Indian Journal of Chemistry
Vol. 59B, February 2020, pp. 271-282

Synthesis, *in vitro* antioxidant and antimicrobial activities of some novel 3-substitued-4-(3-methoxy-4-isobutyryloxybenzylideneamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives

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Received 3 April 2018; accepted (revised) 16 August 2019

In this study, 3-alkyl(aryl)-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones have been reacted with 3-methoxy-4-isobutyryloxybenzaldehyde **2** to afford the corresponding nine new 3-alkyl(aryl)-4-(3-methoxy-4-isobutyryloxybenzylideneamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones **3**. Then, the acetylation reactions of compounds **3** have been investigated and **4** type compounds have been obtained. The compounds **3** have also been treated with morpholine/1-methylpiperazine in the presence of formaldehyde according to the Mannich reaction to synthesize 1-(morpholine-4-yl-methyl)-3-alkyl(aryl)-4-(3-methoxy-4-isobutyryloxy-benzylideneamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones **5**, and 1-(1-methylpiperazin-4-yl-methyl)-3-alkyl(aryl)-4-(3-methoxy-4-isobutyryloxybenzylideneamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones **6**, respectively. The structures of twenty four new compounds have been characterized by IR, ^{1}H and ^{13}C NMR and MS spectroscopic methods. In addition, the newly synthesized compounds have been screened for their antimicrobial activities. Furthermore, these twenty four new compounds have been analyzed for their *in vitro* potential antioxidant activities by three different methods.

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

Antibiotic resistance is recognized as one of the leading public health problems worldwide. The rapid emergence and prevalence of antibiotic resistant pathogens requires a serious effort to identify, develop and design new antibiotics[1]. Considering the importance of heterocyclic compounds in medicinal chemistry, design and synthesis of novel heterocycles can play a significant role in this regard.

Triazoles are heterocyclic compounds that contain three nitrogen atoms. Some of the modern drugs which containing a triazole moiety are alprazolam, triazolam, estazolam (hypnotic, sedative, tranquilizer), trazodone (antidepressant, anxiolytic), trapidil (hypotensive), terconazole (antifungal), hexaconazole (antifungal), etizolam (amnesic, anxiolytic, anticonvulsant, hypnotic, sedative and skeletal muscle relaxant), rilmazafon (hypnotic, anxiolytic) and rizatriptan (antimigrane agent)[2]. 1,2,4-Triazole and 4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives have been found to have a broad spectrum of biological activities[3-9].

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[10]. 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[11]. Mannich bases obtained from 1,2,4-triazole derivatives are reported to possess biological activities such as antifungal[12], antioxidant[13,14], antilipase[15], antibacterial[15-17] properties.

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 morpholine and 1-methylpiperazine ring then to evaluate their antimicrobial and antioxidant activity.

Results and Discussion

In the present study, nine new 3-alkyl(aryl)-4-(3-methoxy-4-isobutyryloxybenzylidene-amino)-4,5-

dihydro-1*H*-1,2,4-triazol-5-ones, five new 1-acetyl-3-alkyl(aryl)-4-(3-methoxy-4-isobutyryloxybenzyli deneamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones, five new 1-(morpholine-4-yl-methyl)-3-alkyl(aryl)-4-(3-methoxy-4-isobutyryloxy-benzylideneamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones and five new 1-(1-methylpiperazine-4-yl-methyl)-3-alkyl(aryl)-4-(3-methoxy-4-isobutyryloxybenzylidene-amino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones synthesized. For that purpose, 3-methoxy-4-isobutyryloxybenzaldehyde[18] **2** was synthesized by the reactions of 4-hydroxy-3-methoxybenzaldehyde with isobutyryl chloride by using triethylamine. The 3-alkyl(aryl)-4-(3-methoxy-4-isobutyryloxybenzylideneamino)-4,5-dihydro-1*H*-1, 2,4-triazol-5-ones **3a-i** were obtained from the reactions of compounds 3-alkyl(aryl)-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones **1a-i** with 3-methoxy-4-isobutyryloxy benzaldehyde **2**. Then, the reactions of compounds **3a, 3b, 3d, 3e** and **3g** with acetic anhydride were investigated and **4** type compounds were prepared. Later, synthesized **3** type compounds were treated with morpholine / 1-methylpiperazine in the presence of formaldehyde according to the Mannich reaction to synthesized **5a, 5b, 5d, 5e, 5g** / **6a, 6b, 6d, 6e, 6g** respectively (Scheme I).

The structures of nine new Schiff bases **3**, five new *N*-acetyl derivatives **4**, ten new *N*-Mannich base derivatives **5** and **6** of **3** type compounds were identified by using IR, ^{1}H NMR, ^{13}C NMR, and MS data.

Antimicrobial Activity

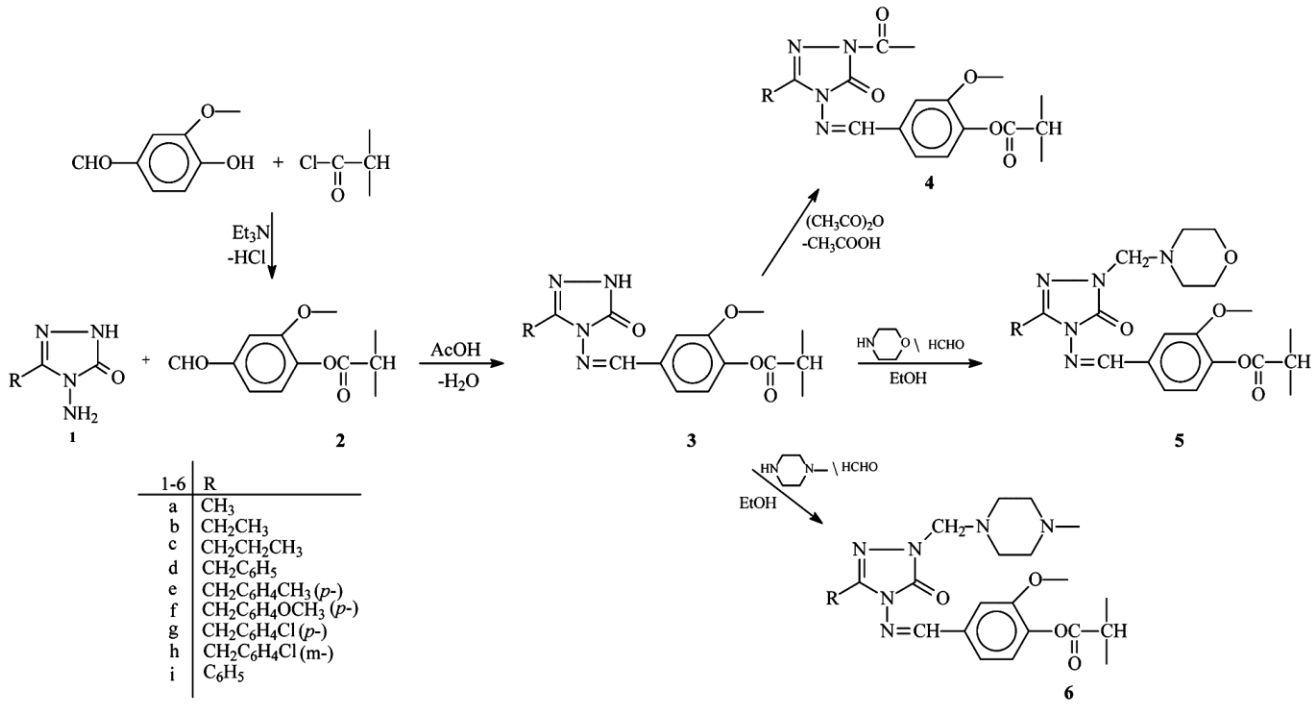
The synthesized compounds were screened for their antimicrobial activities, and some of them were found to possess significant activity (Table I). The compounds **3a**, **3b**, **3d** and **3i** did not display any antimicrobial activity against to all of tested microorganisms. Mannich bases were particularly appeared most active at a better level compared to Schiff bases.

Antioxidant Activity

The antioxidant activities of twenty four new compounds **3a-i**, **4a**, **4b**, **4d**, **4e**, **4g**, **5a**, **5b**, **5d**, **5e**, **5g**, **6a**, **6b**, **6d**, **6e** and **6g** 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 and α-tocopherol. It has been observed that



Scheme I

Table I — Antimicrobial activity of the compounds **3**, **4**, **5** and **6**

Compd	Microorganisms and inhibition zone (mm)					
	Bs	Bc	Pa	Kp	Sa	Ec
3a	–	–	–	–	–	–
3b	–	–	–	–	–	–
3c	16	12	7	–	–	–
3d	–	–	–	–	–	–
3e	11	–	9	–	–	12
3f	8	11	11	10	12	14
3g	13	17	11	–	–	10
3h	13	–	–	8	–	–
3i	–	–	–	–	–	–
4a	–	–	13	12	8	16
4b	11	11	8	11	8	14
4d	7	10	12	–	10	12
4e	10	7	–	9	10	9
4g	8	8	–	7	8	9
5a	19	19	18	21	22	23
5b	17	16	16	13	15	16
5d	18	17	17	20	17	21
5e	18	14	18	21	19	17
5g	14	19	16	14	18	20
6a	23	25	26	24	24	26
6b	22	24	26	25	26	26
6d	21	22	23	26	22	18
6e	19	19	23	25	14	21
6g	11	13	20	14	11	15
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 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 (3261), Neo.: Neomycin (3360), Str.: Streptomycin (3385).

the reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity[19]. 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[20]. 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 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[21]. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule[22]. 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[23]. Antiradical activities of compounds and standard antioxidants such as BHT, BHA and α-tocopherol were determined by using DPPH· method. Scavenging effect values of the compounds with BHT, BHA and α-tocopherol at different concentrations are respectively given in Figure 1 and Figure 2. The newly synthesized compounds which demonstrate increasing scavenging effect with growing concentration, were plotted on the graphs.

The metal chelating effect of these compounds and references decreased in order of α-tocopherol > BHA > BHT > **6a** > **3c** > **3e** > **5b** > **3a** > **3b** > **3i** > **6d**, which were 74.9, 74.3, 65.8, 19.3, 16.0, 12.4, 9.6, 9.3, 8.2, 7.2, 5.3 (%), at the highest concentration, respectively.

Ferrous Iron 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[24]. 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 *p*H[25] and oxidized back through Fenton type reactions with the production

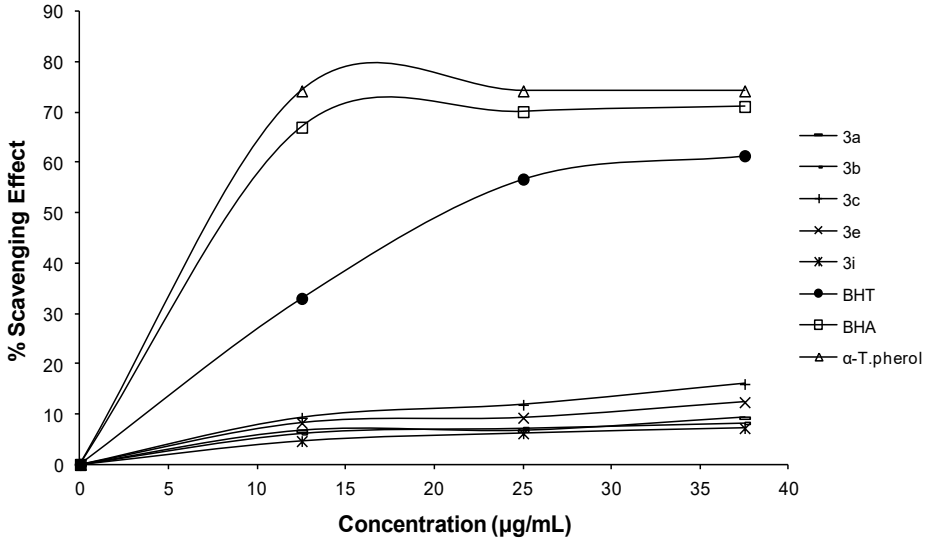


Figure 1 — Scavenging effect of compounds **3a-c**, **3e**, **3i**, BHT, BHA and α-tocopherol at different concentrations (12.5-25-37.5 µg/mL)

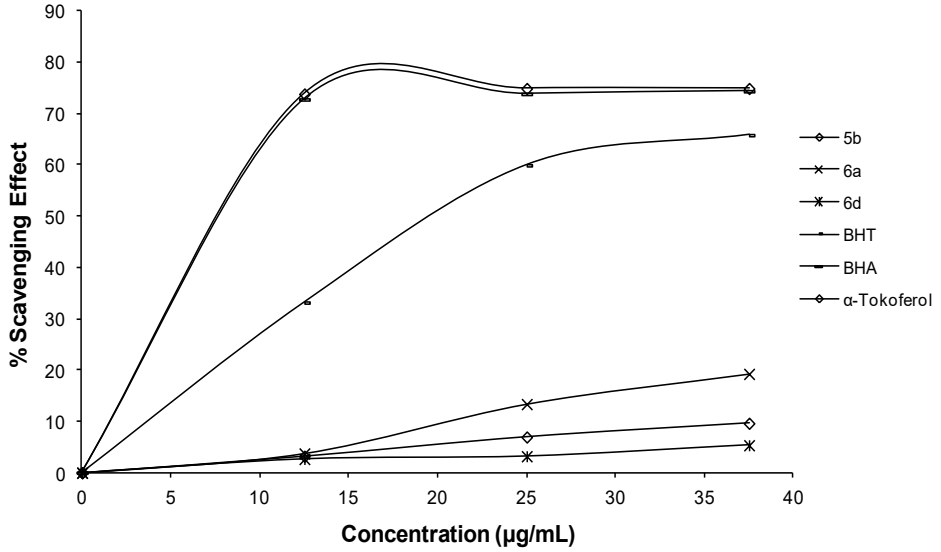


Figure 2 — Scavenging effect of compounds **5b**, **6a**, **6d**, BHT, BHA and α-tocopherol at different concentrations (12.5-25-37.5 µg/mL)

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[26]. Also, the production of highly active ROS such as $O_2^{\cdot -}$, H_2O_2 and $OH^{\cdot}$ is also catalyzed by free iron though Haber-Weiss reactions:

$$O^{\cdot}_2 + H_2O_2 \rightarrow O_2 + OH^- + OH^{\cdot}$$

Among the transition metals, iron is known as the most important lipid oxidation pro-oxidant due to its high reactivity. The ferrous state of iron accelerates lipid oxidation by breaking down the hydrogen and lipid peroxides to reactive free radicals *via* the Fenton reactions: $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^{\cdot}$

Fe^{3+} ion also produces radicals from peroxides, even though the rate is tenfold less than that of Fe^{2+} ion, which is the most powerful pro-oxidant among the various types of metal ions[27]. Ferrous ion chelating activities of the compounds **3**, **4**, **5**, **6**, EDTA and α-tocopherol are respectively shown in Figure 3, Figure 4 and Figure 5.

In this study, metal chelating capacity was significant since it reduced the concentrations of the catalyzing transition metal. It was reported that chelating agents

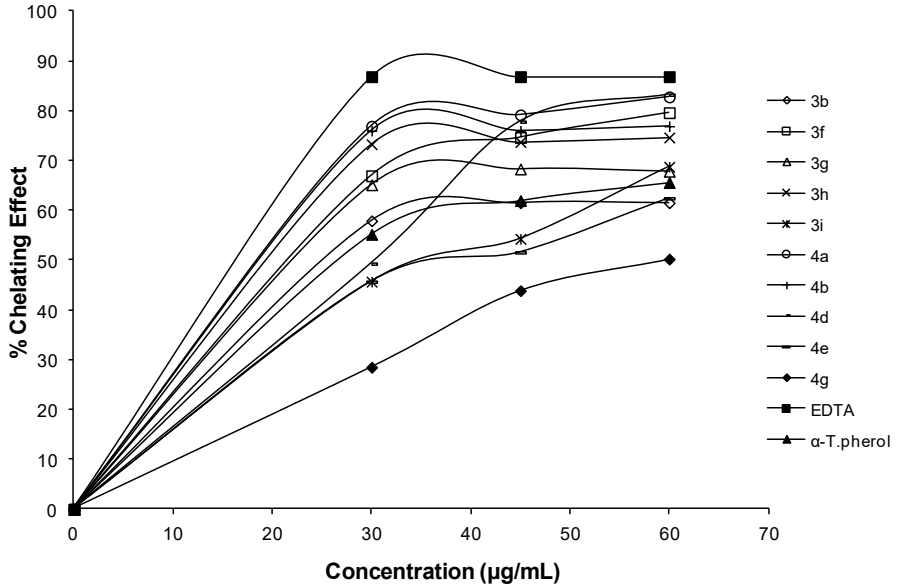


Figure 3 — Metal chelating effect of different amount of the compounds **3**, **4**, EDTA and α-tocopherol on ferrous ions

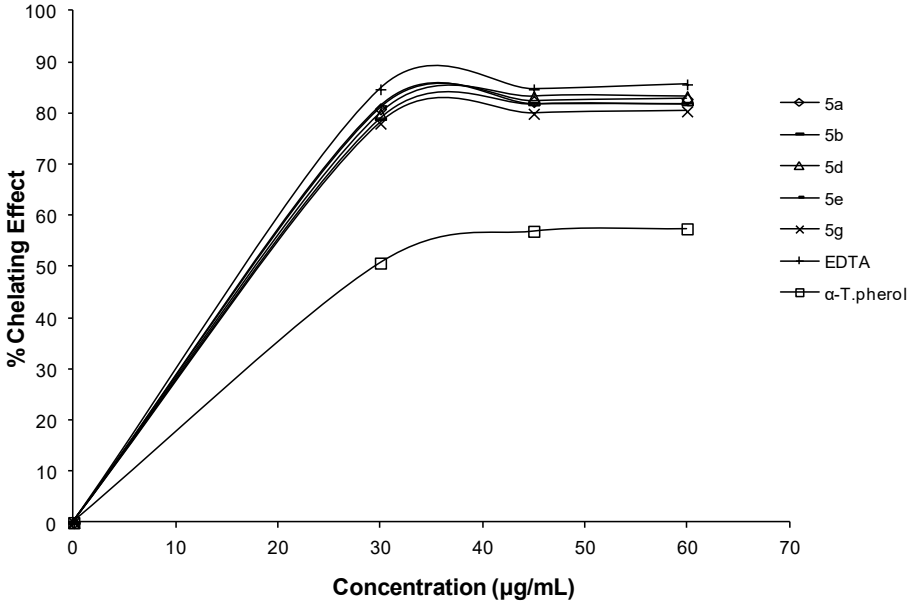


Figure 4 — Metal chelating effect of different amount of the compounds **5**, EDTA and α-tocopherol on ferrous ions

that form σ-bonds with a metal are effective as secondary antioxidants because they reduce the redox potential thereby stabilizing the oxidized form of metal ion[28]. The data obtained from Figure 3, Figure 4 and Figure 5 reveal that the compounds demonstrate a marked capacity for iron binding, except **3c-e**, suggesting that their action as peroxidation protectors may be related to their iron binding capacity. Mannich bases were found to be most active when compared to Schiff bases for all concentrations.

Experimental Section

Chemical reagents used in this study were purchased from Merck AG, Aldrich and Fluka. The starting materials **1a-i** were prepared from the reactions of the corresponding ester ethoxycarbonylhydrazones with an aqueous solution of hydrazine hydrate as described in the literature[29,30]. Melting points were determined in open glass capillaries by using a Stuart SMP-30 melting point apparatus and are uncorrected. The IR spectra were obtained by an ALPHA-P

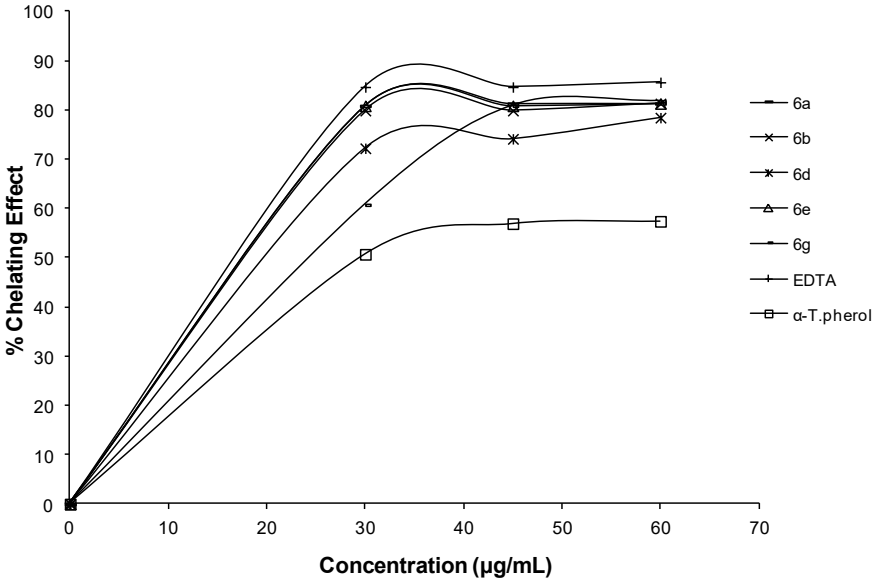


Figure 5 — Metal chelating effect of different amount of the compounds **6**, EDTA and α-tocopherol on ferrous ions

BRUKER FT-IR spectrometer. ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 with TMS as internal standard using a Bruker 400 NMR spectrometer at 400 MHz for ^{1}H and 100 MHz for ^{13}C with TMS as internal standard. Electrospray ionisation mass spectrometry (ESI-MS) was performed on a TSQ Quantum Access Max Triple Stage Quadrupole Mass Spectrometer.

General procedure for the synthesis of compounds 3

4-Hydroxy-3-methoxybenzaldehyde (0.01 mol) dissolved in ethyl acetate (20 mL) was treated with isobutyryl chloride (0.01 mol) and to this solution was slowly added triethylamine (0.01 mol) with stirring at 0-5°C. The process of stirring continued for 2 h, and then the mixture was refluxed for 3 h and filtered. The filtrate was evaporated *in vacuo*, and the crude product was washed with water and recrystallized from ethanol to afford compound **2**[18], m.p.64°C. IR: 2849 and 2737 (CHO), 1759, 1697 (C=O), 1232 (COO) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 1.25 (d, 6H, 2CH_3 J = 6.80 Hz), 2.86 (hept, 1H, CH; J = 6.80 Hz), 3.87 (s, 3H, OCH_3), 7.35 (d, 1H, ArH; J = 7.60 Hz), 7.60 (td, 1H, ArH; J = 8.00 Hz, 1.60 Hz), 7.64 (d, 1H, ArH; J = 1.60 Hz), 9.98 (s, 1H, CHO); ^{13}C NMR (DMSO-d_6): δ 18.77 (2CH_3), 33.04 (CH), 56.01 (OCH_3), [111.50; 123.31(2C); 132.26; 144.50; 151.59] (ArC), 174.31 (COO), 191.80 (CHO). The corresponding compound **1** (0.01 mol) was dissolved in acetic acid (20 mL) and treated with 3-methoxy-4-isobutyryloxy benzaldehyde **2** (0.01 mol). The mixture was refluxed for 2 h and then the solvent evaporated at 50-55°C *in vacuo*. Several recrystallizations of the residue from an appropriate solvent gave pure compounds 3-alkyl(aryl)-4-(3-methoxy-4-isobutyryloxy-benzylideneamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones **3** as colorless crystals.

3-Methyl-4-(3-methoxy-4-isobutyryloxy benzylidene amino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one, 3a: Yield 3.09 g (97.4%). m.p. 159°C. IR: 3170 (NH), 3039 (C=CH), 1750, 1707 (C=O), 1605, 1581 (C=N), 1222 (COO) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 1.25 (d, 6H, 2CH_3; J = 6.92 Hz), 2.30 (s, 3H, CH_3), 2.84 (hept, 1H, CH; J = 6.96 Hz), 3.84 (s, 3H, OCH_3), 7.22 (d, 1H, ArH; J = 8.12 Hz), 7.46 (dd, 1H, ArH; J = 8.20Hz, 0.80 Hz), 7.58 (d, 1H, ArH; J = 1.60 Hz), 9.72 (s, 1H, N=CH), 11,85 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 11.55 (CH_3), 19.19 (2CH_3), 33.65 (CH), 56.43 (OCH_3), [111.82; 120.88; 123.79; 132.81; 142.33; 151.73] (ArC), 144.74 (Triazole C_3), 151.68 (N=CH), 153.31 (Triazole C_5), 174.70 (COO); MS (70 eV): *m/z* (%) 115.11 (% 16), 319.11 $(M+1)^+$ (% 52), 360.11 (100), 637.25 (2M+1, 84).

3-Ethyl-4-(3-methoxy-4-isobutyryloxybenzylidene amino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one, 3b: Yield 3.19 g (96.3%). m.p. 169°C. IR: 3169 (NH), 3052 (C=CH), 1762, 1701 (C=O), 1597 (C=N), 1232 (COO) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 1.21 (t, 3H, CH_2CH_3; J = 7.60 Hz), 1.24 (d, 6H, 2CH_3; J = 7.20 Hz), 2.70 (q, 2H, CH_2CH_3; J = 7.60 Hz), 2.84 (hept, 1H, CH; J = 7.20 Hz), 3.83 (s, 3H, OCH_3), 7.21 (d, 1H, ArH;

J = 8.40 Hz), 7.45 (dd, 1H, ArH; *J* = 8.20 Hz, 1.60 Hz), 7.56 (d, 1H, ArH; *J* = 1.60 Hz), 9.71 (s, 1H, N=CH), 11,84 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 9.98 (CH_2CH_3), 18.48 (CH_2CH_3), 18.72 ($2CH_3$), 33.17 (CH), 56.00 (OCH_3), [111.47; 120.34; 123.36; 132.37; 141.90; 151.35] (ArC), 148.04 (Triazole C_3), 151.29 (N=CH), 153.00 (Triazole C_5), 174.20 (COO); MS (70 eV): *m/z* (%) 115.14 (8), 333.09 (M+1, 52), 374.12 (96), 665.29 (2M+1, 100).

3-*n*-Propyl-4-(3-methoxy-4-isobutyryloxy benzylideneamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one, 3c: Yield 3.24 g (93.9%). m.p. 133°C. IR: 3169 (NH), 3068 (C=CH), 1754, 1703 (C=O), 1597, 1582 (C=N), 1232 (COO) cm^{-1}; ^{1}H NMR (DMSO-d_6): 0.96 (t, 3H, $CH_2CH_2CH_3$; *J* = 7.60 Hz), 1.24 (d, 6H, $2CH_3$; *J* = 7.20 Hz), 1.70 (sext, 2H, $CH_2CH_2CH_3$; *J* = 7.20 Hz), 2.66 (t, 2H, $CH_2CH_2CH_3$; *J* = 7.20 Hz), 2.84 (hept, 1H, CH; *J* = 7.20 Hz), 3.83 (s, 3H, OCH_3), 7.21 (d, 1H, ArH; *J* = 8.00 Hz), 7.45 (dd, 1H, ArH; *J* = 8.40 Hz, 1.60 Hz), 7.56 (d, 1H, ArH; *J* = 2.00 Hz), 9.71 (s, 1H, N=CH), 11,84 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 13.48 ($CH_2CH_2CH_3$), 18.73 ($2CH_3$), 18.95 ($CH_2CH_2CH_3$), 26.69 ($CH_2CH_2CH_3$), 33.17 (CH), 55.98 (OCH_3), [111.49; 120.30; 123.39; 132.38; 141.90; 151.28] (ArC), 146.92 (Triazole C_3), 151.28 (N=CH), 152.98 (Triazole C_5), 174.21 (COO); MS (70 eV): *m/z* (%) 115.12 (22), 143.07 (8), 347.08 (M+1, 40), 388.15 (62), 693.33 (2M+1, 100).

3-Benzyl-4-(3-methoxy-4-isobutyryloxy benzylideneamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one, 3d: Yield 3.85 g (97.8%); m.p. 154°C. IR: 3154 (NH), 3073 (C=CH), 1758, 1704 (C=O), 1599, 1575 (C=N), 1227 (COO), 758,702 (monosubstituted benzenoid ring) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 1.24 (d, 6H, $2CH_3$; *J* = 6.80 Hz), 2.83 (hept, 1H, CH; *J* = 6.80 Hz), 3.85 (s, 3H, OCH_3), 4.08 (s, 2H, CH_2Ph), 7.19 (d, 1H, ArH; *J* = 8.00 Hz), 7.22-7.24 (m, 1H, ArH), 7.29-7.36 (m, 4H, ArH), 7.37 (dd, 1H, ArH; *J* = 8.00 Hz, 1.60 Hz), 7.49 (d, 1H, ArH; *J* = 1.60 Hz), 9.67 (s, 1H, N=CH), 11,99 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 18.72 ($2CH_3$), 31.22 (CH_2Ph), 33.17 (CH), 55.98 (OCH_3), [110.66; 121.00; 123.32; 132.32; 141.94; 151.26] (ArC), [126.69; 128.44(2C); 128,70 (2C); 135.87] (ArC linked C-3), 146.20 (Triazole C_3), 151.20 (N=CH), 152.38 (Triazole C_5), 174.21 (COO); MS (70 eV): *m/z* (%) 115.13 (86), 143.04 (38), 395.10 (M+1, 40), 436.12 (26), 789.30 (2M+1, 100).

3-*p*-Methylbenzyl-4-(3-methoxy-4-isobutyryloxy benzylideneamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one, 3e: Yield 3.99 g (97.9%). m.p. 151°C. IR: 3167 (NH), 3072 (C=CH), 1750, 1709 (C=O), 1595, 1575 (C=N), 1238 (COO), 865 (1,4-disubstituted benzenoid ring) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 1.24 (d, 6H, $2CH_3$; *J* = 6.80 Hz), 2.24 (s, 3H, $PhCH_3$), 2.83 (hept, 1H, CH; *J* = 6.80 Hz), 3.83 (s, 3H, OCH_3), 4.01 (s, 2H, CH_2Ph), 7.11 (d, 2H, ArH; *J* = 8.00 Hz), 7.19 (d, 2H, ArH; *J* = 8.00 Hz), 7.21 (d, 1H, ArH; *J* = 8.00 Hz), 7.38 (dd, 1H, ArH; *J* = 8.00 Hz, 1.60 Hz), 7.49 (d, 1H, ArH; *J* = 1.60 Hz), 9.66 (s, 1H, N=CH), 11,96 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 18.73 ($2CH_3$), 20.57 ($PhCH_3$), 30.83 (CH_2Ph), 33.17 (CH), 55.97 (OCH_3), [110.64; 121.02; 123.33; 132.34; 141.93; 151.76] (ArC), [128.57(2C); 129.01(2C); 132.75; 135.76] (ArC linked C-3), 146.35 (Triazole C_3), 151.21 (N=CH), 152.33 (Triazole C_5), 174.22 (COO); MS (70 eV): *m/z* (%) 115.10 (14), 143.03 (8), 395.10 (M+1, 100), 450.17 (26), 817.39 (2M+1, 66).

3-*p*-Methoxybenzyl-4-(3-methoxy-4-isobutyryloxy benzylideneamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one, 3f: Yield 3.94 g (94.3%); m.p. 171°C. IR: 3163 (NH), 3031 (C=CH), 1759, 1695 (C=O), 1611, 1581 (C=N), 1245 (COO), 836 (1,4-disubstituted benzenoid ring) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 1.24 (d, 6H, $2CH_3$; *J*=7.20 Hz), 2.84 (hept, 1H, CH; *J* =7.20 Hz), 3.70 (s, 3H, *p*-OCH_3), 3.84 (s, 3H, OCH_3), 3.99 (s, 2H, CH_2Ph), 6.87 (d, 2H, ArH; *J*=8.80 Hz), 7.20 (d, 1H, ArH; *J*=8.40 Hz), 7.24 (d, 2H, ArH; *J*=8.40 Hz), 7.39 (dd, 1H, ArH; *J*=8.40 Hz, 1.60 Hz), 7.51 (d, 1H, ArH; *J*=1.60 Hz), 9.67 (s, 1H, N=CH), 11,95 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 18.73 ($2CH_3$), 30.34 (CH_2Ph), 33.17 (CH), 50.00 (*p*-OCH_3), 56.00 (OCH_3), [110.74; 120.97; 123.34; 132.35; 141.93; 151.28] (ArC), [113.87(2C); 127.62; 129.76(2C); 158.07] (ArC linked C-3), 146.51 (Triazole C_3), 151.22 (N=CH), 152.42 (Triazole C_5), 174.23 (COO); MS (70 eV): *m/z* (%) 115.09 (44), 143.12 (100), 425.12 (M+1, 86), 466.12 (22), 849.36 (2M+1, 48).

3-*p*-Chlorobenzyl-4-(3-methoxy-4-isobutyryloxy benzylideneamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one, 3g: Yield 4.16 g (97.3%). m.p. 177°C. IR: 3169 (NH), 3061 (C=CH), 1755, 1700 (C=O), 1598, 1578 (C=N), 1267 (COO), 852 (1,4-disubstituted benzenoid ring) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 1.24 (d, 6H, $2CH_3$; *J* = 6.80 Hz), 2.84 (hept, 1H,CH; *J* = 6.80 Hz), 3.83 (s, 3H, OCH_3), 4.09 (s, 2H, CH_2Ph), 7.20 (d, 2H, ArH; *J* = 8.00 Hz), 7.34-7.37 (m, 3H, ArH), 7.38 (dd, 1H, ArH; *J* = 8.40 Hz, 2.00 Hz), 7.48 (d, 1H, ArH; *J* = 1.60 Hz), 9.67 (s, 1H, N=CH), 12.01 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 18.72 ($2CH_3$), 30.52 (CH_2Ph), 33.17 (CH), 55.99 (OCH_3), [110.73; 120.99;

123.34; 132.27; 141.97; 151.27] (ArC), [128.38 (2C); 130.62 (2C); 131.40; 134.87] (ArC linked C-3), 145.86 (Triazole C_3), 151.19 (N=CH), 152.54 (Triazole C_5), 174.21 (COO); MS (70 eV): *m/z* (%) 115.11 (100), 143.10 (74), 429.04 (M+1, 26), 470.09 (10).

3-*m*-Chlorobenzyl-4-(3-methoxy-4-isobutyryloxy benzylideneamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one, 3h: Yield 4.08 g (95.3%). m.p. 179°C. IR: 3130 (NH), 3052 (C=CH), 1755, 1709 (C=O), 1595, 1574 (C=N), 1267 (COO), 792 (1,3-disubstituted benzenoid ring) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 1.24 (d, 6H, $2CH_3$; *J* = 6.80 Hz), 2.83 (hept, 1H, CH; *J* = 6.80 Hz), 3.84 (s, 3H, OCH_3), 4.11 (s, 2H, CH_2Ph), 7.20 (d, 1H, ArH; *J* = 8.00 Hz), 7.28-7.35 (m, 3H, ArH), 7.38 (dd, 1H, ArH; *J* = 8.00 Hz, 1.60 Hz), 7.44-7.45 (m, 1H, ArH), 7.50 (d, 1H, ArH; *J* = 1.60 Hz), 9.67 (s, 1H, N=CH), 12.01 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 18.72 ($2CH_3$), 30.74 (CH_2Ph), 33.17 (CH), 56.00 (OCH_3), [110.66; 121.07; 123.32; 132.25; 141.99; 151.29] (ArC), [126.75; 127.48; 128.76; 130.28; 132.95; 138.30] (ArC linked C-3), 145.69 (Triazole C_3), 151.17 (N=CH), 152.56 (Triazole C_5), 174.20 (COO); MS (70 eV): *m/z* (%) 115.12 (100), 143.12 (76), 429.16 (M+1, 16), 470.13 (8).

3-Phenyl-4-(3-methoxy-4-isobutyryloxybenzylidene amino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one, 3i: Yield 3.69 g (97.2%); m.p. 172°C. IR: 3159 (NH), 3056 (C=CH), 1754, 1700 (C=O), 1584 (C=N), 1264 (COO), 771 and 694 (monosubstituted benzenoid ring) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 1.24 (d, 6H, $2CH_3$; *J* = 7.20 Hz), 2.84 (hept, 1H, CH; *J* = 7.20 Hz), 3.81 (s, 3H, OCH_3), 7.23 (d, 1H, ArH; *J* = 8.40 Hz), 7.43 (dd, 1H, ArH; *J* = 8.40 Hz, 2.00 Hz), 7.52 (d, 1H, ArH; *J* = 2.00 Hz), 7.53-7.56 (m, 3H, ArH), 7.91-7.94 (m, 2H, ArH), 9.66 (s, 1H, N=CH), 12.38 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 18.73 ($2CH_3$), 33.17 (CH), 55.90 (OCH_3), [111.26; 120.88; 123.47; 132.20; 142.10; 151.31] (ArC), [126.59; 127.98 (2C); 128.48 (2C); 130.13] (ArC linked C-3), 144.55 (Triazole C_3), 151.31 (N=CH), 155.40 (Triazole C_5), 174.22 (COO); MS (70 eV): *m/z* (%) 115.11 (96), 143.07 (80), 381.08 (M+1, 70), 422.17 (34), 761.27 (2M+1, 100).

General procedure for the synthesis of compounds 4

The corresponding compound **3** (0.01 mol) was refluxed with acetic anhydride (15 mL) for 30 min. After addition of absolute ethanol (50 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 EtOH gave pure compounds **4a, 4b, 4d, 4e** and **4g** as colourless crystals.

1-Acetyl-3-methyl-4-(3-methoxy-4-isobutyryloxy benzylideneamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one, 4a: Yield 2.66 g (74%). m.p. 152°C. IR: 3068 (C=CH), 1742, 1729 (C=O), 1584 (C=N), 1261 (COO) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 1.24 (d, 6H, $2CH_3$; *J* = 6.80 Hz), 2.37 (s, 3H, CH_3), 2.49 (s, 3H, $COCH_3$), 2.84 (hept, 1H, CH; *J* = 6.80 Hz), 3.84 (s, 3H, OCH_3), 7.24 (d, 1H, ArH; *J* = 8.00 Hz), 7.49 (dd, 1H, ArH; *J* = 8.00 Hz, 1.60 Hz), 7.61 (d, 1H, ArH; *J* = 1.60 Hz), 9.58(s, 1H, N=CH); ^{13}C NMR (DMSO-d_6): δ 11.21 (CH_3), 18.72 ($2CH_3$), 23.42 ($COCH_3$), 33.18 (CH), 56.08 (OCH_3), [111.60; 121.01; 123.46; 131.79; 142.35; 151.36] (ArC), 146.70 (Triazole C_3), 147.87 (N=CH), 155.24 (Triazole C_5), 166.00 ($COCH_3$), 174.18 (COO); MS (70 eV): *m/z* (%) 115.10 (10), 361.05 (M+1, 78), 743.24 (2M+23, 100).

1-Acetyl-3-ethyl-4-(3-methoxy-4-isobutyryloxy benzylideneamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one, 4b: Yield 2.72 g (72.8%). m.p. 125°C. IR: 3076 (C=CH), 1727 (C=O), 1583 (C=N), 1247 (COO) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 1.24 (d, 6H, $2CH_3$; *J* = 7.20 Hz), 1.25 (t, 3H, CH_2CH_3; *J* = 6.80 Hz), 2.50 (s, 3H, $COCH_3$), 2.77 (q, 2H, CH_2CH_3; *J* = 7.20 Hz), 2.84 (hept, 1H, CH; *J* = 6.80 Hz), 3.84 (s, 3H, OCH_3), 7.24 (d, 1H, ArH; *J* = 8.40 Hz), 7.48 (dd, 1H, ArH; *J* = 8.40 Hz, 1.60 Hz), 7.60 (d, 1H, ArH; *J* = 1.60 Hz), 9.57 (s, 1H, N=CH); ^{13}C NMR (DMSO-d_6): δ 9.41 (CH_2CH_3), 18.52 (CH_2CH_3), 18.72 ($2CH_3$), 23.43 ($COCH_3$), 33.18 (CH), 56.05 (OCH_3), [111.61; 120.89; 123.47; 131.82; 142.34; 151.36] (ArC), 148.09 (Triazole C_3), 150.18 (N=CH), 155.21 (Triazole C_5), 165.95 ($COCH_3$), 174.17 (COO); MS (70 eV): *m/z* (%) 375.10 (M+1, 100), 771.27 (2M+23, 66).

1-Acetyl-3-benzyl-4-(3-methoxy-4-isobutyryloxy benzylideneamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one, 4d: Yield 3.35g (76.9%). m.p. 107°C. IR: 3062 (C=CH), 1754(C=O), 1607, 1578 (C=N), 1248 (COO), 765 and 709 (monosubstituted benzenoid ring) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 1.24 (d, 6H, $2CH_3$; *J* = 6.80 Hz), 2.51 (s, 3H, $COCH_3$), 2.84 (hept, 1H, CH; *J* = 6.80 Hz), 3.83 (s, 3H, OCH_3), 4.16 (s, 2H, CH_2Ph), 7.21 (d, 1H, ArH; *J* = 8.00 Hz), 7.24-7.26 (m, 1H, ArH), 7.31-7.38 (m, 4H, ArH), 7.41 (dd, 1H, ArH; *J* = 8.00 Hz, 1.60 Hz), 7.51 (d, 1H, ArH; *J* = 2.00 Hz), 9.54 (s, 1H, N=CH); ^{13}C NMR

(DMSO-d_6): δ 18.72 ($2CH_3$), 23.51 ($COCH_3$), 31.16 (CH_2Ph), 33.17 (CH), 56.04 (OCH_3), [110.79; 121.55; 123.42; 131.80; 142.35; 151.32] (ArC), [126.94; 128.51(2C); 128,89(2C); 134.76] (ArC linked C-3), 148.02 (Triazole C_3), 148.23 (N=CH), 154.40 (Triazole C_5), 165.97 ($COCH_3$), 174.18 (COO); MS (70 eV): *m/z* (%) 115.14 (48), 143.10 (30), 437.10 (M+1, 100).

1-Acetyl-3-*p*-methylbenzyl-4-(3-methoxy-4-isobutyryloxybenzylideneamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one, 4e: Yield 3.10 g (68.9 %). m.p. 118°C. IR: 3079 (C=CH), 1749, 1725 (C=O), 1610, 1576 (C=N), 1250 (COO), 844 (1,4-disubstituted benzenoid ring) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 1.24 (d, 6H, $2CH_3$; *J* = 7.20 Hz), 2.25 (s, 3H, $PhCH_3$), 2.51 (s, 3H, $COCH_3$), 2.84 (hept, 1H, CH; *J* = 6.80 Hz), 3.84 (s, 3H, OCH_3), 4.10 (s, 2H, CH_2Ph), 7.12 (d, 2H, ArH; *J* = 8.00 Hz), 7.22 (d, 1H, ArH; *J* = 8.40 Hz), 7.26 (d, 2H, ArH; *J* = 8.00 Hz), 7.41 (dd, 1H, ArH; *J* = 8.40 Hz, 2.00 Hz), 7.51 (d, 1H, ArH; *J* = 2.00 Hz), 9.54 (s, 1H, N=CH); ^{13}C NMR (DMSO-d_6): δ 18.12 ($2CH_3$), 20.59 ($PhCH_3$), 23.51 ($COCH_3$), 30.78 (CH_2Ph), 33.17 (CH), 56.01 (OCH_3), [110.76; 121.56; 123.42; 131.61; 142.34; 151.32] (ArC), [128.77(2C); 129.07(2C); 131.82; 136.07] (ArC linked C-3), 148.01 (Triazole C_3), 148.37 (N=CH), 154.34 (Triazole C_5), 165.77 (*C*OCH3), 174.19 (COO); MS (70 eV): *m/z* (%) 115.10 (84), 143.16 (18), 451.17 (M+1, 100).

1-Acetyl-3-*p*-chlorobenzyl-4-(3-methoxy-4-isobutyryloxybenzylideneamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one, 4g: Yield 3.52 g (75 %). m.p. 156°C. IR: 3076 (C=CH), 1751, 1720 (C=O), 1610, 1578 (C=N), 1244 (COO), 862 (1,4-disubstituted benzenoid ring) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 1.24 (d, 6H, $2CH_3$; *J* = 7.20 Hz), 2.51 (s, 3H, $COCH_3$), 2.84 (hept, 1H, CH; *J* = 7.20 Hz), 3.83 (s, 3H, OCH_3), 4.18 (s, 2H, CH_2Ph), 7.22 (d, 1H, ArH; *J* = 8.00 Hz), 7.38-7.43 (m, 5H, ArH), 7.50 (d, 1H, ArH; *J* = 1.60 Hz), 9.55 (s, 1H, N=CH); ^{13}C NMR (DMSO-d_6): δ 18.72 ($2CH_3$), 23.50 ($COCH_3$), 30.47 (CH_2Ph), 33.17 (CH), 56.05 (OCH_3), [110.83; 121.54; 123.44; 131.77; 142.37; 151.34] (ArC), [128.44 (2C); 130.83 (2C); 131.66; 133.80] (ArC linked C-3), 147.93 (Triazole C_3), 148.01 (N=CH), 154.49 (Triazole C_5), 165.95 ($COCH_3$), 174.19 (COO); MS (70 eV): *m/z* (%) 115.11 (84), 143.07 (100), 471.11 (M+1, 38).

General procedure for the synthesis of compounds 5

The corresponding compound **3** (0.01 mol) was dissolved in 100 mL of ethanol followed by addition of morpholine (0.015 mol) and formaldehyde (0.02 mol). The reaction mixture was refluxed for 3 h. 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 **5** as colourless crystals.

1-(Morpholine-4-yl-methyl)-3-methyl-4-(3-methoxy-4-isobutyryloxybenzylidene-amino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one, 5a: Yield 3.20 g (76.9%). m.p. 136°C. IR: 3067 (C=CH), 1762, 1691 (C=O), 1601, 1577 (C=N), 1237 (COO) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 1.24 (d, 6H, $2CH_3$; *J* = 7.20 Hz), 2.32 (s, 3H, CH_3), 2.58 (t, 4H, CH_2NCH_2; *J* = 4.40 Hz), 2.84 (hept, 1H, CH; *J* = 7.20 Hz), 3.56 (t, 4H, CH_2OCH_2; *J* = 4.40 Hz), 3.84 (s, 3H, OCH_3), 4.54 (s, 2H, NCH_2), 7.23 (d, 1H, ArH; *J* = 8.40 Hz), 7.46 (dd, 1H, ArH; *J* = 8.40 Hz, 2.00 Hz), 7.59 (d, 1H, ArH; *J* = 1.60 Hz), 9.69 (s, 1H, N=CH); ^{13}C NMR (DMSO-d_6): δ 10.95 (CH_3), 18.73 ($2CH_3$), 33.18 (CH), 49.97 (CH_2NCH_2), 56.06 (OCH_3), 65.92 (NCH_2), 66.05 (CH_2OCH_2), [111.50; 120.69; 123.40; 132.13; 142.08; 151.32] (ArC), 143.12 (Triazole C_3), 150.23 (N=CH), 153.79 (Triazole C_5), 174.20 (COO); MS (70 eV): *m/z* (%) 118.11 (24), 129.09 (24), 132.13 (38), 418.13 (M+1, 100), 459.13 (6).

1-(Morpholine-4-yl-methyl)-3-ethyl-4-(3-methoxy-4-isobutyryloxybenzylidene-amino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one, 5b: Yield 3.33 g (77.4%). m.p. 154°C. IR: 3077 (C=CH), 1763, 1692 (C=O), 1611, 1578 (C=N), 1235 (COO) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 1.23 (d, 6H, $2CH_3$; *J* = 7.20 Hz), 1.24 (t, 3H, CH_2CH_3; *J*=6.80 Hz), 2.59 (t, 4H, CH_2NCH_2; *J* = 4.40 Hz), 2.74 (q, 2H, CH_2CH_3; *J* = 7.60 Hz), 2.84 (hept, 1H, CH; *J* = 7.20 Hz), 3.56 (t, 4H, CH_2OCH_2; *J* = 4.40 Hz), 3.84 (s, 3H, OCH_3), 4.55 (s, 2H, NCH_2), 7.23 (d, 1H, ArH; *J* = 8.00 Hz), 7.46 (dd, 1H, ArH; *J* = 8.00 Hz, 1.60 Hz), 7.58 (d, 1H, ArH; *J* = 1.60 Hz), 9.69 (s, 1H, N=CH); ^{13}C NMR (DMSO-d_6): δ 9.96 (CH_2CH_3), 18.38 (CH_2CH_3), 18.72 ($2CH_3$), 33.18 (CH), 49.98 (CH_2NCH_2), 56.03 (OCH_3), 65.96 (NCH_2), 66.03 (CH_2OCH_2), [111.52; 120.56; 123.40; 132.17; 142.07; 151.32] (ArC), 146.83 (Triazole C_3), 150.35 (N=CH), 153.75 (Triazole C_5), 174.19 (COO); MS (70 eV): *m/z (*%) 118.11 (12), 129.10 (44), 132.13 (28), 432.15 (M+1, 100).

1-(Morpholine-4-yl-methyl)-3-benzyl-4-(3-methoxy-4-isobutyryloxybenzylidene-amino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one, 5d: Yield 3.58g (72.6%). m.p. 131°C. IR: 3068 (C=CH), 1758, 1696 (C=O), 1596, 1578 (C=N), 1246 (COO), 747 and 697

(monosubstituted benzenoid ring) cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.24 (d, 6H, $2CH_3$; *J* = 7.20 Hz), 2.60 (t, 4H, CH_2NCH_2; *J* = 4.40 Hz), 2.84 (hept, 1H, CH; *J* = 7.20 Hz), 3.57 (t, 4H, CH_2OCH_2; *J* = 4.40 Hz), 3.83 (s, 3H, OCH_3), 4.12 (s, 2H, CH_2Ph), 4.59 (s, 2H, NCH_2), 7.21 (d, 1H, ArH; *J* = 8.00 Hz), 7.23-7.25 (m, 1H, ArH), 7.31-7.36 (m, 4H, ArH), 7.38 (dd, 1H, ArH; *J* = 8.00 Hz, 1.60 Hz), 7.49 (d, 1H, ArH; *J* = 1.60 Hz), 9.64 (s, 1H, N=CH); ^{13}C NMR (DMSO-d_6): δ 18.72 ($2CH_3$), 31.05 (CH_2Ph), 33.17 (CH), 50.00 (CH_2NCH_2), 56.00 (OCH_3), 66.04 (NCH_2+ CH_2OCH_2), [110.68; 121.22; 123.34; 132.11; 142.10; 151.29] (ArC), [126.77; 128.50(2C); 128,63(2C); 135.70] (ArC linked C-3), 144.91 (Triazole C_3), 150.24 (N=CH), 153.07 (Triazole C_5), 174.18 (COO); MS (70 eV): *m/z* (%) 118.11 (40), 129.10 (64), 132.13 (60), 494.20 (M+1, 100).

1-(Morpholine-4-yl-methyl)-3-*p*-methylbenzyl-4-(3-methoxy-4-isobutyryloxy-benzylideneamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one, 5e: Yield 3.69 g (72.8 %). m.p. 122°C. IR: 3072 (C=CH), 1756, 1695 (C=O), 1613, 1579 (C=N), 1243 (COO), 860 (1,4-disubstituted benzenoid ring) cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.24 (d, 6H, $2CH_3$; *J* = 6.80 Hz), 2.24 (s, 3H, $PhCH_3$), 2.59 (t, 4H, CH_2NCH_2; *J* = 4.40 Hz), 2.84 (hept, 1H, CH; *J* = 6.80 Hz), 3.57 (t, 4H, CH_2OCH_2; *J* = 4.40 Hz), 3.84 (s, 3H, OCH_3), 4.06 (s, 2H, CH_2Ph), 4.58 (s, 2H, NCH_2),7.12 (d, 2H, ArH; *J* = 8.00 Hz), 7.21 (d, 1H, ArH; *J* = 8.00 Hz), 7.23 (d, 2H, ArH; *J* = 8.00 Hz), 7.39 (dd, 1H, ArH; *J* = 8.40 Hz, 1.60 Hz), 7.50 (d, 1H, ArH; *J* = 1.60 Hz), 9.64 (s, 1H, N=CH); ^{13}C NMR (DMSO-d_6): δ 18.72 ($2CH_3$), 20.56 ($PhCH_3$), 30.65 (CH_2Ph), 33.17 (CH), 49.99 (CH_2NCH_2), 55.98 (OCH_3), 66.03 (NCH_2+ CH_2OCH_2), [110.67; 121.23; 123.36; 132.13; 142.08; 151.28] (ArC), [128.52(2C); 129.07(2C); 132.57; 135.88] (ArC linked C-3), 145.06 (Triazole C_3), 150.24 (N=CH), 153.01 (Triazole C_5), 174.20 (COO); MS (70 eV): *m/z* (%) 118.11 (36), 129.10 (56), 132.13 (36), 508.21 (M+1, 100).

1-(Morpholine-4-yl-methyl)-3-*p*-chlorobenzyl-4-(3-methoxy-4-isobutyryloxy-benzylideneamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one, 5g: Yield 3.89 g (73.8 %). m.p. 125°C. IR: 3062 (C=CH), 1763, 1697 (C=O), 1594, 1576 (C=N), 1245 (COO), 860 (1,4-disubstituted benzenoid ring) cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.24 (d, 6H, $2CH_3$; *J* = 7.20 Hz), 2.59 (t, 4H, CH_2NCH_2; *J* = 4.40 Hz), 2.84 (hept, 1H, CH; *J* = 6.80 Hz), 3.57 (t, 4H, CH_2OCH_2; *J* = 4.40 Hz), 3.83 (s, 3H, OCH_3), 4.13 (s, 2H, CH_2Ph), 4.58 (s, 2H, NCH_2), 7.21 (d, 1H, ArH; *J* = 8.00 Hz), 7.36-7.42 (m, 5H, ArH), 7.49 (d, 1H, ArH; *J* = 2.00 Hz), 9.65 (s, 1H, N=CH); ^{13}C NMR (DMSO-d_6): δ 18.72 ($2CH_3$), 30.51 (CH_2Ph), 33.17 (CH), 49.97 (CH_2NCH_2), 56.01 (OCH_3), 66.03 (NCH_2), 66.09 (CH_2OCH_2), [110.77; 121.21; 123.37; 132.06; 142.13; 151.30] (ArC), [128.45 (2C); 130.58 (2C); 131.49; 134.70] (ArC linked C-3), 144.59 (Triazole C_3), 150.33 (N=CH), 153.24 (Triazole C_5), 174.19 (COO); MS (70 eV): *m/z* (%) 118.11 (48), 129.10 (84), 132.10 (48), 528.18 (M+1, 100).

General procedure for the synthesis of compounds 6

The corresponding compound 3 (0.01 mol) was dissolved in 100 mL of ethanol followed by addition of 1-methylpiperazin (0.015 mol) and formaldehyde (0.02 mol). 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 **6** as colourless crystals.

1-(1-Methylpiperazin-4-yl-methyl)-3-methyl-4-(3-methoxy-4-isobutyryloxy-benzylideneamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one, 6a: Yield 3.88 g (90.4%). m.p. 88°C. IR: 3071 (C=CH), 1756, 1691 (C=O), 1600, 1579 (C=N), 1247 (COO) cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.24 (d, 6H, $2CH_3$), 2.15 (s, 3H, NCH_3), 2.30 (s, 3H, CH_3), 2.34 (m, 4H, ($2CH_2$; *J* = 4.40 Hz), 2.61 (m, 4H, $2CH_2$), 2.85 (hept, 1H, CH; *J* = 6.80 Hz), 3.85 (s, 3H, OCH_3), 4.55 (s, 2H, NCH_2), 7.23 (d, 1H, ArH; *J* = 8.00 Hz), 7.47 (dd, 1H, ArH; *J* = 8.00 Hz, 2.00 Hz), 7.60 (d, 1H, ArH; *J* = 2.00 Hz), 9.71 (s, 1H, N=CH); ^{13}C NMR (DMSO-d_6): δ 10.90 (CH_3), 18.71 ($2CH_3$), 33.18 (CH), 45.49 (NCH_3), 49.37 ($2CH_2$), 54.60 ($2CH_2$), 56.02 (OCH_3), 65.72 (NCH_2), [111.46; 120.65; 123.65; 132.14; 142.82; 151.31] (ArC), 143.79 (Triazole C_3), 150.06 (N=CH), 153.63 (Triazole C_5), 174.16 (COO); MS (70 eV): *m/z* (%) 113.10 (38), 431.13 (M+1, 100).

1-(1-Methylpiperazin-4-yl-methyl)-3-ethyl-4-(3-methoxy-4-isobutyryloxy-benzylideneamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one, 6b: Yield 3.49 g (78.8%). m.p. 116°C; IR: 3065 (C=CH), 1758, 1791 (C=O), 1611, 1579 (C=N), 1235 (COO), 749 (1,2-disubstituted benzenoid ring) cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.23 (t, 3H, CH_2CH_3; *J* = 7.20 Hz), 1.24 (d, 6H, $2CH_3$; *J* = 7.20 Hz), 2.14 (s, 3H, NCH_3), 2.30 (m, 4H, $2CH_2$), 2.60 (m, 4H, $2CH_2$), 2.74 (q, 2H, CH_2CH_3; *J* = 7.60 Hz), 2.84 (hept, 1H, CH; *J* = 7.20

Hz), 3.84 (s, 3H, OCH_3), 4.55 (s, 2H, NCH_2), 7.22 (d, 1H, ArH; *J* = 8.00 Hz), 7.46 (dd, 1H, ArH; *J* = 8.00 Hz, 1.60 Hz), 7.58 (d, 1H, ArH; *J* = 1.60 Hz), 9.69 (s, 1H, N=CH); ^{13}C NMR (DMSO-d_6): δ 9.99 (CH_2CH_3), 18.72 (CH_2CH_3), 18.88 ($2CH_3$), 33.18 (CH), 45.67 (NCH_3), 49.39 ($2CH_2$), 54.54 ($2CH_2$), 56.02 (OCH_3), 65.75 (NCH_2), [111.52; 120.54; 123.39; 132.18; 142.05; 151.31] (ArC), 146.69 (Triazole C_3), 150.31 (N=CH), 153.67 (Triazole C_5), 174.19 (COO); MS (70 eV): *m/z* (%) 113.11 (32), 445.16 (M+1, 100).

1-(1-Methylpiperazin-4-yl-methyl)-3-benzyl-4-(3-methoxy-4-isobutyryloxy-benzylideneamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one, 6d: Yield 4.37g (86.34%). m.p. 101°C. IR: 3082 (C=CH), 1758, 1694 (C=O), 1608, 1575 (C=N), 1237 (COO), 747, 698 (monosubstituted benzenoid ring) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 1.23 (d, 6H, $2CH_3$; *J* = 7.20 Hz), 2.14 (s, 3H, NCH_3), 2.31 (m, 4H, $2CH_2$), 2.62 (m, 4H, 2CH2), 2.84 (hept, 1H, CH; *J* = 7.20 Hz), 3.83 (s, 3H, OCH_3), 4.11 (s, 2H, CH_2Ph), 4.59 (s, 2H, NCH_2), 7.22-7.27 (m, 2H, ArH), 7.30-7.36 (m, 4H, ArH), 7.38 (dd, 1H, ArH; *J* = 8.40 Hz, 1.60 Hz), 7.49 (d, 1H, ArH; *J* = 1.60 Hz), 9.65 (s, 1H, N=CH); ^{13}C NMR (DMSO-d_6): δ 18.72 ($2CH_3$), 31.02 (CH_2Ph), 33.17 (CH), 45.67 (NCH_3), 49.40 ($2CH_2$), 54.49 ($2CH_2$), 55.99 (OCH_3), 65.98 (NCH_2), [110.69; 121.20; 123.34; 132.13; 142.09; 151.26] (ArC), [126.76; 128.45(2C); 128,66(2C); 135.73] (ArC linked C-3), 144.77 (Triazole C_3), 150.20 (N=CH), 153.00 (Triazole C_5), 174.18 (COO); MS (70 eV): *m/z* (%) 113.12 (30), 507.20 (M+1, 100).

1-(1-Methylpiperazin-4-yl-methyl)-3-*p*-methylbenzyl-4-(3-methoxy-4-isobutyryl-oxybenzylideneamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one, 6e: Yield 3.77 g (72.55 %). m.p. 149°C. IR: 3078 (C=CH), 1753, 1696 (C=O), 1613, 1579 (C=N), 1239 (COO), 868 (1,4-disubstituted benzenoid ring) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 1.24 (d, 6H, $2CH_3$; *J* = 7.20 Hz), 2.13 (s, 3H, NCH_3), 2.24 (s, 3H, $PhCH_3$), 2.30 (m, 4H, $2CH_2$), 2.60 (m, 4H, $2CH_2$), 2.84 (hept, 1H, CH; *J* = 6.80 Hz), 3.83 (s, 3H, OCH_3), 4.05 (s, 2H, CH_2Ph), 4.58 (s, 2H, NCH_2), 7.12 (d, 2H, ArH; *J* = 8.00 Hz), 7.20 (d, 2H, ArH; *J* = 8.00 Hz), 7.22 (d, 1H, ArH; *J* = 8.00 Hz), 7.38 (dd, 1H, ArH; *J* = 8.00 Hz, 1.60 Hz), 7.50 (d, 1H, ArH; *J* = 2.00 Hz), 9.64 (s, 1H, N=CH); ^{13}C NMR (DMSO-d_6): δ 18.73 ($2CH_3$), 20.56 ($PhCH_3$), 30.65 (CH_2Ph), 33.18 (CH), 45.74 (NCH_3), 49.45 ($2CH_2$), 54.53 ($2CH_2$), 55.99 (OCH_3), 65.88 (NCH_2), [110.71; 121.21; 123.36; 132.15; 142.08; 151.29] (ArC), [128.50 (2C); 129.07 (2C); 132.60; 135.87] (ArC linked C-3), 144.92 (Triazole C_3), 150.20 (N=CH), 152.98 (Triazole C_5), 174.20 (COO); MS (70 eV): *m/z* (%) 113.12 (30), 521.24 (M+1, 100).

1-(1-Methylpiperazin-4-yl-methyl)-3-*p*-chlorobenzyl-4-(3-methoxy-4-isobutyryloxybenzylideneamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one, 6g: Yield 3.97 g (73.48 %). m.p. 107°C. IR: 3069 (C=CH), 1758, 1697 (C=O), 1611, 1577 (C=N), 1236 (COO), 868 (1,4-disubstituted benzenoid ring) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 1.24 (d, 6H, $2CH_3$; *J* = 7.20 Hz), 2.13 (s, 3H, NCH_3), 2.29 (m, 4H, $2CH_2$), 2.60 (m, 4H, $2CH_2$), 2.84 (hept, 1H, CH; *J* = 7.20 Hz), 3.83 (s, 3H, OCH_3), 4.13 (s, 2H, CH_2Ph), 4.57 (s, 2H, NCH_2), 7.21 (d, 2H, ArH; *J* = 8.00 Hz), 7.35-7.41 (m, 2H, ArH), 7.38 (d, 2H, ArH; *J* = 8.00 Hz), 7.48 (d, 1H, ArH; *J* = 1.60 Hz), 9.65 (s, 1H, N=CH); ^{13}C NMR (DMSO-d_6): δ 18.72 ($2CH_3$), 30.34 (CH_2Ph), 33.17 (CH), 45.73 (NCH_3), 49.42 ($2CH_2$), 54.51 ($2CH_2$), 56.01 (OCH_3), 65.92 (NCH_2), [110.76; 121.19; 123,37; 132.08; 142.10; 151.29] (ArC), [128.46 (2C); 130.56 (2C); 131.48; 134.73] (ArC linked C-3), 144,45 (Triazole C_3), 150,18 (N=CH), 153,17 (Triazole C_5), 174.20 (COO); MS (70 eV): *m/z* (%) 113.12 (98), 541.19 (M+1, 100).

Antimicrobial activity

All bacterial and yeast strains were obtained from the company of Microbiological Environmental Protection Laboratories (France) and were as follows: *Bacillus subtilis* (ATCC 11774), *Bacillus cereus* (ATCC 11778), *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumonia* (ATCC 4352). Simple susceptibility screening test using agar well diffusion method was used[31,32]. 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 (10 μg) for bacteria, streptomycin and

fluconazole (5 μg) for yeast were used as positive controls, DMSO was used as solved control.

Antioxidant Activity: Chemicals

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[33] as explained in the literature[14].

Free Radical Scavenging Activity

Free radical scavenging activity of compounds was measured by DPPH·, using the method of Blois[34] as explained in the literature[14].

Metal Chelating Activity

The chelation of ferrous ions by the synthesized compounds and standards were estimated by the method of Dinis *et al*.[35] as explained in the literature[14].

Acknowledgements

The authors thank F. Aytemiz for studies on antimicrobial activities.

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