ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Synthesis, Characterization, Antioxidant and Antimicrobial Activities and DNA Damage of Some Novel 2-[3-alkyl (aryl)-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl]-phenoxyacetic acids in Human Lymphocytes.

Esra Çiftçi[1], Murat Beytur[2]*, Mustafa Calapoğlu[1], Özlem Gürsoy-Kol[2], Muzaffer Alkan[3], Vehbi Atahan Toğay[4], Sevda Manap[2], and Haydar Yüksek[2].

[1] Süleyman Demirel University, Faculty of Arts and Sciences, Department of Chemistry, 32000 Isparta, Turkey
[2] Kafkas University, Faculty of Arts and Sciences, Department of Chemistry, 36100 Kars, Turkey
[3] Kafkas University, Faculty of Education, Primary-Science Education, 36100 Kars, Turkey
[4] Süleyman Demirel University, Faculty of Medicine, Department of Medicinal Biology, 32000 Isparta, Turkey

ABSTRACT

In this study, nine novel 2-[3-alkyl(aryl)-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl] phenoxyacetic acids (**2**) were obtained by the reactions of 3-alkyl-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones with 2-formylphenoxy acetic acid. Their structures were characterized by using ^{1}H-NMR, ^{13}C-NMR and IR spectral data together with elemental analysis. The synthesized novel compounds were analyzed for their in vitro antioxidant activity methods such as reducing power, free radical scavenging and metal chelating activity. Besides, agar hole methods were used to investigate the antibacterial properties of the **2** type compounds against the strains of *B. subtilis*, B. *cereus*, *P. aeruginosa, K. Pneumoniae*, *S. aureus*, and *E. coli*. Finally, the alkaline comet assay was used to investigate genotoxicity potential of the synthesized compounds in the peripheral blood lymphocytes (PBLs).
Keywords: 1,2,4-triazol-5-one, Antioxidant, Antibacterial, Comet assay.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

1,2,4-Triazole nucleus and its derivatives a considerable component of heterocyclic structures which demonstrate diverse biological activities such as antifungal [1], antimicrobial [2, 3], antioxidant [4, 5], antiviral [6], anti-inflammatory [7], anticancer [8-10], anticonvulsant activities [11] and antibacterial [12, 13].

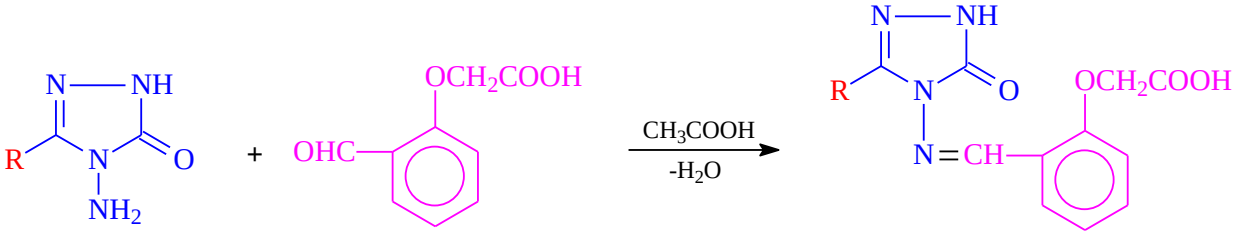
Several compounds possessing 1,2,4-triazole nucleus are clinically used drugs such as fluconazole, itraconazole, terconazole [14, 15], etizolam [16] and furacylin [17]. The numerous researches are reported that reporting the method of synthesizing Schiff bases and different biological activities this compounds such as antimicrobial, antitumor and antioxidant. The results of the previous studies indicated that the Schiff bases possess equable to noteworthy activities against diverse fungal strains and bacterial, which might be owing to azomethine linkage present in such compounds [18-23].

Antioxidants have the capacity to protect organisms and cells from damage induced by oxidative stress; therefore, considerable research has been conducted to examine this feature. Natural sources that provide the active components for preventing or reducing the impact of oxidative stress on cells have been used [24].

Exogenous chemicals and endogenous metabolic processes in the human body or in food systems might produce highly reactive free radicals, especially oxygen, which are capable of oxidizing biomolecules that can result in cell death and tissue damage. Oxidative damage has a pathological role in serious human diseases (e.g., emphysema, cirrhosis, atherosclerosis and arthritis). Furthermore, a variety of pathophysiological processes, such as inflammation, diabetes, genotoxicity and cancer, stem from the excessive generation of reactive oxygen species (ROS) induced by various stimuli that exceed the antioxidant capacity of the organism [25]. Even several natural sources of these active components have been used to synthesize and obtain effective new antioxidative compounds. It is well-known that 1,2,4-triazole derivatives present an antitumor activity on many cancer types, such as leukemia, non-small cell lung, colon, melanoma, ovarian, renal, prostate, and breast cancers. Several in vitro studies were performed for this purpose in recent years [26-28].

A broad range of *in-vivo* and *in-vitro* detection of deoxyribonucleic acid (DNA) damage at the level of an individual eukaryotic cell have been used for genotoxicity assessment of chemicals. Single cell gel electrophoresis (SCGE) or the comet assay method is extremely sensitive for evaluation DNA damage formation and repair at the level of the individual eukaryotic cell. Comet assay determines the percentage of DNA breaks and repair them [29]. The comet results could provide useful data concerning the genotoxic potential of the triazole derivative compounds.

In the present study, the newly synthesized 1,2,4-triazole derivatives (Scheme 1) were investigated by using diverse antioxidant methods such as: Free radical scavenging, metal chelating activities and reducing power. In addition, the alkaline comet assay method was used to investigate genotoxicity potential of the synthesized novel compounds in the peripheral blood lymphocytes (PBLs). Structures of all the synthesized compounds were elucidated with ^{1}H-NMR, ^{13}C-NMR, IR spectral methods and elemental analysis.



1 **2**

Scheme 1 Synthesis route of compounds 1,2(a-i) 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

ISSN: 0975-8585

MATERIAL AND METHODS

Chemical reagents and apparatus

Chemical reagents used in the study were supplied from Sigma (Sigma-Aldrich GmbH, Germany), Fluka (Switzerland) and Merck AG, (Germany). The starting compounds (**1**) were obtained from the reactions of the corresponding ester ethoxycarbonylhydrazones with hydrazine hydrate (37 %) as indicated in the literature [30, 31]. 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) at 400 MHz and 100 MHz, respectively. Elemental analyses were carried out on a LECO, CHNS-932 for H, C, and N. The IR spectra were created using Alpha-P Bruker FT-IR spectrometer (Germany).

General procedure for the synthesis of compounds 2

The compounds **1** (0.01 mol) dissolved in acetic acid (15 mL) were treated 2-formylphenoxy acetic acid **2** (0.01 mol). The mixtures were refluxed for 1.5 h and then evaporated at 50-55 °C *in vacuo*. Several recrystallizations of the residue from appropriate solvent gave pure compounds **2a-i** as colorless crystals.

2-(3-Methyl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl)-phenoxyacetic Acid (2a)

Yield 2.61 g (94.63 %). mp. 270 ºC. IR (KBr): 3246-2486 (OH), 3170 (NH), 1706 (C=O), 1597 (C=N), 1255 (COO), 755 (1,2-disubstituted benzenoid ring) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 2.28 (s, 3H, CH_3), 4.86 (s, 2H, OCH_2), 7.04 (d, 1H, ArH; *J*=8.40 Hz), 7.08 (t, 1H, ArH; *J*=7.20 Hz), 7.48 (t, 1H, ArH; *J*=7.20 Hz), 7.94 (d, 1H, ArH; *J*=7.60 Hz), 10.06 (s, 1H, N=CH), 11.81 (s, 1H, NH), 13.15 (s, 1H, COOH). ^{13}C-NMR (DMSO-d_6): δ 11.64 (CH_3), 65.28 (OCH_2), 113.27; 121.73; 122.38; 126.18; 133.26; 157.59 (ArC), 144.85 (Triazole C_3), 149.61 (N=CH), 151.69 (Triazole C_5), 170.39 (COOH). Anal. Calcd. for $C_{12}H_{12}N_4O_4$ (276.25): C: 52.17; H: 4.38; N: 20.28, Found: C: 52.25; H: 4.58; N: 20.12.

2-(3-Ethyl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl)-phenoxyacetic Acid (2b)

Yield 2.79 g (96.08 %). mp. 227 ºC. IR (KBr): 3196-2471 (OH), 3160 (NH), 1712 (C=O), 1599 (C=N), 1258 (COO), 758 (1,2-disubstituted benzenoid ring) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 1.22 (t, 3H, $CH_2\underline{CH_3}$; *J*=7.60 Hz), 2.69 (q, 2H, $\underline{CH_2}CH_3$; *J*=7.60 Hz), 4.86 (s, 2H, OCH_2), 7.04 (d, 1H, ArH; *J*=8.40 Hz), 7.08 (t, 1H, ArH; *J*=8.00 Hz), 7.46-7.50 (m, 1H, ArH), 7.91 (d, 1H, ArH; *J*=8.00 Hz), 10.06 (s, 1H, N=CH), 11.84 (s, 1H, NH), 13.14 (s, 1H, COOH). ^{13}C-NMR (DMSO-d_6): δ 10.57 ($CH_2\underline{C}H_3$), 19.05 ($\underline{C}H_2CH_3$), 65.28 (OCH_2), 113.28; 121.77; 122.41; 126.10; 133.25; 157.59 (ArC), 148.61 (Triazole C_3), 149.59 (N=CH), 151.84 (Triazole C_5), 170.40 (COOH). Anal. Calcd. for $C_{13}H_{14}N_4O_4$ (290.28): C: 53.79; H: 4.86; N: 19.30, Found: C: 55.61; H: 5.19; N: 16.39.

2-(3-n-Propyl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl)-phenoxyacetic Acid (2c)

Yield 2.90 g (95.43 %). mp. 247 ºC. IR (KBr): 3269-2639 (OH), 3172 (NH), 1710 (C=O), 1592 (C=N), 1256 (COO), 759 (1,2-disubstituted benzenoid ring) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 0.96 (t, 3H, $CH_2CH_2\underline{CH_3}$; *J*=7.60 Hz), 1.69 (sext, 2H, $CH_2\underline{CH_2}CH_3$; *J*=7.60 Hz), 2.65 (t, 2H, $\underline{CH_2}CH_2CH_3$; *J*=7.60 Hz), 4.86 (s, 2H, OCH_2), 7.04 (d, 1H, ArH; *J*=8.40 Hz), 7.09 (t, 1H, ArH; *J*=7.60 Hz), 7.48 (t, 1H, ArH; *J*=7.60 Hz), 7.92 (d, 1H, ArH; *J*=7.60 Hz), 10.05 (s, 1H, N=CH), 11.85 (s, 1H, NH), 13.15 (s, 1H, COOH). ^{13}C-NMR (DMSO-d6): δ 13.96 ($CH_2CH_2\underline{C}H_3$), 19.41 ($CH_2\underline{C}H_2CH_3$), 27.24 ($\underline{C}H_2CH_2CH_3$), 65.28 (OCH_2), 113.29; 121.80; 122.41; 126.07; 133.26; 157.60 (ArC), 147.47 (Triazole C_3), 149.60 (N=CH), 151.78 (Triazole C_5), 170.40 (COOH). Anal. Calcd. for $C_{14}H_{16}N_4O_4$ (304.31): C: 55.26; H: 5.30; N: 18.41, Found: C: 55.25; H: 5.56; N: 18.16.

2-(3-Benzyl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl)-phenoxyacetic Acid (2d)

Yield 3.30 g (93.89 %). mp. 274 ºC. IR (KBr): 3279-2509 (OH), 3176 (NH), 1710, 1680 (C=O), 1596 (C=N), 1256 (COO), 817 ve 694 (monosubstituted benzenoid ring), 760 (1,2-disubstituted benzenoid ring) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 4.07 (t, 2H, CH_2Ph), 4.84 (s, 2H, OCH_2), 7.02 (d, 1H, ArH; *J*=8.40 Hz), 7.07 (t, 1H, ArH; *J*=7.60 Hz), 7.21-7.25 (m, 1H, ArH), 7.29-7.33 (m, 4H, ArH), 7.46 (t, 1H, ArH; *J*=7.60 Hz), 7.86 (d, 1H, ArH; *J*=7.60 Hz), 10.03 (s, 1H, N=CH), 11.97 (s, 1H, NH), 13.12 (s, 1H, COOH). ^{13}C-NMR (DMSO-d_6): δ 31.56 (CH_2Ph), 65.25 (OCH_2), 113.27; 121.76; 122.32; 126.12; 127.16; 128.91 (2C); 129.22 (2C); 133.28; 136.34, 157.60 (ArC), 146.77 (Triazole C_3),

149.31 (N=CH), 151.69 (Triazole C_5), 170.37 (COOH). Anal. Calcd. for $C_{18}H_{16}N_4O_4$ (352.35): C: 61.36; H: 4.58; N: 15.90, Found: C: 61.30; H: 4.87; N: 15.85.

2-(3-p-Methyl benzyl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl)-phenoxyacetic Acid (2e)

Yield 3.40 g (92.96 %). mp. 282 ºC. IR (KBr): 3304-2648 (OH), 3181 (NH), 1706, 1681 (C=O), 1595 (C=N), 1242 (COO), 879 (1,4-disubstituted benzenoid ring), 756 (1,2-disubstituted benzenoid ring) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 2.25 (s, 3H, *p*-PhCH_3), 4.01 (s, 2H, CH_2Ph), 4.84 (s, 2H, OCH_2), 7.02 (d, 1H, ArH; *J*=8.40 Hz), 7.08 (t, 1H, ArH; *J*=7.60 Hz), 7.11 (d, 2H, ArH; *J*=7.60 Hz), 7.20 (d, 2H, ArH; *J*=8.00 Hz), 7.47 (t, 1H, ArH; *J*=8.40 Hz), 7.87 (d, 1H, ArH; *J*=7.60 Hz), 10.02 (s, 1H, N=CH), 11.95 (s, 1H, NH), 13.13 (s, 1H, COOH). ^{13}C-NMR (DMSO-d_6): δ 21.08 (*p*-PhCH_3), 31.16 (CH_2Ph), 65.25 (OCH_2), 113.26; 121.76; 122.33; 126.12; 129.09 (2C); 129.48 (2C); 133.22; 133.27; 136.21, 157.60 (ArC), 146.92 (Triazole C_3), 149.26 (N=CH), 151.69 (Triazole C_5), 170.38 (COOH). Anal. Calcd. for $C_{19}H_{18}N_4O_4$ (366.38): C: 62.29; H: 4.95; N: 15.29, Found: C: 61.95; H: 5.12; N: 15.30.

2-(3-p-Methoxybenzyl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl)-phenoxyacetic Acid (2f)

Yield 3.62 g (94.75 %). mp. 254 ºC. IR (KBr): 3254-2511 (OH), 3166 (NH), 1712 (C=O), 1584 (C=N), 1247 (COO), 880 (1,4-disubstituted benzenoid ring), 759 (1,2-disubstituted benzenoid ring) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 3.71 (s, 3H, OCH_3), 3.99 (s, 2H, CH_2Ph), 4.83 (s, 2H, OCH_2), 6.87 (d, 2H, ArH; *J*=8.40 Hz), 7.02 (d, 1H, ArH; *J*=8.80 Hz), 7.08 (t, 1H, ArH; *J*=7.60 Hz), 7.24 (d, 2H, ArH; *J*=8.40 Hz), 7.47 (t, 1H, ArH; *J*=8.40 Hz), 7.89 (d, 1H, ArH; *J*=7.60 Hz), 10.03 (s, 1H, N=CH), 11.94 (s, 1H, NH), 13.11 (s, 1H, COOH). ^{13}C-NMR (DMSO-d_6): δ 30.70 (CH_2Ph), 55.47 (OCH_3), 65.31 (OCH_2), 113.27; 114.33 (2C); 121.75; 122.33; 126.13; 128.09; 130.29 (2C); 133.27; 157.63; 158.52 (ArC), 147.08 (Triazole C_3), 149.30 (N=CH), 151.71 (Triazole C_5), 170.40 (COOH). Anal. Calcd. for $C_{19}H_{18}N_4O_5$ (382.38): C: 59.68; H: 4.74 N; 14.65, Found: C: 58.93; H: 4.99; N: 14.49.

2-(3-p-Chlorobenzyl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl)-phenoxyacetic Acid (2g)

Yield 3.72 g (96.37 %). mp. 260 ºC. IR (KBr): 3277-2483 (NH), 3158 (NH), 1706 (C=O), 1597 (C=N), 1244 (COO), 850 (1,4-disubstituted benzenoid ring), 750 (1,2-disubstituted benzenoid ring) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 4.08 (s, 2H, CH_2Ph), 4.84 (s, 2H, OCH_2), 7.03 (d, 1H, ArH; *J*=8.40 Hz), 7.07 (t, 1H, ArH; *J*=7.60 Hz), 7.34-7.39 (m, 4H, ArH), 7.47 (t, 1H, ArH; *J*=8.40 Hz), 7.85 (d, 1H, ArH; *J*=7.60 Hz), 10.03 (s, 1H, N=CH), 11.99 (s, 1H, NH), 13.13 (s, 1H, COOH). ^{13}C-NMR (DMSO-d_6): δ 30.88 (CH_2Ph), 65.26 (OCH_2), 113.28; 121.77; 122.28; 126.15; 128.85 (2C); 131.17 (2C); 131.86; 133.22; 135.35; 157.62 (ArC), 146.44 (Triazole C_3), 149.42 (N=CH), 151.67 (Triazole C_5), 170.37 (COOH). Anal. Calcd. for $C_{18}H_{15}N_4O_4Cl$ (386.79): C: 55.89; H: 3.91; N: 14.48, Found: C: 56.14; H: 4.18; N: 13.88.

2-(3-m-Chlorobenzyl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl)-phenoxyacetic Acid (2h)

Yield 3.59 g (92.97 %). mp. 260 ºC. IR (KBr): 3218-2509 (OH), 3175 (NH), 1715 (C=O), 1593 (C=N), 1262 (COO), 801 ve 682 (1,3-disubstituted benzenoid ring), 765 (1,2-disubstituted benzenoid ring) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 4.10 (s, 2H, CH_2Ph), 4.83 (s, 2H, OCH_2), 7.02 (d, 1H, ArH; *J*=8.40 Hz), 7.06 (t, 1H, ArH; *J*=7.60 Hz), 7.27-7.29 (m, 3H, ArH), 7.44 (s, 1H, ArH), 7.47 (t, 1H, ArH; *J*=8.40 Hz), 7.85 (d, 1H, ArH), 10.03 (s, 1H, N=CH), 11.10 (s, 1H, NH), 13.13 (s, 1H, COOH). ^{13}C-NMR (DMSO-d_6): δ 31.20 (CH_2Ph), 65.34 (OCH_2), 113.29; 121.69; 122.27; 126.13; 127.20; 128.03; 129.43; 130.73; 133.26; 133.29; 138.72; 157.66 (ArC), 146.27 (Triazole C_3), 149.38 (N=CH), 151.66 (Triazole C_5), 170.39 (COOH). Anal. Calcd. for $C_{18}H_{15}N_4O_4Cl$ (386.79): C: 55.89; H: 3.91; N: 14.48, Found: C: 55.30; H: 4.26; N: 13.92.

2-(3-Phenyl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl)-phenoxyacetic Acid (2i)

Yield 3.28 g (97.14 %). mp. 292 ºC. IR (KBr): 3208-2362 (OH), 3159 (NH), 1708 (C=O), 1597 (C=N), 1229 (COO), 801 ve 678 (monosubstituted benzenoid ring), 758 (1,2-disubstituted benzenoid ring) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 4.88 (s, 2H, OCH_2), 7.06 (d, 1H, ArH; *J*=8.40 Hz), 7.07 (t, 1H, ArH; *J*=8.00 Hz), 7.49 (t, 1H, ArH; *J*=8.00 Hz), 7.52-7.56 (m, 3H, ArH), 7.85 (d, 1H, ArH; *J*=7.60 Hz), 7.89-7.92 (m, 1H, ArH), 10.01 (s, 1H, N=CH), 12.38 (s, 1H, NH), 13.15 (s, 1H, COOH). ^{13}C-NMR (DMSO-d_6): δ 65.36 (OCH_2), 113.43; 121.89; 122.19; 126.30; 127.19; 128.48 (2C); 128.97 (2C); 130.53; 133.55; 157.78 (ArC), 145.16 (Triazole C_3), 151.83 (N=CH), 152.17 (Triazole C_5), 170.38 (COOH). Anal. Calcd. for $C_{17}H_{14}N_4O_4$ (338.32): C: 60.35; H: 4.17; N: 16.56, Found: C: 60.08; H: 4.43; N: 16.41.

Antioxidant activity: Chemicals

Butylated hydroxytoluene (BHT) was obtained from E. Merck (Germany). Ferrous chloride, α-tocopherol, 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 (Germany).

Reducing power

The reducing power of all the title compounds was determined according to the method of Oyaizu [4, 5, 32]. 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 (50–250 µg/mL), 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 [33].

Free radical scavenging activity

Free radical scavenging activity of all the synthesized compounds was measured 1,1-diphenyl-2-picryl-hydrazyl (DPPH), using the method of Blois [4, 5, 34]. 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 [35]. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [36]. The reduction capability of DPPH radicals was determined by decrease in its absorbance at 517 nm induced by antioxidants. The absorption maximum of a stable DPPH radical in ethanol was at 517 nm. 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· is usually used as a substrate to evaluate antioxidative activity of antioxidants [37].

Metal chelating activity

The chelation of ferrous ions by the synthesized compounds and standards were estimated by the method of literature [38] as explained in [4, 5]. 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 [39]. 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 [40] 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 [41]. Also, the production of highly active ROS such as $O_2^{\cdot-}$, H_2O_2 and OH·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 [42]. 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 [43].

It was reported that the compounds with structures containing two or more of the functional groups, such as -OH, -SH, -COOH, $-PO_3H_2$, C=O, $-NR_2$, -S-, -O- in a favorable structure–function configuration should have chelation activity [44-46]. In this respect, L-carnitine may chelate the ferrous ions with hydroxyl and carboxylate groups [46].

Antimicrobial activity

Simple susceptibility screening test using agar-well diffusion method [47] as adapted earlier [48] was used for determination of antimicrobial activities of **2a-i** compounds. All test microorganisms were obtained from the Microbiologics Environmental Protection Laboratories Company in France and are as follows; *B. subtilis* ATCC11774, *B. cereus* ATCC11778, *E. coli* ATCC259222, *K. pneumoniae* ATCC4352, *P. aeruginosa* ATCC27853, *S. aureus* ATCC6538. 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. As solved control was used dimethylsulphoxide (DMSO).

Comet Assay

The Synthesized compounds were provided as a powder, which was reconstituted in DMSO, aliquoted and maintained frozen at −20 °C until use. Each aliquot was thawed only once. When incubated with cells, the final solution contains no more than 1% DMSO. The same volume of DMSO was also added to control cells for each experiment in which the synthesized compounds were used.

For each experiment, approximately 3 ml heparinized whole blood was collected by venous puncture from one 22-year-old non-smoking male volunteer not exposed to radiation or drugs. Lymphocytes were isolated by Histopaque (Sigma). Blood was layered onto Histopaque at a ratio of 1:1. Blood was then centrifuged at 2000 RPM for 20min at room temperature. The white lymphocyte layer was removed and then used for further experiments. Cell concentrations were adjusted to approximately $2x10^5$ /ml in the PBS. The cells were suspended in PBS at a ratio of 1:1 and total volume of 1 ml. Each reaction contained 50 uL suspension, varying microliter amounts of the compounds investigated, and PBS buffer in a total volume of 1 ml. The cells were incubated for 1 h at 37 °C in an incubator together with negative, positive control and BHA samples. After incubation the lymphocytes were harvested by centrifugation at 3500 RPM for 3 min at 4 °C. After harvest the cells were suspended in 75 uL LMA for embedding on slides. Triplicate experiments were carried out with blood samples from the same donor collected at different time intervals. Each experiment included a positive control, which was hydrogen peroxide at 100 uM. An aliquot of cells was used to check for viability by Trypan blue exclusion.

The comet assay was conducted under alkali conditions according to literature [49]. All chemicals used were obtained from Sigma, St. Louis, MO, USA. Briefly, A 75 µL of the mixture were than layered on the slides precoated with on 1 % normal melting point (NMP) agarose and immediately covered with a coverslip and then kept for 10 min in a refrigerator to solidify. The embedded cells were lysed in a precooled lytic solution (2.5 M NaCl, 100 mM Na_2-EDTA, 10 mM Tris, pH 10, with 10% DMSO and 1% Triton X-100 added fresh) at 4 °C for 75 min, rinsed and equilibrated in an alkaline electrophoresis buffer (0.3 M NaOH and 1 mM EDTA, pH 13). Electrophoresis was performed in an ice bath at 25 V and 300 mA for 20 min, and the slides were neutralized with 0.4 M Tris–HCl (pH 7.5), stained with ethidium bromide (20 µg/mL) for 5 min in the dark and analyzed using an fluorescence microscope (Zeiss Axio Imager A1). The DNA contents in the head and tail were quantified using the "Comet score" from Open Comet.

Statistical analysis:

Statistical analysis was performed using the commercial software package IBM SPSS Statistics 20.0 (IBM Corporation, Armonk, NY, USA). Statistical comparison of the results from the DMSO-treated negative control,

H_2O_2-treated positive control, BHA and test groups treated with compunds were performed by one-way analysis of variance (ANOVA), and post hoc analysis of group differences was carried out by the least significant difference (LSD) test. All values were expressed as Mean ± SD. Statistical significance was set at $p<0.05$.

RESULTS AND DISCUSSION

Chemistry

The structures of nine novel 2-[3-alkyl(aryl)-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl]-phenoxyacetic acid **2a-i** were characterized by using elemental analyses and IR, ^{1}H-NMR, ^{13}C-NMR and elemental analysis (Scheme 1).

Antioxidant activity

The antioxidant activities of nine new compounds **2a-i** 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 this paper, all the title compounds showed lower absorbance than standard antioxidants in made measurements at 700 nm. Therefore, Total reductive capability wasn't observed for reducing metal ion complexes to their lower oxidation state or for any electron transfer reaction. Hereby, all the compounds did not exhibit a reductive activity.

DPPH· radical scavenging activity

Antiradical activities of compounds and standard antioxidants such as BHA, BHT and α-tocopherol were determined by using DPPH· method at 517 nm. Scavenging effect values of compounds **2a-i,** BHA and α-tocopherol at various concentrations (12.5–37.5 µg/mL), are given Figure **1**. The radical scavenging effect of the compounds and the standards were found as following order: α-tocopherol > BHA > BHT > **2b** > **2d** > **2g** > **2e** > **2i**

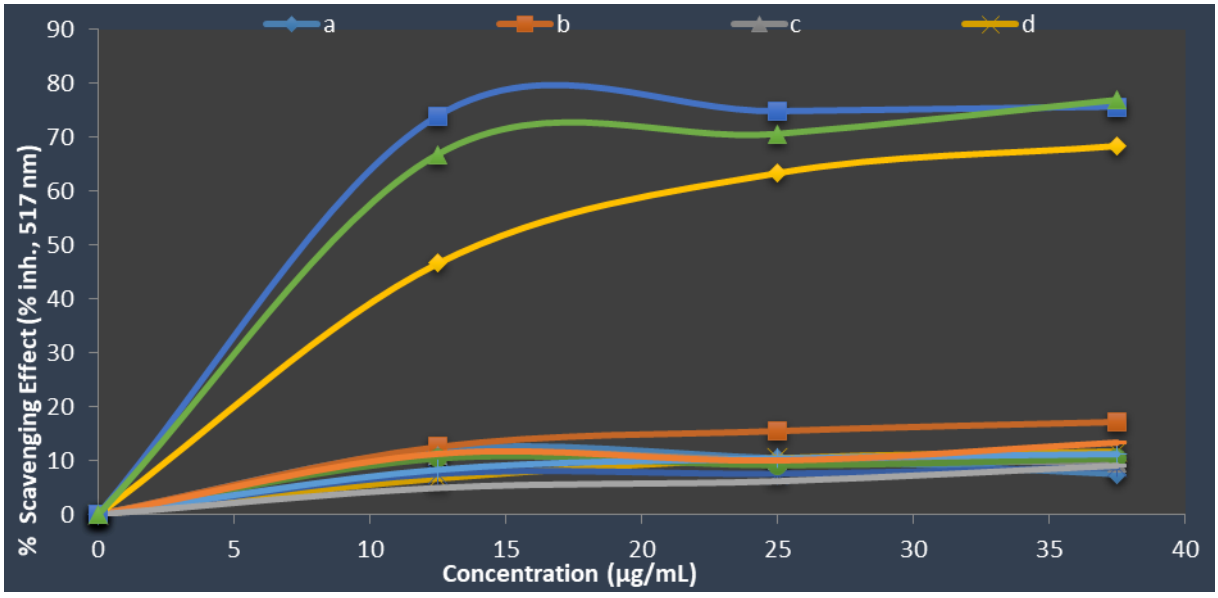


Figure 1- Scavenging effect of compounds 2a-i, BHT, BHA and α-tocopherol at different concentrations (12.5, 25, 37.5 µg/mL).

Metal Chelating Activity

Metal chelating activities of synthesized compounds and standards indicates in Figure 2 as % inhibition at 562 nm. The values obtained from Figure **2** reveal that the metal chelating effects of the newly synthesized

ISSN: 0975-8585

compounds **2d**, **2f**, **2g**, **2h** and **2i** were concentration-dependent although **2a**, **2b**, **2c** and **2e** were not. Ferrous ion chelating activity of the novel compounds and standards increased in the order of **2g** < **2i** = **2f** < **2h** < **2d** < α-tocopherol < EDTA. Moreover, the compound-iron complex may be active, because it can occur metal-catalyzed reactions.

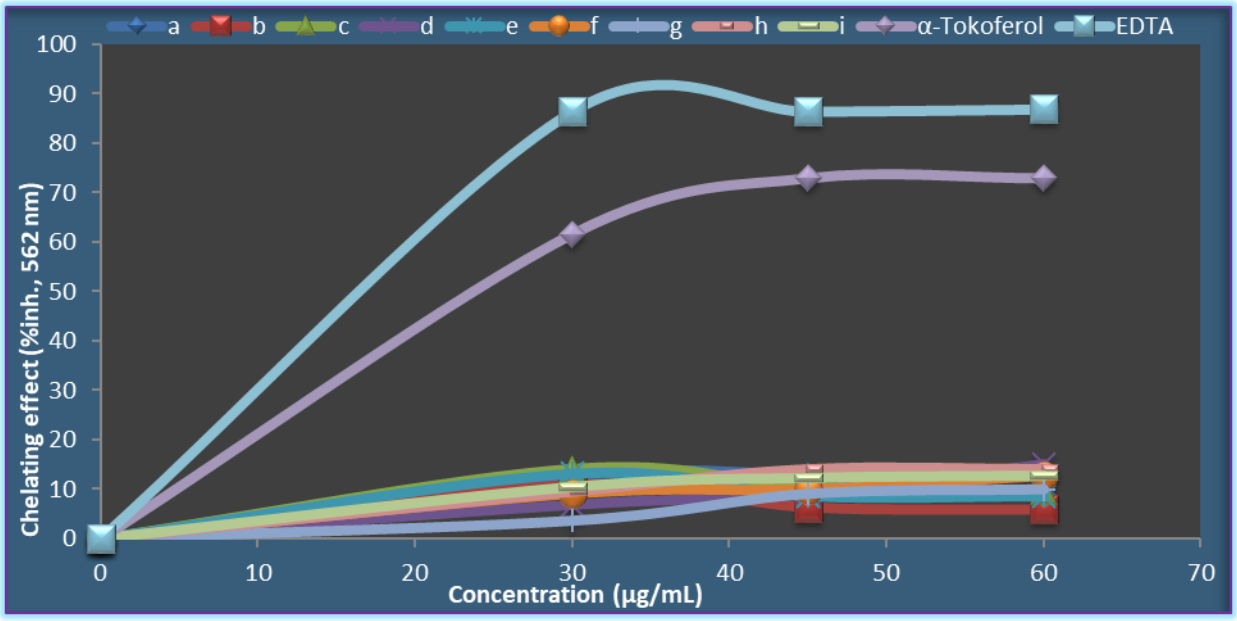


Figure 2- Metal chelating activities in different concentrations of the 2 type compounds (2a-I), EDTA and α-tocopherol (30, 45, 60 µg/mL).

Antimicrobial activity

The observed data for the antimicrobial activity of **2a-i** compounds were given in Table **1** and Figure **3**. The data reveal that, the highest zone diameter was obtained from **2c** compound against three different stains; *Bacillus subtilis* (ATCC 10978), *Pseudomonas aeruginosa* (ATCC 43288) and *Klepsiella pneumoniae* (ATCC 4352). The screening data also indicate that compounds **2f** and **2h** were found to be effective against *Pseudomonas aeruginosa* (ATCC 43288) strain.

Figure 3- The zone diameters obtained against *Escherichia coli* (ATCC 25922) with agar well diffusion method

ISSN: 0975-8585

Table 1: Screening for antimicrobial activity of the 2 type compounds

Compound	Microorganisms and inhibition zone (mm)					
	Bs (A)	Bc (B)	Pa (C)	Kp(D)	Sa (E)	Ec (F)
2a (1)	-	-	**+**	-	-	-
2b (4)	-	-	**+**	-	**+**	**+**
2c (5)	**++**	**+**	**++**	**++**	-	-
2d (3)	-	-	**+**	**+**	**+**	-
2e (6)	**+**	**+**	**+**	**+**	-	**+**
2f (16)	-	-	**++**	+	-	-
2g (7)	**+**	**+**	**+**	-	-	**+**
2h (15)	-	-	**++**	**+**	**+**	-
2i (2)	-	-	**+**	**+**	**+**	-

Results were interpreted in terms of diameter of the inhibition zone: (-): <5,5 mm; (+): 5,5-10 mm; (++): 11-16 mm; (+++): ≥ 17 mm. Bs: *Bacillus subtilis* (ATCC 10978), Bc: *Bacillus cereus* (ATCC 11778), Pa: *Pseudomonas aeruginosa (*ATCC 43288), Kp: *Klepsiella pneumoniae* (ATCC 4352), Sa: *Staphylococcus aureus* (ATCC 6538), Ec: *Escherichia coli* (ATCC 25922).

Comet assay

The Comet Assay is a rapid, quantitative technique in which visual evidence of DNA damage in eukaryotic cells can be measured. It is based on the quantification of denaturized DNA fragments that migrate out of the cell nucleus during electrophoresis. Treated with compounds and BHA cause DNA damage or prevent DNA damage in human peripheral lymphocyte samples, compared to non-treated lymphocyte samples. When DNA is broken in treated cells, it forms a tail that moves away from the unbroken DNA (the head). The cell viability after the compound treatment was more than 80%, as examined with trypan blue dye. The amount of DNA damage in cells was estimated from comet tail length as the extent of migration of the genetic material. Furthermore, the percentage of DNA in the tail (tail intensity) has been shown to be proportional to the frequency of DNA strand breaks [50]. Tail moment is a simple descriptor calculated by the computerized image analysis system considering both the migration tail length as well as the fraction of DNA migrated in the tail [51]. The mean values (±SEM.) of tail length, tail intensity and tail moment in human peripheral lymphocytes following in vitro incubated at 37 ^{0}C for 1 hour treatment with 10, 50 and 100 µM concentrations of the negative control, BHA and compound **2a-i** are shown in Table **2**.

The effect of these compounds against human peripheral lymphocytes was evaluated through the alkaline single cell gel electrophoresis assay. The images of cells treated with BHA, **2a**, **2f**, **2g**, **2h** and **2i** showed a significantly dose-dependent increased formation of comets, compared with those of untreated cells. There was dose-dependent highest increase in tail length, tail intensity and tail moment when treated with compound **2g** presented maximum DNA damage among the compounds studied. None of the compound **2c**, **2d** and **2e** tested exhibited significantly DNA damage compared to negative control cells.

In this study, BHA was used as synthetic standard antioxidant. BHA are generally considered non-genotoxic, the DNA damage observed in this experiment may be related to their indirect action on DNA via ROS mechanism. It is widely considered that TBHQ; an *O*-demethylated metabolite of BHA probably produces many of the toxic effects ascribed to BHA. It is also postulated that BHA and TBHQ act through ROS induced oxidative damage including DNA damage [52].

According to the comet study data, **2f** and **2g** compounds formed by the addition of the chlorine group to compound **2d** resulted in a highest genotoxic activities in all concentration tested (Figure **4** D). The replacement of the *chlorine* group (compound **2f**) by a *methoxy* group (compound **2h**) has led to decline of genotoxicity at only the highest concentration (100 µM) tested (Figure **4** E). The conjugation the triazole ring of the aromatic phenyl group of compound **2d** (compound **2i**) resulted in an increase in DNA damage at 100 µM concentration tested, compared to compound **2d** (Figure **4** F). As a result, chlorination and methoxylation of phenyl group of triazole derivative compounds may increase reactivity of the molecules with cytochrome P450 and also lead to induction of DNA damage as a promutagens to cause a number of events, including activation,

ISSN: 0975-8585

formation of stable electrophilic intermediates and interaction of stable electrophiles with DNA to form covalent adducts, intercalating products, etc.

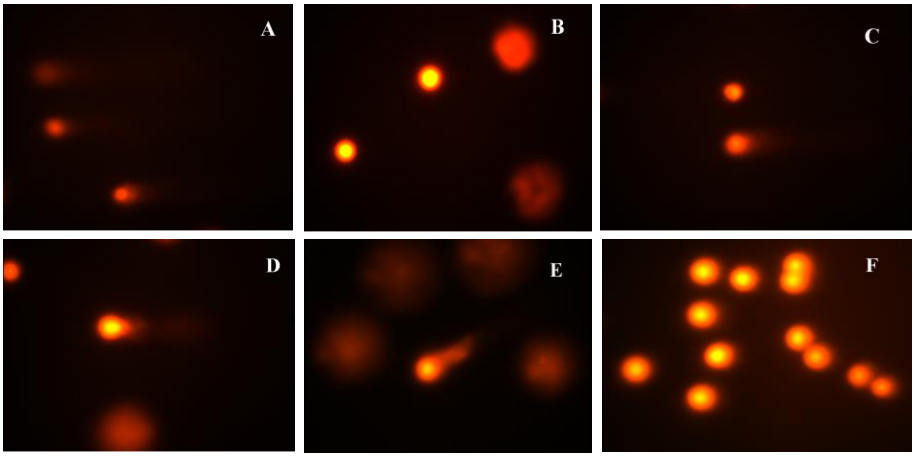


Figure 4- Photographic illustrations of the comet assay after 1-h incubation; (A) H_2O_2-*treated peripheral blood lymphocytes* (positive control). (B) Untreated cells (negative control cells). (C) Treated cells with BHA (synthetic standard antioxidant). (D) Treated cells with 2f. (E) Treated cells with 2h. (F) Treated cells with 2i.

Table 2: DNA damage in human peripheral lymphocytes treated with BHA and compound 2a-i compared with H_2O_2-treated positive control cells and DMSO-treated negative control cells. Data represent mean values (±SEM) of tail length, tail intensity and tail moment of the alkaline comet assay and refer to 150 scores/concentration (50 scores/experiment, three experiments)

Treatment group		Comet assay parameter		
Compound	**Concentration**	**Tail length**	**Tail intensity**	**Tail moment**
Negative (solvent) control	10 µl/ml DMSO	2.04±0.37	4,27±0.44	0.06±0.01
Positive control	100 µM H_2O_2	15,70±0.85***	20,43±1,06***	0.88±0.02***
BHA	10 µM	2.13±0,48	4,14±0,,37	0,04±0,01
	50µM	5.65±0,56**	9,56±1,34*	0,36±0,05***
	100µM	7.43±0,66***	12,04±2,72***	0,46±0,05***
2a	10 µM	3.15±0,26	5,72±0,25	0.08±0.06
	50µM	7.44±0,61***	11,59±0,72***	0.28±0.06***
	100µM	4.85±0,62*	8,96±1,23*	0.24±0.01***
2b	10 µM	2,23±0.19	4,76±0,22	0.08±0.01
	50µM	4,46±0.,78	6,28±0,55	0.15±0.04
	100µM	4,54±0.81	7,66±0,71	0,23±0,06**
2c	10 µM	4,24±0.,79	7,41±0,88	0,23±0,06**
	50µM	2,61±0.78	4,66±0,50	0,09±0,01
	100µM	2,00±0.65	4,99±0,57	0,07±0,02
2d	10 µM	2,18±0.,45	4,26±0,50	0,09±0,02
	50µM	3,50±0.71	7,64±0,,76	0,17±0,04
	100µM	3,00±0.73	6,26±0,48	0,11±0,02
2e	10 µM	2,64±0,78	4,90±0,64	0.08±0.02
	50µM	2,07±0.59	4,50±0,,48	0.07±0.03

ISSN: 0975-8585

Treatment group		Comet assay parameter		
Compound	Concentration	Tail length	Tail intensity	Tail moment
	100µM	1,25±0,60	3,42±0,62	0.03±0.02
2f	10 µM	5,56±1,02**	7,22±0,44	0.37±0.06***
	50µM	6,09±0,71***	8,86±0,,51	0,41±0.05***
	100µM	6,97±0,28***	10,47±0,86*	0,45±0.05***
2g	10 µM	7,41±0,33***	11,17±0,42**	0,45±0.05***
	50µM	7,89±0,28***	12,33±0,,54***	0,53±0.07***
	100µM	6,08±0,63***	9,11±0,73*	0,39±0.05***
2h	10 µM	6,90±0,52***	10,52±0,57**	0,43±0.04***
	50µM	6,25±0,61***	9,01±0,,80	0,38±0.03***
	100µM	2,71±0,45	5,50±0,53	0.08±0.02
2i	10 µM	2,00±0,55	4,68±0,47	0,10±0.02
	50µM	4,21±0,0,88	7,78±0,,63	0,19±0.04*
	100µM	5,12±0,0,46**	8,56±0,61	0,29±0.04***

*p<0.05; **p<0.01; ***p<0.001, significance of DNA damage in synthesized compounds and H_2O_2 treated peripheral lymphocytes compared with negative control cells.

CONCLUSION

In the present study, a series of nine novel 1,2,4-triazol-5-one derivatives were synthesized by the reactions of 1 type compounds with 2-formylphenoxyacetic acid in acetic acid. The different spectral techniques and the elemental analysis confirmed the structure of the compounds. Compounds **2d, 2f** and **2h** demonstrate a marked capacity for antioxidant activity. The data reported with regard to the observed radical scavenging and metal chelating activities of the studied compounds could prevent redox cycling. From observed data for the antimicrobial activity of titled compounds, **2c**, **2f** and **2h** compounds were found to be effective against *Pseudomonas aeruginosa* strain. Also compound **2c** showed moderate activity against *Bacillus subtilis* and *Klebsiella pneumoniae*. Finally, the comet assay showed that four 1,2,4-triazol-5-one derivatives, **2a**, **2f**, **2h**, **2i** and BHA exhibited genotoxic activity in human peripheral blood lymphocytes in a dose dependent manner. The presence of chlorine atom and a methoxy group has drastically increased the genotoxic activity of **2f**, **2g** and **2h** as compared to the bulky phenyl group. Our results (Table **2** and Figure **3**) support the view that the comet assay is a suitable and highly sensitive method for detecting DNA damage induced by triazole derived compounds. Especially, 2-(3-*p*-methoxybenzyl-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl)-phenoxyacetic acid (**2f**) and 2-(3-m-chlorobenzyl-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl)-phenoxyacetic acid (**2h**) compounds have demonstrated significant activity in the antioxidant, antimicrobial and comet assay tests. Design and synthesis of novel small molecules, (specifically **2f** and **2h** compounds), can play specifically a protective role in biological systems and in modern medicinal chemistry. These results may also provide some guidance for the development of novel triazole-based therapeutic target.

ACKNOWLEDGEMENTS

This work was supported by the Scientific Research Projects Coordination Unit of Süleyman Demirel University (Project Number: 3751-YL2-13).

REFERENCES

[1] Kahveci B, Ozil M, Mentese E, Bekircan O, Buruk K, Russ J Org Chem., 2008, 44 1816-1820.
[2] Isloor AM, Kalluraya B, Shetty P, Eur J Med Chem, 2009, 44 3784-3787.
[3] Nadkarni BA, Kamat VR, Khadse BG, Arzneimittel-Forschung-Drug Research, 2001, 51 569-573.
[4] Arslantas A, Yuksek H, Gursoy-Kol O, Ocak Z, Tomruk Z, Calapoglu M, Asian J Chem, 2012, 24 3327-3334.
[5] Kol OG, Yuksek H, Islamoglu F, Rev Chim-Bucharest, 2012, 63 1103-1111.

[6] Henen MA, El Bialy SAA, Goda FE, Nasr MNA, Eisa HM, Med Chem Res, 2012, 21 2368-2378.
[7] Uzgoren-Baran A, Tel BC, Sarigol D, Ozturk EI, Kazkayasi I, Okay G, Ertan M, Tozkoparan B, Eur J Med Chem, 2012, 57 398-406.
[8] Holla BS, Poojary KN, Rao BS, Shivananda MK, Eur J Med Chem, 2002, 37 511-517.
[9] Ibrahim DA, Eur J Med Chem, 2009, 44 2776-2781.
[10] Padmavathi V, Reddy GS, Padmaja A, Kondaiah Ali-Shazia P, Eur J Med Chem, 2009, 44 2106-2112.
[11] Zhang CB, Yang CW, Deng XQ, Quan ZS, Med Chem Res, 2012,21 3294-3300.
[12] Yuksek H, Demirbas A, Ikizler A, Johansson CB, Celik C, Ikizler AA, Arzneimittelforschung, 1997, 47 405-409.
[13] Pitucha M, Olender A, Wujec M, Borowski P, Mardarowicz M, J Chin Chem Soc-Taip, 2010, 57 260-265.
[14] Sztanke K, Tuzimski T, Rzymowska J, Pasternak K, Kandefer-Szerszen M, Eur J Med Chem, 2008, 43, 404-419.
[15] Haber J, Cas Lek Cesk, 2001, 140, 596-604.
[16] Shiroki M, Tahara T, Araki K, in, Japan, 1975, pp. 71.
[17] Povelista FE, Gural AG, in, Moscow, 1973.
[18] Holla BS, Veerendra B, Shivananda MK, Poojary B, Eur J Med Chem, 2003, 38, 759-767.
[19] Bayrak H, Demirbas A, Bektas H, Karaoglu SA, Demirbas N, Turk J Chem, 2010, 34, 835-846.
[20] Chandramouli MR, Shivanand TB, Bheemachari RH, Udupi J, Journal of Chemical and Pharmaceutical Research, 2012, 4, 1151-1159.
[21] Wahi AK, Singh AK, Singh A, Der Pharma Chemica, 2011, 3, 146-154.
[22] Cicekli SU, Onkol T, Ozgen S, Sahin MF, Rev Roum Chim, 2012, 57, 187-195.
[23] Khanmohammadi H, Erfantalab M, Azimi G, Spectrochim Acta A, 2013, 105, 338-343.
[24] Hussain HH, Babic G, Durst T, Wright JS, Flueraru M, Chichirau A, Chepelev LL, J Org Chem, 2003, 68, 7023-7032.
[25] McClements DJ, Decker EA, Journal of Food Science, 2000, 65, 1270-1282.
[26] Al-Soud YA, Al-Dweri MN, Al-Masoudi NA, Farmaco, 2004, 59, 775-783.
[27] Mavrova A, Wesselinova D, Tsenov YA, Denkova P, Eur J Med Chem, 2009, 44, 63-69.
[28] Bekircan O, Kahveci B, Kucuk M, Turk J Chem, 2006, 30, 29-40.
[29] Collins AR, Mol Biotechnol, 2004, 26, 249-261.
[30] Ikizler AA, Un R, Chimica Acta Turcica, 1979, 7, 269-290.
[31] Ikizler AA, Yuksek H, Org Prep Proced Int, 1993, 25, 99-105.
[32] Oyaizu M, Japanese Journal of Nutrition, 1986, 44, 307-315.
[33] Meir S, Kanner J, Akiri B, Philosophhadas S, J Agr Food Chem, 1995, 43, 1813-1819.
[34] Blois MS, Nature, 1958, 181, 1199-1200.
[35] Baumann J, Wurn G, Bruchlausen V, Naunyn-Schmiedeberg's Archives of Pharmacology, 1979, 308 R27.
[36] Soares JR, Dinis TCP, Cunha AP, Almeida LM, Free Radical Research, 1997, 26 469-478.
[37] Duh PD, Tu YY, Yen GC, Food Sci Technol-Leb, 1999, 32, 269-277.
[38] Dinis TCP, Madeira VMC, Almeida LM, A, Arch Biochem Biophys, 1994, 315, 161-169.
[39] Yamaguchi F, Ariga T, Yoshimura Y, Nakazawa H, J Agr Food Chem, 2000, 48, 180-185.
[40] Strlic M, Radovic T, Kolar J, Pihlar B, J Agr Food Chem, 2002, 50, 6313-6317.
[41] Finefrock A., Bush AI, Doraiswamy PM, J Am Geriatr Soc, 2003,51, 1143-1148.
[42] Calis I, Hosny M, Khalifa T, Nishibe S, Phytochemistry, 1993, 33, 1453-1456.
[43] Gordon MH, Food Antioxidants, University of Reading, UK, 1990.
[44] Lindsay RC, Food Chemistry, Third Edition ed., Marcel Dekker, Inc. New York 1996.
[45] Yuan YV, Bone DE, Carrington MF, Food Chem, 2005, 91 485-494.
[46] Gulcin I, Life Sci, 2006, 78, 803-811.
[47] Perez C, Pauli M, Bazerque P, Acta Biologiae et Medecine Experimentaalis, 1990,15, 113-115.
[48] Ahmad I, Mehmood Z, Mohammad F, Journal of Ethnopharmacology, 1998, 62, 183-193.
[49] Singh NP, Mccoy MT, Tice RR, Schneider EL, Exp Cell Res, 1988, 175, 184-191.
[50] Olive PL, Banath JP, Durand RE, H Radiat Res, 1990, 122 86-94.
[51] Villarini M, Moretti M, Pasquini R, Scassellati-Sforzolini G, Fatigoni C, Marcarelli M, Monarca S, Rodríguez AV, Toxicology, 1998, 130 129-139.
[52] Iverson F, Cancer Lett, 1995, 93 49-54.