



SYNTHESIS, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF NOVEL 4-(2-CINNAMOYLOXYBENZYLIDENAMINO)-4,5-DIHYDRO-1H-1,2,4-TRIAZOL-5-ONE DERIVATIVES

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ABSTRACT

In this study, nine novel 3-alkyl(aryl)-4-(2-cinnamoyloxy)-benzylidenamino-4,5-dihydro-1H-1,2,4-triazol-5-ones (**3a-i**), five novel 1-acetyl-3-alkyl(aryl)-4-(2-cinnamoyloxy)-benzylidenamino-4,5-dihydro-1H-1,2,4-triazol-5-ones (**4a-b**, **4e**, **4g**) and six novel 1-(morpholine-4-yl-methyl)-3-alkyl(aryl)-4-(2-cinnamoyloxy)-benzylidenamino-4,5-dihydro-1H-1,2,4-triazol-5-ones (**5a-e**, **5g**) were obtained and identified by spectral data. In addition, the newly synthesized twenty novel compounds were tested for their antioxidant activities using three different methods. Besides, the new compounds were screened for their antimicrobial properties.

KEYWORDS

Mannich base, Schiff base, 4,5-dihydro-1H-1,2,4-triazol-5-one, antioxidant activity, antimicrobial activity

INTRODUCTION

The Mannich reaction, a multi-component condensation uniting structurally diverse substrates containing an active hydrogen atom at least, an amine reagent and an aldehyde component lead to a group of the product named as Mannich baseⁱ. Mannich bases have several practices in the pharmaceutical industry and the other ones, like petroleum, dyes, food industries and cosmetics, etc. The Mannich reaction's main advantage is that it enables two dissimilar molecules to be bonded together in a single-stepⁱⁱ. Some Mannich bases acquired from derivatives of 1,2,4-triazoles have been known to possess antioxidant, antilipase, antifungal and antibacterial activities^{iii-v}.

A triazole refers to one of the heterocycles, is a five-membered ring system of three nitrogen with two carbon atoms. Some drugs which have the triazole nucleus are trazodone (anxiolytic and antidepressant), triazolam, alprazolam, estazolam (sedative, tranquilizer and hypnotic), terconazole (antifungal), etizolam (anxiolytic, amnesic, sedative, hypnotic, skeletal muscle

relaxant and anticonvulsant), trapidil (hypotensive), rilmazafon (anxiolytic and hypnotic), hexaconazole (antifungal) and rizatriptan (antimigraine)^{vi}. Some 1,2,4-triazoles and 4,5-dihydro-1*H*-1,2,4-triazol-5-ones have been reported to show a wide spectrum of pharmacological profile^{vii-ix}.

Antimicrobial resistance (AMR) is one of the serious global health threats. The rapid emergence and spread of drug-resistant bacteria require an important effort to design and identify new antibiotics^x. Given the circumstances, design, develop and obtain of novel heterocyclic compounds could play a major role. In this regard, an approach was made here to synthesize 1,2,4-triazoles incorporating a morpholine ring, after that their antioxidant and antimicrobial properties were evaluated.

EXPERIMENTAL

Synthesis – General procedures

Chemical reagents were obtained from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany), Merck AG (Darmstadt, Germany) and Fluka (Buchs, Switzerland). Melting points of titled compounds were identified using an Electrothermal digital melting point apparatus (United Kingdom) with open glass capillaries and 0.1 °C sensitivity. The infrared spectra were collected on a Perkin–Elmer Instruments Spectrum One FT-IR spectrometer (potassium bromide disks; Perkin Elmer, UK) in 400-4000 cm⁻¹ intervals, by scanning 24 times, the spectrum is created with its average. ¹³C and ¹H NMR spectra were measured in DMSO-d₆ with reference to TMS as internal standard on a Bruker Ultrashield spectrophotometer (Germany) at 100 MHz and 400 MHz, respectively.

*General method for the synthesis of 3-alkyl(aryl)-4-(2-cinnamoyloxy)-benzylidenamino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (3)*

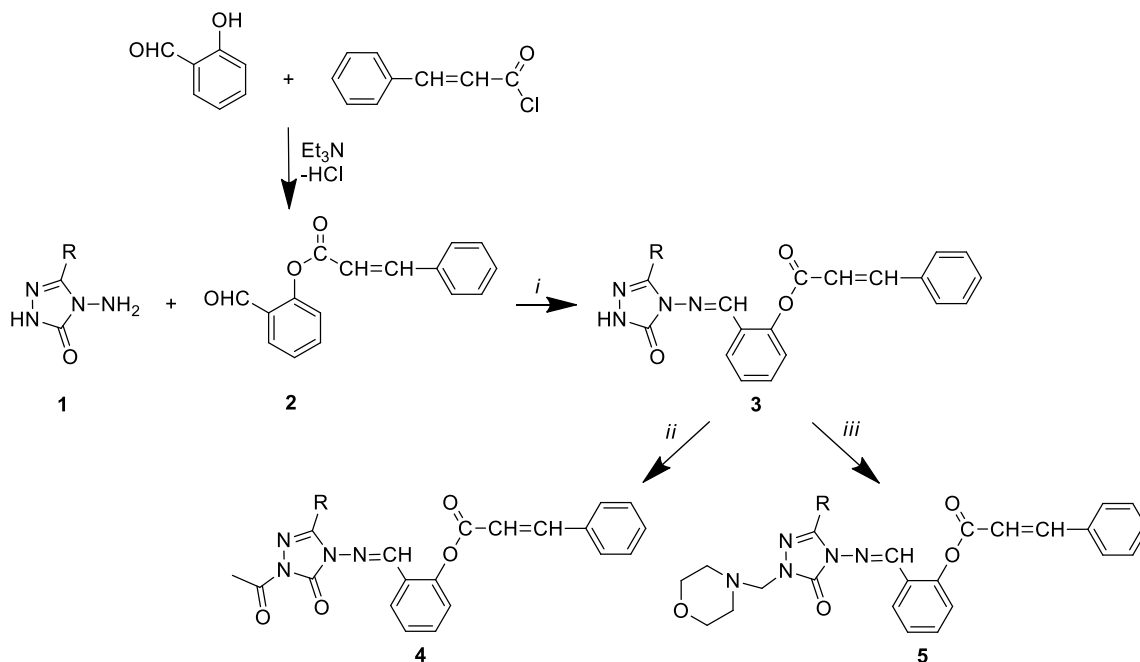
2-Hydroxybenzaldehyde (0.01 mol) dissolved in ethyl ethanoate (100 mL) was reacted with cinnamoyl chloride (0.01 mol). Triethylamine (0.01 mol) in 10 mL ethyl acetate was added to this solution slowly by stirring at 0-5 °C for 2 hours. After that, the mixture was refluxed for 3 hours and filtered. Then the filtrate was evaporated *in vacuo*, the crude product was washed with water and recrystallized from ethyl ethanoate-petroleum ether (1:3) to obtain novel compound **2**^{xi}. The related compound **1** (0.01 mol) was dissolved in ethanoic acid (20 mL) and then treated with 2-cinnamoyloxybenzaldehyde (**2**) (0.01 mol). After that, the mixture was refluxed for 1.5 hours and evaporated at 50-55 °C *in vacuo*. Several recrystallizations of the residue from ethyl alcohol gave pure colorless crystals as compounds **3** (**Scheme- 1**).

*General method for the synthesis of 1-acetyl-3-alkyl(aryl)-4-(2-cinnamoyloxy)-benzylidenamino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (4)*

The related compound **3** (0.01 mol) was refluxed with ethanoic anhydride (15 mL) for half an hour. Then absolute ethyl alcohol (50 mL) was added, the mixture was refluxed for one hour. The solution was evaporated *in vacuo* at 40-45 °C and the pure compounds **4** were obtained by several recrystallizations of the residue from ethanol (**Scheme- 1**).

*General method for the synthesis of 1-(morpholine-4-yl-methyl)-3-alkyl(aryl)-4-(2-cinnamoyloxy)-benzylidenamino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (5)*

The solution of the compound **3** (5 mmol) in absolute ethanol was added formaldehyde (% 37, 10 mmol) and an amine, morpholine (6 mmol). The reaction mixture was refluxed for four hours and then, the mixture was left at room temperature overnight. After cooling the mixture in the refrigerator, the solid formed was obtained by filtration. The filtrate was washed with cold ethyl alcohol and recrystallized from ethyl alcohol (**Scheme- 1**).



i) AcOH, reflux, 1h; ii) Ac₂O, reflux; iii) CH₂O, morpholine, reflux

a) R = CH₃, b) R = CH₂CH₃, c) R = CH₂CH₂CH₃, d) R = CH₂C₆H₅, e) R = CH₂C₆H₄CH₃ (*p*-),
f) R = CH₂C₆H₄OCH₃ (*p*-), g) R = CH₂C₆H₄Cl (*p*-), h) R = CH₂C₆H₄Cl (*m*-), i) R = C₆H₅

Scheme – 1

Antioxidant activity

In this part, the newly synthesized compounds and butylated hydroxytoluene (BHT), α -tocopherol, butylated hydroxyanisole (BHA), and ethylenediaminetetraacetic acid (EDTA) as standard antioxidants were evaluated for their antioxidant activities using three tests. BHT was obtained from E. Merck (Darmstadt, Germany). α -Tocopherol, ferrous chloride, 3-(2-pyridyl)-5,6-bis(phenylsulfonic acid)-1,2,4-triazine (ferrozine), 1,1-diphenyl-2-picryl-hydrazyl (DPPH \cdot), BHA, EDTA, and trichloroacetic acid (TCA) were purchased from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany).

Reducing power

The reducing power of the new compounds was estimated according to Oyaizu method^{xii}. The samples with different concentrations (50-250 μ g/mL) in DMSO (1 mL) were mixed with potassium ferricyanide (1%, 2.5 mL) and phosphate buffer (0.2 M, 2.5 mL, pH = 6.6). The mixture was incubated for 20 min. at 50°C. Then a portion of TCA (10%, 2.5 mL) 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 FeCl₃ (0.1%, 0.5 mL) and distilled water (2.5 mL). Then the absorbance was measured in a spectrophotometer at 700 nm.

Free radical scavenging activity

The free radical scavenging activity of the newly synthesized compounds was determined according to the method of Blois^{xiii}. Shortly, 0.1 mM solution of DPPH \cdot in ethanol was prepared. Then 1 mL of the solution was added to the sample solutions in 3 mL DMSO at different concentrations (50-250 μ g/mL). After the mixture was shaken vigorously, allowed to remain at the room temperature for 30 minutes. Then, the absorbance was measured in a spectrophotometer at 517 nm. The DPPH \cdot scavenging effect was calculated by the formula:

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

A₀ is the absorbance of the control reaction, A₁ is the absorbance in the presence of the samples or standards. The control samples did not contain the analyte.

Metal chelating activity

The chelation of ferrous ions by the newly synthesized compounds was measured according to the method of Dinis *et al.*^{xiv}. Shortly, the samples of different concentrations (50-250 µg/mL) were added to a solution of FeCl₂ (2 mM, 0.05 mL). The reaction was initiated by the addition of 0.2 mL, 5 mM ferrozine. Then the mixture was shaken vigorously, allowed to remain at room temperature for 10 minutes. After the mixture had reached equilibrium, the absorbance of the solution was measured in a spectrophotometer at 562 nm. The percentage of inhibition of the ferrozine-Fe²⁺ complex formation was calculated by the following equation:

$$\% \text{ Inhibition} = (A_0 - A_1/A_0) \times 100$$

A₀ is the absorbance of the control, and A₁ is the absorbance in the presence of the samples or standards. The control samples did not contain the analyte.

Antimicrobial activity

Antimicrobial properties of the synthesized compounds were screened. Bacterial strains used in the antibacterial studies were *Escherichia coli* (ATCC-25922), *Bacillus cereus* (ATCC-11778), *Bacillus subtilis* (ATCC-11774), *Klebsiella pneumonia* (ATCC-4352), *Pseudomonas aeruginosa* (ATCC-27853) and *Staphylococcus aureus* (ATCC-6538) standard strains. Used strains were acquired from the Microbiological Environmental Protection Laboratories (France). The test preferred in the study was the simple susceptibility screening test using the agar well diffusion method^{xv,xvi}, as explained in the literature^{viii}. All the new compounds were weighed and dissolved in DMSO to prepare extract stock solution of 1 mg/mL. Antibiotics used for comparison purposes in the study were Ampicillin X3261, Neomycin X3385 and Streptomycin X3385 discs. Antibiotic discs were purchased from Oxoid (UK).

RESULTS AND DISCUSSIONS

Experimental and spectroscopic data of the newly synthesized compounds **3a-i**, **4a-c**, **4e**, **4g**, **5a-e** and **5g** are presented in **Tables 1–3**.

Table –1. Physical and IR (cm⁻¹) data of the compounds **3-5**

Comp.	Yield, %	mp, °C	IR spectrum, cm ⁻¹
3a	99.0	206	756 and 707 (monosubstituted benzene), 1299 (COO), 1597 (C=N), 1634 (C=C), 1725, 1694 (C=O), 3064 (=CH), 3172 (NH)
3b	98.5	189	756 and 680 (monosubstituted benzene), 1288 (COO), 1590 (C=N), 1631 (C=C), 1706 (C=O), 3057 (=CH), 3175 (NH)
3c	98.4	182	755 and 694 (monosubstituted benzene), 1287 (COO), 1587 (C=N), 1630 (C=C), 1732, 1703 (C=O), 3059 (=CH), 3180 (NH)
3d	88.8	169	760 and 701 (monosubstituted benzene), 1284 (COO), 1586 (C=N), 1632 (C=C), 1733, 1707 (C=O), 3060 (=CH), 3175 (NH)
3e	99.4	184	758 and 707 (monosubstituted benzene), 822 (1,4-disubstituted benzene), 1285 (COO), 1597 (C=N), 1631 (C=C), 1747, 1699 (C=O), 3060 (=CH), 3153 (NH)
3f	98.8	185	761 and 682 (monosubstituted benzene), 806 (1,4-disubstituted benzene), 1299 (COO), 1597 (C=N), 1630 (C=C), 1737, 1696 (C=O), 3064 (=CH), 3156 (NH)
3g	97.1	176	759 and 708 (monosubstituted benzene), 797 (1,4-disubstituted benzene), 1277 (COO), 1597 (C=N), 1634 (C=C), 1709 (C=O), 3062 (=CH), 3187 (NH)

3h	97.6	169	757 and 682 (monosubstituted benzene), 864 and 790 (1,3-disubstituted benzene), 1281 (COO), 1580 (C=N), 1631 (C=C), 1707 (C=O), 3057 (=CH), 3180 (NH)
3i	97.6	177	758 and 684 (monosubstituted benzene), 1238 (COO), 1595 (C=N), 1628 (C=C), 1728, 1697 (C=O), 3057 (=CH), 3167 (NH)
4a	87.2	192	749 and 701 (monosubstituted benzene), 1271 (COO), 1605 (C=N), 1622 (C=C), 1737, 1716 (C=O), 3067 (=CH)
4b	61.5	154	759 and 699 (monosubstituted benzene), 1276 (COO), 1607 (C=N), 1625 (C=C), 1717 (C=O), 3064 (=CH)
4c	62.3	129	761 and 699 (monosubstituted benzene), 1266 (COO), 1603 (C=N), 1632 (C=C), 1729, 1713 (C=O), 3068 (=CH)
4e	83.1	178	766 and 685 (monosubstituted benzene), 823 (1,4-disubstituted benzene), 1298 (COO), 1601 (C=N), 1636 (C=C), 1730, 1716 (C=O), 3070 (=CH)
4g	86.8	177	767 and 685 (monosubstituted benzene), 823 (1,4-disubstituted benzene), 1299 (COO), 1603 (C=N), 1637 (C=C), 1737, 1717 (C=O), 3067 (=CH)
5a	85.6	163	761 and 702 (monosubstituted benzene), 1269 (COO), 1598 (C=N), 1631 (C=C), 1723, 1689 (C=O), 3036 (=CH)
5b	84.1	138	762 and 702 (monosubstituted benzene), 1266 (COO), 1593 (C=N), 1630 (C=C), 1723, 1694 (C=O), 3039 (=CH)
5c	72.6	121	763 and 702 (monosubstituted benzene), 1263 (COO), 1605 (C=N), 1630 (C=C), 1724, 1696 (C=O), 3041 (=CH)
5d	71.4	135	764 and 703 (monosubstituted benzene), 1265 (COO), 1602 (C=N), 1632 (C=C), 1736, 1712 (C=O), 3063 (=CH)
5e	83.3	157	764 and 680 (monosubstituted benzene), 811 (1,4-disubstituted benzene), 1264 (COO), 1601 (C=N), 1633 (C=C), 1735, 1711 (C=O), 3081 (=CH)
5g	81.7	147	762 and 682 (monosubstituted benzene), 802 (1,4-disubstituted benzene), 1263 (COO), 1601 (C=N), 1633 (C=C), 1735, 1716 (C=O), 3055 (=CH)

Table – 2. ¹H NMR Data of the compounds **3-5** (δ/ppm in DMSO-d₆)

Comp.	Chemical shifts
3a	11.82 (NH, 1H, singlet), 9.92 (N=CH, 1H, singlet), Arom. H: [8.02 (1H, doublet of doublets, <i>J</i> =7.60, 1.60Hz), 7.85-7.82 (2H, multiplet), 7.60-7.64 (1H, multiplet), 7.52-7.43 (4H, multiplet), 7.38 (1H, doublet, <i>J</i> =8.40Hz)], 7.94 (=CH, 1H, doublet, <i>J</i> =16.00 Hz), 6.95 (=CH, 1H, doublet, <i>J</i> =16.00Hz), 2.25 (CH ₃ , 3H, singlet).
3b	11.86 (NH, 1H, singlet), 9.93 (N=CH, 1H, singlet), Arom. H: [8.01 (1H, doublet of doublets, <i>J</i> =8.00, 1.60Hz), 7.86-7.81 (2H, multiplet), 7.64-7.60 (1H, multiplet), 7.55-7.42 (4H, multiplet), 7.38 (1H, doublet, <i>J</i> =8.40Hz)], 7.95 (=CH, 1H, doublet, <i>J</i> =16.00Hz), 6.96 (=CH, 1H, doublet, <i>J</i> =16.40 Hz), 2.65 (CH ₂ CH ₃ , 2H, quartet, <i>J</i> =7.60Hz), 1.16 (CH ₂ CH ₃ , 3H, triplet, <i>J</i> =7.60Hz).
3c	11.85 (NH, 1H, singlet), 9.91 (N=CH, 1H, singlet), Arom. H: [8.00 (1H, doublet of doublets, <i>J</i> =7.60, 1.60Hz), 7.87-7.83 (2H, multiplet), 7.64-7.60 (1H, multiplet), 7.51-7.44 (4H, multiplet), 7.37 (1H, doublet, <i>J</i> =8.40Hz)], 7.94 (=CH, 1H, doublet, <i>J</i> =16.00 Hz), 6.95 (=CH, 1H, doublet, <i>J</i> =16.00Hz), 2.62 (CH ₂ CH ₂ CH ₃ , 3H, triplet,

	$J=7.20\text{Hz}$), 1.64 ($\text{CH}_2\text{CH}_2\text{CH}_3$, 2H, sextet, $J=7.20\text{Hz}$), 0.90 ($\text{CH}_2\text{CH}_2\text{CH}_3$, 3H, triplet, $J=7.20\text{Hz}$).
3d	11.73 (NH, 1H, singlet), 9.90 (N=CH, 1H, singlet), Arom. H: [7.97 (1H, doublet of doublets, $J=7.60$, 1.60Hz), 7.83-7.81 (2H, multiplet), 7.63-7.59 (1H, multiplet), 7.49-7.34 (9H, multiplet), 7.25-7.21 (1H, multiplet)], 7.93 (=CH, 1H, doublet, $J=16.00\text{Hz}$), 6.94 (=CH, 1H, doublet, $J=16.00\text{Hz}$), 4.04 (CH_2Ph , 2H, singlet).
3e	11.95 (NH, 1H, singlet), 9.92 (N=CH, 1H, singlet), Arom. H: [7.99 (1H, doublet of doublets, $J=8.00$, 1.20Hz), 7.81-7.77 (2H, multiplet), 7.64-7.59 (1H, multiplet), 7.48-7.43 (4H, multiplet), 7.37 (1H, doublet, $J=8.00\text{Hz}$), 7.18 (2H, doublet, $J=8.00\text{Hz}$), 7.10 (2H, doublet, $J=7.60\text{Hz}$)], 7.93 (=CH, 1H, doublet, $J=16.00\text{Hz}$), 6.94 (=CH, 1H, doublet, $J=16.00\text{Hz}$), 3.98 (CH_2Ph , 2H, singlet), 2.24 (PhCH_3 , 3H, singlet).
3f	11.94 (NH, 1H, singlet), 9.89 (N=CH, 1H, singlet), Arom. H: [8.00 (1H, doublet of doublets, $J=8.00$, 1.60Hz), 7.83-7.81 (2H, multiplet), 7.64-7.60 (1H, multiplet), 7.51-7.44 (4H, multiplet), 7.37 (1H, doublet, $J=8.40\text{Hz}$), 7.21 (2H, doublet, $J=8.80\text{Hz}$), 6.86 (2H, doublet, $J=8.40\text{Hz}$)], 7.93 (=CH, 1H, doublet, $J=16.00\text{Hz}$), 6.94 (=CH, 1H, doublet, $J=16.00\text{Hz}$), 3.96 (CH_2Ph , 2H, singlet), 3.70 (OCH_3 , 3H, singlet).
3g	11.99 (NH, 1H, singlet), 9.90 (N=CH, 1H, singlet), Arom. H: [7.97 (1H, doublet of doublets, $J=8.00$, 1.60Hz), 7.83-7.81 (2H, multiplet), 7.64-7.60 (1H, multiplet), 7.51-7.31 (9H, multiplet)], 7.93 (=CH, 1H, doublet, $J=16.00\text{Hz}$), 6.94 (=CH, 1H, doublet, $J=16.00\text{Hz}$), 4.05 (CH_2Ph , 2H, singlet).
3h	11.99 (NH, 1H, singlet), 9.89 (N=CH, 1H, singlet), Arom. H: [7.98 (1H, doublet of doublets, $J=7.60$, 1.60Hz), 7.83-7.81 (2H, multiplet), 7.64-7.60 (1H, multiplet), 7.49-7.24 (9H, multiplet)], 7.92 (=CH, 1H, doublet, $J=16.00\text{Hz}$), 6.94 (=CH, 1H, doublet, $J=16.00\text{Hz}$), 4.07 (CH_2Ph , 2H, singlet).
3i	12.37 (NH, 1H, singlet), 9.86 (N=CH, 1H, singlet), Arom. H: [7.95 (1H, doublet of doublets, $J=8.00$, 1.60Hz), 7.80-7.78 (2H, multiplet), 7.90-7.87 (2H, multiplet), 7.53-7.38 (8H, multiplet), 7.66-7.59 (1H, multiplet)], 7.91 (=CH, 1H, doublet, $J=16.00\text{Hz}$), 6.86 (=CH, 1H, doublet, $J=16.00\text{Hz}$).
4a	9.76 (N=CH, 1H, singlet), Arom. H: [8.06 (1H, doublet of doublets, $J=8.00$, 1.20Hz), 7.87-7.84 (2H, multiplet), 7.66 (1H, triplet of doublets, $J=8.00$, 1.60 Hz), 7.54-7.48 (4H, multiplet), 7.41 (1H, doublet, $J=8.00\text{Hz}$)], 7.94 (=CH, 1H, doublet, $J=16.00\text{Hz}$), 6.97 (=CH, 1H, doublet, $J=16.00\text{Hz}$), 2.45 (COCH_3 , 3H, singlet), 2.27 (CH_3 , 3H, singlet).
4b	9.75 (N=CH, 1H, singlet), Arom. H: [8.02 (1H, doublet of doublets, $J=8.00$, 1.60Hz), 7.81-7.86 (2H, multiplet), 7.67 (1H, triplet of doublets, $J=8.00$, 1.60Hz), 7.52-7.47 (4H, multiplet), 7.40 (1H, doublet, $J=8.40\text{Hz}$)], 7.95 (=CH, 1H, doublet, $J=16.00\text{Hz}$), 6.96 (=CH, 1H, doublet, $J=16.00\text{Hz}$), 2.71 (CH_2CH_3 , 2H, quartet, $J=7.60\text{Hz}$), 2.44 (COCH_3 , 3H, singlet), 1.19 (CH_2CH_3 , 3H, triplet, $J=7.60\text{Hz}$).
4c	9.76 (N=CH, 1H, singlet), Arom. H: [8.02 (1H, doublet of doublets, $J=7.60$, 1.60Hz), 7.86-7.83 (2H, multiplet), 7.65 (1H, triplet of doublets, $J=8.00$, 1.60Hz), 7.50-7.45 (4H, multiplet), 7.40 (1H, doublet, $J=8.00\text{Hz}$)], 7.95 (=CH, 1H, doublet, $J=16.00\text{Hz}$), 6.95 (=CH, 1H, doublet, $J=16.00\text{Hz}$), 2.66 ($\text{CH}_2\text{CH}_2\text{CH}_3$, 3H, triplet, $J=7.20\text{Hz}$), 2.44 (COCH_3 , 3H, singlet), 1.68 ($\text{CH}_2\text{CH}_2\text{CH}_3$, 2H, sextet, $J=7.60\text{Hz}$), 0.93 ($\text{CH}_2\text{CH}_2\text{CH}_3$, 3H, triplet, $J=7.60\text{Hz}$).
4e	9.74 (N=CH, 1H, singlet), Arom. H: [7.99 (1H, doublet of doublets, $J=7.60$, 1.60Hz), 7.84-7.82 (2H, multiplet), 7.65 (1H, triplet of doublets, $J=8.00$, 1.60Hz), 7.49-7.44 (4H, multiplet), 7.40 (1H, doublet, $J=8.40\text{Hz}$), 7.23 (2H, doublet,

	$J=8.00\text{Hz}$), 7.12 (2H, doublet, $J=8.00\text{Hz}$), 7.94 (=CH, 1H, doublet, $J=16.00\text{Hz}$), 6.94 (=CH, 1H, doublet, $J=16.00\text{Hz}$), 4.07 (CH ₂ Ph, 2H, singlet), 2.43 (COCH ₃ , 3H, singlet), 2.25 (PhCH ₃ , 3H, singlet).
4g	9.75 (N=CH, 1H, singlet), Arom. H: [7.98 (1H, doublet of doublets, $J=8.00$, 1.60Hz), 7.84-7.82 (2H, multiplet), 7.65 (1H, triplet of doublets, $J=8.00$, 1.60Hz), 7.50-7.46 (4H, multiplet), 7.41-7.38 (5H, multiplet)], 7.94 (=CH, 1H, doublet, $J=16.00\text{Hz}$), 6.95 (=CH, 1H, doublet, $J=16.40\text{Hz}$), 4.14 (CH ₂ Ph, 2H, singlet), 2.43 (COCH ₃ , 3H, singlet).
5a	9.90 (N=CH, 1H, singlet), Arom. H: [8.04 (1H, doublet, $J=8.00\text{Hz}$), 7.85-7.83 (2H, multiplet), 7.65-7.61 (1H, multiplet), 7.49-7.44 (4H, multiplet), 7.38 (1H, doublet, $J=8.00\text{Hz}$)], 7.94 (=CH, 1H, doublet, $J=16.00\text{Hz}$), 6.95 (=CH, 1H, doublet, $J=16.00\text{Hz}$), 4.47 (NCH ₂ N, 2H, singlet), 3.51 (CH ₂ OCH ₂ , 4H, triplet, $J=4.80\text{Hz}$), 2.53 (CH ₂ NCH ₂ , 4H, triplet, $J=4.80\text{Hz}$), 2.28 (CH ₃ , 3H, singlet).
5b	9.89 (N=CH, 1H, singlet), Arom. H: [8.00 (1H, doublet of doublets, $J=8.00$, 1.20Hz), 7.86-7.83 (2H, multiplet), 7.63 (1H, multiplet), 7.49-7.46 (4H, multiplet), 7.38 (1H, doublet, $J=8.00\text{Hz}$)], 7.94 (=CH, 1H, doublet, $J=16.00\text{Hz}$), 6.95 (=CH, 1H, doublet, $J=16.00\text{Hz}$), 4.48 (NCH ₂ N, 2H, singlet), 3.51 (CH ₂ OCH ₂ , 4H, triplet, $J=4.40\text{Hz}$), 2.69 (CH ₂ CH ₃ , 2H, quartet, $J=7.60\text{Hz}$), 2.53 (CH ₂ NCH ₂ , 4H, triplet, $J=4.40\text{Hz}$), 1.17 (CH ₂ CH ₃ , 3H, triplet, $J=7.60\text{Hz}$).
5c	9.89 (N=CH, 1H, singlet), Arom. H: [8.01 (1H, doublet of doublets, $J=8.00$, 1.60Hz), 7.87-7.83 (2H, multiplet), 7.64-7.60 (1H, multiplet), 7.49-7.46 (4H, multiplet), 7.38 (1H, doublet, $J=8.00\text{Hz}$)], 7.94 (=CH, 1H, doublet, $J=16.00\text{Hz}$), 6.95 (=CH, 1H, doublet, $J=16.00\text{Hz}$), 4.49 (NCH ₂ N, 2H, singlet), 3.51 (CH ₂ OCH ₂ , 4H, triplet, $J=4.40\text{Hz}$), 2.65 (CH ₂ CH ₂ CH ₃ , 3H, triplet, $J=7.20\text{Hz}$), 2.53 (CH ₂ NCH ₂ , 4H, triplet, $J=4.40\text{Hz}$), 1.66 (CH ₂ CH ₂ CH ₃ , 2H, sextet, $J=7.20\text{Hz}$), 0.91 (CH ₂ CH ₂ CH ₃ , 3H, triplet, $J=7.20\text{Hz}$).
5d	9.87 (N=CH, 1H, singlet), Arom. H: [7.98 (1H, doublet of doublets, $J=8.00$, 1.60Hz), 7.83-7.81 (2H, multiplet), 7.64-7.60 (1H, multiplet), 7.49-7.31 (9H, multiplet), 7.25-7.22 (1H, multiplet)], 7.93 (=CH, 1H, doublet, $J=16.00\text{Hz}$), 6.93 (=CH, 1H, doublet, $J=16.00\text{Hz}$), 4.51 (NCH ₂ N, 2H, singlet), 4.09 (CH ₂ Ph, 2H, singlet), 3.51 (CH ₂ OCH ₂ , 4H, triplet, $J=4.40\text{Hz}$), 2.53 (CH ₂ NCH ₂ , 4H, triplet, $J=4.40\text{Hz}$).
5e	9.86 (N=CH, 1H, singlet), Arom. H: [7.99 (1H, doublet, $J=7.20$ Hz), 7.83-7.81 (2H, multiplet), 7.63-7.61 (1H, multiplet), 7.48-7.43 (4H, multiplet), 7.37 (1H, doublet, $J=8.00$ Hz), 7.19 (2H, doublet, $J=8.00\text{Hz}$), 7.11 (2H, doublet, $J=7.60\text{Hz}$)], 7.92 (=CH, 1H, doublet, $J=16.00\text{Hz}$), 6.93 (=CH, 1H, doublet, $J=16.00\text{Hz}$), 4.50 (NCH ₂ N, 2H, singlet), 4.03 (CH ₂ Ph, 2H, singlet), 3.51 (CH ₂ OCH ₂ , 4H, triplet, $J=4.40\text{Hz}$), 2.53 (CH ₂ NCH ₂ , 4H, triplet, $J=4.40\text{Hz}$), 2.25 (PhCH ₃ , 3H, singlet).
5g	9.88 (N=CH, 1H, singlet), Arom. H: [7.98 (1H, doublet, $J=7.20\text{Hz}$), 7.83-7.81 (2H, multiplet), 7.64-7.61 (1H, multiplet), 7.48-7.43 (4H, multiplet), 7.38-7.32 (5H, multiplet)], 7.93 (=CH, 1H, doublet, $J=16.00\text{Hz}$), 6.93 (=CH, doublet, 1H, $J=16.00\text{Hz}$), 4.50 (NCH ₂ N, 2H, singlet), 4.10 (CH ₂ Ph, 2H, singlet), 3.51 (CH ₂ OCH ₂ , 4H, triplet, $J=4.40\text{Hz}$), 2.52 (CH ₂ NCH ₂ , 4H, triplet, $J=4.40\text{Hz}$).

Table – 3. ^{13}C NMR Data of the compounds **3-5** (δ /ppm in DMSO- d_6)

Comp.	Chemical shifts
3a	11.01 (CH_3), [116.42, 123.48, 125.99, 126.48, 126.91, 128.69 (2C), 128.94 (2C), 130.98, 132.39, 133.67, 144.15, 149.70] (ArC + CH=CH), 147.08 (Triazole C_3), 148.15 (Triazole C_5), 151.11 (N=CH), 164.67 (COO).
3b	9.89 (CH_2CH_3), 18.43 (CH_2CH_3), [116.45, 123.54, 126.03, 126.52, 127.10, 128.69 (2C), 128.95 (2C), 130.99, 132.99, 133.70, 147.07, 149.67] (ArC + CH=CH), 147.91 (Triazole C_3), 148.35 (Triazole C_5), 151.31 (N=CH), 164.68 (COO).
3c	13.13 ($\text{CH}_2\text{CH}_2\text{CH}_3$), 18.82 ($\text{CH}_2\text{CH}_2\text{CH}_3$), 26.65 ($\text{CH}_2\text{CH}_2\text{CH}_3$), [116.42, 123.54, 126.01, 126.54, 127.08, 128.69 (2C), 128.94 (2C), 130.98, 132.39, 133.67, 146.75, 149.65] (ArC + CH=CH), 147.17 (Triazole C_3), 147.45 (Triazole C_5), 151.22 (N=CH), 164.64 (COO).
3d	30.86 (CH_2Ph), [116.29, 123.44, 126.21, 126.50, 126.89, 128.70 (2C), 128.91 (2C), 130.98, 132.46, 133.64, 146.09, 149.83] (ArC + CH=CH), [126.62, 128.33 (2C), 128.61 (2C), 135.57] (ArC linked to C_3), 147.17 (Triazole C_3), 147.45 (Triazole C_5), 151.22 (N=CH), 164.64 (COO).
3e	20.51 (PhCH_3), 30.47 (CH_2Ph), [116.28, 123.44, 125.96, 126.42, 126.51, 128.70 (2C), 128.90 (2C), 130.98, 132.47, 133.64, 146.24, 149.84] (ArC + CH=CH), [128.56 (2C), 128.90 (2C), 132.47, 135.68] (ArC linked to C_3), 147.16 (Triazole C_3), 147.71 (Triazole C_5), 151.12 (N=CH), 164.66 (COO).
3f	30.00 (CH_2Ph), 54.90 (OCH_3), [116.29, 123.45, 125.97, 126.48, 126.53, 128.69 (2C), 128.91 (2C), 130.98, 132.45, 133.64, 146.41, 149.63] (ArC + CH=CH), [113.74 (2C), 127.32, 129.76 (2C), 157.97] (ArC linked to C_3), 147.16 (Triazole C_3), 147.75 (Triazole C_5), 151.13 (N=CH), 164.66 (COO).
3g	30.18 (CH_2Ph), [116.29, 123.46, 125.90, 126.52, 128.69 (2C), 128.91 (2C), 130.64, 130.98, 132.49, 133.63, 145.76, 149.83] (ArC + CH=CH), [128.25 (2C), 130.64 (2C), 131.32, 134.53] (ArC linked to C_3), 147.16 (Triazole C_3), 147.92 (Triazole C_5), 151.10 (N=CH), 164.65 (COO).
3h	30.48 (CH_2Ph), [116.29, 123.47, 125.90, 126.48, 128.66, 128.69 (2C), 128.91 (2C), 130.98, 132.51, 133.62, 145.59, 149.83] (ArC + CH=CH), [126.51, 127.51, 128.86, 128.99, 132.82, 137.93] (ArC linked to C_3), 147.16 (Triazole C_3), 147.86 (Triazole C_5), 151.08 (N=CH), 164.64 (COO).
3i	[116.30, 123.56, 125.82, 126.39, 126.79, 128.66 (2C), 128.89 (2C), 130.94, 132.64, 133.64, 144.51, 150.76] (ArC + CH=CH), [126.59, 127.92 (2C), 128.41 (2C), 130.03] (ArC linked to C_3), 146.99 (Triazole C_3), 149.92 (Triazole C_5), 151.22 (N=CH), 164.65 (COO).
4a	11.21 (CH_3), 23.42 (COCH_3), [116.44, 123.71, 125.57, 126.68, 127.11, 128.82 (2C), 129.05 (2C), 131.14, 133.07, 133.70, 146.57, 150.02] (ArC + CH=CH), 147.31 (Triazole C_3), 147.89 (Triazole C_5), 150.20 (N=CH), 164.72 (COO), 166.01 (COCH_3).
4b	9.31 (CH_2CH_3), 18.50 (CH_2CH_3), 23.35 (COCH_3), [116.36, 123.65, 125.48, 126.59, 127.27, 128.71 (2C), 128.95 (2C), 131.03, 132.95, 133.61, 147.18, 149.94] (ArC + CH=CH), 148.03 (Triazole C_3), 149.86 (Triazole C_5), 150.32 (N=CH), 164.60 (COO), 165.85 (COCH_3).
4c	13.31 ($\text{CH}_2\text{CH}_2\text{CH}_3$), 18.25 ($\text{CH}_2\text{CH}_2\text{CH}_3$), 23.36 (COCH_3), 26.54 ($\text{CH}_2\text{CH}_2\text{CH}_3$), [116.35, 123.63, 125.49, 126.60, 127.20, 128.72 (2C), 128.74 (2C), 131.04, 132.94, 133.62, 147.20, 149.90] (ArC + CH=CH), 147.99 (Triazole C_3), 148.82 (Triazole C_5), 150.37 (N=CH), 164.59 (COO), 165.86 (COCH_3).

4e	20.62 (PhCH ₃), 23.50 (COCH ₃), 30.52 (CH ₂ Ph), [116.31, 123.64, 125.54, 126.67, 128.84 (2C), 129.05 (2C), 131.13, 131.42, 133.08, 133.67, 147.38, 149.65] (ArC + CH=CH), [128.84 (2C), 129.01 (2C), 131.42, 136.06], 148.06 (Triazole C ₃), 148.30 (Triazole C ₅), 150.13 (N=CH), 164.70 (COO), 165.93 (COCH ₃).
4g	23.49 (COCH ₃), 30.23 (CH ₂ Ph), [116.31, 123.76, 125.51, 126.68, 128.39, 128.82 (2C), 129.02 (2C), 131.14, 133.46, 133.56, 147.39, 149.74] (ArC + CH=CH), [128.39 (2C), 130.91 (2C), 131.67, 133.66] (ArC linked to C ₃), 147.87 (Triazole C ₃), 148.06 (Triazole C ₅), 150.13 (N=CH), 164.70 (COO), 165.90 (COCH ₃).
5a	10.94 (CH ₃), 49.92 (CH ₂ NCH ₂), 65.90 (NCH ₂ N), 65.98 (CH ₂ OCH ₂), [116.52, 123.58, 125.93, 126.57, 127.06, 128.77 (2C), 129.02 (2C), 131.07, 132.62, 133.76, 143.10, 149.88] (ArC + CH=CH), 147.18 (Triazole C ₃), 148.66 (Triazole C ₅), 150.24 (N=CH), 164.69 (COO).
5b	9.97 (CH ₂ CH ₃), 18.38 (CH ₂ CH ₃), 49.93 (CH ₂ NCH ₂), 65.94 (NCH ₂ N), 65.98 (CH ₂ OCH ₂), [116.54, 123.63, 125.94, 126.62, 127.27, 128.77 (2C), 129.02 (2C), 131.07, 132.61, 133.76, 146.81, 149.62] (ArC + CH=CH), 147.16 (Triazole C ₃), 148.91 (Triazole C ₅), 150.37 (N=CH), 164.70 (COO).
5c	13.32 (CH ₂ CH ₂ CH ₃), 18.79 (CH ₂ CH ₂ CH ₃), 26.44 (CH ₂ CH ₂ CH ₃), 49.93 (CH ₂ NCH ₂), 65.89 (NCH ₂ N), 66.00 (CH ₂ OCH ₂), [116.52, 123.62, 125.94, 126.63, 127.21, 128.77 (2C), 129.01 (2C), 131.07, 132.61, 133.76, 145.60, 149.83] (ArC + CH=CH), 147.17 (Triazole C ₃), 148.97 (Triazole C ₅), 150.31 (N=CH), 164.69 (COO).
5d	30.80 (CH ₂ Ph), 49.93 (CH ₂ NCH ₂), 65.98 (NCH ₂ N), 66.03 (CH ₂ OCH ₂), [116.36, 123.53, 125.88, 126.55, 126.59, 128.79 (2C), 129.00 (2C), 131.08, 132.69, 133.73, 144.92, 150.03] (ArC + CH=CH), [126.78, 128.48 (2C), 128.69 (2C), 135.57] (ArC linked to C ₃), 147.29 (Triazole C ₃), 148.26 (Triazole C ₅), 150.26 (N=CH), 164.69 (COO).
5e	20.58 (PhCH ₃), 30.39 (CH ₂ Ph), 49.94 (CH ₂ NCH ₂), 65.99 (NCH ₂ N + CH ₂ OCH ₂), [116.39, 123.54, 125.89, 126.58, 127.10, 128.79 (2C), 129.05 (2C), 131.08, 132.68, 133.75, 145.05, 150.02] (ArC + CH=CH), [128.57 (2C), 129.00 (2C), 132.40, 135.87] (ArC linked to C ₃), 147.28 (Triazole C ₃), 148.26 (Triazole C ₅), 150.26 (N=CH), 164.70 (COO).
5g	30.13 (CH ₂ Ph), 49.91 (CH ₂ NCH ₂), 65.98 (NCH ₂ N), 66.06 (CH ₂ OCH ₂), [116.39, 123.55, 125.84, 126.61, 126.68, 128.79 (2C), 129.00 (2C), 131.08, 132.63, 133.72, 144.61, 150.02] (ArC + CH=CH), [128.41 (2C), 130.65 (2C), 131.50, 134.48] (ArC linked to C ₃), 147.28 (Triazole C ₃), 148.45 (Triazole C ₅), 150.25 (N=CH), 164.69 (COO).

Antioxidant activity

Total reductive capability using the potassium ferricyanide reduction method

Reducing power of the new compounds was evaluated by the level of conversion of the Fe³⁺/ferricyanide complex to the Fe²⁺/ferrous form. High absorbance at 700 nm specifies high reducing power. The results were compared with standard antioxidant molecules. The reductive capacity of a compound may serve as an important indicator of its potential antioxidant activity^{xvii}. In the study, all the amounts of the compounds did show lower absorbance than the blank. Therefore, no activities were observed to take part in any electron transfer reaction. Shortly, compounds **3a-i**, **4a-c**, **4e**, **4g**, **5a-e**, **5g** did not show reductive activities.

DPPH• radical scavenging activity

The radical scavenging activity of new compounds was measured by DPPH• via the method of Blois^{xiii}. The activity of antioxidant molecules on DPPH• scavenging was thought to be because

of their hydrogen donating effect^{xviii}. DPPH is a stable free radical molecule that accepts hydrogen radical or an electron to become a diamagnetic molecule^{xix}. The reductive capability of DPPH[•] was determined by a decrease in its absorbance at 517 nm induced by antioxidants. The decrease in absorbance of DPPH[•] was caused by antioxidant molecules, because of the reaction between radical progress and antioxidants, which resulted in the scavenging of the radical by hydrogen donation. It is visually apparent as discolouration from purple to yellow. Therefore, DPPH radical is usually used as a substrate to evaluate the antioxidative ability of antioxidant molecules^{xx}. BHA, BHT, and α -tocopherol were used as a reference to potential antioxidants. The newly synthesized compounds **3a-i**, **4a-c**, **4e**, **4g**, **5a-e**, **5g** did not show any radical scavenging activity.

Ferrous ion chelating activity

The chelation of ferrous ions by the new compounds and standard antioxidants was estimated by the method of Dinis et al.^{xiv}. 3-(2-Pyridyl)-5,6-bis(phenylsulfonic acid)-1,2,4-triazine (ferrozine) can form quantitatively complexes with Fe^{2+} . The complex formation is disrupted in the presence of chelating agents with the ending that the red color of the complex is reduced. Therefore, measurement of colour reduction allows estimation of the chelating effect of the coexisting chelator^{xxi}. Transition metal ions have a key role in the generation of oxygen-free radicals in living organisms. The ferric ion is the relatively biologically inactive form of iron. Nevertheless, it can be reduced to the active one, Fe^{2+} , depending on the situations. The conditions are in particular pH^{xxii} and oxidized back through Haber-Weiss reaction with the production of superoxide anions or Fenton type reaction with hydroxyl radical. The formation of the radicals may lead to protein modification, lipid peroxidation, and also DNA damage. The chelating agents may not activate metal ions and potentially inhibit the metal dependent processes^{xxiii}.

Chelating effects of the compounds **3a-i**, **4a-c**, **4e**, **4g**, **5a-e**, **5g**, α -tocopherol, EDTA are shown in the figures below (**Figures 1–3**). Low absorbance at 562 nm shows high metal chelating activity. The figures disclose that the novel 1,2,4-triazole derivatives indicate a significant activity for iron-binding. However, metal chelating ability of the compounds **5a-e** and **5g** were concentration dependent, the other compounds were not. Therefore, the Mannich bases (**5**) have a significant capacity for iron-binding, suggesting that their action as peroxidation protectors may be related to their iron binding capacity. Also, the compound-iron complex may be active since it can participate in ironcatalyzed reactions.

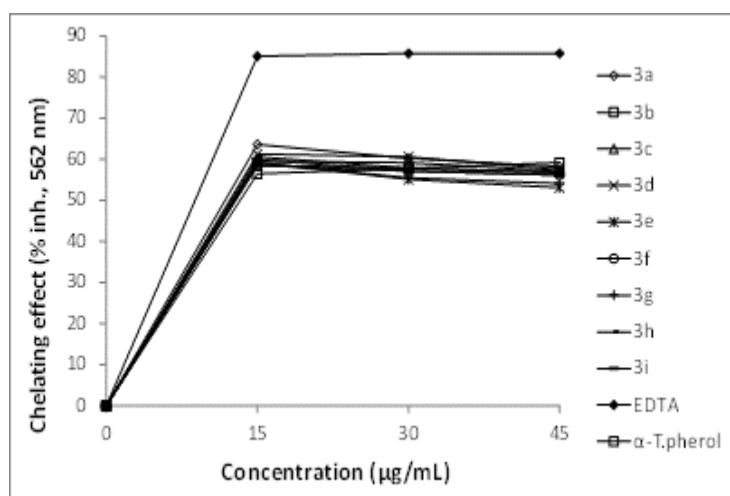


Figure 1. Chelating effect of the compounds **3a-i** and standard antioxidants.

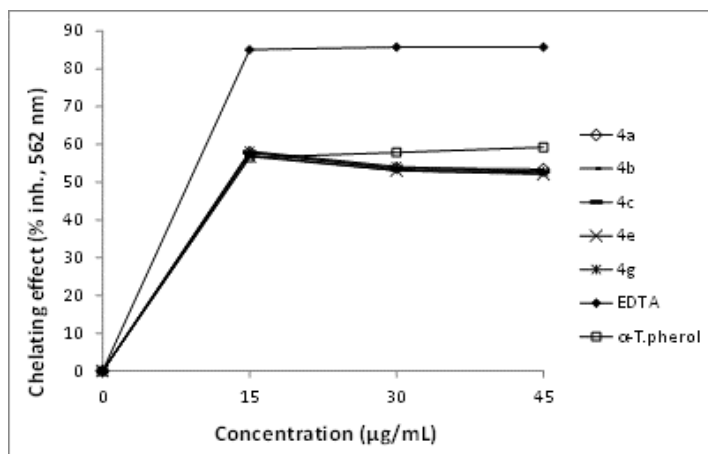


Figure 2. Chelating effect of the compounds **4a-c**, **4e**, **4g** and standard antioxidants.

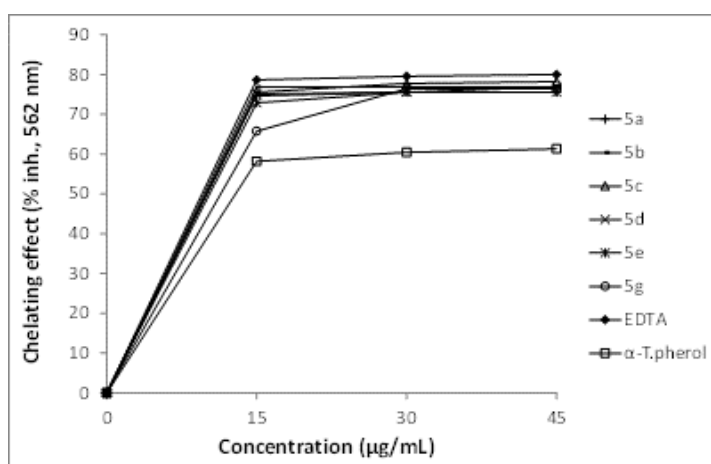


Figure 3. Chelating effect of the compounds **5a-e**, **5g** and standard antioxidants.

Antimicrobial activity

Results were interpreted in terms of the diameter of inhibition zone:

(-): < 5.5 mm (No activity)

(+): 5.5-10 mm (Low level of activity)

(++): 11-16 mm (Moderate activity)

(+++): ≥ 17 mm (High level of activity)

In the paper, the compounds **3** and **4** type Schiff bases displayed no antimicrobial capacity against all the tested microorganisms. Some of the newly synthesized **5** type Mannich bases were found to possess noticeable activity as seen in **Table 4**. The data reveals that the highest zone diameter was obtained from the compound **5b** against *Staphylococcus aureus*. A much better level of activity was shown by the compounds **5a-5d**.

Table – 4. Screening data for antimicrobial activities of **5** type compounds

Compound	Microorganisms and inhibition zone (mm)					
	Ec	Bc	Bs	Kp	Pa	Sa
5a	12	12	14	16	11	14
5b	17	16	14	15	16	19
5c	15	18	16	14	18	17
5d	13	14	12	14	14	18
5e	10	14	10	9	9	13
5g	13	12	9	11	10	14

Amp.	34	36	33	35	36	37
Neo.	16	17	17	16	17	13
Str.	10	12	12	11	12	21

Ec: *Escherichia coli* (ATCC-25922), Bc: *Bacillus cereus* (ATCC-11778), Bs: *Bacillus subtilis* (ATCC-11774), Kp: *Klebsiella pneumoniae* (ATCC-4352), Pa: *Pseudomonas aeruginosa* (ATCC-27853), Sa: *Staphylococcus aureus* (ATCC-6538), Amp.: Ampicillin (X3261), Neo.: Neomycin (X3360), Str.: Streptomycin (X3385).

CONCLUSIONS

In this study, the structures of twenty 4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives were identified using IR, ¹H NMR and ¹³C NMR spectral data, and these obtained values were compatible with literature. New compounds were designed for their *in vitro* antimicrobial, and antioxidant capacity. From the screening results, some of the Mannich bases showed important activity against the tested microorganisms. Besides, all the compounds demonstrate a significant ability for chelating effect but Mannich bases (**5**) were found more active than Schiff bases (**3** and **4**). The data reported about the studied compounds could improve the new triazole-based therapeutic target. The design and synthesis of novel small molecules can play specifically an important role in modern medicinal chemistry. Also, the results may supply several guidance for the progress of new triazole-based therapeutic targets.

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