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Research Article

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In Vitro Antimicrobial and Antioxidant Properties of Some Novel *N,N´*-Bis-{3-alkyl-4-[3-(2-methylbenzoxy)-4-methoxybenzylideneamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-on-1-yl-methyl}-piperazines

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Abstract In this study, 3-alkyl-4-[3-(2-methylbenzoxy)-4-methoxybenzylidenamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**3**) were treated with piperazine in the presence of formaldehyde by the Mannich reaction to obtain novel *N,N´*-bis-{3-alkyl-4-[3-(2-methylbenzoxy)-4-methoxybenzylideneamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-on-1-yl-methyl}-piperazines **(4)**. The structures of new compounds were characterized from IR, ^{1}H NMR and ^{13}C NMR spectral data. In addition, *in vitro* antibacterial activities of the new compounds were evaluated against six bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus* and *Klepsiella pneumonia* according to agar well diffusion method. Furthermore, the newly synthesized compounds were analyzed for their *in vitro* potential antioxidant activities in three different methods; including 1,1-diphenlyl-2-picryl-hydrazyl free radical (DPPH·) scavenging, reducing activity by Fe^{+3} – Fe^{+2} transformation and ferrous metal (Fe^{+2}) chelating activities.

Keywords: *1,2,4-Triazol-5-one, Mannich base, antimicrobial activity, antioxidant activity*

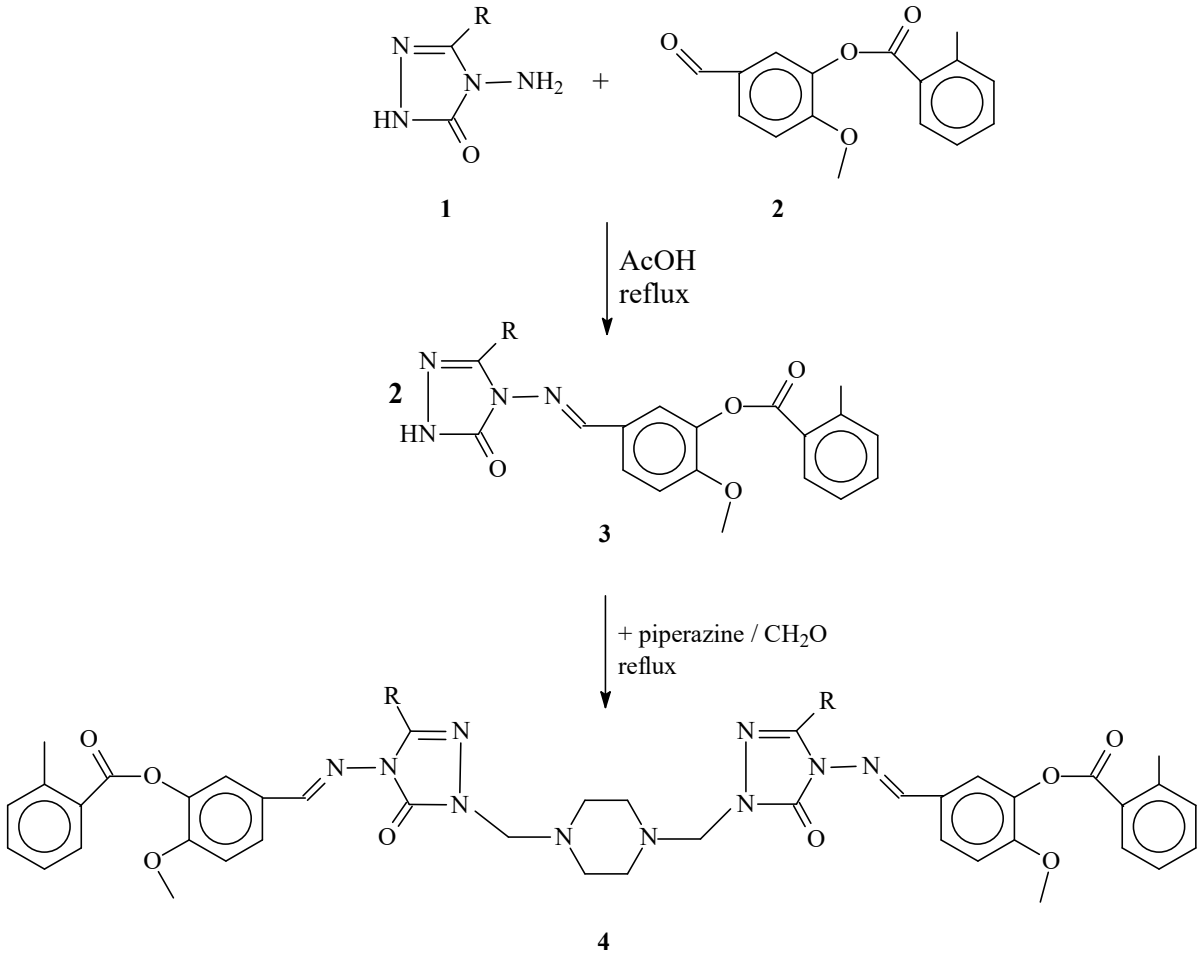
Introduction

The classical Mannich reaction, a three-component condensation between structurally diverse substrates containing at least one active hydrogen atom, an aldehyde component and an amine reagent leads to a class of compounds known as Mannich bases [1]. Mannich bases have applications in the pharmaceutical field and in other industries, such as the petroleum, the cosmetics, the dyes, and the food industries, etc. The principal advantage of the Mannich reaction is that it enables two different molecules to be bonded together in one step [2]. Mannich bases acquired from 1,2,4-triazole derivatives are reported to possess biological activities including, antifungal, antioxidant, antilipase and antibacterial properties [3-6].

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 [7–10]. 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 piperazine ring and to evaluate their antimicrobial and antioxidant activity.

In the present study, a series of *N,N´*-bis-{3-alkyl-4-[3-(2-methylbenzoxy)-4-methoxybenzylideneamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-on-1-yl-methyl}-piperazines **(4)** were synthesized from the reactions of 3-alkyl-4-[3-(2-methylbenzoxy)-4-methoxybenzylidenamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**3**) with piperazine in the presence of formaldehyde. The starting compounds 3-alkyl-4-[3-(2-methylbenzoxy)-4-methoxybenzylidenamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**3**) were prepared from the reactions of 3-alkyl-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**1**) with 3-(2-methylbenzoxy)-4-methoxybenzaldehyde (**2**) as described in the literature (Scheme **1**)

[11]. The structures of new compounds were identified by using IR, ^{1}H NMR and ^{13}C NMR spectral data. In addition, *in-vitro* antimicrobial properties of novel heterocyclic compounds were investigated and evaluated against six different microorganisms with agar well diffusion method. On the other hand, the synthesized newly synthesized compounds were analyzed for their *in vitro* potential antioxidant activities in three different methods.



a) R = CH_3, b) R = CH_2CH_3, c) R = $CH_2C_6H_5$, d) R = $CH_2C_6H_4CH_3$ (*p*-), e) R = $CH_2C_6H_4Cl$ (*p*-), f) R = C_6H_5

*Scheme 1: Synthetic route of compounds **4***

Materials and Methods

Chemistry

Chemical reagents used in the study were supplied from Sigma (Sigma-Aldrich GmbH, Germany), Fluka (Switzerland) and Merck AG, (Germany). 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-d6) using a Bruker spectrometer (Germany) at 400 MHz and 100 MHz, respectively.

General Procedure for the Synthesis of *N,N´*-bis-{3-alkyl-4-[3-(2-methylbenzoxy)-4-methoxybenzylideneamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-on-1-yl-methyl}-piperazines (4)

The corresponding compound **3** (10 mmol) was dissolved in 100 mL of ethanol followed by addition of piperazine (5 mmol) and formaldehyde (37%, 15mmol). The reaction mixture was refluxed for 3 hours. After standing at RT overnight, the solid was filtered and crystallized from ethanol. The solid was recrystallized from the same solvent and purified by drying *in vacuo* to obtain pure compounds **4** as colourless crystals.

***N,N´*-Bis-{3-methyl-4-[3-(2-methylbenzoxy)-4-methoxybenzylideneamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-on-1-yl-methyl}-piperazine (4a)**

Yield: 88%, m.p. 264-266 °C; IR (cm^{-1}) υ_{max}: 1737, 1690 (C=O), 1606, 1570 (C=N), 1271 (COO), 750 (*o*-disubstitue benzenoid ring); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2,34 (s, 6H, $2CH_3$), 2,68 (s, 6H, $2PhCH_3$), 2,77 (s, 8H, $4CH_2$), 3,89 (s, 6H, $2OCH_3$), 4,62 (s, 4H, $2NCH_2N$), 7,05 (d, 2H, ArH, *J* = 8.4 Hz), 7,26 (s, 2H, ArH), 7,33 (t, 2H, ArH, *J* = 7.6 Hz), 7,47-7,51 (m, 2H, ArH), 7,57 (dd, 2H, ArH, *J* = 8.4, 2.0 Hz), 7,69 (d, 2H, ArH, *J* = 2.0 Hz), 8,18-8,20 (m, 2H, ArH), 9,77 (s, 2H, 2N=CH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 11,38 ($2CH_3$), 21,73 ($2PhCH_3$), 49,95 ($4CH_2$), 56,09 ($2OCH_3$), 66,27 ($2NCH_2N$), [112,17 (2C), 121,23 (2C), 125,89 (2C), 127,02 (2C), 128,33 (2C), 128,44 (2C), 131,34 (2C), 131,86 (2C), 132,70 (2C), 140,56 (2C), 141.27 (2C), 153.03 (2C)] (ArC), 143,78 (2Triazole C-3), 151,10 (2Triazole C-5), 154,00 (2N=CH), 165,24 (2COO).

***N,N´*-Bis-{3-ethyl-4-[3-(2-methylbenzoxy)-4-methoxybenzylideneamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-on-1-yl-methyl}-piperazine (4b)**

Yield: 93%, m.p. 261-263 °C; IR (cm^{-1}) υ_{max}: 1739, 1693 (C=O), 1606, 1587 (C=N), 1272 (COO), 761 (*o*-disubstitue benzenoid ring); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1,28 (t, 6H, $2CH_2CH_3$, *J* = 7.6 Hz), 2,68 (s, 6H, $2PhCH_3$), 2,74 (q, 4H, $2CH_2CH_3$, *J* = 7.6 Hz), 2,78 (s, 8H, $4CH_2$), 3,89 (s, 6H, $2OCH_3$), 4,65 (s, 4H, $2NCH_2N$), 7,05 (d, 2H, ArH, *J* = 8.4 Hz), 7,26 (s, 2H, ArH), 7,33 (t, 2H, ArH, *J* = 7.6 Hz), 7,47-7,51 (m, 2H, ArH), 7,57 (dd, 2H, ArH, *J* = 8.4, 2.0 Hz), 7,68 (d, 2H, ArH, *J* = 2.0 Hz), 8,18-8,21 (m, 2H, ArH), 9,77 (s, 2H, 2N=CH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 10,52 ($2CH_2\underline{C}H_3$), 19,10 ($2\underline{C}H_2CH_3$), 21,73 ($2PhCH_3$), 49,98 ($4CH_2$), 56,09 ($2OCH_3$), 66,29 ($2NCH_2N$), [112,19 (2C), 121,21 (2C), 125,89 (2C), 127,11 (2C), 128,25 (2C), 128,45 (2C), 131,34 (2C), 131,86 (2C), 132,69 (2C), 140,56 (2C), 141.27 (2C), 152.95 (2C)] (ArC), 147,71 (2Triazole C-3), 151,26 (2Triazole C-5), 154,04 (2N=CH), 165,23 (2COO).

***N,N´*-Bis-{3-benzyl-4-[3-(2-methylbenzoxy)-4-methoxybenzylideneamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-on-1-yl-methyl}-piperazine (4c)**

Yield: 84%, m.p. 256-258 °C; IR (cm^{-1}) υ_{max}: 1740, 1695 (C=O), 1607, 1578 (C=N), 1242 (COO), 762 (*o*-disubstitue benzenoid ring), 762 and 702 (monosubstitue benzenoid ring); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2,69 (s, 6H, $2PhCH_3$), 2,78 (s, 8H, $4CH_2$), 3,89 (s, 6H, $2OCH_3$), 4,06 (s, 4H, $2CH_2Ph$), 4,67 (s, 4H, $2NCH_2N$), 7,04 (d, 2H, ArH, *J* = 8.4 Hz), 7.19-7.23 (m, 2H, ArH), 7,26-7.36 (m, 12H, ArH), 7,48-7,50 (m, 4H, ArH), 7,52 (d, 2H, ArH, *J* = 1.6 Hz), 8,18-8,20 (m, 2H, ArH), 9,70 (s, 2H, 2N=CH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 21,72 ($2PhCH_3$), 31,82 ($2CH_2Ph$), 49,98 ($4CH_2$), 56,09 ($2OCH_3$), 66,48 ($2NCH_2N$), [112,21 (2C), 121,53 (2C), 125,91 (2C), 127,00 (2C), 128,03 (2C), 128,52 (2C), 131,31 (2C), 131,86 (2C), 132,69 (2C), 140,49 (2C), 141.21 (2C), 152.91 (2C)] (ArC), [127,00 (2C), 128,62 (4C), 128,90 (4C), 135,37 (2C)] (ArC linked to C-3), 145,52 (2Triazole C-3), 151,15 (2Triazole C-5), 154,04 (2N=CH), 165,23 (2COO).

***N,N´*-Bis-{3-(*p*-methylbenzyl)-4-[3-(2-methylbenzoxy)-4-methoxybenzylideneamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-on-1-yl-methyl}-piperazine (4d)**

Yield: 94%, m.p. 257-259 °C; IR (cm^{-1}) υ_{max}: 1740, 1698 (C=O), 1610, 1588 (C=N), 1243 (COO), 813 (*p*-disubstitue benzenoid ring), 776 (*o*-disubstitue benzenoid ring); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2,27 (s, 6H, $2PhCH_3$-*p*), 2,69 (s, 6H, $2PhCH_3$-*o*), 2,78 (s, 8H, $4CH_2$), 3,89 (s, 6H, $2OCH_3$), 4,02 (s, 4H, $2CH_2Ph$), 4,66 (s, 4H, $2NCH_2N$), 7,03-7.09 (m, 6H, ArH), 7.33-7.36 (m, 4H, ArH), 7,48-7,53 (m, 4H, ArH), 7,56 (d, 2H, ArH, *J* = 2.0 Hz), 8,18-8,20 (m, 2H, ArH), 9,70 (s, 2H, 2N=CH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 21,00 ($2PhCH_3$-*p*), 21.73 ($2PhCH_3$-*o*), 31,41 ($2CH_2Ph$), 49,98 ($4CH_2$), 56,09 ($2OCH_3$), 66,45 ($2NCH_2N$), [112,20 (2C), 121,54 (2C), 125,91 (2C), 127,06 (2C), 128,03 (2C), 128,79 (2C), 131,32 (2C), 131,87 (2C), 132,69 (2C), 140,49 (2C), 141.22 (2C), 152.85 (2C)] (ArC), [128,52 (4C), 129,31 (4C), 132,25 (2C), 136,59 (2C)] (ArC linked to C-3), 145,73 (2Triazole C-3), 151,16 (2Triazole C-5), 154,01 (2N=CH), 165,23 (2COO).

***N,N´*-Bis-{3-(*p*-chlorobenzyl)-4-[3-(2-methylbenzoxy)-4-methoxybenzylideneamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-on-1-yl-methyl}-piperazine (4e)**

Yield: 94%, m.p. 258-260 °C; IR (cm^{-1}) υ_{max}: 1735, 1698 (C=O), 1609, 1588 (C=N), 1241 (COO), 809 (*p*-disubstitue benzenoid ring), 775 (*o*-disubstitue benzenoid ring); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2,69 (s, 6H, 2PhCH_3), 2,77 (s, 8H, 4CH_2), 3,89 (s, 6H, 2OCH_3), 4,02 (s, 4H, 2CH_2Ph), 4,66 (s, 4H, 2NCH_2N), 7,05 (d, 2H, ArH, *J* = 8.8 Hz), 7.26 (m, 8H, ArH), 7.33-7.37 (m, 4H, ArH), 7,48-7,54 (m, 4H, ArH), 7,59 (d, 2H, ArH, *J* = 1.6 Hz), 8,19-8,27 (m, 2H, ArH), 9,70 (s, 2H, 2N=CH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 21.73 (2PhCH_3), 31,19 (2CH_2Ph), 49,96 (4CH_2), 56,11 (2OCH_3), 66,49 (2NCH_2N), [112,25 (2C), 121,42 (2C), 125,94 (2C), 126,83 (2C), 128,12 (2C), 128,44 (2C), 131,34 (2C), 131,89 (2C), 132,74 (2C), 140,54 (2C), 141.27 (2C), 153.17 (2C)] (ArC), [128,80 (4C), 130,27 (4C), 132,99 (2C), 133,76 (2C)] (ArC linked to C-3), 145,07 (2Triazole C-3), 151,08 (2Triazole C-5), 154,15 (2N=CH), 165,22 (2COO).

***N,N´*-Bis-{3-phenyl-4-[3-(2-methylbenzoxy)-4-methoxybenzylideneamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-on-1-yl-methyl}-piperazine (4f)**

Yield: 90%, m.p. 269-271 °C; IR (cm^{-1}) υ_{max}: 1743, 1695 (C=O), 1610, 1573 (C=N), 1242 (COO), 767 (*o*-disubstitue benzenoid ring), 767 and 687 (monosubstitue benzenoid ring); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2,67 (s, 6H, 2PhCH_3), 2,87 (s, 8H, 4CH_2), 3,89 (s, 6H, 2OCH_3), 4,79 (s, 4H, 2NCH_2N), 7,06 (d, 2H, ArH, *J* = 8.4 Hz), 7.31-7.34 (m, 4H, ArH), 7.45-7.50 (m, 8H, ArH), 7,60-7,63 (m, 4H, ArH), 7,92-7.94 (m, 4H, ArH), 8,16-8,18 (m, 2H, ArH), 9,74 (s, 2H, 2N=CH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 21.70 (2PhCH_3), 50,04 (4CH_2), 56,11 (2OCH_3), 66,77 (2NCH_2N), [112,28 (2C), 121,84 (2C), 125,89 (2C), 126,90 (2C), 128,38 (2C), 128,50 (2C), 131,31 (2C), 131,84 (2C), 132,66 (2C), 140,53 (2C), 141.20 (2C), 154.18 (2C)] (ArC), [126,52 (2C), 128,20 (4C), 128,58 (4C), 130,17 (2C)] (ArC linked to C-3), 144,27 (2Triazole C-3), 151,39 (2Triazole C-5), 154,90 (2N=CH), 165,20 (2COO).

Antimicrobial activity

The antibacterial properties of the newly synthesized compounds were investigated by measuring the inhibition zone diameters. The bacterial strains required for the study, which are common infectious agents, were obtained from France. Microbiological Environmental Protection Laboratories has been reported as the company where these bacteria were produced. In the antibacterial investigation of the study, three different gram-positive bacteria strains (*Bacillus subtilis* ATCC-11774; *Bacillus cereus* ATCC-11778; *Staphylococcus aureus* ATCC-6538) and three different gram-negative bacterial strains (*Pseudomonas aeruginosa* ATCC-27853; *Klebsiella pneumonia* ATCC-4352; *Escherichia coli* ATCC-25922) were selected. Agar well diffusion method was preferred as the appropriate method for antibacterial effect studies [12,13]. Details on the application of the method were given literature [8,10]. Streptomycin (3385), Ampicillin (3261) and Neomycin (3360) as standard antibiotics were used to compare the effect levels of the synthesized compounds. DMSO was used as solved control.

Antioxidant activity

Butylated hydroxytoluene (BHT) was obtained from E. Merck. Ferrous chloride, α-tocopherol, 1,1-diphenyl-2-picryl-hydrazyl (DPPH·), 3-(2-pyridyl)-5,6-bis(phenylsulfonic acid)-1,2,4-triazine (ferrozine), butylated hydroxyanisole (BHA), ethylenediaminetetraacetic acid (EDTA) and trichloroacetic acid (TCA) were obtained from Sigma–Aldrich.

Reducing power: The reducing power of the synthesized compounds was determined according to the method of Oyaizu [14]. Different concentrations of the samples (50-250 µg/mL) in DMSO (1 mL) were mixed with phosphate buffer (2.5 mL, 0.2 M, pH = 6.6) and potassium ferricyanide (2.5 mL, 1%). The mixture was incubated at 50°C for 20 min and afterwards a portion (2.5 mL) of trichloroacetic acid (10%) was added to the mixture, which was centrifuged for 10 min at 1000 x g. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%), and then the absorbance at 700 nm was measured in a spectrophometer. Higher absorbance of the reaction mixture indicated greater reducing power.

Free radical scavenging activity: Free radical scavenging activity of compounds was measured by DPPH., using the method of Blois [15]. Briefly, 0.1 mM solution of DPPH· in ethanol was prepared, and this solution (1 mL) was added to sample solutions in DMSO (3 mL) at different concentrations (50-250 µg/mL). The mixture was shaken vigorously and allowed to remain at the room temperature for 30 min. Then, the absorbance was measured at 517 nm in a spectrophometer. The lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The DPPH· concentration (mM) in the reaction medium was calculated from the following calibration curve and determined by linear regression (R: 0.997): Absorbance = (0.0003 × DPPH·) – 0.0174.
The capability to scavenge the DPPH radical was calculated by using the following equation: DPPH· scavenging effect (%) = $(A_0 – A_1/A_0) \times 100$, where A_0 is the absorbance of the control reaction, and A_1 is the absorbance in the presence of the samples or standards.

Metal chelating activity: The chelation of ferrous ions by the synthesized compounds and standards were estimated by the method of Dinis et al [16]. Shortly, the synthesized compounds (50-250 µg mL^{-1}) were added to a 2 mM solution of $FeCl_2$ (0.05 mL). The reaction was initiated by the addition of 5 mM ferrozine (0.2 mL), and then the mixture was shaken vigorously and left remaining at the room temperature for 10 min. After the mixture had reached equilibrium, the absorbance of the solution was measured at 562 nm in a spectrophotometer. All tests and analyses were carried out in triplicate and averaged. The percentage of inhibition of ferrozine-Fe^{2+} complex formation was given by the formula: Inhibition% = $(A_0 - A_1/A_0) \times 100$, where A_0 is the absorbance of the control, and A_1 is the absorbance in the presence of the samples or standards. The control did not contain compound or standard.

Results and Discussion

Chemistry

In the present work, nine novel *N,N′*-bis-{3-alkyl-4-[3-(2-methylbenzoxy)-4-methoxybenzylideneamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-on-1-yl-methyl}-piperazines **(4)** were synthesized. The structures of compounds **4** were identified by using IR, ^{1}H NMR and ^{13}C NMR spectral data.

Antimicrobial activity

The microbiological results are summarized in Table 1.

Table 1: Antimicrobial activity of the compounds 4a-4f

	Microorganisms and inhibition zone (mm)					
Compounds	**Bs**	**Bc**	**Pa**	**Kp**	**Sa**	**Ec**
4a	16 (++)	**19 (+++)**	16 (++)	11 (++)	13 (++)	13 (++)
4b	14 (++)	13 (++)	14 (++)	-	10 (+)	16 (++)
4c	**19 (+++)**	15 (++)	11 (++)	-	**17 (+++)**	11 (++)
4d	10 (+)	13 (++)	13 (++)	-	10 (+)	**17 (+++)**
4e	12 (++)	14 (++)	14 (++)	-	10 (+)	**19 (+++)**
4f	**21 (+++)**	**17 (+++)**	15 (++)	10 (+)	14 (++)	11 (++)
Amp.	33	36	36	35	37	34
Neo.	17	17	17	16	13	16
Str.	12	12	12	11	21	10

Bs: *Bacillus subtilis* (ATCC-11774), Bc: *Bacillus ce*reus (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 (X3385), Str.: Streptomycin (X3385).

When the synthesized compounds are examined in general, a higher effect is observed against Gram-positive bacteria. While high effect of compounds **4c,f** was observed against *B. subtilis*, moderate effect of compounds **4a,b,e** was determined. It was determined that only compound **4d** showed low effect. The effects of compounds **4a,f** were high against *B. cereus* strain. A moderate effect was observed in compounds **4b-e**. Compound **4c** showed high efficacy for the other Gram-positive bacteria, *S.aureus*, while moderate efficacy was obtained for compounds **4a,f**. The effect of the remaining compounds **4b,d,e** were low.
Considering the Gram-negative bacteria *E.coli*, the high effect of compounds **4d,e** was observed. The effect of the other compounds **4a-c,f** was moderate. The effect of all compounds was moderate against *P. aeruginosa* strain. *K.*

pneumoniae was observed as the most resistant bacteria. Only moderate effect of compound **4a** and low effect of compound **4f** was determined.

When compared with standard antibiotics, effect values close to Neomycin and Streptomycin were obtained. For some compounds, however, the higher efficacy than standard antibiotics was an encouraging result.

Evaluation of results according to inhibition diameter: <5.5 mm negative effect (-); 5.5-10 mm low effect (+); 11-16 mm moderate effect (++); ≥17 mm high effect (+++) [17].

Antioxidant activity

The antioxidant activities of fifteen new compounds **4** were determined. Several methods have been used to determine antioxidant activities and the methods used in the study are given below:

Total reductive capability using the potassium ferricyanide reduction method

The reductive capabilities of compounds were assessed by the extent of conversion of the Fe^{3+} / ferricyanide complex to the Fe^{2+} / ferrous form. The reducing powers of the compounds were observed at different concentrations, and results were compared with BHT, BHA and α-tocopherol. It has been observed that the reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity [18]. The antioxidant activity of putative antioxidant has been attributed to various mechanisms, among which are prevention chain initiation, binding of transition metal ion catalyst, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging [19]. In the study, examined compounds did not show the reductive activities. In other words, all the amount of the compounds showed lower absorbance than standard antioxidants such as BHT, BHA and α-tocopherol. Hence, no activities were observed to reduce metal ions complexes to their lower oxidation state or to take part in any electron transfer reaction.

DPPH radical scavenging activity

The model of scavenging the stable DPPH radical model is a widely used method to evaluate antioxidant activities in a relatively short time compared with other methods. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability [20]. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [21]. The reduction capability of DPPH radicals was determined by decrease in its absorbance at 517 nm induced by antioxidants. The absorption maximum of a stable DPPH radical in ethanol was at 517 nm. The decrease in absorbance of DPPH radical was caused by antioxidants because of reaction between antioxidant molecules and radical, progresses, which resulted in the scavenging of the radical by hydrogen donation. It is visually noticeable as a discoloration from purple to yellow. Hence, DPPH· is usually used as a substrate to evaluate antioxidative activity of antioxidants [22]. Antiradical activities of compounds and standard antioxidants such as BHT, BHA and α-tocopherol were determined by using DPPH· method. The newly synthesized compounds did not show any scavenging effect.

Ferrous ion chelating activity

The chelating effect towards ferrous ions by the compounds and standards was determined. Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of chelating agents, the complex formation is disrupted with the result that the red colour of the complex is decreased. Measurement of colour reduction therefore allows estimation of the chelating activity of the coexisting chelator [23]. Transition metals have pivotal role in the generation oxygen free radicals in living organism. The ferric iron (Fe^{3+}) is the relatively biologically inactive form of iron. However, it can be reduced to the active Fe^{2+}, depending on condition, particularly pH [24] and oxidized back through Fenton type reactions with the production of hydroxyl radical or Haber-Weiss reactions with superoxide anions. The production of these radicals may lead to lipid peroxidation, protein modification and DNA damage. Chelating agents may not activate metal ions and potentially inhibit the metal-dependent processes [25].

Ferrous ion chelating activities of the compounds **4**, EDTA and α-tocopherol are shown in Figure **1**. The data obtained from the figure reveal that the metal chelating effects of the compounds **4** were concentration-dependent. Also, the compounds **4** demonstrate a marked capacity for iron binding. The metal chelating effect of these compounds and references decreased in order of EDTA > **4f** > **4d** ≈ **4e** > **4a** > **4b** > **4c** > α-tocopherol, which were 79.7, 77.1, 76.7, 76.2, 75.3, 71.4, 59.9 (%), at the highest concentration, respectively.

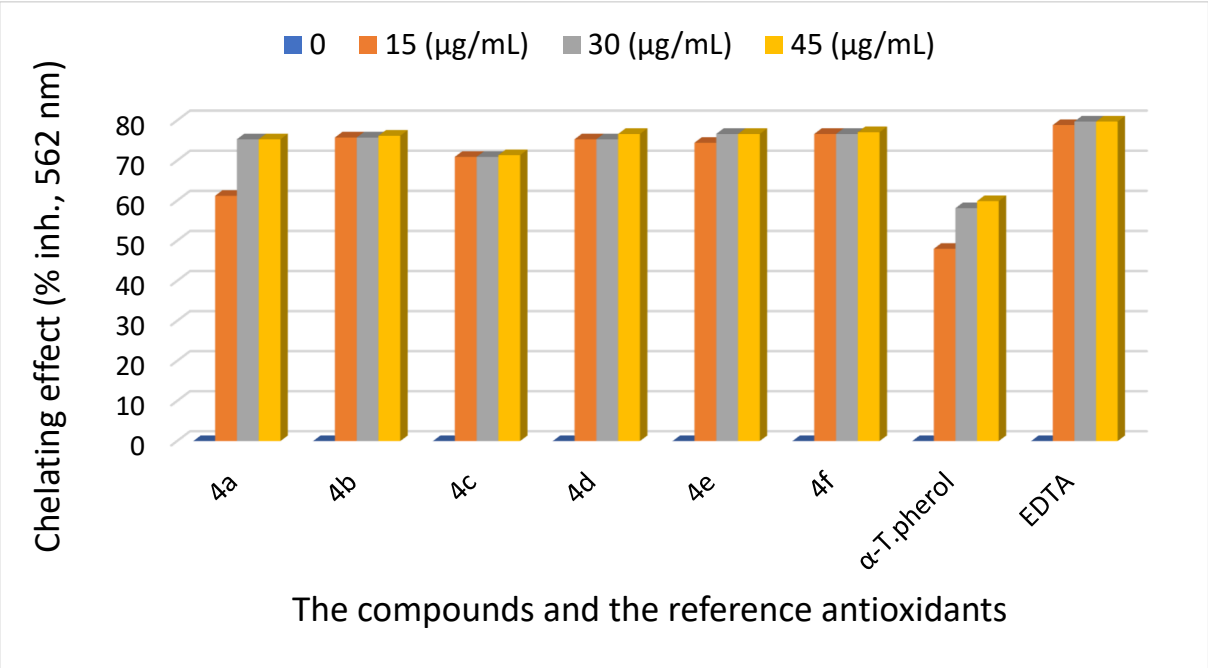


*Figure 1: Metal chelating effect of different amount of the compounds **4**, EDTA and α-tocopherol on ferrous ions.*

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References

[1]. G. Roman, Eur. J. Med. Chem, 2015; 89: 743-816.
[2]. M. Tramontini, L. Angiolini, Mannich Bases: Chemistry and Uses, CRC Press, Boca Raton, 1994.
[3]. S. Ceylan, S. Med. Chem. Res, 2016; 25: 1958–1970.
[4]. S. Boy, A. Aras, F. Türkan, O. Akyıldırım, M. Beytur, H. Sedef Karaman, S. Manap, H. Yüksek, Chem. Biodivers, 2021; 18: e2100433.
[5]. S. Manap, Ö. Gürsoy-Kol, M. Alkan, H. Yüksek, Indian J. Chem. - Section B, 2020; 59(2): 271–282.
[6]. S. Boy, F. Türkan, M. Beytur, A. Aras, O. Akyıldırım, H. Sedef Karaman, H. Yüksek, Bioorg. Chem. 2021; 107: 104524.
[7]. P. B. Miniyar, A. A. Mahajan, S. N. Mokale, M. U. Shah, A. S. Kumar, G. U. Chaturbhuj, Arabian J. Chem, 2017; 10(3): 295–299.
[8]. O. Aktaş-Yokuş, H. Yüksek, S. Manap, F. Aytemiz, M. Alkan, M. Beytur, O. Gürsoy-Kol, Bulgarian Chem. Commun, 2017; 49: 98-106.
[9]. H. Yüksek, O. Akyıldırım, M. L. Yola, Ö. Gürsoy-Kol, M. Çelebier, D. Kart, Archiv der Pharmazie, 2013; 346: 470–480.
[10]. O. Aktas-Yokus, H. Yuksek, O. Gursoy-Kol, S. Alpay-Karaoglu, Med. Chem. Res, 2015; 4: 2813–2824.
[11]. H. Yüksek, O. Kutanis, G. Özdemir, M. Beytur, S. Kara, Ö. Gürsoy-Kol, M. Alkan, Res. J. Pharm. Biol. Chem. Sci, 2018; 9(4): 501–512.
[12]. C. Perez, M. Pauli, P. Bazerque, Acta. Biol. Med. Exp, 1990; 15: 113.
[13]. Ahmad, Z. Mehmood, F. Mohammed, J. Ethnopharmacol, 1998; 62: 183.
[14]. M. Oyaizu, The Japanese Journal of Nutrition and Dietetics, 1986; 44(17): 307–315.
[15]. M. Blois, Nature, 1958; 181(4617): 1199–1200.

[16]. D. C. P. Dinis, V. M. C. Madeira, L. M. Almeida, Archives of Biochemistry and Biophysics, 1994; 315(1): 161–169.
[17]. N. Demirbaş, A. Ş. Karaoğlu, A. Demirbaş, K. Sancak, Eur. J. Med. Chem, 2004; 39: 793-804.
[18]. S. Meir, J. Kanner, B. Akiri, S. Philosoph-Hadas, Journal of Agricultural and Food Chemistry, 1995; 43(7): 1813–1819.
[19]. Yildirim, A. Mavi, A. A. Kara, J. Agr. Food Chem, 2001; 49: 4083-4089.
[20]. J. Baumann, G. Wurn, V. N-S. Bruchlausen, Archives of Pharmacology, 1979; 308: R27.
[21]. J. R. Soares, T. C. P. Dinis, A. P. Cunha, L. M. Almeida, Free Radical Research, 1997; 26(5): 469–478.
[22]. P. D. Duh, Y. Y. Tu, G. C. Yen, LWT - Food Science and Technology, 1999; 32(5): 269–277.
[23]. F. Yamaguchi, T. Ariga, Y. Yoshimura, H. Nakazawa, J. Agr. Food Chem, 2000; 48(2): 180–185.
[24]. M. Strlič, T. Radovič, J. Kolar, B. Pihlar, Agr. Food Chem, 2002; 50(22): 6313–6317.
[25]. E. Finefrock, A. I. Bush, P. M. Doraiswamy, Journal of the American Geriatrics Society, 2003; 51: 1143–1148.