ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Synthesis, In Vitro Antioxidant and Antimicrobial Activities of Some Novel 2-Methoxy-4-[(3-substitue-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl)azomethine]phenyl 2-methylbenzoate Derivatives.

Haydar Yüksek[1], Osman Kutanis[1], Gül Özdemir[1], Murat Beytur[1], Savaş Kara[1], Özlem Gürsoy-Kol[1]*, and Muzaffer Alkan[2].

[1]Department of Chemistry, Kafkas University, 36100 Kars, Turkey.
[2]Education Faculty, Kafkas University, 36100 Kars, Turkey.

ABSTRACT

In the present study, nine novel 2-methoxy-4-[(3-alkyl/aryl-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl)azomethine]phenyl 2-methylbenzoates (**4a-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-(2-methylbenzoxy)-4-methoxybenzaldehyde (**3**) by using triethylamine. Then, the acetylation reactions of compounds **4a–e** and **4g** were investigated and **5** type compounds synthesized. The structures of fifteen new compounds were established from IR, ^{1}H NMR, ^{13}C NMR, UV and mass 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. Furthermore, the synthesized compounds were screened for their antimicrobial activities. Considering both the antioxidant and the antimicrobial evaluation, compound **4d** exhibited the moderate effect.

Keywords: 4,5-dihydro-1*H*-1,2,4-triazol-5-one, Schiff base, acetylation, antioxidant activity, antimicrobial activity.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

The balance between pro-oxidants and antioxidants reflects the morphological consequences. Oxidative stress can occur with an imbalance in favor of pro-oxidants and/or against antioxidants. This situation may cause cellular dysfunction or death [1]. Scientists in various disciplines have become more interested in naturally-occurring antioxidant and also related synthetic derivatives that could provide active components to prevent or reduce the impact of oxidative stress [2].

Moreover, emerging antibiotic resistance is recognized as one of the most significant public health problems of the last few decades. The rapid emergence and prevalence of antibiotic resistant pathogens requires a serious effort to identify, develop and design new antibiotics [3]. Design and synthesis of novel heterocycles can play a significant role because of their importance in medicinal chemistry.

Triazoles are heterocyclic compounds that contain three nitrogen atoms. Some of the modern drugs which containing a triazole moiety are alprazolam, triazolam, estazolam (hypnotic, sedative, tranquilizer), trazodone (antidepressant, anxiolytic), trapidil (hypotensive), terconazole (antifungal), hexaconazole (antifungal), etizolam (amnesic, anxiolytic, anticonvulsant, hypnotic, sedative and skeletal muscle relaxant), rilmazafon (hypnotic, anxiolytic) and rizatriptan (antimigrane agent) [4]. 1,2,4-Triazole and 4,5-dihydro-1H-1,2,4-triazol-5-one derivatives have been found to have a broad spectrum of biological activities [5-12].

MATHERIALS AND METHODS

Chemical reagents used in this paper were bought from Merck AG, Aldrich and Fluka. Melting points were taken using an Electrothermal Melting-point Apparatus in an open capillary tube and were not corrected. The infrared spectra were recorded on a Perkin Elmer Instruments Spectrum One FT-IR spectrophotometer. ^{1}H and ^{13}C NMR spectra were determined in deuterated dimethyl sulfoxide with TMS as internal standard using a Bruker Ultrashield spectrophotometer at 200 MHz and 50 MHz, respectively. UV absorption spectra were evaluated in 10 mm quartz cells between 200 and 400 nm using a PG Instruments Ltd T80 UV/Vis spectrometer. Extinction coefficients (ε) are clarified in $L \cdot mol^{-1} \cdot cm^{-1}$.

EXPERIMENTAL SECTION

Chemistry

General procedure for the synthesis of 2-methoxy-4-[(3-alkyl/aryl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl)azomethine]phenyl 2-methylbenzoates (4)

3-Hydroxy-4-methoxybenzaldehyde (0.01 mol) dissolved in ethyl acetate (100 mL) was reacted with 2-methylbenzoyl chloride (0.01 mol), and to this solution was slowly mixed triethylamine (0.01 mol) by stirring at 0-5 °C. Stirring was continued for 2 h, and after that the mixture was refluxed for 3 hours and filtered. The filtrate was evaporated *in vacuo*, and the crude product was washed with water and recrystallized from ethyl acetate-petroleum ether (1:3) to afford novel compound **3**. Yield: 98; m.p. 78°C; IR (cm^{-1}) υ_{max}: 2821 and 2739 (CHO), 1725, 1679 (C=O), 1274 (COO), 727 (1,2-disubstituted benzenoid ring). Then the corresponding compound **2** (0.01 mol) was dissolved in ethanoic acid (20 mL) and by treated 3-(2-methylbenzoxy)-4-methoxybenzaldehyde **3** (0.01 mol). The mixture was refluxed for 1.5 hours and then evaporated at 50-55 °C *in vacuo*. A few recrystallizations of the residue from ethanol gave pure compounds **4a-i** as uncolored crystals.

2-Methoxy-4-[(3-methyl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl)azomethine]-phenyl 2-methylbenzoate (4a)

White solid; yield: 98%; m.p. 206°C; IR (cm^{-1}) υ_{max}: 3173 (NH), 1747, 1690 (C=O), 1600 (C=N), 1273 (COO), 730 (1,2-disubstituted benzenoid ring); ^{1}H-NMR (200 MHz, DMSO-d_6) (ppm) δ H: 2.27 (s, 3H, CH_3), 2.59 (s, 3H, $PhCH_3$), 3.87 (s, 3H, OCH_3), 7.32 (d, 1H, ArH; *J* = 8.48 Hz), 7.40-7.44 (m, 2H, ArH), 7.58-7.61 (m, 1H, ArH), 7.75-7.78 (m, 2H, ArH), 8.08 (d, 1H, ArH; *J* = 7.76 Hz), 9.68 (s, 1H, N=CH), 11.80 (s, 1H, NH); ^{13}C NMR (50 MHz, DMSO-d_6) (ppm) δ C: 11.59 (CH_3), 21.57 ($PhCH_3$), 56.69 (OCH_3), [113.42, 121.59, 126.70, 126.91, 128.52, 128.61, 131.24, 132.32, 133.48, 140.26, 140.52, 153.33] (Ar-C), 144.73 (Triazol-C_3), 151.76 (Triazol-C_5), 154.03

(N=CH), 165.15 (COO); UV [Etanol, λ_{max}, nm (ε, L.mol^{-1}.cm^{-1})]: 307 (23.031), 230 (25.336); ESI–MS m/z: 395, 384, 367(M+1), 359, 341, 318, 303, 288, 259, 244, 243, 242(100), 223, 222, 215, 186, 156.

2-Methoxy-4-[(3-ethyl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl)azomethine]-phenyl 2-methylbenzoate (4b)

White solid; yield: 96%; m.p. 184°C; IR (cm^{-1}) υ_{max}: 3182 (NH), 1737, 1680 (C=O), 1587 (C=N), 1269 (COO), 759 (1,2-disubstituted benzenoid ring); ^{1}H-NMR (200 MHz, DMSO-d_6) (ppm) δ H: 1.20 (t, 3H, CH_2CH_3; *J* = 7.40 Hz), 2.59 (s, 3H, $PhCH_3$), 2.68 (q, 2H, $\underline{CH_2}CH_3$; *J* = 7.40 Hz), 3.87 (s, 3H, OCH_3), 7.32 (d, 1H, ArH; *J* = 9.04 Hz), 7.40-7.44 (m, 2H, ArH), 7.58-7.60 (m, 1H, ArH), 7.75-7.77 (m, 2H, ArH), 8.08 (d, 1H, ArH; *J* = 7.52 Hz), 9.67 (s, 1H, N=CH), 11.82 (s, 1H, NH); ^{13}C NMR (50 MHz, DMSO-d_6) (ppm) δ C: 10.46 ($CH_2\underline{CH_3}$), 19.02 ($\underline{CH_2}CH_3$), 21.57 ($PhCH_3$), 56.70 (OCH_3), [113.48, 121.62, 126.72, 126.94, 128.52 (2C), 131.25, 132.33, 133.49, 140.27, 140.52, 153.45] (Ar-C), 148.50 (Triazol-C_3), 151.90 (Triazol-C_5), 154.04 (N=CH), 165.14 (COO); UV [Etanol, λ_{max}, nm (ε, L.mol^{-1}.cm^{-1})]: 305 (24.238), 289 (22.057), 231 (22.026), 227 (25.905), 211 (20.794); ESI–MS m/z: 431, 404(M+1+23), 403(M+23), 381(M+1), 377, 363, 336, 309, 299, 282, 268, 244, 243, 242(100), 227, 200, 173, 159.

2-Methoxy-4-[(3-n-propyl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl)azomethine]-phenyl 2-methylbenzoate (4c)

White solid; yield: 94%; m.p. 196°C; IR (cm^{-1}) υ_{max}: 3165 (NH), 1735, 1682 (C=O), 1586 (C=N), 1265 (COO), 757 (1,2-disubstituted benzenoid ring); ^{1}H-NMR (200 MHz, DMSO-d_6) (ppm) δ H: 0.93 (t, 3H, $CH_2CH_2CH_3$; *J* = 7.36 Hz), 1.68 (sext, 2H, $CH_2\underline{CH_2}CH_3$; *J* = 7.08 Hz), 2.59 (s, 3H, $PhCH_3$), 2.65 (t, 2H, $\underline{CH_2}CH_2CH_3$; *J* = 7.08 Hz), 3.88 (s, 3H, OCH_3), δ 7.29-7.34 (m, 1H, ArH), 7.40-7.44 (m, 2H, ArH), 7.58-7.62 (m, 1H, ArH), 7.73-7.75 (m, 2H, ArH), 8.08-8.10 (m, 1H, ArH), 9.67 (s, 1H, N=CH), 11.83 (s, 1H, NH); ^{13}C NMR (50 MHz, DMSO-d_6) (ppm) δ C: 13.42 ($CH_2CH_2\underline{CH_3}$), 18.80 ($CH_2\underline{CH_2}CH_3$), 21.06 ($PhCH_3$), 26.62 ($\underline{CH_2}CH_2CH_3$), 56.19 (OCH_3), [112.99, 121.17, 126.20, 126.43, 127.93, 128.03, 130.74, 131.82, 132.97, 139.76, 140.02, 152.99] (Ar-C), 146.84 (Triazol-C_3), 151.33 (Triazol-C_5), 153.53(N=CH), 164.64 (COO); UV [Etanol, λ_{max}, nm (ε, L.mol^{-1}.cm^{-1})]: 306 (21.234), 233 (23.166); ESI–MS m/z: 443, 436, 418(M+1+23), 417(M+23), 412, 395(M+1), 371, 352, 335, 308, 279, 263, 243, 242(100), 222, 185, 153.

2-Methoxy-4-[(3-benzyl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl)azomethine]-phenyl 2-methylbenzoate (4d)

White solid; yield: 94%; m.p. 225°C; IR (cm^{-1}) υ_{max}: 3162 (NH), 1735, 1699 (C=O), 1600 (C=N), 1275 (COO), 764 (1,2-disubstituted benzenoid ring), 764 and 701 (monosubstituted benzenoid ring); ^{1}H-NMR (200 MHz, DMSO-d_6) (ppm) δ H: 2.59 (s, 3H, $PhCH_3$), 3.87 (s, 3H, OCH_3), 4.06 (s, 2H, CH_2Ph), 7.18-7.22 (m, 1H, ArH), 7.26-7.33 (m, 5H, ArH), 7.41-7.45 (m, 2H, ArH), 7.60 (t, 1H, ArH; *J* = 7.44 Hz), 7.70-7.73 (m, 2H, ArH), 8.08 (d, 1H, ArH; *J* = 8.08 Hz), 9.62 (s, 1H, N=CH), 11.95 (s, 1H, NH); ^{13}C NMR (50 MHz, DMSO-d_6) (ppm) δ C: 21.55 ($PhCH_3$), 31.59 (CH_2Ph), 56.70 (OCH_3), [113.47, 121.80, 126.75, 126.90, 128.83, 129.30, 130.74, 131.18, 132.34, 140.20, 140.45, 152.97] (Ar-C), [127.14, 128.44 (2C), 128.60 (2C), 136.33] (Ar-C linked C-3), 146.69 (Triazol-C_3), 151.74 (Triazol-C_5), 154.04 (N=CH), 165.20 (COO); UV [Etanol, λ_{max}, nm (ε, L.mol^{-1}.cm^{-1})]: 307 (23.000), 234 (26.093), 214 (21.288); ESI–MS m/z: 499, 475, 465(M+23), 443(M+1), 431, 429, 404, 377, 363, 349, 336, 309, 299, 295, 267, 263, 243, 242(100), 227, 200, 173, 159.

2-Methoxy-4-[(3-p-methylbenzyl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl)azomethine]-phenyl 2-methylbenzoate (4e)

White solid; yield: 98%; m.p. 221°C; IR (cm^{-1}) υ_{max}: 3165 (NH), 1735, 1699 (C=O), 1603 (C=N), 1273 (COO), 827 (1,4-disubstituted benzenoid ring), 763 (1,2-disubstituted benzenoid ring); ^{1}H-NMR (200 MHz, DMSO-d_6) (ppm) δ H: 2.21 (s, 3H, *p*-$PhCH_3$), 2.61 (s, 3H, *o*-$PhCH_3$), 3.87 (s, 3H, OCH_3), 4.05 (s, 2H, CH_2Ph), 7.07 (d, 2H, ArH; *J* = 7.56 Hz), 7.19 (d, 2H, ArH; *J* = 7.76 Hz), 7.30 (d, 1H, ArH; *J* = 8.56 Hz), 7.42-7.46 (m, 2H, ArH), 7.59-7.63 (m, 1H, ArH), 7.69-7.73 (m, 2H, ArH), 8.09 (d, 1H, ArH; *J* = 8.28 Hz), 9.61 (s, 1H, N=CH), 11.92 (s, 1H, NH); ^{13}C NMR (50 MHz, DMSO-d_6) (ppm) δ C: 20.53 ($PhCH_3$), 21.06 ($PhCH_3$-*o*), 30.74 (CH_2Ph), 56.22 (OCH_3), [112.99, 121.25, 126.26, 126.43, 128.66 (2C), 131.86, 132.72, 133.02, 139.70, 139.77, 152.42] (Ar-C), [127.99, 128.09, 128.90 (2C), 130.70, 135.66] (Ar-C linked C-3), 146.33 (Triazol-C_3), 151.24 (Triazol-C_5), 153.53 (N=CH), 164.69 (COO); UV [Etanol, λ_{max}, nm (ε, L.mol^{-1}.cm^{-1})]: 308 (15.635), 222 (21.395), 212 (20.500), 210 (20.333); ESI–MS m/z: 479(M+23), 458, 457(M+1), 443, 410, 399, 371, 343, 309, 263, 244, 243, 242(100), 205, 192, 153.

2-Methoxy-4-[(3-p-methoxybenzyl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl)azomethine]-phenyl 2-methylbenzoate (4f)

White solid; yield: 99%; m.p. 188°C; IR (cm^{-1}) υ_{max}: 3170 (NH), 1734, 1701 (C=O), 1606 (C=N), 1273 (COO), 830 (1,4-disubstituted benzenoid ring), 764 (1,2-disubstituted benzenoid ring); ^{1}H-NMR (200 MHz, DMSO-d_6) (ppm) δ H: 2.61 (s, 3H, $PhCH_3$), 3.67 (s, 3H, *p*-$PhOCH_3$), 3.87 (s, 3H, OCH_3), 3.98 (s, 2H, CH_2Ph), 6.83 (d, 2H, ArH; *J* = 8.08 Hz), 7.23 (d, 2H, ArH; *J* = 7.96 Hz), 7.30 (d, 1H, ArH; *J* = 8.80 Hz), 7.42-7.44 (m, 2H, ArH), 7.59 (t, 1H, ArH; *J* = 7.60 Hz), 7.73 (m, 2H, ArH), 8.09 (d, 1H, ArH; *J* = 7.48 Hz), 9.63 (s, 1H, N=CH), 11.93 (s, 1H, NH); ^{13}C NMR (50 MHz, DMSO-d_6) (ppm) δ C: 21.56 ($PhCH_3$), 30.75 (CH_2Ph), 55.38 (*p*-$PhOCH_3$), 56.68 (OCH_3), [113.45, 121.79, 126.73, 126.94, 128.45, 128.58, 131.21, 132.33, 133.48, 140.22, 140.50, 152.94,] (Ar-C), [114.22 (2C), 128.07, 130.38 (2C), 158.50] (Ar-C linked C-3), 147.00 (Triazol-C_3), 151.76 (Triazol-C_5), 154.03 (N=CH), 165.20 (COO); UV [Etanol, λ_{max}, nm (ε, $L.mol^{-1}.cm^{-1}$)]: 307 (31.729), 234 (35.965), 231 (35.400), 227 (34.294), 214 (28.659); ESI–MS m/z: 496(M+1+23), 495(M+23), 490, 474(M+2), 473(M+1, 100), 457, 443, 430, 396, 395, 386, 363, 343, 331, 317, 291, 275, 263, 243, 242(65), 239, 191, 153.

2-Methoxy-4-[(3-p-chlorobenzyl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl)azomethine]-phenyl 2-methylbenzoate (4g)

White solid; yield: 99%; m.p. 172°C; IR (cm^{-1}) υ_{max}: 3171 (NH), 1731, 1698 (C=O), 1601 (C=N), 1273 (COO), 828 (1,4-disubstituted benzenoid ring), 760 (1,2-disubstituted benzenoid ring); ^{1}H-NMR (200 MHz, DMSO-d_6) (ppm) δ H: 2.60 (s, 3H, $PhCH_3$), 3.87 (s, 3H, OCH_3), 4.07 (s, 2H, CH_2Ph), 7.30 (d, 1H, ArH; *J* = 8.48 Hz), 7.34-7.36 (m, 4H, ArH), 7.41-7.45 (m, 2H, ArH), 7.58-7.60 (m, 1H, ArH), 7.70-7.73 (m, 2H, ArH), 8.09 (d, 1H, ArH; *J* = 7.28 Hz), 9.63 (s, 1H, N=CH), 11.96 (s, 1H, NH); ^{13}C NMR (50 MHz, DMSO-d_6) (ppm) δ C: 21.07 ($PhCH_3$), 30.41 (CH_2Ph), 56.21 (OCH_3), [112.98, 121.25, 126.24, 126.36, 128.26 (2C), 131.33, 131.84, 133.00, 139.72, 139.99, 152.55] (Ar-C), [128.07, 130.72 (2C), 130.75 (2C), 134.80] (Ar-C linked C-3), 145.86 (Triazol-C_3), 151.22 (Triazol-C_5), 153.57 (N=CH), 164.68 (COO); UV [Etanol, λ_{max}, nm (ε, $L.mol^{-1}.cm^{-1}$)]: 308 (19.660), 290 (18.284), 229 (26.266), 226 (26.339), 221 (25.045), 214 (22.339); ESI–MS m/z: 499, 477(M+2), 475(M), 443, 408, 391, 357, 333, 311, 299, 283, 244, 243, 242(100), 222, 207, 192, 168.

2-Methoxy-4-[(3-m-chlorobenzyl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl)azomethine]-phenyl 2-methylbenzoate (4h)

White solid; yield: 94%; m.p. 231°C; IR (cm^{-1}) υ_{max}: 3157 (NH), 1737, 1700 (C=O), 1598 (C=N), 1274 (COO), 793 and 685 (1,3-disubstituted benzenoid ring), 759 (1,2-disubstituted benzenoid ring); ^{1}H-NMR (200 MHz, DMSO-d_6) (ppm) δ H: 2.60 (s, 3H, $PhCH_3$), 3.87 (s, 3H, OCH_3), 4.08 (s, 2H, CH_2Ph), 7.26-7.34 (m, 4H, ArH), 7.41-7.45 (m, 3H, ArH), 7.60 (t, 1H, ArH; *J* = 7.40 Hz), 7.71-7.72 (m, 2H, ArH), 8.09 (d, 1H, ArH; *J* = 7.72 Hz), 9.64 (s, 1H, N=CH), 11.97 (s, 1H, NH); ^{13}C NMR (50 MHz, DMSO-d_6) (ppm) δ C: 21.09 ($PhCH_3$), 30.72 (CH_2Ph), 56.22 (OCH_3), [112.93, 120.96, 126.36, 126.66, 128.32(2C), 131.84, 132.85, 133.00, 138.21, 139.79, 152.55] (Ar-C), [126.23, 127.52, 128.05, 129.04, 130.17, 130.72] (Ar-C linked C-3), 145.69 (Triazol-C_3), 151.21 (Triazol-C_5), 153.60 (N=CH), 164.63 (COO); UV [Etanol, λ_{max}, nm (ε, $L.mol^{-1}.cm^{-1}$)]: 309 (21.663), 290 (18.940), 230 (26.000), 225 (26.189), 212 (22.762); ESI–MS m/z: 499, 477(M+2), 443, 409, 381, 353, 325, 311, 293, 283, 260, 251, 243, 242(100), 232, 223, 207, 195, 165.

2-Methoxy-4-[(3-phenyl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl)azomethine]-phenyl 2-methylbenzoate (4i)

White solid; yield: 96%; m.p. 197°C; IR (cm^{-1}) υ_{max}: 3152 (NH), 1730, 1707 (C=O), 1608 (C=N), 1277 (COO), 765 (1,2-disubstituted benzenoid ring), 765 and 686 (monosubstituted benzenoid ring); ^{1}H-NMR (200 MHz, DMSO-d_6) (ppm) δ H: 2.59 (s, 3H, $PhCH_3$), 3.88 (s, 3H, OCH_3), 7.34 (d, 1H, ArH; *J* = 8.40 Hz), 7.42-7.44 (m, 2H, ArH), 7.52-7.53 (m, 3H, ArH), 7.57-7.62 (m, 1H, ArH), 7.71-7.72 (m, 1H, ArH), 7.78-7.80 (m, 1H, ArH), 7.89-7.92 (m, 2H, ArH), 8.07 (d, 1H, ArH; *J* = 7.80 Hz), 9.58 (s, 1H, N=CH), 12.36 (s, 1H, NH); ^{13}C NMR (50 MHz, DMSO-d_6) (ppm) δ C: 21.52 ($PhCH_3$), 56.75 (OCH_3), [113.68, 122.37, 126.62, 126.72, 128.57 (2C), 131.19, 132.31, 133.47, 140.20, 140.45, 154.26] (Ar-C), [127.16, 128.36(2C), 128.99 (2C), 130.55] (Ar-C linked C-3), 145.00 (Triazol-C_3), 151.86 (Triazol-C_5), 156.71 (N=CH), 165.16 (COO); UV [Etanol, λ_{max}, nm (ε, $L.mol^{-1}.cm^{-1}$)]: 308 (19.911), 280 (21.627), 233 (29.941), 222 (23.137); ESI–MS m/z: 487, 461, 451(M+23), 429(M+1), 402, 391, 368, 354, 311, 299, 283, 244, 243, 242(100), 222, 207, 192, 168.

General procedure for the synthesis of 2-methoxy-4-[(1-acetyl-3-alkyl/aryl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl)azomethine]phenyl 2-methylbenzoates (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**.

2-Methoxy-4-[(1-acetyl-3-methyl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl)azomethine]-phenyl 2-methylbenzoate (5a)

White solid; yield: 66%; m.p. 176°C; IR (cm^{-1}) υ_{max}: 1760, 1740, 1691 (C=O), 1608 (C=N), 1271 (COO), 729 (1,2-disubstituted benzenoid ring); ^{1}H-NMR (200 MHz, DMSO-d_6) (ppm) δ H: 2.35 (s, 3H, CH_3), 2.51 (s, 3H, CO$\underline{CH_3}$), 2.59 (s, 3H, PhCH_3), 3.89 (s, 3H, OCH_3), 7.34 (d, 1H, ArH; *J* = 8.32 Hz), 7.40-7.44 (m, 2H, ArH), 7.60 (t, 1H, ArH; *J* = 7.56 Hz), 7.80-7.83 (m, 2H, ArH), 8.09 (d, 1H, ArH; *J* = 7.80 Hz), 9.55 (s, 1H, N=CH); ^{13}C NMR (50 MHz, DMSO-d_6) (ppm) δ C: 11.73 (CH_3), 21.58 (PhCH_3), 23.93 (CO$\underline{CH_3}$), 56.76 (OCH_3), [113.51, 121.91, 126.34, 126.73, 128.46, 129.12, 131.26, 132.35, 133.53, 140.29, 140.55, 154.47] (Ar-C); 147.21 (Triazol-C_3), 148.41 (Triazol-C_5), 155.56 (N=CH), 165.12 (COO), 166.50 ($\underline{CO}CH_3$); UV [Etanol, λ_{max}, nm (ε, L.mol^{-1}.cm^{-1})]: 307 (23.091), 291 (25.403), 232 (30.673), 227 (29.142), 214 (24.530); ESI–MS m/z: 485, 483, 482, 473, 454, 432(M+1+23), 431(M+23), 427, 426, 410, 409(M+1), 408(M), 367, 344, 332, 306, 271, 258, 243, 242(100), 230, 216, 203, 190, 154, 153.

2-Methoxy-4-[(1-acetyl-3-ethyl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl)azomethine]-phenyl 2-methylbenzoate (5b)

White solid; yield: 66%; m.p. 189°C; IR (cm^{-1}) υ_{max}: 1767, 1734, 1699 (C=O), 1608 (C=N), 1267 (COO), 735 (1,2-disubstituted benzenoid ring); ^{1}H-NMR (200 MHz, DMSO-d_6) (ppm) δ H: 1.24 (t, 3H, CH_2CH_3; *J* = 7.44 Hz), 2.51 (s, 3H, $COCH_3$), 2.59 (s, 3H, PhCH_3), 2.76 (q, 2H, CH_2CH_3; *J* = 7.52 Hz), 3.89 (s, 3H, OCH_3), 7.35 (d, 1H, ArH; *J* = 9.08 Hz), 7.40-7.44 (m, 2H, ArH), 7.60 (t, 1H, ArH; *J* = 7.56 Hz), 7.80-7.82 (m, 2H, ArH), 8.09 (d, 1H, ArH; *J* = 7.96 Hz), 9.54 (s, 1H, N=CH); ^{13}C NMR (50 MHz, DMSO-d_6) (ppm) δ C: 9.92 ($CH_2\underline{CH_3}$), 19.04 ($\underline{CH_2}CH_3$), δ 21.58 (PhCH_3), 23.93 (CO$\underline{CH_3}$), 56.76 (OCH_3), [113.51, 121.95, 126.36, 126.75, 128.46, 129.05, 131.27, 132.36, 133.55, 140.31, 140.55, 154.48] (Ar-C), 148.66 (Triazol-C_3), 150.71 (Triazol-C_5), 155.76 (N=CH), 165.10 (COO), 166.40 ($\underline{CO}CH_3$); UV [Etanol, λ_{max}, nm (ε, L.mol^{-1}.cm^{-1})]: 307 (23.798), 232 (27.307), 230 (27.250), 222 (25.328); ESI–MS m/z: 487, 486, 468, 461, 445(M+23), 441, 440, 424, 423(M+1), 422(M), 390, 381, 362, 335, 313, 288, 263, 244, 243, 242(100), 230, 153.

2-Methoxy-4-[(1-acetyl-3-n-propyl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl)azomethine]-phenyl 2-methylbenzoate (5c)

White solid; yield: 66%; m.p. 125°C; IR (cm^{-1}) υ_{max}: 1762, 1736, 1692 (C=O), 1609 (C=N), 1242 (COO), 733 (1,2-disubstituted benzenoid ring); ^{1}H-NMR (200 MHz, DMSO-d_6) (ppm) δ H: 0.98 (t, 3H, $CH_2CH_2CH_3$; *J* = 7.36 Hz), 1.73 (sext, 2H, $CH_2\underline{CH_2}CH_3$; *J* = 7.40 Hz), 2.51 (s, 3H, $COCH_3$), 2.59 (s, 3H, PhCH_3), 2.72 (t, 2H, $\underline{CH_2}CH_2CH_3$; *J* = 7.40 Hz), 3.89 (s, 3H, OCH_3), 7.34 (d, 1H, ArH; *J* = 8.88 Hz), 7.40-7.45 (m, 2H, ArH), 7.60 (t, 1H, ArH; *J* = 7.52 Hz), 7.79-7.82 (m, 2H, ArH), 8.09 (d, 1H, ArH; *J* = 7.76 Hz), 9.53 (s, 1H, N=CH); ^{13}C NMR (50 MHz, DMSO-d_6) (ppm) δ C: 13.40 ($CH_2CH_2\underline{CH_3}$), 18.34 ($CH_2\underline{CH_2}CH_3$), 21.06 (PhCH_3), 23.43 (CO$\underline{CH_3}$), 26.59 ($\underline{CH_2}CH_2CH_3$), 56.26 (OCH_3), [113.07, 121.49, 125.84, 126.22, 127.97, 128.40, 130.74, 131.83, 133.01, 139.80, 140.05, 153.97] (Ar-C), 148.07 (Triazol-C_3), 149.04 (Triazol-C_5), 155.24 (N=CH), 164.61 (COO), 165.96 ($\underline{CO}CH_3$); UV [Etanol, λ_{max}, nm (ε, L.mol^{-1}.cm^{-1})]: 307 (24.574), 232 (27.960), 228 (27.614), 212 (22.594); ESI–MS m/z: 500, 482, 459(M+23), 455, 454, 438, 437(M+1), 436(M), 396, 395, 386, 366, 334, 320, 292, 263, 244, 243, 242(100), 230, 216, 183, 153.

2-Methoxy-4-[(1-acetyl-3-benzyl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl)azomethine]-phenyl 2-methylbenzoate (5d)

White solid; yield: 68%; m.p. 207°C; IR (cm^{-1}) υ_{max}: 1762, 1732, 1693 (C=O), 1603 (C=N), 1244 (COO), 729 (1,2-disubstituted benzenoid ring), 765 and 702 (monosubstituted benzenoid ring); ^{1}H-NMR (200 MHz,

DMSO-d_6) (ppm) δ H: 2.51 (s, 3H, $COCH_3$), 2.60 (s, 3H, $PhCH_3$), 3.88 (s, 3H, OCH_3), 4.15 (s, 2H, CH_2Ph), 7.24-7.45 (m, 8H, ArH), 7.60-7.62 (m, 1H, ArH), 7.74-7.77 (m, 2H, ArH), 8.08 (d, 1H, ArH; *J* = 7.28 Hz), 9.51 (s, 1H, N=CH); ^{13}C NMR (50 MHz, DMSO-d_6) (ppm) δ C: 21.07 ($PhCH_3$), 23.52 ($CO\underline{CH_3}$), 31.04 (CH_2Ph), 56.27 (OCH_3), [113.05, 121.59, 125.85, 126.28, 126.89, 128.41, 130.70, 131.87, 133.05, 139.72, 139.99, 153.96] (Ar-C), [128.02, 128.41 (2C), 129,01 (2C), 134.72] (Ar-C linked C-3), 148.31 (Triazol-C_3), 150.20 (Triazol-C_5), 154.57 (N=CH), 164.67 (COO), 166.50 ($\underline{CO}CH_3$); UV [Etanol, λ_{max}, nm (ε, L.mol^{-1}.cm^{-1})]: 308 (11.666), 225 (15.980), 217 (15.949), 212 (16.929); ESI–MS m/z: 487, 486(M+1), 485(M), 464, 443, 427, 399, 371, 362, 343, 319, 285, 263, 244, 243, 242(100), 230, 205, 154, 153.

2-Methoxy-4-[(1-acetyl-3-p-methylbenzyl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl)azo-methine]-phenyl 2-methylbenzoate (5e)

White solid; yield: 66%; m.p. 206°C; IR (cm^{-1}) υ_{max}: 1763, 1735, 1694 (C=O), 1604 (C=N), 1248 (COO), 832 (1,4-disubstituted benzenoid ring), 760 (1,2-disubstituted benzenoid ring); ^{1}H-NMR (200 MHz, DMSO-d_6) (ppm) δ H: 2.22 (s, 3H, *p*-$PhCH_3$), 2.51 (s, 3H, $COCH_3$), 2.61 (s, 3H, *o*-$PhCH_3$), 3.88 (s, 3H, OCH_3), 4.09 (s, 2H, CH_2Ph), 7.09 (d, 2H, ArH), 7.24 (d, 2H, ArH), 7.33 (d, 1H, ArH), 7.44-7.46 (m, 2H, ArH), 7.61 (m, 1H, ArH), 7.72-7.78 (m, 2H, ArH), 8.09 (d, 1H, ArH), 9.50 (s, 1H, N=CH); ^{13}C NMR (50 MHz, DMSO-d_6) (ppm) δ C: 21.05 ($PhCH_3$-*o*), 21.58 ($PhCH_3$-*p*), 24.02 ($CO\underline{CH_3}$), 31.19 (CH_2 Ph), 56.77 (OCH_3), [113.54, 122.03, 126.38, 126.78, 128.50, 129.01, 131.23, 132.38, 133.57, 140.23, 140.52, 154.45] (Ar-C), [129.38 (2C), 129,47 (2C), 132.07, 136.47] (Ar-C linked C-3), 148.56 (Triazol-C_3), 148.94 (Triazol-C_5), 155.01 (N=CH), 165.16 (COO), 166.50 ($\underline{CO}CH_3$); UV [Etanol, λ_{max}, nm (ε, L.mol^{-1}.cm^{-1})]: 308 (25.607), 228 (33.312), 225 (33.900), 215 (29.750), 211 (27.538); ESI–MS m/z: 537, 521(M+23), 517, 516(100), 500, 499(M+1), 489, 458, 457, 434, 408, 386, 351, 324, 306, 277, 243, 242, 213, 172, 153.

2-Methoxy-4-[(1-acetyl-3-p-chlorobenzyl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl)azo-methine]-phenyl 2-methylbenzoate (5g)

White solid; yield: 82%; m.p. 216°C; IR (cm^{-1}) υ_{max}: 1763, 1731, 1694 (C=O), 1603 (C=N), 1246 (COO), 832 (1,4-disubstituted benzenoid ring), 728 (1,2-disubstituted benzenoid ring); ^{1}H-NMR (200 MHz, DMSO-d_6) (ppm) δ H: δ 2.51 (s, 3H, $COCH_3$), 2.61 (s, 3H, $PhCH_3$), 3.88 (s, 3H, OCH_3), 4.16 (s, 3H, CH_2Ph), 7.32-7.46 (m, 7H, ArH), 7.61 (t, 1H, ArH), 7.74-7.77 (d, 2H, ArH), 8.01 (d, 1H, ArH), 9.51 (s, 1H, N=CH); ^{13}C NMR (50 MHz, DMSO-d_6) (ppm) δ C: 21.58 ($PhCH_3$), 24.00 ($CO\underline{CH_3}$), 30.86 (CH_2Ph), 56.78 (OCH_3), [113.55, 122.03, 124.00, 126.32, 126.77, 128.82, 131,23, 132.09, 133.56, 140.20, 140.60, 153.00] (Ar-C), [129.05 (2C), 131.47 (2C), 132.37, 134.23] (Ar-C linked C-3), 147.20 (Triazol-C_3), 148.50 (Triazol-C_5), 154.48 (N=CH), 164.50 (COO), 166.50 ($\underline{CO}CH_3$); UV [Etanol, λ_{max}, nm (ε, L.mol^{-1}.cm^{-1})]: 308 (21.700), 232 (27.900), 228 (28.730), 219 (25.960); ESI–MS m/z: 582, 564, 557, 538, 536, 521, 520, 519(M+2), 518, 477, 443, 424, 390, 371, 326, 314, 287, 244, 243, 242(100), 230, 190.

Biological protocols

Antioxidant activity: Chemicals

Butylated hydroxytoluene (BHT) was obtained 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), ethylenediaminetetraacetic acid (EDTA) and trichloroacetic acid (TCA) were obtained from Sigma–Aldrich.

Reducing power

The reducing power of the synthesized compounds was determined according to the method of Oyaizu [13]. 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 [14]. 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. [15]. Shortly, the synthesized compounds (50-250 µg mL^{-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.

Antimicrobial activity

All bacterial and yeast strains were obtained from the company of Microbiological Environmental Protection Laboratories (France) and were as follows: *Bacillus Substilis* (ATCC 11774)*, Bacillus Cereus* (ATCC 11778)*, Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 25922)*, Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumonia* (ATCC 4352). Simple susceptibility screening test using agar well diffusion method was used [16,17]. All the newly synthesized compounds were weighed and dissolved in dimethylsulphoxide (DMSO) to prepare extract stock solution of 1 mg/ml.

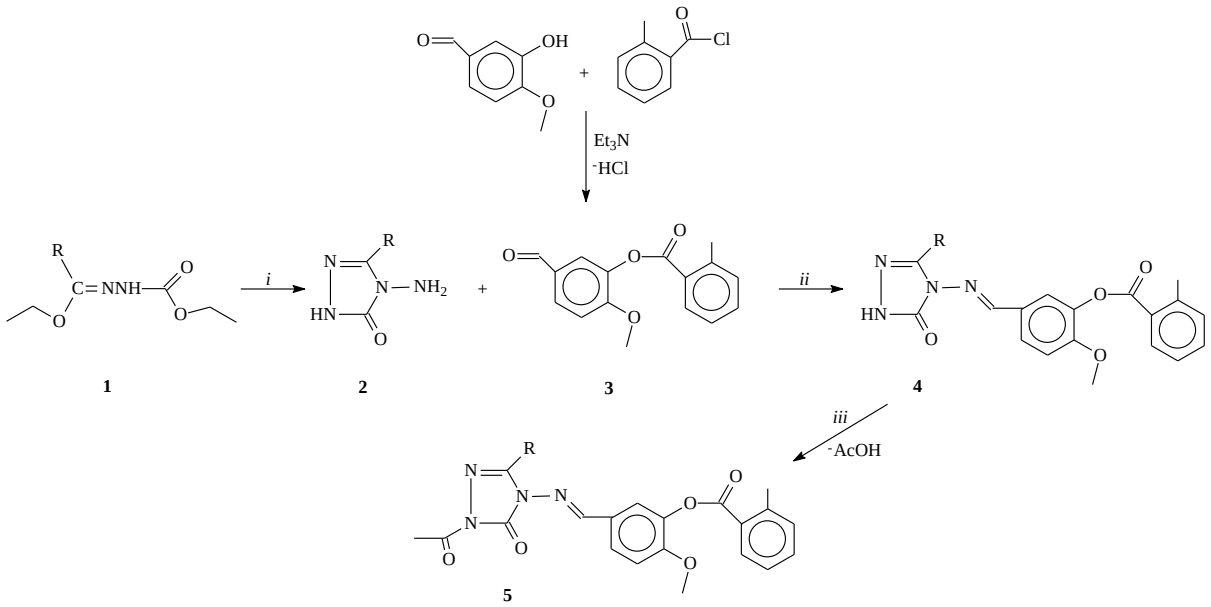
Each microorganism was suspended in Mueller-Hinton Broth and diluted to 106 colony forming unit (cfu) per ml. They were “flood-inoculated” onto the surface of Mueller Hinton Agar and then dried. Five-millimeter diameter wells were cut from the agar using a sterile cork-borer, and 250–5000 µg/50 µl of 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. Ampicillin, neomycin and streptomycin were standard antibacterial and antifungal agents, DMSO was used as solved control.

RESULTS AND DISCUSSION

Chemistry

The 2-methoxy-4-[(3-alkyl/aryl-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl)azomethine]-phenyl 2-methylbenzoates **4a-i** were obtained from the reactions of compounds 3-alkyl(aryl)-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones **2a-i** with 3-(2-methylbenzoxy)-4-methoxybenzaldehyde **3** which were synthesized by the reactions of 3-hydroxy-4-methoxybenzaldehyde with 2-methylbenzoyl chloride by using triethylamine. Then, the reactions of compounds **4a-e** and **4g** with acetic anhydride were investigated, and compounds **5a-e** and **5g** were prepared (Scheme 1).

ISSN: 0975-8585



i) $N_2H_4.H_2O$, reflux, 6 h; *ii*) AcOH, reflux, 1 h; *iii*) 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 = $CH_2C_6H_4OCH_3$ (*p*-), g) R = $CH_2C_6H_4Cl$ (*p*-), h) R = $CH_2C_6H_4Cl$ (*m*-), i) R = C_6H_5

Scheme 1: Synthetic route of compounds 3, 4 and 5

The structures of nine new 2-methoxy-4-[(3-alkyl/aryl-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl)azomethine]phenyl 2-methylbenzoates **4a-i** compounds, and five new 2-methoxy-4-[(1-acetyl-3-alkyl/aryl-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl)azomethine]-phenyl 2-methyl-benzoates **5a-5e** and **5g** compounds were identified by using IR, ^{1}H NMR, ^{13}C NMR, UV and mass spectral data.

Antioxidant activity

The antioxidant activities of fifteen new compounds **4a-i**, **5a-5e** and **5g** 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

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 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 [18]. 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 [19]. In the study, examined compounds did not show the reductive activities. In other words, all the amount of the compounds showed lower absorbance than standard antioxidants such as BHT, BHA and α-tocopherol. Hence, no activities were observed to reduce metal ions complexes to their lower oxidation state or to take part in any electron transfer reaction.

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 [20]. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [21]. The

reduction capability of DPPH radicals was determined by decrease in its absorbance at 517 nm induced by antioxidants. The absorption maximum of a stable DPPH radical in ethanol was at 517 nm. The decrease in absorbance of DPPH radical was caused by antioxidants because of 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, $DPPH^{\cdot}$ is usually used as a substrate to evaluate antioxidative activity of antioxidants [22]. Antiradical activities of compounds and standard antioxidants such as BHT, BHA and α-tocopherol were determined by using $DPPH^{\cdot}$ method. The newly synthesized compounds did not show any scavenging effect.

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 [23]. 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 [24] 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 [25]. 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:

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

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 [26]. Ferrous ion chelating activities of the compounds **4**, **5**, BHT, BHA and α-tocopherol are respectively shown in Figures **1** and **2**.

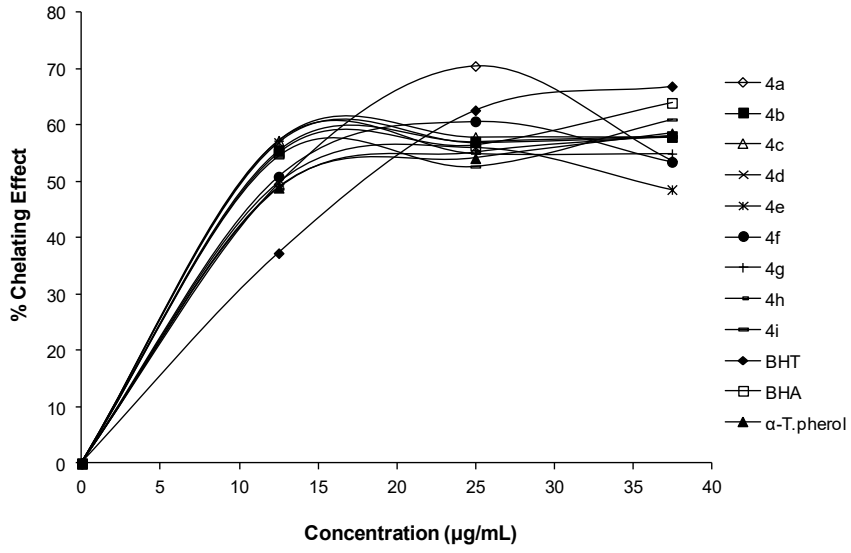


Figure 1: Metal chelating effect of different amount of the compounds 4, BHT, BHA and α-tocopherol on ferrous ions.

ISSN: 0975-8585

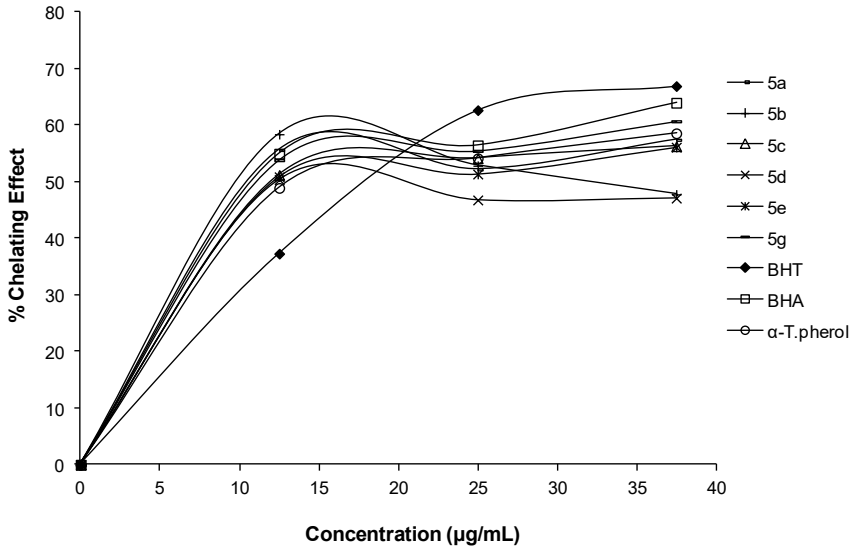


Figure 2: Metal chelating effect of different amount of the compounds 5, BHT, BHA and α-tocopherol on ferrous ions.

In this study, metal chelating capacity was significant since it reduced the concentrations of the catalyzing transition metal. It was reported that chelating agents that form σ-bonds with a metal are effective as secondary antioxidants because they reduce the redox potential thereby stabilizing the oxidized form of metal ion [27]. The data obtained from Figures **1** and **2** reveal that the metal chelating effects of the compounds **4b-d**, **4h**, **5c** and **5g** were concentration-dependent, the other compounds were not. Thus, the compounds **4b-d**, **4h**, **5c** and **5g** demonstrate a marked capacity for iron binding. The metal chelating effect of these compounds and references decreased in order of BHT > BHA > 5g > α-tocopherol > 4b ≈ 4h > 4c ≈ 4d > 5c, which were 66.9, 64.0, 60.7, 58.6, 58.1, 57.9, 56.3 (%), at the highest concentration, respectively.

Antimicrobial activity

The microbiological results are summarized in Table I. Microbiology results are not promising; only compounds **4d** and **4g** showed moderate antimicrobial activity against to all of tested microorganisms, except *Escherichia coli*. The data also reveal that, the highest zone diameter was obtained from compound **4d** against *Staphylococcus aureus*. The other active compounds are **4b** (against to *Bacillus cereus* and *Staphylococcus aureus*); **5a**, **5c**, **5e** and **5g** (against to *Bacillus subtilis*).

Table I: Antimicrobial activity of the compounds 4 and 5.

Compounds	Microorganisms and inhibition zone (mm)					
	Bs	Bc	Pa	Kp	Sa	Ec
4a	-	-	-	-	-	-
4b	-	9	-	-	9	-
4c	-	-	-	-	-	-
4d	14	15	14	15	**17**	-
4e	-	-	-	-	-	-
4f	-	-	-	-	-	-
4g	12	13	9	12	13	-

4h	-	-	-	-	-	-
4i	-	-	-	-	-	-
5a	11	-	-	-	-	-
5b	-	-	-	-	-	-
5c	15	-	-	-	-	-
5d	-	-	-	-	-	-
5e	13	-	-	-	-	-
5g	13	-	-	-	-	-
Ampicillin	33	36	36	35	37	34
Neomycin	17	17	17	16	13	16
Streptomycin	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 (X3261), Neo.: Neomycin (X3360), Str.: Streptomycin (X3385).

The compounds **4a**, **4c**, **4e**, **4f**, **4h**, **4i**, **5b** and **5d** did not display any antimicrobial activity against to all of tested microorganisms.

CONCLUSION

In the present study, new 1,2,4-triazole derivatives (**4a-i, 5a-e** and **5g**) were designed and synthesized. Their structures were identified using IR, ^{1}H NMR, ^{13}C NMR, UV and MS spectral data. The entire target compounds were also investigated for their antioxidant and antimicrobial potential. Considering both the antimicrobial and the antioxidant evaluation, compound **4d** exhibited the moderate effect. The promising *in vitro* antioxidant and antimicrobial activity of the compound has encouraged us to further study of novel agents.

ACKNOWLEDGEMENTS

The authors thank F. Aytemiz for antimicrobial activities.

REFERENCES

[1] Oxidants, Antioxidants and Free Radicals, edited by Baskin S, Salem H. CRC Press, 1997, p. 228.
[2] Hussain HH, Babic G, Durst T, Wright J, Flueraru M, Chichirau A, Chepelev LLJ. Org Chem 2003; 68: 7023-7032.
[3] Antimicrobials: New and Old Molecules in the Fight Against Multi-Resistant Bacteria, edited by Marinelli F, Genilloud O. Springer-Verlag, Berlin, 2014, p.142.
[4] Sahu JK, Ganguly S, Kaushik A. Chin J Nat Med 2013; 11: 456-465.
[5] Singh RJ, Singh DK. Asian J Chem 2010; 22: 2664-2668.
[6] Yüksek H, Akyıldırım O, Yola ML, Gürsoy-Kol Ö, Çelebier M, Kart D. Arch Pharm Chem Life Sci 2013; 346: 470-480.
[7] Kaczor AA, Pitucha M, Karczmarzyk Z, Wysocki W, Rzymowska J, Matosiuk D. Med Chem. 2013; 9: 313-328.
[8] Aktaş-Yokuş Ö, Yüksek H, Gürsoy-Kol Ö, Alpay-Karaoğlu Ş. Med Chem Res 2015; 24: 2813-2824.
[9] Thakkar SS, Thakor P, Doshi H, Ray A. Bioorg Med Chem 2017; 25: 4064-4075.

[10] Aktaş-Yokuş Ö, Yüksek H, Manap S, Aytemiz F, Alkan M, Beytur M, Gürsoy-Kol Ö. Bulg Chem Commun 2017; 49(S1): 98-106.
[11] Khalid W, Badshah A, Khan A, Nadeem H, Ahmed S. Chem Cent J 2018; 12: 1-16.
[12] Abuelhassan AH, Badran MM, Hassan HA, Abdelhamed D, Elnabtity S, Aly OM. Med Chem Res 2018; 27: 928-938.
[13] Oyaizu M. Jpn J Nutr 1986; 44: 307-315.
[14] Blois MS. Nature 1958; 181: 1199-1200.
[15] Dinis TCP, Madeira VMC, Almeida LM. Arch Biochem Biophys 1994; 315: 161-169.
[16] Perez C, Pauli M, Bazerque P. Acta Biol Med Exp 1990; 15: 113-115.
[17] Ahmad I, Mehmood Z, Mohammed F. J Ethnopharmacol 1998; 62: 183-193.
[18] Meir S, Kanner J, Akiri B, Philosophhadas S. J Agr Food Chem 1995; 43: 1813-1819.
[19] Yildirim A, Mavi A, Kara AA. J Agr Food Chem 2001; 49: 4083-4089.
[20] Baumann J, Wurn G, Bruchlausen V. N-S Arch Pharmacol 1979; 308: R27.
[21] Soares JR, Dinis TCP, Cunha AP, Almeida LM. Free Radical Res 1997; 26: 469-478.
[22] Duh PD, Tu YY, Yen GC. Food Sci Technol-Leb 1999; 32: 269-277.
[23] Yamaguchi F, Ariga T, Yoshimura Y, Nakazawa H. J Agr Food Chem 2000; 48: 180-185.
[24] Strlic M, Radovic T, Kolar J, Pihlar B. J Agr Food Chem 2002; 50: 6313-6317.
[25] Finefrock AE, Bush AI, Doraiswamy PM. J Am Geriatr Soc 2003; 51: 1143-1148.
[26] Calis I, Hosny M, Khalifa T, Nishibe S. Phytochemistry 1993; 33: 1453-1456.
[27] Gordon MH. Food Antioxidants. Elsevier, London, 1990.