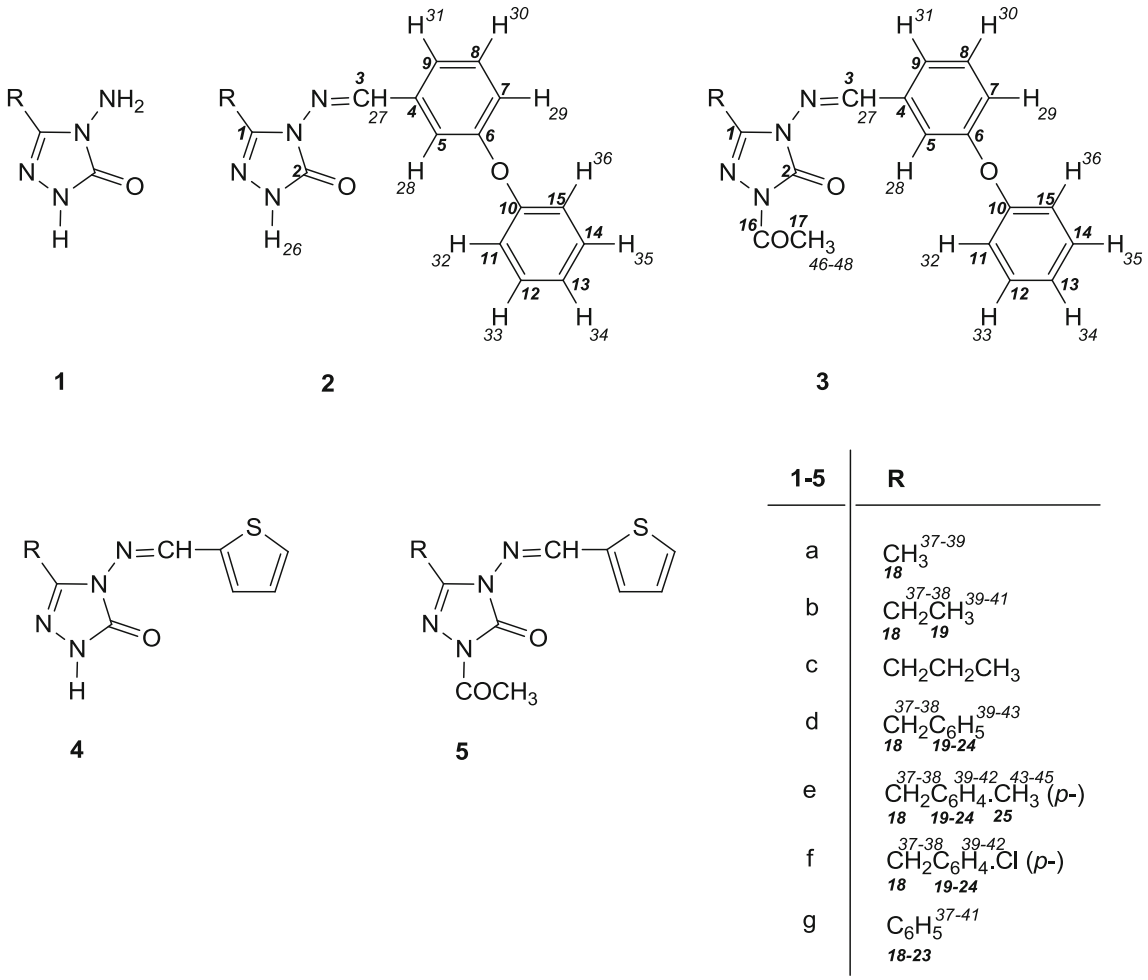

Fig. 1 Synthesized (**2**, **3**) and the known (**4**, **5**) compounds

(1H, s, H-26), 9.69 (1H, s, H-27), 7.60 (1H, d, H-31), 7.50 (2H, dd, H-33, H-35), 7.45 (1H, s, H-28), 7.39 (1H, dd, H-30), 7.20 (1H, dd, H-34), 7.15 (2H, d, H-32, H-36), 7.05 (1H, d, H-29), 2.22 (3H, s, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz,): $\delta = 157.8$ (C, C-6), 156.8 (C, C-10), 153.3 (C, C-2), 151.6 (CH, C-3), 144.8 (C, C-1), 136.0 (C, C-4), 131.4 (CH, C-8), 130.8 (CH, C-12, C-14), 124.4 (CH, C-13), 123.5 (CH, C-9), 121.8 (CH, C-5), 119.5 (CH, C-11, C-15), 117.0 (CH, C-7), 11.6 (CH_3); ESI–MS *m/z* 317 [M + 23] (29), 295 [M + 1] (10), 104 (100); Anal. Calcd. for $C_{16}H_{14}N_4O_2$: C, 65.30; H, 4.79; N, 19.04. Found: C, 65.63; H, 4.93; N, 18.98.

*3-Ethyl-4-(3-phenoxy-benzylidenamino-4,5-dihydro-1H-1,2,4-triazol-5-one (**2b**)* Yield 86.9; mp 171 °C; UV (EtOH) λ_{max} (log ε) 294 (4.12), 225 (4.31), 259 (3.17) nm; IR (KBr) υ_{max} 3,173, 1,713, 1,610, 1,591, 772 and 693 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz,): $\delta = 11.85$ (1H, s, H-26), 9.68 (1H, s, H-27), 7.58 (1H, d, H-31), 7.50 (2H, dd, H-33, H-35), 7.45 (1H, s, H-28), 7.38 (1H, dd, H-30), 7.20 (1H, dd, H-34), 7.16 (2H, d, H-32, H-36), 7.06 (1H, d, H-29), 2.63 (2H, q, CH_2CH_3), 1.17 (3H, t, CH_2CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz,): $\delta = 157.8$ (C, C-6), 156.4 (C, C-10), 153.2 (C, C-2), 151.8 (CH, C-3), 148.4 (C, C-1), 136.1 (C, C-4), 131.2 (CH, C-8), 130.7 (CH, C-12, C-14), 124.4 (CH, C-13), 123.6 (CH, C-9), 121.8 (CH, C-5), 119.5 (CH, C-11, C-15), 116.9 (CH, C-7), 19.0 (CH_2, CH_2CH_3), 10.5 (CH_3, CH_2CH_3); ESI–MS *m/z* 331 [M + 23] (10), 309 [M + 1] (4), 197 (100); Anal. Calcd. for $C_{17}H_{16}N_4O_2$: C, 66.22; H, 5.23; N, 18.17. Found: C, 66.64; H, 5.64; N. 18.34.

*3-Benzyl-4-(3-phenoxy-benzylidenamino-4,5-dihydro-1H-1,2,4-triazol-5-one (**2d**)* Yield 92.5; mp 167 °C; UV (EtOH) λ_{max} (log ε) 297 (4.00), 224 (4.24), 258 (3.17) nm; IR (KBr) υ_{max} 3173, 1711, 1591, 681 and 591 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz,): $\delta = 12.03$ (1H, s, H-26), 9.66 (1H, s, H-27), 7.50 (1H, d, H-31), 7.46 (2H, dd, H-33, H-35), 7.43 (1H, s, H-28), 7.39 (1H, dd, H-30), 7.24 (2H, dd, H-40, H-42), 7.21 (2H, d, H-39, H-43), 7.20 (1H, dd, H-34), 7.15 (2H, d, H-32, H-36), 7.10 (1H, dd, H-41), 7.08

(1H, d, H-29), 3.96 (2H, s, CH_2); ^{13}C NMR (DMSO-d_6, 100 MHz,): δ = 157.2 (C, C-6), 156.6 (C, C-10), 153.0 (C, C-2), 151.8 (CH, C-3), 146.6 (C, C-1), 136.2 (C, C-4), 136.0 (C, C-19), 131.2 (CH, C-8), 130.6 (CH, C-12, C-14), 129.2 (CH, C-20, C-24), 128.9 (CH, C-21, C-23), 127.2 (CH, C-22), 124.4 (CH, C-13), 124.0 (CH, C-9), 121.9 (CH, C-5), 119.6 (CH, C-11, C-15), 116.1 (CH, C-7), 31.6 (CH_2); ESI–MS *m/z* 393 [M + 23] (14), 371 [M + 1] (22), 181 (100); Anal. Calcd. for $C_{22}H_{18}N_4O_2$: C, 71.34; H, 4.90; N, 15.13. Found: C, 71.57; H, 5.25; N, 15.26.

*3-p-Methylbenzyl-4-(3-phenoxy-benzylidenamino-4,5-dihydro-1H-1,2,4-triazol-5-one (**2e**)* Yield 91.2; mp 166 °C; UV (EtOH) λ_{max} (log ε) 297 (3.92), 223 (4.26), 259 (3.10) nm; IR (KBr) υ_{max} 3173, 1705, 1586, 810, 765 and 685 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz,): δ = 11.98 (1H, s, H-26), 9.65 (1H, s, H-27), 7.51 (1H, d, H-31), 7.47 (2H, dd, H-33, H-35), 7.43 (1H, s, H-28), 7.38 (1H, dd, H-30), 7.21 (2H, d, H-39, H-42), 7.18 (1H, dd, H-34), 7.14 (2H, d, H-32, H-36),7.11 (2H, d, H-40, H-41), 6.99 (1H, d, H-29), 3.88 (2H, s, CH_2), 2.22 (3H, s, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz,): δ = 157.9 (C, C-6), 156.6 (C, C-10), 152.8 (C, C-2), 151.6 (CH, C-3), 146.8 (C, C-1), 136.2 (C, C-4), 136.0 (C, C-22), 133.0 (C, C-19), 131.2 (CH, C-8), 130.7 (CH, C-12, C-14), 129.5 (CH, C-20, C-24), 129.0 (CH, C-21, C-23), 124.4 (C, C-13), 124.1 (CH, C-9), 122.0 (CH, C-5), 119.6 (CH, C-11, C-15), 116.1 (CH, C-7), 31.2 (CH_2), 21.0 (CH_3); ESI–MS *m/z* 407 [M + 23] (39), 385 [M + 1] (45), 359 (100); Anal. Calcd. for $C_{23}H_{20}N_4O_2$: C, 71.86; H, 5.24; N, 14.57. Found: C, 72.09; H, 5.65; N, 14.70.

3-p-Chlorobenzyl-4-(3-phenoxy-benzylidenamino-4,5-dihydro-1H-1,2,4-triazol-5-one (***2f***) Yield 90.8; mp 196 °C; UV (EtOH) λ_{max} (log ε) 294 (4.01), 224 (4.32), 259 (3.16) nm; IR (KBr) υ_{max} 3,175, 1,704, 1,585, 845, 748 and 693 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz,): δ = 12.02 (1H, s, H-26), 9.68 (1H, s, H-27), 7.50 (1H, d, H-31), 7.47 (2H, dd, H-33, H-35), 7.42 (1H, s, H-28), 7.36 (1H, dd, H-30), 7.25 (2H, d, H-39, H-42), 7.19 (1H, dd, H-34), 7.14 (2H, d, H-32, H-36), 7.11 (2H, d, H-40, H-41), 7.06 (1H, d, H-29), 3.97 (2H, s, CH_2); ^{13}C NMR (DMSO-d_6, 100 MHz,): δ = 157.9 (C, C-6), 156.6 (C, C-10), 153.0 (C, C-2), 151.6 (CH, C-3), 146.2 (C, C-1), 135.9 (C, C-4), 135.1 (C, C-22), 131.9 (C, C-19), 131.1 (CH, C-8), 131.0 (CH, C-20, C-24), 130.6 (CH, C-12, C-14), 128.8 (CH, C-21, C-23), 124.4 (C, C-13), 123.9 (CH, C-9), 121.9 (CH, C-5), 119.6 (CH, C-11, C-15), 116.3 (CH, C-7), 30.9 (CH_2); Anal. Calcd. for $C_{22}H_{17}ClN_4O_2$: C, 65.27; H, 4.23; N, 13.84. Found: C, 65.20; H, 4.64; N, 13.89.

*3-Phenyl-4-(3-phenoxy-benzylidenamino-4,5-dihydro-1H-1,2,4-triazol-5-one (**2g**)* Yield 85.4; mp 171 °C; UV (EtOH) λ_{max} (log ε) 279 (4.18), 225 (4.35), 259 (3.22) nm; IR (KBr) υ_{max} 3161, 1701, 1610, 1586, 759 and 687 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz,): δ = 12.40 (1H, s, H-26), 9.67 (1H, s, H-27), 7.82 (2H, d, *J* = 7.98 Hz, H-37, H-41), 7.53 (1H, d, H-31), 7.51 (2H, dd, H-33, H-35), 7.45 (2H, dd, H-38, H-40), 7.41 (1H, s, H-28), 7.38 (1H, dd, H-30), 7.35 (1H, dd, H-39), 7.20 (1H, dd, H-34), 7.15 (1H, d, H-29), 7.06 (2H, d, *J* = 8.36 Hz, H-32, H-36); ^{13}C NMR (DMSO-d_6, 100 MHz,): δ = 158.3 (C, C-6), 156.4 (C, C-10), 155.6 (C, C-2), 151.8 (CH, C-3), 145.0 (C, C-1), 135.8 (C, C-4), 131.2 (CH, C-8), 130.6 (CH, C-12, C-14), 130.4 (CH, C-22), 129.0 (CH, C-20, C-24), 128.4 (CH, C-21, C-23), 127.0 (C, C-19), 124.6 (C, C-13), 123.8 (CH, C-9), 121.8 (CH, C-5), 119.9 (CH, C-11, C-15), 116.0 (CH, C-7); ESI–MS *m/z* 239 (100); Anal. Calcd. for $C_{21}H_{16}N_4O_2$: C, 70.78; H, 4.53; N, 15.72. Found: C, 70.66; H, 4.85; N, 15.75.

*General method for the preparation of 1-acetyl-3-alkyl(aryl)-4-(3-phenoxy-benzylidenamino-4,5-dihydro-1H-1,2,4-triazol-5-ones (**3**)*

The corresponding compound **2** (0.01 mol) was refluxed with acetic anhydride (15 mL) for 0.5 h. After addition of absolute ethanol (50 mL), the mixture was refluxed for 1 h more. Evaporation of the resulting solution at 40–45 °C in vacuo and several recrystallizations of the residue from EtOH gave pure compounds **3a**, **3d**–**g** as colorless crystals.

Spectral data of each compound are given below:

*1-Acetyl-3-methyl-4-(3-phenoxy-benzylidenamino-4,5-dihydro-1H-1,2,4-triazol-5-one (**3a**)* Yield 92.6; mp 121 °C; UV (EtOH) λ_{max} (log ε) 294 (4.09), 224 (4.30), 259 (3.15) nm; IR (KBr) υ_{max} 1,771, 1,731, 1,619, 1,589, 757 and 696 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz,): δ = 9.69 (1H, s, H-27), 7.51 (1H, d, H-31), 7.49 (2H, dd, H-33, H-35), 7.45 (1H, s, H-28), 7.38 (1H, dd, H-30), 7.20 (1H, dd, H-34), 7.16 (2H, d, H-32, H-36), 7.04 (1H, d, H-29), 2.62 (3H, s, $COCH_3$), 2.40 (3H, s, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz,): δ = 166.4 (C=O), 158.1 (C, C-6), 156.6 (C, C-10), 154.8 (C, C-2), 148.7 (CH, C-3), 147.6 (C, C-1), 135.1 (C, C-4), 130.4 (CH, C-8), 130.0 (CH, C-12, C-14), 124.0 (CH, C-13), 123.2 (CH, C-9), 122.0 (CH, C-5), 119.4 (CH, C-11, C-15), 117.4 (CH, C-7), 23.7 (CH_3, $COCH_3$), 11.8 (CH_3); ESI–MS *m/z* 359 [M + 23] (100), 337 [M + 1] (5); Anal. Calcd. for $C_{18}H_{16}N_4O_3$: C, 64.28; H, 4.79; N, 16.66. Found: C, 64.39; H, 5.07; N, 15.74.

*1-Acetyl-3-benzyl-4-(3-phenoxy-benzylidenamino-4,5-dihydro-1H-1,2,4-triazol-5-one (**3d**)* Yield 94.7; mp 160 °C; UV (EtOH) λ_{max} (log ε) 290 (3.97), 223 (4.22), 259 (3.11) nm; IR (KBr) υ_{max} 1,741, 1,726, 1,618, 1,597, 771 and 689 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz,): δ = 9.62 (1H, s, H-27), 7.42 (1H, d, H-31), 7.40 (2H, dd, H-33, H-35), 7.37 (1H, s, H-28), 7.34 (1H, dd, H-30), 7.25 (2H, dd, H-40, H-42), 7.18 (2H, d, H-39, H-43), 7.17 (1H,

dd, H-34), 7.12 (2H, d, H-32, H-36), 7.10 (1H, dd, H-41), 7.07 (1H, d, H-29), 4.07 (2H, s, CH_2), 2.63 (3H, s, $COCH_3$); ^{13}C NMR (DMSO-d_6, 100 MHz,): $\delta = 166.4$ (C=O), 158.2 (C, C-6), 156.6 (C, C-10), 154.7 (C, C-2), 149.4 (C, C-3), 148.7 (C, C-1), 135.0 (C, C-4), 134.0 (C, C-19), 130.4 (CH, C-8), 130.0 (CH, C-12, C-14), 129.1 (CH, C-20, C-24), 128.7 (CH, C-21, C-23), 127.4 (CH, C-22), 124.0 (CH, C-13), 123.6 (CH, C-9), 122.2 (CH, C-5), 119.4 (CH, C-11, C-15), 116.5 (CH, C-7), 32.1 (CH_2), 23.8 (CH_3, $COCH_3$); ESI–MS *m/z* 435 [M + 23] (38), 223 (100); Anal. Calcd. for $C_{24}H_{20}N_4O_3$: C, 69.89; H, 4.89; N, 13.58. Found: C, 69.36; H, 5.20; N, 13.67.

*1-Acetyl-3-p-methylbenzyl-4-(3-phenoxy-benzylidenamino-4,5-dihydro-1H-1,2,4-triazol-5-one (**3e**)* Yield 91.7; mp 182 °C; UV (EtOH) λ_{max} (log ε) 293 (4.00), 224 (4.29), 259 (3.13) nm; IR (KBr) υ_{max} 1,741, 1,726, 1,620, 1,578, 826 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz,): $\delta = 9.60$ (1H, s, H-27), 7.45 (1H, d, H-31), 7.43 (2H, dd, H-33, H-35), 7.40 (1H, s, H-28), 7.37 (1H, dd, H-30), 7.22 (2H, d, H-39, H-42), 7.13 (1H, dd, H-34), 7.09 (2H, d, H-40, H-41), 7.10 (2H, d, H-32, H-36), 7.04 (1H, d, H-29), 4.04 (2H, s, CH_2), 2.63 (3H, s, $COCH_3$), 2.28 (3H, s, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz,): $\delta = 166.5$ (C = O), 159.5 (C, C-6), 158.1 (C, C-10), 156.6 (C, C-2), 154.7 (C, C-3), 148.8 (C, C-1), 137.1 (C, C-22), 135.0 (C, C-4), 131.0 (C, C-19), 130.4 (CH, C-8), 130.0 (CH, C-12, C-14), 129.5 (CH, C-20, C-24), 129.0 (CH, C-21, C-23), 124.1 (CH, C-13), 123.6 (CH, C-9), 122.2 (CH, C-5), 119.4 (CH, C-11, C-15), 116.6 (CH, C-7), 31.9 (CH_2), 24.0 (CH_3, $COCH_3$), 21.1 (CH_3); ESI–MS *m/z* 449 [M + 23] (86), 427 [M + 1] (35), 421 (100); Anal. Calcd. for $C_{25}H_{22}N_4O_3$: C, 70.41; H, 5.20; N, 13.14. Found: C, 70.26; H, 5.64; N, 13.32.

*1-Acetyl-3-p-chlorobenzyl-4-(3-phenoxy-benzylidenamino-4,5-dihydro-1H-1,2,4-triazol-5-one (**3f**)* Yield 88.9; mp 155 °C; UV (EtOH) λ_{max} (log ε) 294 (3.96), 224 (4.30), 259 (3.10) nm; IR (KBr) υ_{max} 1,768, 1,743, 1,620, 1,576, 846, 766 and 689 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz,): $\delta = 9.62$ (1H, s, H-27), 7.46 (1H, d, H-31), 7.40 (2H, dd, H-33, H-35), 7.37 (1H, s, H-28), 7.34 (1H, dd, H-30), 7.22 (2H, d, H-39, H-42), 7.10 (1H, dd, H-34), 7.08 (2H, d, H-32, H-36), 7.12 (2H, d, H-40, H-41), 7.05 (1H, d, H-29), 4.03 (2H, s, CH_2), 2.63 (3H, s, $COCH_3$); ^{13}C NMR (DMSO-d_6, 100 MHz,): $\delta = 166.2$ (C=O), 158.2 (C, C-6), 156.4 (C, C-10), 154.7 (C, C-2), 148.8 (C, C-3), 148.6 (C, C-1), 134.9 (C, C-4), 133.4 (C, C-19), 132.5 (C, C-22), 130.6 (CH, C-8), 130.4 (CH, C-20, C-24), 130.1 (CH, C-12, C-14), 129.0 (CH, C-21, C-23), 124.2 (CH, C-13), 123.8 (CH, C-9), 122.4 (CH, C-5), 119.5 (CH, C-11, C-15), 116.4 (CH, C-7), 31.7 (CH_2), 23.8 (CH_3, $COCH_3$); ESI–MS *m/z* 471 [(M + 2) + 23] (5), 469 [M + 23] 12), 448 [M + 2] (2), 447 [M + 1] (5), 217 (100); Anal. Calcd. for $C_{24}H_{19}ClN_4O_3$: C, 64.50; H, 4.29; N, 12.54. Found: C, 63.63; H, 4.62; N, 12.40.

*1-Acetyl-3-phenyl-4-(3-phenoxy-benzylidenamino-4,5-dihydro-1H-1,2,4-triazol-5-one (**3g**)* Yield 93.9; mp 163 °C; UV (EtOH) λ_{max} (log ε) 278 (4.06), 225 (4.29), 259 (3.16) nm; IR (KBr) υ_{max} 1,732, 1,619, 1,590, 775 and 695 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz,): $\delta = 9.67$ (1H, s, H-27), 7.98 (2H, d, $J = 7.72$ Hz, H-37, H-41), 7.55 (1H, d, H-31), 7.53 (2H, dd, H-33, H-35), 7.42 (2H, dd, H-38, H-40), 7.40 (1H, s, H-28), 7.36 (1H, dd, H-30), 7.35 (1H, dd, H-39), 7.21 (1H, dd, H-34), 7.06 (1H, d, H-29), 7.04 (2H, d, H-32, H-36), 2.68 (3H, s, $COCH_3$); ^{13}C NMR (DMSO-d_6, 100 MHz,): $\delta = 166.9$ (C=O), 158.1 (C, C-6), 156.4 (C, C-10), 156.3 (C, C-2), 148.9 (C, C-3), 147.2 (C, C-1), 135.0 (C, C-4), 131.5 (CH, C-22), 130.4 (CH, C-8), 130.0 (CH, C-12, C-14), 129.1 (CH, C-20, C-24), 128.5 (CH, C-21, C-23), 125.2 (CH, C-19), 124.2 (CH, C-13), 123.5 (CH, C-9), 122.1 (CH, C-5), 119.5 (CH, C-11, C-15), 116.9 (CH, C-7), 24.0 (CH_3, $COCH_3$); ESI–MS *m/z* 421 [M + 23] (100), 399 [M + 1] (65); Anal. Calcd. for $C_{23}H_{18}N_4O_3$: C, 69.34; H, 4.55; N, 14.06. Found: C, 68.90; H, 4.84; N, 14.13.

*3-Methyl-4-(2-thienymethylenamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (**4a**)* m.p.: 175 °C [(Yuksek *et al*., 2011) 174 °C]

*3-Ethyl-4-(2-thienymethylenamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (**4b**)* m.p.: 158 °C [(Yuksek *et al*., 2011) 157 °C]

*3-n-Propyl-4-(2-thienymethylenamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (**4c**)* m.p.: 160 °C [(Yuksek *et al*., 2011) 158 °C]

*3-Benzyl-4-(2-thienymethylenamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (**4d**)* m.p.: 188 °C [(Yuksek *et al*., 2011) 188 °C]

*3-p-Methylbenzyl-4-(2-thienymethylenamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (**4e**)* m.p.: 152 °C [(Yuksek *et al*., 2011) 151 °C]

*3-p-Chlorobenzyl-4-(2-thienymethylenamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (**4f**)* m.p.: 191 °C [(Yuksek *et al*., 2011) 192 °C]

*3-Phenyl-4-(2-thienymethylenamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (**4g**)* m.p.: 162 °C [(Yuksek *et al*., 2011) 164 °C]

*1-Acetyl-3-methyl-4-(2-thienymethylenamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (**5a**)* m.p.: 180 °C [(Yuksek *et al*., 2011) 178 °C]

*1-Acetyl-3-ethyl-4-(2-thienymethylenamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (**5b**)* m.p.: 177 °C [(Yuksek *et al*., 2011) 179 °C]

*1-Acetyl-3-benzyl-4-(2-thienymethylenamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (**5d**)* m.p.: 165 °C [(Yuksek *et al*., 2011) 167 °C]

*1-Acetyl-3-p-methylbenzyl-4-(2-thienymethylenamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (**5e**)* m.p.: 185 °C [(Yuksek *et al*., 2011) 188 °C]

*1-Acetyl-3-p-chlorobenzyl-4-(2-thienymethylenamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (**5f**)* m.p.: 201 °C [(Yuksek *et al*., 2011) 200 °C]

*1-Acetyl-3-phenyl-4-(2-thienymethylenamino)-4,5-dihydro-1H-1,2,4-triazol-5-one (**5g**)* m.p.: 161 °C [(Yuksek *et al*., 2011) 160 °C]

Antioxidant activity

Chemicals

Butylated hydroxytoluene (BHT) was purchased from E. Merck. Ferrous chloride, α-tocopherol, 1,1-diphenyl-2-picryl-hydrazyl (DPPH·), 3-(2-pyridyl)-5,6-bis(phenylsulfonic acid)-1,2,4-triazine (ferrozine), butylated hydroxyanisole (BHA), and trichloracetic acid (TCA) were bought from Sigma (Sigma-Aldrich GmbH, Sternheim,Germany).

Reducing power

The reducing power of the synthesized compounds was determined according to the method (Oyaizu, 1986). 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 after which a portion (2.5 mL) of trichloroacetic acid (10 %) was added to the mixture, which was then centrifuged for 10 min at 1,000×*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 (Blois, 1958). Briefly, 0.1 mM solution of DPPH· in ethanol was prepared, and this solution (1 mL) was added to sample solutions in DMSO (3 mL) at different concentrations (50–250 μg/mL). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then, the absorbance was measured at 517 nm in a spectrophometer. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The DPPH· concentration (mM) in the reaction medium was calculated from the following calibration curve and determined by linear regression (*R*: 0.997):

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

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

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

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 was estimated by the method (Dinis *et al*., 1994). Briefly, the synthesized compounds (50–250 μg/mL) were added to a 2 mM solution of $FeCl_2$ (0.05 mL). The reaction was initiated by the addition of 5 mM ferrozine (0.2 mL), and the mixture was shaken vigorously and left standing at 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. The percentage of inhibition of ferrozine–Fe^{2+} complex formation was given by the formula: % Inhibition = $(A_0 - A_1/A_0) \times 100$, where A_0 is the absorbance of the control, and A_1 is the absorbance in the presence of the samples or standards. The control did not contain compound or standard.

Potentiometric titrations

A Jenway 3040-model ion analyzer and an Ingold pH electrode were used for potentiometric titrations. 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.

Antimicrobial activity

All bacterial and yeast strains were obtained from the Hifzissihha Institute of Refik Saydam (Ankara, Turkey) and were as follows: *Escherichia coli* ATCC 25922, *Yersinia pseudotuberculosis* ATCC 911, *Pseudomonas aeruginosa* ATCC 43288, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Bacillus cereus* 702 Roma, *Mycobacterium smegmatis* ATCC 607, *Candida albicans*

ATCC 60193, *Saccharomyces cerevisiae* RSKK 251. Simple susceptibility screening test using agar well-diffusion method was used (Ahmad *et al*., 1998; Perez *et al*., 1990). Each microorganism was suspended in Mueller–Hinton (MH) (Difco, Detroit, MI) 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) (Difco, Detriot, MI, USA) and then dried. For *C. albicans*, SDA was used. Five-millimeter diameter wells were cut from the agar using a sterile cork-borer, and 50 μL of the sample solutions was 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. Ampicillin (10 μg) and fluconazole (5 μg) were standard antibacterial and antifungal agents, respectively. DMSO was used as solvent control.

Results and discussion

In this study, the structures of six new 3-alkyl(aryl)-4-(3-phenoxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**2a**, **2b**, **2d**–**g**) and five new 1-acetyl-3-alkyl(aryl)-4-(3-phenoxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**3a**, **3d**–**g**) were identified using elemental analysis, IR, ^{1}H NMR, ^{13}C NMR, UV, and MS 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 (Meir *et al*., 1995). 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 (Yildirim *et al*., 2001). In this study, all of the concentrations of the compounds of series **2a**, **b**, **d**–**g** and **3a**, **d**–**g** 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 (Baumann *et al*., 1979). DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares *et al*., 1997). 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 (Duh *et al*., 1999). In the study, antiradical activities of compounds and standard antioxidants such as BHA, BHT, and α-tocopherol were determined using DPPH· method. Figure 2 illustrates that the newly synthesized compounds **2** showed lower activities as a radical scavenger or hydrogen donors. Compounds **3** did not show any activity.

Ferrous ion chelating activity

The chelating effect toward 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 color of the complex is decreased. Measurement of color reduction therefore allows estimation of the chelating activity of the coexisting chelator (Yamaguchi *et al*., 2000). 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 (Strlic *et al*., 2002) 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 (Finefrock *et al*., 2003). 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:

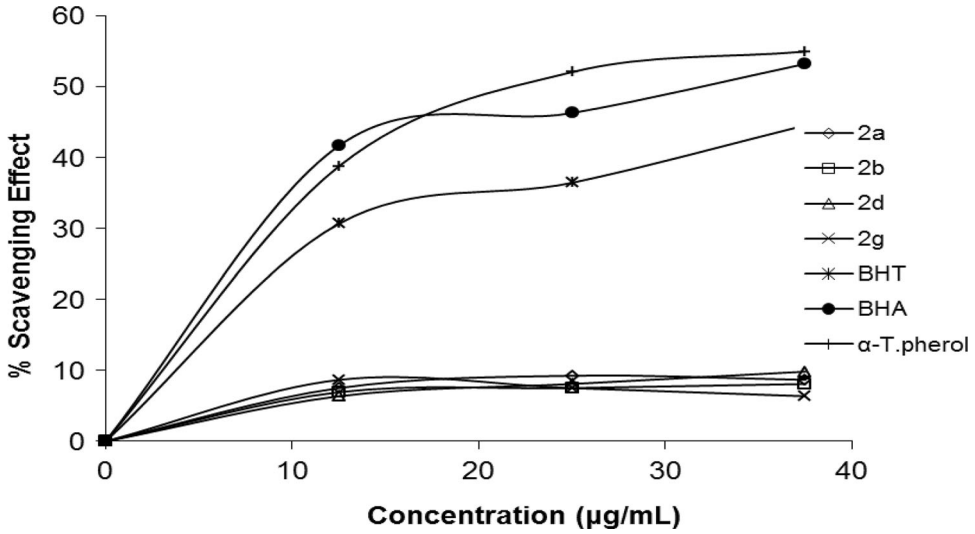

Fig. 2 Scavenging effect of compounds **2**, BHT, BHA and α-tocopherol at different concentrations (12.5–25–37.5 µg/mL)

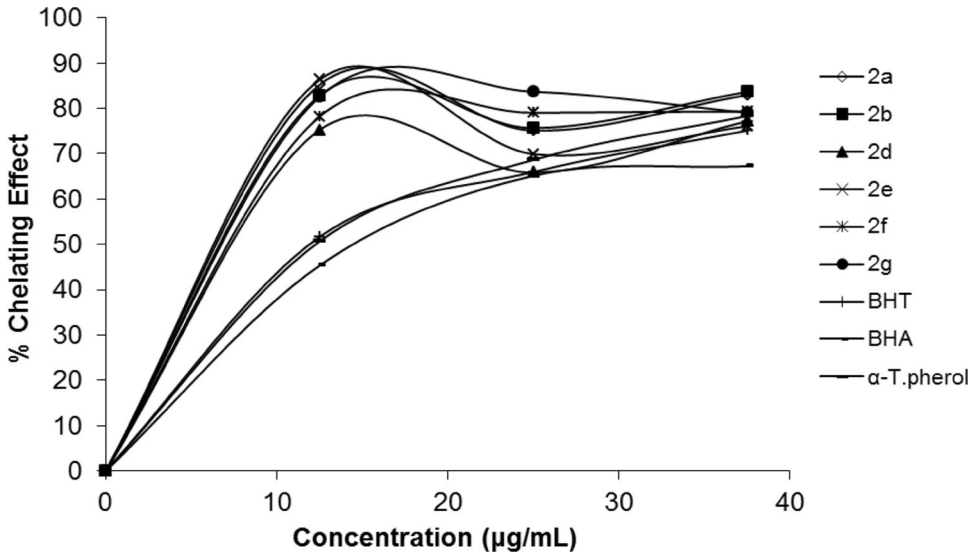

Fig. 3 Metal chelating effect of different amount of the compounds **2**, BHT, BHA, and α-tocopherol on ferrous ions

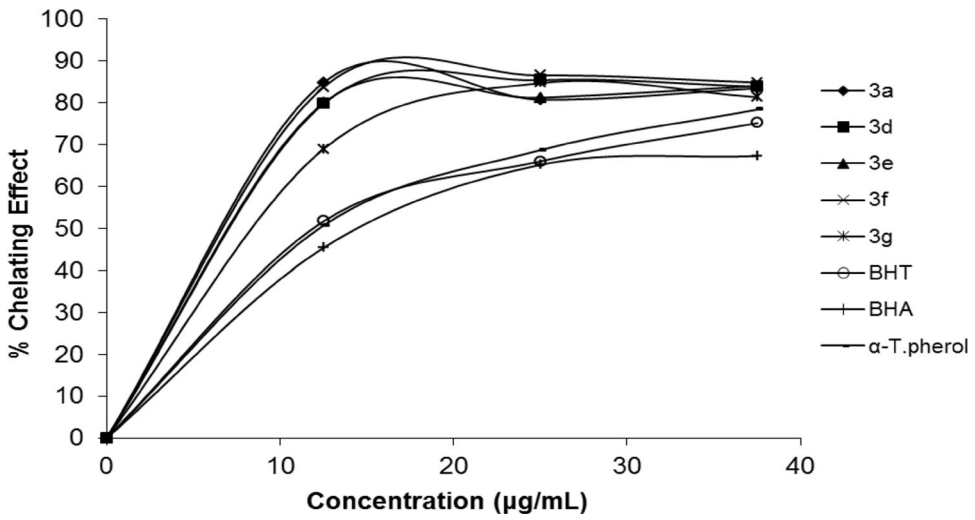

Fig. 4 Metal chelating effect of different amount of the compounds **3**, BHT, BHA, and α-tocopherol on ferrous ions

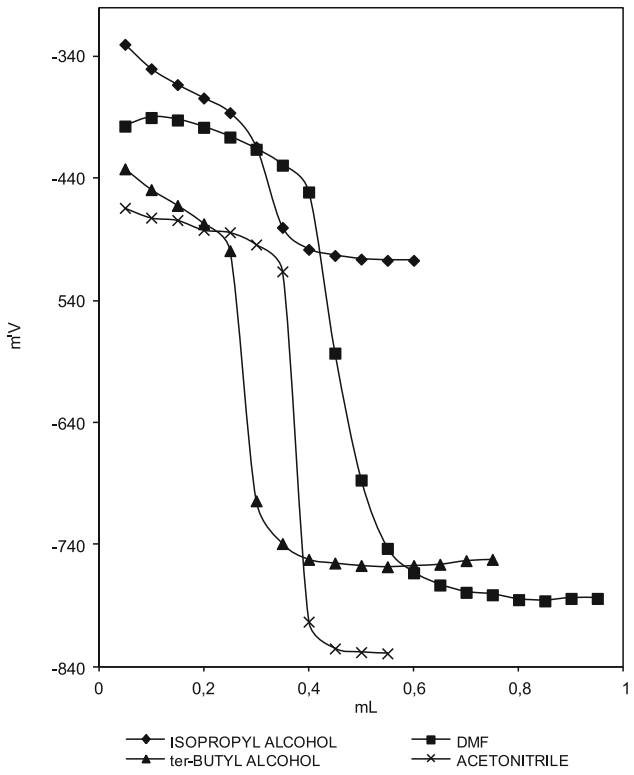

Fig. 5 Potentiometric titration curves of 0.001 M solutions of compound **2a** titrated with 0.05 M TBAH in isopropyl alcohol, *tert*-butyl alcohol, DMF, and acetonitrile at 25 °C

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

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 (Calis *et al*., 1993). 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 (Gordon, 1990).

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 Figs. 3 and 4 reveal that the compounds, especially **2f** and **3d** 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, the free iron is known to have low solubility, and a chelated iron complex has greater solubility in solution, which can be contributed solely by the ligand. Furthermore, the compound-iron complex may also be active, since it can participate in iron-catalyzed reactions.

Potentiometric titrations

In order to determine the pK_a values of the compounds (**2a**, **b**, **d**–**g**), they were titrated potentiometrically with TBAH in four non-aqueous solvents: isopropyl alcohol, *tert*-butyl alcohol, acetonitrile, and DMF. The mV values read in each titration were plotted against 0.05 M TBAH volumes (mL) added, and potentiometric titration curves were obtained for all the cases. From the titration curves, the HNP values were measured, and the corresponding pK_a values were calculated. The data obtained from the potentiometric titrations were interpreted, and the effect of the C-3 substituent in 4,5-dihydro-1*H*-1,2,4-triazol-5-one ring as well as solvent effects was studied (Bahceci *et al*., 2002; Gursoy-Kol *et al*., 2012; Yuksek *et al*., 2011; Yuksek and Gursoy-Kol, 2008).

As an example for the potentiometric titration curves for 0.001 M solutions of compound **2a** titrated with 0.05 M TBAH in isopropyl alcohol, *tert*-butyl alcohol, DMF, and acetonitrile are shown in Fig. 5.

When the dielectric permittivity of solvents is taken into consideration, the acidity order can be given as follows: DMF ($\varepsilon = 36.7$) > acetonitrile ($\varepsilon = 36.0$) > isopropyl

Table 1 HNP and the corresponding pK_a values of compounds **2** in isopropyl alcohol, *tert*-butyl alcohol, DMF, and acetonitrile

Compounds	Isopropyl alcohol		*tert*-Butyl alcohol		DMF		Acetonitrile	
	HNP (mV)	pK_a	HNP (mV)	pK_a	HNP (mV)	pK_a	HNP (mV)	pK_a
2a	−363	13.04	−455	14.78	−398	13.70	−478	15.22
2b	−357	12.95	−495	15.42	−434	14.38	−454	14.67
2d	−345	12.68	−464	14.90	−441	14.46	−433	14.32
2e	−358	12.93	−463	14.80	−422	14.13	−443	14.47
2f	−343	12.55	−456	14.33	−366	13.03	−431	14.23
2g	−331	12.45	−426	14.25	−405	13.84	−459	14.89

Table 2 Screening result for antimicrobial and antifungal activity of the compounds **2**, **3**, **4,** and **5**

Compounds	Microorganisms and inhibition zone (mm)								
	Ec	Yp	Pa	Sa	Ef	Bc	Ms	Ca	Sc
2a	–	–	–	–	–	–	–	–	–
2b	–	–	–	–	–	–	–	–	–
2d	–	–	–	–	–	–	–	–	–
2e	–	–	–	–	–	–	–	–	–
2f	–	–	–	–	–	–	–	–	–
2g	–	–	–	–	–	–	–	–	–
3a	–	–	–	–	–	–	–	–	–
3d	–	–	–	–	–	–	–	–	–
3e	–	–	–	–	–	–	–	–	–
3f	–	–	–	–	–	–	–	–	–
3g	–	–	–	–	–	–	–	–	–
4a	–	–	–	–	–	–	6	–	–
4b	–	–	–	–	–	–	10	–	–
4c	–	–	–	–	–	–	8	–	–
4d	–	–	–	–	–	–	6	–	–
4e	–	–	–	–	–	–	6	–	–
4f	–	–	–	–	–	–	6	–	–
4g	–	–	–	–	–	–	–	–	–
5a	–	–	–	–	–	–	–	–	–
5b	–	–	–	–	–	–	–	–	–
5d	–	–	–	–	–	–	6	10	8
5e	–	–	–	–	–	–	–	–	–
5f	–	–	–	–	–	–	–	–	–
5g	–	–	–	–	–	–	–	–	–
Amp.	10	18	18	35	10	15			
Strep.							35		
Flu.								25	>25

Ec *Escherichia coli* ATCC 25922, Yp: *Yersinia pseudotuberculosis* ATCC 911, Pa: *Pseudomonas aeruginosa* ATCC 43288, Sa: *Staphylococcus aureus* ATCC 25923, Ef: *Enterococcus faecalis* ATCC 29212, Bc: *Bacillus cereus* 702 Roma, Ms: *Mycobacterium smegmatis* ATCC607, Ca: *Candida albicans* ATCC 60193, *Saccharomyces cerevisiae* RSKK 251, Amp: Ampicillin, Strep.: Streptomycin, Flu.: Fluconazole, (–): no activite

alcohol ($\varepsilon = 19.4$) > *tert*-butyl alcohol ($\varepsilon = 12$). As seen in Table 1, the acidity order for compounds **2a** and **2g** is: acetonitrile > *tert*-butyl alcohol > DMF > isopropyl alcohol, for compounds **2b**, **2e** and **2f** are: *tert*-butyl alcohol > acetonitrile > DMF > isopropyl alcohol, for compound **2d** it is: *tert*-butyl alcohol > DMF > acetonitrile > isopropyl alcohol.

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 structure (Bahceci *et al*., 2002; Gunduz, 1998; Gursoy-Kol *et al*., 2012; Yuksek *et al*., 2011; Yuksek and Gursoy-Kol, 2008). Table 1 and Fig. 5 show that the HNP values and corresponding pK_a values obtained from the potentiometric titrations depend on the non-aqueous solvents used and the substituents at C-3, in 4,5-dihydro-1*H*-1,2,4-triazol-5-one ring.

Antimicrobial and antifungal activity

The synthesized compounds were screened for their biological activities, and some of them were found to possess moderate antimicrobial activity (Table 2). The compounds **2** and **3** did not display any antimicrobial activity against to all of tested microorganisms. Although the compounds **4a**–**4f** and **5d** observed the low antituberculos activity against the *Mycobacterium smegmatis,* only one (**5d**) chemical showed antifungal activity against *C. albicans* and *S. cerevisiae*.

Conclusions

In this study, the structures of 11 new 4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives synthesized from the

reactions of **1** type compounds with 3-phenoxybenzaldehyde were identified using elemental analysis, IR, ^{1}H NMR, ^{13}C NMR, UV, and MS spectral data, and these obtained spectral values were seen as compatible with literature (Bahceci *et al*., 2002; Gursoy-Kol *et al*., 2012; Yuksek *et al*., 2011; Yuksek and Gursoy-Kol, 2008). The newly synthesized compounds were screened for their antioxidant activities. All of the compounds demonstrated a marked capacity for the iron binding. The data reported with regard to the observed metal chelating activities of the studied compounds could prevent redox cycling. These new compounds and 13 recently reported 4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives were also screened for their antimicrobial activities. From the screening results, compounds **4b** and **4c** showed moderate activity against the *Mycobacterium smegmatis.*

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