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# Antioxidant and Antimicrobial Activities of Some Newly Synthesized 4-[1-(2,6-Dimethylmorpholin-4-yl-methyl)-3-alkyl(aryl)-4,5-dihydro-1*H*-1,2,4-triazol-5-on-4-yl-azomethine]-2-methoxyphenyl benzoates

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**Abstract**:In this study, 4-(3-alkyl/aryl-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl-azomethine)-2-methoxyphenyl benzoates were treated with 2,6-dimethylmorpholine in the presence of formaldehyde according to the Mannich reaction to synthesize six novel 4-[1-(2,6-dimethylmorpholin-4-yl-methyl)-3-alkyl(aryl)-4,5-dihydro-triazol-5-on-4-yl-azomethine]-2-methoxyphenyl benzoates. The structures of synthesized six novel heterocyclic compounds were characterized by IR, $^{13}$C-NMR and $^{1}$H-NMR spectroscopic methods. The novel 4-[1-(2,6-dimethylmorpholin-4-yl-methyl)-3-alkyl(aryl)-4,5-dihydro-triazol-5-on-4-yl-azomethine]-2-methoxyphenyl benzoates were investigated *in vitro* antioxidant properties by using reducing power, free radical scavenging and metal chelating activity. For the measurement of the reductive ability, $Fe^{3+}$-$Fe^{2+}$ transformation was investigated in the presence of compound using by the method of Oyaizu (1986). The hydrogen atoms or electrons donation ability of the synthesized compound was measured by DPPH$^{\cdot}$ using the method of Blois (1958). The chelating effect of ferrous ions by the compound was determined according to the method of Dinis, Madeira & Almeida (1994). BHT, BHA, EDTA and α-tocopherol were used as reference antioxidant compounds. The new compounds were examined *in-vitro* antimicrobial properties against 6 different microorganisms (*Bacillus substilis* (ATCC11774), *BacilluscCereus* (ATCC11778), *Staphylococcus aureus* (ATCC6538), *Escherichia coli* (ATCC25922), *Pseudomonas aeruginosa* (ATCC27853) and *Klebsiella pneumonia* (ATCC4352)) by the agar well method and the obtained results were evaluated.

**Keywords:** Mannich base, 4,5-dihydro-1*H*-1,2,4-triazol-5-one, Antioxidant, Antimicrobial

## Introduction

Mannich bases have applications the field medicinal chemistry, the product synthetic polymers, the petroleum industry, as products used in water treatment, cosmetics, the dyes industry, etc (Tramontini & Angiolini, 1994). Moreover, Mannich bases have some biological activities such as anticancer (Savariz et al., 2010), antibacterial



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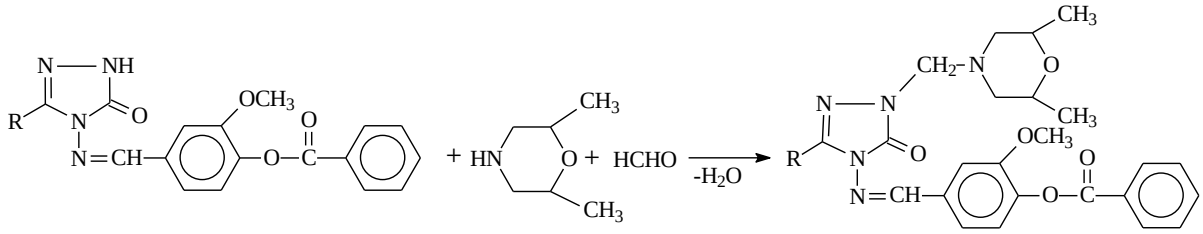
(Maddila & Jonnalagadda, 2012), anti-inflammatory (Liu, Yu, Li, Pang, & Zhao, 2013), anti-HIV (Sriram, Yogeeswari, Dinakaran, & Sowmya, 2008), analgesic (Nithinchandra, Kalluraya, Aamir, & Shabaraya, 2012), antiviral (Chen et al., 2010), antifungal (Ozkan-Daguyan, Sahin, & Koksal, 2013), antitumor (Pati et al., 2008), antidepressant (Köksal & Bilge, 2007) and antioxidant activities (Hamama, Zoorob, Gouda, & Afsah, 2011).
Antioxidants are extensively studied for their capacity to protect organism and cell from damage that is induced by the oxidative stress. A great deal of research has been devoted to the study of different types of natural and synthetic antioxidant. A large number of heterocyclic compounds, containing the 1,2,4-triazole ring, are associated with diverse biological properties such as antioxidant, anti-inflammatory, antimicrobial and antiviral activity. External chemicals and internal 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 by 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 ability of the organism leads to variety of pathophysiological processes like inflammation, diabetes, genotoxicity and cancer (McClements & Decker, 2000).

Triazoles are heterocyclic compounds that contain three nitrogen atoms. 1,2,4-Triazole and 4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives are reported to possess a broad spectrum of biological activities such as analgesic, antibacterial, antioxidant and antiparasitic properties (Aktas-Yokus, Yuksek, Gursoy-Kol, & Alpay-Karaoglu, 2015; Chidananda et al., 2012). Considering about the development of new hetero moieties by combining potential biological active scaffolds, an attempt was made here to obtain 1,2,4-triazoles bearing piperidine ring and to evaluate their antioxidant activity.

In the present paper, 4-(3-alkyl/aryl-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl-azomethine)-2-methoxyphenyl benzoates (**1**) (Koca, Yıldız, & Köçek, 2010) were treated with 2,6-dimethylmorpholine in the presence of formaldehyde according to the Mannich reaction to synthesize six novel 4-[1-(2,6-dimethylmorpholin-4-yl-methyl)-3-alkyl(aryl)-4,5-dihydro-triazol-5-on-4-yl-azomethine]-2-methoxyphenyl benzoates (**2**).




**1** **2**

Scheme 1

## Method

### Chemicals and Apparatus

Chemical reagents used in this paper were bought from Merck AG, Aldrich and Fluka. Melting points were recorded in open glass capillaries using a Stuart SMP30 melting point apparatus and were not corrected. The infrared spectra were recorded on an Alpha-P Bruker FT-IR Spectrometer. $^{1}H$ and $^{13}C$ NMR spectra were determined in deuterated dimethyl sulfoxide with TMS as internal standard using a Bruker Avance III spectrophotometer at 400 MHz and 100 MHz, respectively.

### Synthesis of 4-[1-(2,6-dimethylmorpholin-4-yl-methyl)-3-alkyl(aryl)-4,5-dihydro-triazol-5-on-4-yl-azomethine]-2-methoxyphenyl benzoates: The General Procedure

4-(3-alkyl/aryl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl-azomethine)-2-methoxyphenyl benzoates (**1**) were obtained according to the literature (Koca, Yıldız, & Köçek, 2010). Then, to solution of this compound (**1**) (5 mmol) in absolute ethanol was added 2,6-dimethylmorpholine (6 mmol). The reaction mixture was refluxed for 4 hours. The mixture was left at room temperature for overnight. After cooling the mixture in the refrigerator,

the solid formed was obtained by filtration, washed with cold ethanol and recrystallized from ethanol. Several recrystallizations of the residue from the same solvent gave pure compounds **2** as colourless crystals.

*4-[1-(2,6-dimethylmorpholin-4-yl-methyl)-3-methyl-4,5-dihydro-triazol-5-on-4-yl-azomethine]-2-methoxyphenyl benzoate (2a)*

Yield: 70.58%, m.p. 90°C. IR (KBr, $\upsilon$, $cm^{-1}$): 1741, 1695 (C=O), 1595 (C=N), 1259 (COO), 753 and 705 (monosubstituted benzenoid ring). $^1$H NMR (400 MHz, DMSO-$d_6$): δ 21.05-1.15 (m, 6H, $2CH_3$), 3.87 (s, 3H, $OCH_3$;), 4.58 (m, 4H, $NH_2N$), 2.3-2.08, 2.33-2.37,-2.56-2.78,2.81-3.56(m, 6H), 7.43 (d, 1H, ArH *J*=8.40Hz), 7.55-7.57 (m, 1H, ArH), 7.63-7.69 (m, 3H, ArH), 7.77 (m, 1H, ArH), 8.15-8.17 (m, 2H, ArH), 9.76(s, 1H, N=CH). $^{13}$C NMR (100 MHz, DMSO-$d_6$): δ 10.98 ($CH_3$), 17.90 and 18.93 ($2CH_3$), 55.02 and 55.60 ($2CH_2$), 56.06 ($OCH_3$), 65.26 ($NCH_2N$), 71.03 (2CH), 111.66 (CH), 120.68 (CH); 123.69(CH),128.43(C), 129.04(2CH), 129.85(2CH), 132.49(C), 134.17(CH),141.92(C), 150.28 (C) (ArC), 143.14 (Triazole $C_3$), 151.39 (Triazole $C_5$), 153.60 (N=CH), 163.78 (COO).

*4-[1-(2,6-dimethylmorpholin-4-yl-methyl)-3-ethyl-4,5-dihydro-triazol-5-on-4-yl-azomethine]-2-methoxyphenyl benzoate (2b)*

Yield: 70.14%, m.p. 122°C. IR (KBr, $\upsilon$, $cm^{-1}$): 1741, 1695 (C=O), 1595 (C=N), 1259 (COO), 753 and 705 (monosubstituted benzenoid ring). $^1$H NMR (400 MHz, DMSO-$d_6$): δ 1.05-1.15 (m, 6H, $2CH_3$), 1.26 (t, 3H, $CH_2CH_3$; *J*=7.20 Hz), 2.79 (q, 2H, $CH_2CH_3$; *J*=7.20 Hz), 3.86 (s, 3H, $OCH_3$), 4.57-4.60 (m, 2H, $NCH_2N$), 2.02-2.06, 2.25-2.28, 2.61-2.64, 2.80-2.82, 3.51-3.56 (m, 6H), 7.43 (d, 1H, ArH; *J*=8.00 Hz), 7.54-7.56 (m, 1H, ArH), 7.63-7.67 (m, 3H, ArH), 7.77-7.79 (m, 1H, ArH), 8.15-8.17 (m, 2H, ArH), 9.75 (s, 1H, N=CH). $^{13}$C NMR (100 MHz, DMSO-$d_6$): δ 10.05 ($CH_2\underline{CH_3}$), 17.90 ($CH\underline{CH_3}$), 18.40 ($\underline{CH_2}CH_3$), 18.94 ($CH\underline{CH_3}$), 55.03 and 55.63 ($2CH_2$), 56.05 ($OCH_3$), 66.29 ($NCH_2N$), 71.04 (2CH), 111.67; 120.57; 123.69; 128.43; 129.04 (2CH); 129.86 (2CH); 132.52; 134.18; 141.91; 150.38 (ArC), 146.87 (Triazole $C_3$), 151.39 (Triazole $C_5$), 153.64 (N=CH), 163.78 (COO).

*4-[1-(2,6-dimethylmorpholin-4-yl-methyl)-3-benzyl-4,5-dihydro-triazol-5-on-4-yl-azomethine]-2-methoxyphenyl benzoate (2c)*

Yield: 70.31%, m.p. 147°C. IR (KBr, $\upsilon$, $cm^{-1}$): 1739, 1706 (C=O), 1592 (C=N), 1254 (COO), 750 and 714 (monosubstituted benzenoid ring). $^1$H NMR (400 MHz, DMSO-$d_6$): δ 1.06-1.15 (m, 6H, $2CH_3$), 3.86 (s, 3H, $OCH_3$), 4.15 (s, 2H, $CH_2Ph$), 4.63 (s, 2H, $NCH_2N$), 2.03-2.08, 2.24-2.28, 2.60-2.64, 2.81-2.83, 3.51-3.56 (m,6H), 7.26-7.28 (m, 1H, ArH), 7.33-7.41 (m, 5H, ArH), 7.47-7.48 (m, 1H, ArH), 7.59-7.66 (m, 3H, ArH), 7.77-7.79 (m, 1H, ArH), 8.14-8.17 (m, 2H, ArH), 9.71 (s, 1H, N=CH). $^{13}$C NMR (100 MHz, DMSO-$d_6$): δ 17.89 and 18.93 ($2CH_3$), 31.02 ($CH_2Ph$), 55.07 and 55.67 ($2CH_2$), 56.02 ($OCH_3$), 65.78 ($NCH_2N$), 71.02 (2CH), 110.87; 121.23; 123.64; 126.80; 128.42; 128.50 (2CH); 128.83 (2CH); 129.04(2CH); 129.86 (2CH); 132.46; 134.18; 135.76; 141.95; 150.27 (ArC), 144.92 (Triazole $C_3$), 151.36 (Triazole $C_5$), 153.00 (N=CH), 163.78 (COO).

*4-[1-(2,6-dimethylmorpholin-4-yl-methyl)-3-p-methylbenzyl-4,5-dihydro-triazol-5-on-4-yl-azomethine]-2-methoxyphenyl benzoate (2d)*

Yield: 70.31%, m.p. 129°C. IR (KBr, $\upsilon$, $cm^{-1}$): 1741, 1706 (C=O), 1579 (C=N), 1252 (COO), 868 (1,4-disubstituted benzenoid ring), 748 and 711 (monosubstituted benzenoid ring). $^1$H NMR (400 MHz, DMSO-$d_6$): δ 1.06-1.10 (m) and 1.15 (d, *J*=6.40 Hz) (6H, $2CH_3$), 2.27 (s, 3H, $PhCH_3$), 3.87 (s, 3H, $OCH_3$), 4.09 (s, 2H, $CH_2Ph$), 4.62 (s, 2H, $NCH_2N$), 2.02-2.07, 2.25-2.28, 2.60-2.64, 2.81-2.83, 3.50-3.56 (m,6H), 7.15 (d, 2H, ArH; *J*=8.00 Hz), 7.26 (d, 2H, ArH; *J*=7.60 Hz), 7.41 (d, 1H, ArH; *J*=8.00 Hz), 7.48-7.50 (m, 1H, ArH), 7.59-7.67 (m, 3H, ArH), 7.78-7.80 (m, 1H, ArH), 8.15-8.17 (m, 2H, ArH), 9.70 (s, 1H, N=CH). $^{13}$C NMR (100 MHz, DMSO-$d_6$): δ 17.89 and 18.93 ($2CH_3$), 20.56 ($PhCH_3$), 30.63 ($CH_2Ph$), 55.08 and 55.68 ($2CH_2$), 56.01 ($OCH_3$), 65.76 ($NCH_2N$), 71.02 (2CH), 110.87; 121.24; 123.64; 128.42; 128.54 (2CH); 129.05(2CH) (2CH); 129.86 (2CH); 132.48; 132.63; 134.18; 135.91; 141.94; 150.26 (ArC), 145.07 (Triazol $C_3$), 151.36 (Triazol $C_5$), 152.95 (N=CH), 163.78 (COO).

*4-[1-(2,6-dimethylmorpholin-4-yl-methyl)-3-p-methoxybenzyl-4,5-dihydro-triazol-5-on-4-yl-azomethine]-2-methoxyphenyl benzoate (2e)*

Yield: 74.60%, m.p. 113°C. IR (KBr, $\upsilon$, $cm^{-1}$): 1740, 1706 (C=O), 1595 (C=N), 1247 (COO), 868 (1,4-disubstituted benzenoid ring), 747 and 7134 (monosubstituted benzenoid ring). $^1$H NMR (400 MHz, DMSO-$d_6$): δ 1.05-1.07 (m) and 1.15 (d) (6H, $2CH_3$), 3.87 (s, 3H, $OCH_3$), 4.07 (s, 2H, $CH_2Ph$), 4.62 (s, 2H, $NCH_2N$), 2.02-2.07, 2.28-2.32, 2.60-2.64, 2.80-2.83, 3.54-3.58 (m, 6H), 6.90 (d, 2H, ArH; *J*=8.80 Hz ), 7.30 (d, 2H, ArH; *J*=8.40 Hz), 7.42 (d, 1H, ArH; *J*=8.00 Hz), 7.49-7.51 (m, 1H, ArH), 7.61-7.67 (m, 3H, ArH), 7.78-7.79 (m, 1H, ArH), 8.15-8.17 (m, 2H, ArH), 9.71 (s, 1H, N=CH). $^{13}$C NMR (100 MHz, DMSO-$d_6$): δ 17.89 and 18.93 ($2CH_3$), 30.16 ($CH_2Ph$), 56.03 ($OCH_3$), 65.76 ($NCH_2N$), 71.02 (2CH), 110.95; 113.96 (2CH), 121.19; 123.65; 127.49; 128.43; 129.04 (2CH); 129.73 (2CH); 129.86 (2CH); 132.49; 134.48; 141.95; 150.27; 158.16 (ArC), 145.22 (Triazole $C_3$), 151.37 (Triazole $C_5$), 153.02 (N=CH), 163.79 (COO).

*4-[1-(2,6-dimethylmorpholin-4-yl-methyl)-3-p-chlorobenzyl-4,5-dihydro-triazol-5-on-4-yl-azomethine]-2-methoxyphenyl benzoate (2f)*

Yield: 72.13%, m.p. 127°C. IR (KBr, $\upsilon$, $cm^{-1}$): 1740, 1705 (C=O), 1596 (C=N), 1252 (COO), 868 (1,4-disubstituted benzenoid ring), 746 and 711 (monosubstituted benzenoid ring). $^1$H NMR (400 MHz, DMSO-$d_6$): δ 1.05-1.14 (m, 6H, $2CH_3$), 3.86 (s, 3H, $OCH_3$), 4.17 (s, 2H, $CH_2Ph$), 4.62 (s, 2H, $NCH_2N$), 2.02-2.07, 2.28-2.32, 2.62-2.66, 2.80-2.85, 3.54-3.57 (m,6H), 7.40-7.42 (m, 5H, ArH), 7.48-7.50 (m, 1H, ArH), 7.58 (m, 1H, ArH), 7.63-7.67 (m, 2H, ArH), 7.78-7.79 (m, 1H, ArH), 8.14-8.17 (m, 2H, ArH), 9.72 (s, 1H, N=CH). $^{13}$C NMR (100 MHz, DMSO-$d_6$): δ 17.88 and 18.93 ($2CH_3$), 30.34 ($CH_2Ph$), 55.05 and 55.65 ($2CH_2$), 56.03 ($OCH_3$), 65.81 ($NCH_2N$), 71.02 (2CH), 110.95; 121.22; 123.65; 128.44; 129.04(2CH); 129.86 (2CH); 130.59 (2CH); 130.76 (2CH), 131.51; 132.41; 134.18; 134.77; 141.98; 150.26 (ArC), 144.61 (Triazole $C_3$), 151.37 (Triazole $C_5$), 153.16 (N=CH), 163.77 (COO).

**Antioxidant Activity**

*Chemicals*

Butylated hydroxytoluene (BHT), ferrous chloride, DPPH., α-tocopherol, 3- butylated hydroxyanisole (BHA), (2-pyridyl)-5,6-bis(phenylsulfonic acid)-1,2,4-triazine (ferrozine) and trichloroacetic acid (TCA) were obtained from E. Merck or Sigma.

*Reducing Power*

The reducing power of the compounds **2a-i** was determined using the method of Oyaizu (1986). Different concentrations of the samples (50-250 µg/mL) in DMSO (1 mL) were mixed with phosphate buffer (2.5 mL, 0.2 M, pH = 6.6) and potassium ferricyanide (2.5 mL, 1%). The mixture was incubated at 50$^o$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 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 effect of the compounds **2a-f** was estimated by DPPH$^{\cdot}$, by the method of Blois (1958). 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 µg/mL). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm in a spectrophometer. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The DPPH$^{\cdot}$ concentration (mM) in the reaction medium was calculated from the following calibration curve and determined by linear regression (R: 0.997):

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

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

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

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 chelating of ferrous ions by the compounds **2a-f** and references was measured according to the method of Dinis et al., (1994). Briefly, the synthesized compounds (30–60 μg/mL) were added to a 2 mM solution of $FeCl_2$ (0.05 mL). The reaction was initiated by the addition of 5 mM ferrozine (0.2 mL), and then 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 measured at 562 nm in a spectrophotometer. All tests 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.

***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), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumonia* (ATCC 4352), *Staphylococcus aureus* (ATCC 6538) and *Escherichia coli* (ATCC 25922). Simple susceptibility screening test using agar well diffusion method was used (Perez, Pauli, & Bazerque, 1990, Ahmad, Mehmood, & Mohammed, 1998). 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

In this study, the structures of six new 4-[1-(2,6-dimethylmorpholin-4-yl-methyl)-3-alkyl(aryl)-4,5-dihydro-triazol-5-on-4-yl-azomethine]-2-methoxyphenyl benzoates (**2a-f**) were characterized with IR, $^1$H NMR and $^{13}$C NMR spectral data.

**Antioxidant Activity**

The antioxidant capacities of nine newly synthesized compounds **2a-f** were determined. Different processes have been used to identify antioxidant capacities. The processes used in the paper are clarified below:

*Reducing Power*

The reducing power of the compounds **2** was determined. The reductive capabilities of compounds are assessed by the extent of conversion of the $Fe^{3+}$/ferricyanide complex to the $Fe^{2+}$/ferrous form. The reducing powers of the compounds were observed at different concentrations, and results were compared with BHA, BHT and α-

tocopherol. The reducing capacity of a compound may serve as a significant indicator for its potential antioxidant activity (Meir, Kanner, Akiri, & Philosoph-Hadas, 1995). The antioxidant activity of a putative antioxidant has been attributed to various mechanisms, among which are prevention chain initiation, binding of transition metal ion catalyst, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging (Yildirim, Mavi, & Kara, 2001). 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 Radical Scavenging Activity*

Free radical scavenging effect of the compounds **2** was estimated by DPPH radical model. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability (Baumann, Wurn, & Bruchlausen, 1979). DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares, Dinis, Cunha, & Almeida, 1997). The reduction capability of DPPH radicals was determined by decrease in its absorbance at 517 nm induced by antioxidants. BHT, BHA and α-tocopherol were used as a reference to antioxidant compounds. Compounds **2a-f** did not show any ability.

*Iron Binding Capacity*

The chelating of ferrous ions by the compounds **2** and references was measured. 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 (Yamaguchi, Ariga, Yoshimura, & Nakazawa, 2000). The transition metals ions play an important role as catalysts of oxidative process, leading to formation of hydroxyl radicals and hydroperoxide decomposition reaction via Fenton chemistry (Halliwell, 1996). The production of these radicals may lead to lipid peroxidation, protein modification and DNA damage. Chelating agents are effective as secondary antioxidants because they potentially inhibit the metal-dependent processes thereby stabilizing the oxidized form of the metal ion (Finefrock, Bush, & Doraiswamy, 2003). Iron binding activities of the compounds **2**, BHT, BHA and α-tocopherol are shown in Figure 2.

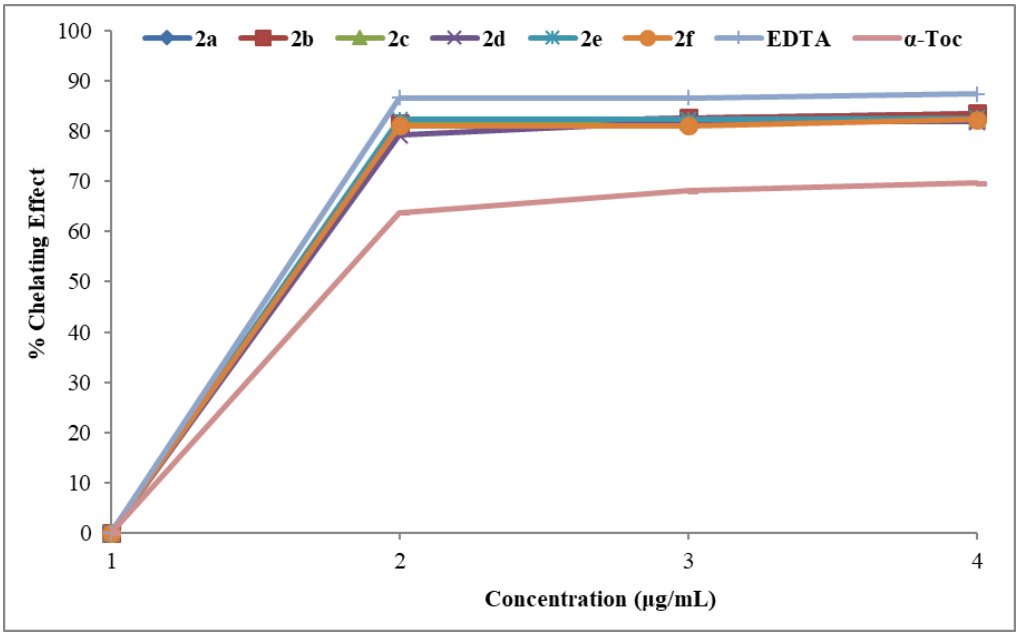



Figure 1. Metal chelating effect of the compounds 2a-f, EDTA and α-tocopherol on ferrous ions

In the current paper, high iron binding capacity of synthesized compounds would be beneficial in retarding metal-chelating oxidation. The data acquired from Figure 1 disclose that the metal chelating effects of all the

compounds (**2)** were significant and concentration-dependent. The metal chelating effect of the compounds and references decreased in order of EDTA > 2a > 2b = 2e > 2c = 2d > 2f > α-tocopherol which were 86.6, 82.7, 82.3, 82.3, 81.9, 81.9, 81.1, 68.1 (%), at the 30 µg/mL, respectively.

**Antimicrobial Activity**

The microbiological results are summarized in Table I. Microbiology results are promising; all the compounds showed very good antimicrobial activity against to Bacillus subtilis (ATCC-11774) and Bacillus cereus (ATCC-11778). However, not all compounds showed any activity against other microorganisms.

Table 1. Antimicrobial activity of the compounds 2.

| Code | Compounds | Bs | Bc | Pa | Kp | Sa | Ec |
|---|---|---|---|---|---|---|---|
| 1 | 2a | 18 | 21 | - | - | - | - |
| 2 | 2b | 21 | 23 | - | - | - | - |
| 3 | 2c | 22 | 20 | - | - | - | - |
| 4 | 2d | 18 | 16 | - | - | - | - |
| 5 | 2e | 19 | 17 | - | - | - | - |
| 6 | 2f | 21 | 20 | - | - | - | - |
| A | Ampicillin X3261 | 33 | 36 | 36 | 35 | 37 | 34 |
| N | Neomycin X3385 | 17 | 17 | 17 | 16 | 13 | 16 |
| S | Streptomycin X3385 | 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).

## Conclusion

In the present study, new Mannich bases derivatives with 1,2,4-triazole moiety (**2a-f**) were designed and synthesized. Their structures were identified using IR, $^{1}$H NMR and $^{13}$C NMR spectral data. The entire target compounds were also investigated for their antioxidant and antimicrobial potential. All of the compounds demonstrate a marked ability for metal chelating activity. The data reported with regard to the observed metal chelating activities of the studied compounds could prevent redox cycling. The results may also give several advices for the improvement of new triazole-based therapeutic target. Also, The synthesized compounds showed very good antimicrobial activity against to *Bacillus subtilis* and *Bacillus cereus*.

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