Synthesis and *In Vitro* Antioxidant Evaluation of Some Novel 4, 5-Dihydro-1*H*-1, 2, 4-triazol-5-one Derivatives

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Abstract: In this study, thirteen novel 4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives were synthesized and characterized by elemental analyses and IR, ¹H NMR, ¹³C NMR and UV spectral data. The synthesized compounds were analyzed for their *in vitro* potential antioxidant activities in three different methods. Those antioxidant activities were compared to standard antioxidants such as BHA, BHT and α -tocopherol. The data reveal that the compounds demonstrate a marked capacity for iron binding, especially **5a** and **7c**. In addition to this, the compounds **4** were titrated potentiometrically with TBAH in non-aqueous solvents because of the weak acidic properties of 4,5-dihydro-1*H*-1,2,4-triazol-5-one ring N-H group.

Keywords: 1,2,4-Triazol-5-one, Schiff base, Synthesis, Antioxidant activity, Acidity.

Introduction

Compounds containing 1,2,4-triazole ring have been reported to possess different biological activities such as antimicrobial, antifungal, anti-inflammatory, antioxidant, antiviral, anticancer, analgesic, and anticonvulsant activity depending on the substituents in the ring system¹⁻⁹. Moreover several articles reporting the synthesis of some *N*-arylidenamino-4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives have been published^{5,10-14}.

Besides, antioxidants have become one of the major areas of scientific research. Antioxidants are extensively studied for their capacity to protect organism and cell from damage that are induced by oxidative stress. Scientists in many different disciplines 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 cell¹⁵. 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 significantly pathological role in human diseases. 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 variety of pathophysiological processes such as inflammation, diabetes, genotoxicity and cancer¹⁶. In the present study, due to a wide range applications and 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) free radical scavenging, reducing power and metal chelating activities.

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, therefore, 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 p*K*_a values of the compounds were determined^{5,10-13,17,18}.

Experimental

Chemical reagents and all solvents used in this study were purchased from Merck AG, Aldrich and Fluka. The starting materials (**2a-g**) were prepared from the reactions of the corresponding ester ethoxycarbonylhydrazones (**1a-g**) with an aqueous solution of hydrazine hydrate as described in the literature^{14,19}. Melting points were determined in open glass capillaries using an Electrothermal 9100 digital melting point apparatus and are uncorrected. The IR spectra were obtained on a Perkin-Elmer Instruments Spectrum One FT-IR spectrometer. ¹H NMR and ¹³C NMR spectra were recorded in deuterated dimethyl sulfoxide with TMS as internal standard using a Varian Mercury spectrometer at 200 MHz and 50 MHz, respectively. UV absorption spectra were measured in 10 mm quartz cells between 200 and 400 nm using a Shimadzu-1201 UV/VIS spectrometer. Extinction coefficients (ϵ) are expressed in L·mol⁻¹·cm⁻¹. Elemental analyses were carried out on an ECS 4010 Costech Instruments Elemental Combustion System (CHNS-O) for C, H, and N. 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 trichloroacetic acid (TCA) were bought from Sigma.

General procedure for the synthesis of compounds 4

3-Hydroxybenzaldehyde (0.01 mol) was dissolved in ethyl acetate (15 mL) was treated with 4-nitrobenzoyl chloride (0.01 mol) and to this solution was added triethylamine (0.01 mol) slowly with stirring at 0-5 °C. Stirring was continued for 2 h, then the mixture was refluxed for 4 h and filtered. The filtrate was evaporated *in vacuo* and the crude product was washed with water and recrystallized from ethanol to afford compound **3**, m.p. 133 °C; IR (KBr) (ν , cm⁻¹): CHO 2854, 2752; C=O 1727, 1699; NO₂ 1525 and 1350; COO 1235, 1,3-disubstituted benzenoid ring 778 and 712. The corresponding compound **2** (0.01 mol) was dissolved in acetic acid (15 mL) and treated with 3-(4-nitrobenzoxo)benzaldehyde (**3**) (0.01 mol). The mixture was refluxed for 1.5 h and then evaporated at 50-55 °C *in vacuo*. Several recrystallizations of the residue from AcOH-H₂O (1:3) gave pure compounds (**4**) as colorless crystals.

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

Yield 2.81 g (96%). M.p. 270 °C. IR (KBr): NH 3178; C=O 1748, 1703; C=N 1607, 1577; NO₂ 1525 and 1347; COO 1261; 1,4-disubstituted benzenoid ring 842; 1,3-disubstituted

benzenoid ring 798 and 714 cm^{-1} . ^1H NMR (DMSO- d_6): δ 2.24 (s, 3H, CH_3), 7.43-7.77 (m, 4H, Ar-H), 8.28-8.38 (m, 4H, Ar-H), 9.72 (s, 1H, N=CH), 11.82 (s, 1H, NH). ^{13}C NMR (DMSO- d_6): δ 10.91 (CH_3), 119.92, 123.77 (2C), 124.58, 126.04, 130.16, 131.16 (2C), 134.11, 135.18, 150.66, 151.04 (arom-C), 144.17 (triazole C_3), 150.39 (N=CH), 152.01 (triazole C_5), 162.93 (C=O). UV λ_{max} (ϵ): 256 (4488), 209 (17049) nm. Anal. Calcd. for $\text{C}_{17}\text{H}_{13}\text{N}_5\text{O}_5$ (367.32): C, 55.59; H, 3.57; N, 19.07. Found: C, 55.65; H, 3.77; N, 19.06.

3-Ethyl-4-[3-(4-nitrobenzoxy)benzylidenamino]-4,5-dihydro-1H-1,2,4-triazol-5-one (4b)

Yield 2.41 g (63%). M.p. 226 $^{\circ}\text{C}$. IR (KBr): NH 3178; C=O 1736, 1705; C=N 1607, 1592; NO_2 1527 and 1349; COO 1263; 1,4-disubstituted benzenoid ring 842; 1,3-disubstituted benzenoid ring 793 and 713 cm^{-1} . ^1H NMR (DMSO- d_6): δ 1.17 (t, 3H, CH_3), 2.64 (q, 2H, CH_2), 7.44-7.76 (m, 4H, Ar-H), 8.29-8.39 (m, 4H, Ar-H), 9.72 (s, 1H, N=CH), 11.84 (s, 1H, NH). ^{13}C NMR (DMSO- d_6): δ 9.80 (CH_3), 18.32 (CH_2), 119.96, 123.79 (2C), 124.59, 125.96, 130.19, 131.19 (2C), 134.14, 135.21, 150.69, 151.21 (arom-C), 147.91 (triazole C_3), 150.42 (N=CH), 152.12 (triazole C_5), 162.94 (C=O). UV λ_{max} (ϵ): 256 (19476), 213 (22229) nm.

3-Benzyl-4-[3-(4-nitrobenzoxy)benzylidenamino]-4,5-dihydro-1H-1,2,4-triazol-5-one (4c)

Yield 4.35 g (98%). M.p. 216 $^{\circ}\text{C}$. IR (KBr): NH 3164; C=O 1745, 1714; C=N 1591; NO_2 1522 and 1349; COO 1266; 1,4-disubstituted benzenoid ring 823; 1,3-disubstituted benzenoid ring 784 and 713 cm^{-1} ; monosubstituted benzenoid ring 760 and 688 cm^{-1} . ^1H NMR (DMSO- d_6): δ 4.04 (s, 2H, CH_2), 7.16-7.32 (m, 5H, Ar-H), 7.44-7.73 (m, 4H, Ar-H), 8.34-8.43 (m, 4H, Ar-H), 9.70 (s, 1H, N=CH), 12.00 (s, 1H, NH). ^{13}C NMR (DMSO- d_6): δ 30.98 (CH_2), 120.12, 123.90 (2C), 124.59, 126.00, 126.59, 128.30 (2C), 128.72 (2C), 130.24, 131.23 (2C), 134.17, 135.16, 135.62, 150.66, 151.05 (arom-C), 146.15 (triazole C_3), 150.51 (N=CH), 151.91 (triazole C_5), 162.97 (C=O). UV λ_{max} (ϵ): 258 (9254), 212 (14492) nm.

3-p-Methylbenzyl-4-[3-(4-nitrobenzoxy)benzylidenamino]-4,5-dihydro-1H-1,2,4-triazol-5-one (4d)

Yield 3.31 g (73%). M.p. 229 $^{\circ}\text{C}$. IR (KBr): NH 3175; C=O 1747, 1713; C=N 1589, 1576; NO_2 1523 and 1352; COO 1266; 1,4-disubstituted benzenoid ring 840, 819; 1,3-disubstituted benzenoid ring 785 and 714 cm^{-1} . ^1H NMR (DMSO- d_6): δ 2.17 (s, 3H, CH_3), 3.97 (s, 2H, CH_2), 7.03 (d, 2H, Ar-H, $J=8.06$ Hz), 7.16 (d, 2H, Ar-H, $J=8.09$ Hz), 7.44-7.72 (m, 4H, Ar-H), 8.32-8.42 (m, 4H, Ar-H), 9.68 (s, 1H, N=CH), 11.97 (s, 1H, NH). ^{13}C NMR (DMSO- d_6): δ 20.42 (CH_3), 30.58 (CH_2), 120.06, 123.86 (2C), 124.54, 125.99, 128.56 (2C), 128.83 (2C), 130.21, 131.20 (2C), 132.47, 134.14, 135.17, 135.62, 150.64, 151.04 (arom-C), 144.26 (triazole C_3), 150.49 (N=CH), 151.86 (triazole C_5), 162.94 (C=O). UV λ_{max} (ϵ): 257 (20261), 264 (7550), 214 (27364) nm. Anal. Calcd. for $\text{C}_{24}\text{H}_{19}\text{N}_5\text{O}_5$ (457.45): C, 63.02; H, 4.19; N, 15.31. Found: C, 62.94; H, 4.97; N, 15.41.

3-p-Chlorobenzyl-4-[3-(4-nitrobenzoxy)benzylidenamino]-4,5-dihydro-1H-1,2,4-triazol-5-one (4e)

Yield 4.65 g (97%). M.p. 219 $^{\circ}\text{C}$. IR (KBr): NH 3173; C=O 1744, 1716; C=N 1591, 1575; NO_2 1525 and 1354; COO 1264; 1,4-disubstituted benzenoid ring 829, 810; 1,3-disubstituted benzenoid ring 794 and 713 cm^{-1} . ^1H NMR (DMSO- d_6): δ 4.04 (s, 2H, CH_2), 7.30-7.33 (m, 4H, Ar-H), 7.48-7.73 (m, 4H, Ar-H), 8.37-8.43 (m, 4H, Ar-H), 9.60 (s, 1H, N=CH), 12.01 (s, 1H, NH). ^{13}C NMR (DMSO- d_6): δ 30.31 (CH_3), 120.05, 123.86 (2C), 124.62, 126.08, 128.20 (2C), 130.25, 130.66 (3C), 131.23 (2C), 134.17, 134.56, 135.10, 150.66, 151.02 (arom-C), 145.80 (triazole C_3), 150.49 (N=CH), 151.95 (triazole C_5), 162.97 (C=O). UV λ_{max} (ϵ): 258 (13520), 214 (22700) nm.

3-Phenyl-4-[3-(4-nitrobenzoxy)benzylidenamino]-4,5-dihydro-1H-1,2,4-triazol-5-one (4f)

Yield 3.71 g (86%). M.p. 247 °C. IR (KBr): NH 3159; C=O 1736, 1707; C=N 1607, 1577; NO₂ 1526 and 1351; COO 1264; 1,4-disubstituted benzenoid ring 841; 1,3-disubstituted benzenoid ring 803 and 712 cm⁻¹; monosubstituted benzenoid ring 771 and 695 cm⁻¹. ¹H NMR (DMSO-d₆): δ 7.45-7.86 (m, 9H, Ar-H), 8.26-8.36 (m, 4H, Ar-H), 9.67 (s, 1H, N=CH), 12.39 (s, 1H, NH). ¹³C NMR (DMSO-d₆): δ 120.60, 123.75 (2C), 124.78, 125.72, 126.39, 127.80 (2C), 128.37 (2C), 129.97, 130.26, 131.14 (2C), 134.08, 134.91, 150.64, 151.15 (arom-C), 144.41 (triazole C₃), 150.37 (N=CH), 155.02 (triazole C₅), 162.86 (C=O). UV λ_{max} (ε): 259 (13175), 210 (15433) nm. Anal. Calcd. for C₂₂H₁₅N₅O₅ (429.39): C, 61.54; H, 3.52; N, 16.31. Found: C, 61.70; H, 3.53; N, 16.21.

3-Cyclopropyl-4-[3-(4-nitrobenzoxy)benzylidenamino]-4,5-dihydro-1H-1,2,4-triazol-5-one (4g)

Yield 3.85 g (98%). M.p. 215 °C. IR (KBr): NH 3175; C=O 1737, 1707; C=N 1607, 1578; NO₂ 1525 and 1350; COO 1260; 1,4-disubstituted benzenoid ring 842; 1,3-disubstituted benzenoid ring 785 and 713 cm⁻¹. ¹H NMR (DMSO-d₆): δ 0.87-0.95 (m, 4H, CH₂CH₂), 2.06-2.20 (m, 1H, CH), 7.47-7.82 (m, 4H, Ar-H), 8.37 (s, 4H, Ar-H), 9.75 (s, 1H, N=CH), 11.81 (s, 1H, NH). ¹³C NMR (DMSO-d₆): δ 5.32, 6.57 (aliph-C), 120.00, 123.86 (2C), 124.73, 126.18, 130.25, 131.24 (2C), 134.17, 135.21, 150.71, 151.16 (arom-C), 148.17 (triazole C₃), 150.46 (N=CH), 152.53 (triazole C₅), 163.00 (C=O). UV λ_{max} (ε): 259 (2870), 209 (13810) nm.

General procedure for the synthesis of compounds 5

The corresponding compound **4** (0.01 mol) was refluxed with acetic anhydride (20 mL) for 0.5 h. After the addition of absolute ethanol (100 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 ethyl alcohol gave pure compounds **5** as colorless needles.

1-Acetyl-3-methyl-4-[3-(4-nitrobenzoxy)benzylidenamino]-4,5-dihydro-1H-1,2,4-triazol-5-one (5a)

Yield 4.00 g (98%). M.p. 225 °C. IR (KBr): C=O 1737, 1720; C=N 1619, 1598; NO₂ 1528 and 1349; COO 1262; 1,4-disubstituted benzenoid ring 836; 1,3-disubstituted benzenoid ring 765 and 714 cm⁻¹. ¹H NMR (DMSO-d₆): δ 2.33 (s, 3H, CH₃), 2.46 (s, 3H, COCH₃), 7.54-7.84 (m, 4H, Ar-H), 8.37 (s, 4H, Ar-H), 9.61 (s, 1H, N=CH). ¹³C NMR (DMSO-d₆): δ 11.12 (CH₃), 23.37 (COCH₃), 120.32, 123.90 (2C), 125.27, 126.51, 130.40, 131.28 (2C), 134.19, 134.66, 146.64, 150.74 (arom-C), 147.72 (triazole C₃), 150.50 (N=CH), 154.22 (triazole C₅), 163.03, 165.95 (2C=O). UV λ_{max} (ε): 255 (29204), 220 (16347) nm.

1-Acetyl-3-benzyl-4-[3-(4-nitrobenzoxy)benzylidenamino]-4,5-dihydro-1H-1,2,4-triazol-5-one (5c)

Yield 4.00 g (83%). M.p. 191 °C. IR (KBr): C=O 1732; C=N 1605, 1585; NO₂ 1524 and 1349; COO 1257; 1,4-disubstituted benzenoid ring 840; 1,3-disubstituted benzenoid ring 806 and 709 cm⁻¹; monosubstituted benzenoid ring 763 and 706 cm⁻¹. ¹H NMR (DMSO-d₆): δ 2.47 (s, 3H, COCH₃), 4.12 (s, 2H, CH₂), 7.19-7.37 (m, 5H, Ar-H), 7.52-7.75 (m, 4H, Ar-H), 8.33-8.43 (m, 4H, Ar-H), 9.58 (s, 1H, N=CH). ¹³C NMR (DMSO-d₆): δ 23.44 (COCH₃), 30.94 (CH₂), 120.41, 123.94 (2C), 125.14, 126.38, 126.87, 128.38 (2C), 128.96 (2C), 130.35, 131.25 (2C), 134.14, 134.53, 134.66, 147.87, 150.68 (arom-C), 148.21 (triazole C₃), 150.50 (N=CH), 153.66 (triazole C₅), 162.98, 165.89 (2C=O). UV λ_{max} (ε): 256 (24585), 215 (24195) nm. Anal. Calcd. for C₂₅H₁₉N₅O₆ (485.46): C, 61.85; H, 3.94; N, 14.43. Found: C, 61.81; H, 3.91; N, 14.49.

1-Acetyl-3-p-Chlorobenzyl-4-[3-(4-nitrobenzoxy)benzylidenamino]-4,5-dihydro-1H-1,2,4-triazol-5-one (5e)

Yield 4.57 g (88%). M.p. 199 °C. IR (KBr): C=O 1781, 1766, 1735; C=N 1606, 1577; NO₂ 1525 and 1368; COO 1258; 1,4-disubstituted benzenoid ring 841, 804; 1,3-disubstituted benzenoid ring 785 and 714 cm⁻¹. ¹H NMR (DMSO-d₆): δ 2.48 (s, 3H, COCH₃), 4.14 (s, 2H, CH₂), 7.35 (s, 4H, Ar-H), 7.53-7.77 (m, 4H, Ar-H), 8.40 (s, 4H, Ar-H), 9.59 (s, 1H, N=CH). ¹³C NMR (DMSO-d₆): δ 23.44 (COCH₃), 30.27 (CH₂), 120.34, 123.95 (2C), 125.20, 126.53, 128.28 (2C), 130.39, 130.91 (2C), 131.29 (2C), 131.55, 133.55, 134.16, 134.64, 147.92, 150.70 (arom-C), 147.92 (triazole C₃), 150.53 (N=CH), 153.70 (triazole C₅), 163.01, 165.85 (2C=O). UV λ_{max} (ε): 257 (6707), 207 (9133) nm.

1-Acetyl-3-phenyl-4-[3-(4-nitrobenzoxy)benzylidenamino]-4,5-dihydro-1H-1,2,4-triazol-5-one (5f)

Yield 4.06 g (86%). M.p. 209 °C. IR (KBr): C=O 1740; C=N 1607, 1585; NO₂ 1527 and 1349; COO 1256; 1,4-disubstituted benzenoid ring 843; 1,3-disubstituted benzenoid ring 788 and 710 cm⁻¹; monosubstituted benzenoid ring 770 and 689 cm⁻¹. ¹H NMR (DMSO-d₆): δ 2.54 (s, 3H, COCH₃), 7.49-7.60 (m, 5H, Ar-H), 7.73-7.91 (m, 4H, Ar-H), 8.28-8.39 (m, 4H, Ar-H), 9.52 (s, 1H, N=CH). ¹³C NMR (DMSO-d₆): δ 23.47 (COCH₃), 120.95, 123.86 (2C), 125.04, 125.41, 126.06, 128.57 (4C), 130.45, 131.24 (3C), 134.10, 134.37, 145.88, 150.69 (arom-C), 147.98 (triazole C₃), 150.44 (N=CH), 157.25 (triazole C₅), 162.93, 166.14 (2C=O). UV λ_{max} (ε): 259 (12677), 205 (12200) nm.

General procedure for the synthesis of compounds 7

The corresponding compound **2** (0.01 mol) was dissolved in 2 N NaOH (10 mL) and treated with dimethyl sulphate (2.08 mL). After stirring of the mixture at room temperature for 1 h, the solid formed was filtered, washed with cold water (15 mL) and dried *in vacuo*. Several recrystallizations of the residue from an appropriate solvent gave pure compounds **6** as colourless needles. Then compound **6** (0.01 mol) was dissolved in acetic acid (15 mL) and treated with 3-(4-nitrobenzoxy) benzaldehyde **3** (0.01 mol). The mixture was refluxed for 1.5 h and then evaporated at 50-55 °C *in vacuo*. Several recrystallizations of the residue from ethyl alcohol gave pure compounds **7** as colorless crystals.

1-Methyl-3-benzyl-4-[3-(4-nitrobenzoxy)benzylidenamino]-4,5-dihydro-1H-1,2,4-triazol-5-one (7c)

Yield 3.10 g (68%). M.p. 163 °C. IR (KBr): C=O 1742, 1709; C=N 1602, 1570; NO₂ 1523 and 1347; COO 1259; 1,4-disubstituted benzenoid ring 839; 1,3-disubstituted benzenoid ring 784 and 711 cm⁻¹; monosubstituted benzenoid ring 762 and 680 cm⁻¹. ¹H NMR (DMSO-d₆): δ 3.32 (s, 3H, NCH₃), 4.04 (s, 2H, CH₂), 7.28 (m, 5H, Ar-H), 7.49-7.73 (m, 4H, Ar-H), 8.37 (m, 4H, Ar-H), 9.67 (s, 1H, N=CH). ¹³C NMR (DMSO-d₆): δ 30.70 (CH₂), 31.80 (NCH₃), 120.27, 123.92 (2C), 124.78, 126.05, 126.63, 128.32 (2C), 128.80 (2C), 130.25, 131.25 (2C), 134.17, 134.96, 135.38, 144.64, 150.68 (arom-C), 149.25 (triazole C₃), 150.50 (N=CH), 152.09 (triazole C₅), 163.05 (C=O). UV λ_{max} (ε): 259 (26187), 219 (19760) nm.

1-Methyl-3-phenyl-4-[3-(4-nitrobenzoxy)benzylidenamino]-4,5-dihydro-1H-1,2,4-triazol-5-one (5f)

Yield 3.00 g (68%). M.p. 192 °C. IR (KBr): C=O 1733, 1707; C=N 1607, 1576; NO₂ 1525 and 1350; COO 1263; 1,4-disubstituted benzenoid ring 841; 1,3-disubstituted benzenoid ring

780 and 713 cm^{-1} ; monosubstituted benzenoid ring 773 and 686 cm^{-1} . ^1H NMR (DMSO- d_6): δ 3.43 (s, 3H, NCH_3), 7.47-7.63 (m, 5H, Ar-H), 7.71-7.84 (m, 4H, Ar-H), 8.28-8.39 (m, 4H, Ar-H), 9.64 (s, 1H, $\text{N}=\text{CH}$). ^{13}C NMR (DMSO- d_6): δ 32.20 (NCH_3), 120.92, 123.84 (2C), 125.10, 125.81, 127.85 (2C), 128.49 (2C), 130.15, 130.35, 130.70, 131.21 (2C), 134.20, 134.72, 147.20, 150.40 (arom-C), 149.50 (triazole C_3), 150.67 ($\text{N}=\text{CH}$), 155.80 (triazole C_5), 163.05 ($\text{C}=\text{O}$). UV λ_{max} (ϵ): 261 (28133), 217 (20933) nm. Anal. Calcd. for $\text{C}_{23}\text{H}_{17}\text{N}_5\text{O}_5$ (443.42): C, 62.30; H, 3.86; N, 15.79. Found: C, 62.31; H, 3.86; N, 15.78.

Reducing power

The reducing power of the synthesized compounds (except **5f**) was determined according to the method of Oyaizu²⁰. Different concentrations of the samples (50-250 $\mu\text{g/mL}$) 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 $^\circ\text{C}$ 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 1000 $\times$ 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 spectrophotometer. Higher absorbance of the reaction mixture indicated greater reducing power.

Free radical scavenging activity

Free radical scavenging activity of compounds was measured by DPPH $^\cdot$ using the method of Blois²¹. Briefly, 0.1 mM solution of DPPH $^\cdot$ in ethanol was prepared, and this solution (1 mL) was added to sample solutions in DMSO (3 mL) at different concentrations (50-250 $\mu\text{g/mL}$). 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 spectrophotometer. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The DPPH $^\cdot$ concentration (mM) in the reaction medium was calculated from the following calibration curve and determined by linear regression (R : 0.997): Absorbance = 0.0003 $\times$ DPPH $^\cdot$ - 0.0174. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH}^\cdot \text{ scavenging effect (\%)} = (A_0 - A_1/A_0) \times 100 \quad (1)$$

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*²². Briefly, the synthesized compounds (50-250 $\mu\text{g/mL}$) 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 room temperature for 10 min. After the mixture had reached equilibrium, the absorbance of the solution was then measured at 562 nm in a spectrophotometer. All test and analyses were run in triplicate and averaged. The percentage of inhibition of ferrozine- Fe^{2+} complex formation was given by the formula: % Inhibition = $(A_0 - A_1/A_0) \times 100$, where A_0 is the absorbance of the control, and A_1 is the absorbance in the presence of the samples or standards. The control did not contain compound or standard.

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.

Statistical analysis

Statistical significance of differences between the mean activities of the subgroups of compounds was determined by utilizing ANOVA for variables with normal distributions and the Kruskal Wallis test for variables not normally distributed. All results are presented as means $\pm$ standard deviation (SD). Differences were considered significant at $p < 0.05$. SPSS computer software was used for all statistical calculations.

Results and Discussion

The 3-alkyl(aryl)-4-[3-(4-nitrobenzoxy)-benzylidenamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-one (**4a-g**) were prepared. The starting compounds 3-alkyl(aryl)-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**2a-g**) were prepared from the reactions of the corresponding ester ethoxycarbonylhydrazones (**1a-g**) with an aqueous solution of hydrazine hydrate as described in the literature^{14,19,23}. Compounds **4** were obtained from the reactions of compounds **2** with 3-(4-nitrobenzoxy)benzaldehyde (**3**) which were synthesized by the reactions of 3-hydroxybenzaldehyde with 4-nitrobenzoyl chloride by using triethylamine. Then the reactions of compounds **4a**, **4c**, **4e**, and **4f** with acetic anhydride were investigated and compounds **5a**, **5c**, **5e**, and **5f** were prepared. Moreover, the reactions of compounds **2c** and **2f** with NaOH/dimethyl sulphate gave compounds **6c**, **6f**. Finally, compounds **7c**, **7f** were synthesized by the reactions of compounds **6c**, **6f** and compound **3** (Scheme 1).

Scheme 1. Synthesis route of compounds **2 – 7**

i) Et₃N, AcOEt, 0-5 °C; ii) N₂H₄, H₂O, reflux, 6 h; iii) AcOH, reflux, 1 h; iv) Ac₂O, reflux, 1 h; v) 2N NaOH, (CH₃)₂SO₄, a) R = CH₃, b) R = CH₂CH₃, c) R = CH₂C₆H₅, d) R = CH₂C₆H₄.CH₃ (*p*-), e) R = CH₂C₆H₄.Cl (*p*-), f) R = C₆H₅, g) R = cyclopropyl.

The structures of seven new 3-alkyl(aryl)-4-[3-(4-nitrobenzoxy)-benzylidenamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-one **4a-g**, four new 1-acetyl-3-alkyl(aryl)-4-[3-(4-nitrobenzoxy)benzylidenamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-one **5a**, **5c**, **5e**, **5f** and two new 1-methyl-3-alkyl(aryl)-4-[3-(4-nitrobenzoxy)benzylidenamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-one **7c**, **7f** were identified using IR, ^1H NMR, ^{13}C NMR, UV and elemental analyses data.

Antioxidant activity

The antioxidant activities of 13 new compounds **4a-g**, **5a**, **5c**, **5e**, **5f**, **7c**, **7f** were determined. The structure-activity relationship studies of compounds were done by statistical analysis. Several methods are used to determine antioxidant activities. The methods used in the study are given below:

Total reductive capability using the potassium ferricyanide reduction method

The reductive capabilities of compounds are assessed by the extent of conversion of the Fe^{3+} / ferricyanide complex to the Fe^{2+} / ferrous form using the method of Oyaizu²⁰. The reducing powers of the compounds were observed at different concentrations, and results were compared with BHA, BHT and α -tocopherol. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity²⁴. 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 scavenging²⁵. In this study, all of the amounts of the compounds showed lower absorbance than blank. Hence, no activities were observed to reduce metal ions complexes to their lower oxidation state or to take part in any electron transfer reaction. In other words, compounds did not show the reductive activities.

DPPH $\cdot$ 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 ability²⁶. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule²². 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 caused by antioxidants, because of reaction between antioxidant molecules and radical, progresses, which result 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 antioxidants²⁷. In the study, antiradical activities of compounds and standard antioxidants such as BHA and α -tocopherol were determined by using DPPH $\cdot$ method. Scavenging effect values of compounds **4**, and **5**, **7**, BHA and α -tocopherol at different concentrations are given Figures 1, 2 respectively. All the compounds tested with this method showed lower absorbance than absorbance of the control reaction and higher absorbance than that of the standard antioxidant reactions. The data obtained in the study indicate that the newly synthesized compounds showed mild activities as a radical scavenger, indicating that it has moderate activities as hydrogen donors.

Figure 1. Scavenging effect of compounds **4a-g**, BHA and α -tocopherol at different concentrations (12.5-25-37.5 $\mu\text{g/mL}$).

Figure 2. Scavenging effect of compounds **5a**, **5c**, **5e**, **5f**, **7c**, **7f**, BHA and α -tocopherol at different concentrations (12.5-25-37.5 $\mu\text{g/mL}$).

Ferrous ion chelating activity

The chelating effect towards ferrous ions by the compounds and standards was determined according to the method of Dinis²⁸. 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 chelator²⁹. 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 ³⁰ 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 processes³¹. Also, the production of highly active ROS such as $\text{O}_2^{\cdot-}$, H_2O_2 and $\text{OH}^{\cdot}$ is also catalyzed by free iron through Haber-Weiss reactions:

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^{3+} ion also produces radicals from peroxides, although the rate is tenfold less than that of Fe^{2+} ion, which is the most powerful pro-oxidant among the various types of metal ions³². Ferrous ion chelating activities of the compounds **4**, and **5**, **7**, BHT and α -tocopherol are shown in Figures 3 and 4, respectively. 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 ion³³. The data obtained from Figures 3 and 4 reveal that the compounds, especially **5a** and **7c**, 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, 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.

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

Figure 4. Metal chelating effect of different amount of the compounds **5a**, **5c**, **5e**, **5f**, **7c**, **7f**, BHT and α -tocopherol on ferrous ions.

The structure-activity relationship studies of compounds revealed that, when compared with compounds **4**, *N*-acetyl substitution of 4,5-dihydro-1*H*-1,2,4-triazol-5-one ring in compounds **5** decreased the reducing activity but increased the scavenging activity significantly. On the other hand, compounds **7** that have *N*-methyl substituted showed a significant increase in their reducing power when compared with *N*-acetyl substituted compounds **5**, and also significant increase in the scavenging activity of them when compared with unsubstituted compounds **4**. These results are shown in Table 1. Hence, these modifications included acetylation and methylation of the compounds **4** may be affected their electron transfer ability and hydrogen donating ability.

Table 1. The comparison of reducing power, DPPH[•] scavenging, and chelating activities of compounds **4**, **5**, and **7**.

Compounds	4 (n=21)	5 (n=12)	7 (n=6)
Reducing power, $\mu\text{g/mL}$, $A_{700\text{ nm}}$	$0,051 \pm 0,028^a$	$0,027 \pm 0,019^b$	$0,037 \pm 0,015^a$
DPPH [•] scavenging activity, $\mu\text{g/mL}$, $A_{517\text{ nm}}$, % inhibition	$31,44 \pm 3,68^a$	$42,01 \pm 1,74^b$	$42,28 \pm 0,71^b$
Metal chelating effect, $\mu\text{g/mL}$, $A_{517\text{ nm}}$, % inhibition	$30,03 \pm 12,82^a$	$39,50 \pm 25,41^a$	$42,51 \pm 26,24^a$

Each value is expressed as mean $\pm$ standard deviation of sum of the three different concentration measurements. Means with different letters within a row are significantly different ($p < 0.05$).

In conclusion, the data here reported could be of the possible interest because of the observed radical scavenging and metal chelating activities of the studied compounds could prevent redox cycling. Design and synthesis of novel small molecules which can specifically protective role in biological systems are in perspective in modern medicinal chemistry. These results may also provide some guidance for the development of novel triazole-based therapeutic target.

Potentiometric titrations

In order to determine the pK_a values of the compounds **4a-g** they were titrated potentiometrically with TBAH in four non-aqueous solvents: isopropyl alcohol, *tert*-butyl alcohol, acetone 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 was 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 were studied^{10-13,17,18,34}. 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 constant³⁵⁻³⁷.

As an example, the potentiometric titration curves for 0.001 M solutions of compounds **4a-g** titrated with 0.05 M TBAH in DMF is shown in Figure 5.

When the dielectric permittivity of solvents is taken into consideration, the acidity order can be given as follows: DMF ($\epsilon=36.7$) > acetone ($\epsilon=36$) > isopropyl alcohol ($\epsilon=19.4$) > *tert*-butyl alcohol ($\epsilon=12$). As seen in Table 2, the acidity order for compounds **4a** and **4f** is: isopropyl alcohol > DMF > *tert*-butyl alcohol > acetone and for compounds **4b**, **4d**, **4e**, and **4g** it is: isopropyl alcohol > DMF > acetone > *tert*-butyl alcohol, while the order for compound **4c** is: isopropyl alcohol > *tert*-butyl alcohol > DMF > acetone. In isopropyl

alcohol, all these compounds show the strongest acidic properties. Compounds **4a**, **4c**, and **4f** show the weakest acidic properties in acetone and for compounds **4b**, **4d**, **4e**, and **4g** it is in *tert*-butyl alcohol. This situation may be attributed to the hydrogen bonding between the negative ions formed and the solvent molecules in the amphiprotic neutral solvents.

Figure 5. Potentiometric titration curves of 0.001 *M* solutions of compounds **4a-g** titrated with 0.05 *M* TBAH in DMF at 25 °C.

Table 2. The HNP and the corresponding pK_a values of compounds **4a-g** in isopropyl alcohol, *tert*-butyl alcohol, DMF and acetone.

Comp.	Isopropyl alcohol		<i>tert</i> -Butyl alcohol		DMF		Acetone	
	HNP mV	pK_a	HNP mV	pK_a	HNP mV	pK_a	HNP mV	pK_a
4a	-415	13,83	-560	16,78	-507	14,58	-602	17,58
4b	-382	13,27	-579	16,97	-448	13,55	-570	16,84
4c	-373	12,78	-460	14,64	-462	14,81	-521	15,86
4d	-380	12,84	-550	16,16	-567	15,59	-539	16,00
4e	-361	12,47	-562	16,41	-536	15,08	-533	16,24
4f	-353	12,52	-501	15,39	-529	14,88	-517	15,68
4g	-402	13,26	-561	16,28	-506	14,35	-505	15,37

The degree to which a pure solvent ionizes was represented by its *autoprotolysis constant*, K_{HS} .

For the above reaction the constant is defined by

$$K_{HS} = [H_2S^+][S^-]$$

Autoprotolysis is an acid-base reaction between identical solvent molecules in which some act as an acid and others as a base. Consequently, the extent of an autoprotolysis reaction depends both on the intrinsic acidity and the intrinsic basicity of the solvent. The importance of the autoprotolysis constant in titrations lies in its effect on the completeness of a titration reaction³⁸. The exchange of the pK_a values with autoprotolysis constant and

dielectric constant are given in Figure 6. 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^{10-13,17,18,34}. Table 2 shows 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.

Figure 6. The variation of the pK_a values for synthesized compounds **4a-g** with dielectric and autoprotolysis constant

Conclusion

1,2,4-Triazoles have broad spectrum of biological activities. The synthesis and *in vitro* antioxidant evaluation of new 4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives are described. All of the compounds demonstrate a marked capacity for iron binding.

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