



Synthesis, characterization and investigation of antimicrobial and antioxidant activities of some new 2-[(4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl)azomethine]phenyl 4-nitrobenzoate derivatives

H Yüksek^a, A Berkyürek^a, S Manap^a, G Özdemir^a, M Beytur^a, H Balseven^a, M Alkan^b, F Aytemiz^a & Ö Gürsoy-Kol^{*a}

^a Department of Chemistry, Kafkas University, Kars, 36100, Turkey

^b Education Faculty, Kafkas University, Kars, 36100, Turkey

E-mail: ozlemgursoy@gmail.com

Received 24 September 2020; accepted (revised) 23 December 2021

The synthesis of 2-[[3-alkyl(aryl)-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl]azomethine]-phenyl 4-nitrobenzoates **3** from the reactions of 3-alkyl(aryl)-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones **1** with 2-(4-nitrobenzoxy)-benzaldehyde **2** is described. The acetylation reactions of the compounds **3** giving compounds **4** have been investigated. The compounds **3** have also been treated with 4-piperidinecarboxamide / piperazine in the presence of formaldehyde according to the Mannich reaction to synthesize 2-[[1-(4-piperidinecarboxamide-1-yl-methyl)-3-alkyl(aryl)-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl]azomethine]-phenyl 4-nitrobenzoates **5** and *N,N'*-bis-[[3-alkyl(aryl)-4-[2-(4-nitrobenzoxy)-benzylideneamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-on-1-yl-methyl]-piperazines **6**. The newly synthesized compounds have been characterized using IR, ¹H and ¹³C NMR, UV-Vis and mass spectral data. In addition, the newly synthesized compounds have been screened for their antimicrobial activities. Furthermore, these thirty one new compounds have been analyzed for their *in vitro* potential antioxidant activities by three different methods.

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

Triazoles are five-membered heterocyclic compounds containing three nitrogen atoms. There are many commercial drugs which contained 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), rilmazafone (hypnotic, anxiolytic) and rizatriptan (antimigraine agent)¹ (Figure 1). 1,2,4-Triazole and also 4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives have been known to possess antioxidant, anti-inflammatory, antitumor and antibacterial activities²⁻⁵.

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 leads to a group of the product named as Mannich base⁶. Mannich bases have several practices in the pharmaceutical field 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-step⁷. Some Mannich bases acquired from derivatives of

1,2,4-triazoles have been reported to show a wide spectrum of pharmacological profile⁸⁻¹¹.

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 antibiotics¹². 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 4-piperidinecarboxamide and piperazine ring then to evaluate their antimicrobial and antioxidant activity. In this regard, ten new 2-[[3-alkyl(aryl)-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl]azomethine]-phenyl 4-nitrobenzoates **3**, ten new 2-[[1-acetyl-3-alkyl(aryl)-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl]azomethine]-phenyl 4-nitrobenzoates **4**, six new 2-[[1-(4-piperidinecarboxamide-1-yl-methyl)-3-alkyl(aryl)-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl]-azomethine]-phenyl 4-nitrobenzoates **5** and five new *N,N'*-bis-[[3-alkyl(aryl)-4-[2-(4-nitrobenzoxy)-benzylideneamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-on-1-yl-methyl]-piperazines **6** were synthesized. The structures of thirty one new compounds were identified by using IR, ¹H NMR, ¹³C NMR, UV and MS data. In addition, the antimicrobial activity of newly synthesized compounds determined.

Scheme I — The reactions route of the synthesized compounds

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

Compd	Microorganisms and inhibition zone (mm)					
	Bs	Bc	Pa	Kp	Sa	Ec
5b	12	11	12	12	18	13
5c	14	10	15	12	17	14
5d	13	14	12	14	17	12
5e	16	14	16	16	19	17
5f	15	9	13	11	12	10
5g	17	13	13	17	16	18
6a	–	9	12	13	10	–
6d	13	11	14	11	12	–
6g	–	12	12	–	11	–
6i	11	11	11	12	10	–
6j	–	9	10	–	10	–
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).

concentration-dependent. Finally, Mannich bases were found to be most active when compared to Schiff bases.

Experimental Section

Chemical reagents used in this paper were bought from Merck AG, Aldrich and Fluka. Melting points

were taken using an Electrothermal Melting-point Apparatus in open capillary tubes and were not corrected. The infrared spectra were recorded on a Perkin Elmer Instruments Spectrum One FT-IR spectrophotometer. ¹H and ¹³C NMR spectra were determined in deuterated dimethyl sulfoxide with TMS

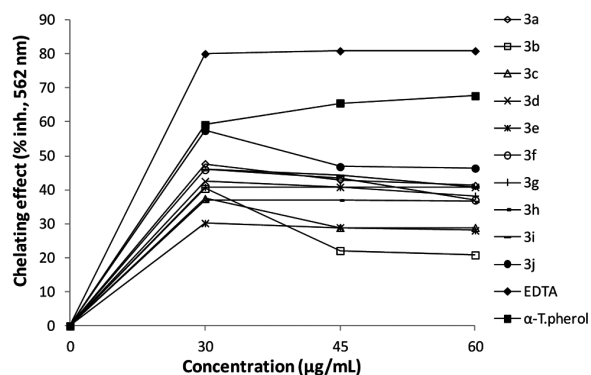


Figure 2 — Metal chelating effect of the compounds **3**, EDTA and α -tocopherol on ferrous ions

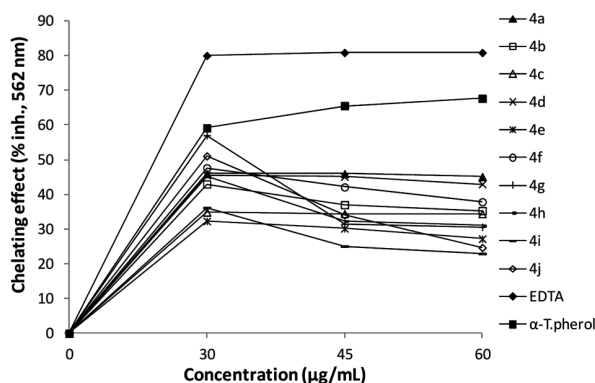


Figure 3 — Metal chelating effect of the compounds **4**, EDTA and α -tocopherol on ferrous ions

as internal standard using a Bruker Ultrashield spectrophotometer at 300 MHz and 75 MHz, respectively. Electrospray ionization mass spectrometry (ESI-MS) was performed on a TSQ Quantum Access Max Triple Stage Quadrupole Mass Spectrometer. UV absorption spectra were evaluated in 10 mm quartz cells between 200 and 400 nm using a PG Instruments Ltd T80 UV/Vis spectrometer. Extinction coefficients (ϵ) are clarified in $\text{L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$. The starting compounds 3-alkyl(aryl)-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones **1a-j** were obtained from the reactions of the corresponding ester ethoxycarbonylhydrazones with an aqueous solution of hydrazine hydrate as explained in the literature^{13,14}.

General procedure for the synthesis of 2-[(3-alkyl(aryl)-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl)azomethine]-phenyl 4-nitrobenzoates, **3**

2-Hydroxybenzaldehyde (0.01 mol) dissolved in ethyl acetate (100 mL) was reacted with 4-nitrobenzoyl chloride (0.01 mol), and to this solution was slowly mixed triethylamine (0.01 mol) by stirring at 0-5 °C. Stirring was continued for 2 h, and after that the

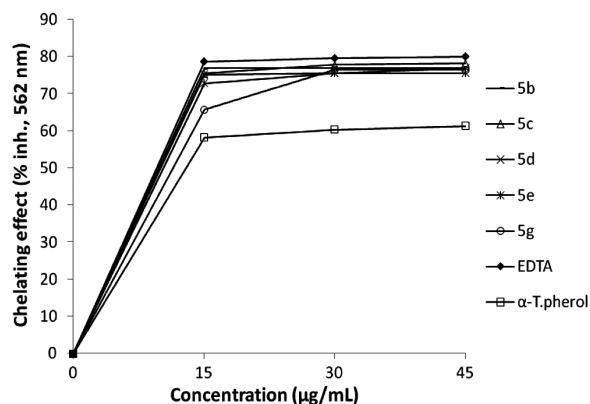


Figure 4 — Metal chelating effect of the compounds **5**, EDTA and α -tocopherol on ferrous ions

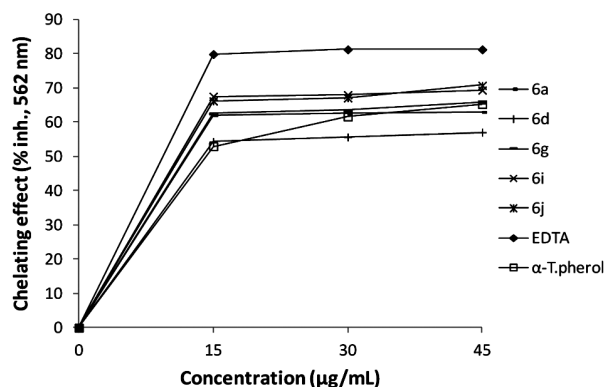


Figure 5 — Metal chelating effect of the compounds **6**, EDTA and α -tocopherol on ferrous ions

mixture was refluxed for 3 hours and filtered. The filtrate was evaporated *in vacuo*, and the crude product was washed with water and recrystallized from ethyl acetate-petroleum ether (1:3) to afford novel compound **2**¹⁵. Yield: 92%; mp 123°C; IR (ATR, cm^{-1}) ν_{max} : 2840 and 2766 (CHO), 1739, 1696 (C=O), 1520 and 1346 (NO_2), 1254 (COO), 771 (1,2-disubstituted benzenoid ring). Then the corresponding compound **2** (0.01 mol) was dissolved in ethanoic acid (20 mL) and by treated 2-(4-nitrobenzoxy)-benzaldehyde **2** (0.01 mol). The mixture was refluxed for 1.5 hours and then evaporated at 50-55°C *in vacuo*. A few recrystallizations of the residue from ethanoic acid-water (1:3) gave pure compounds **3a-j** as uncolored crystals.

2-[(3-Methyl-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl)azomethine]-phenyl 4-nitrobenzoate, **3a**: White solid; yield: 98%; mp 292-294 °C. IR (ATR, cm^{-1}) ν_{max} 3154 (NH), 1743, 1706 (C=O), 1605 (C=N), 1525 and 1354 (NO_2), 1262 (COO), 843 (1,4-disubstituted benzenoid ring), 755 (1,2-disubstituted benzenoid ring). ¹HNMR (300 MHz, DMSO-d_6) δ : 2.10 (3H, s, CH_3), 7.52-7.86 (3H, m, ArH), 8.06 (1H, m, ArH), 8.44 (4H,

m, ArH), 9.92 (1H, s, N = CH), 11.78 (1H, s, NH) ppm. ^{13}C NMR (75 MHz, DMSO- d_6) δ : 10.86 (CH₃), [123.54, 123.99 (2C), 124.05, 125.86, 127.76, 131.47 (2C), 132.27, 132.60, 149.37, 151.10] (Ar-C), 148.97 (triazole-C₃), 149.45 (triazole-C₅), 151.30 (N = CH), 163.00 (COO) ppm. UV [Ethanol, λ_{max} , nm (ϵ , L.mol⁻¹.cm⁻¹): 304 (12.675), 270 (16.860)]. Mass spectrum (ESI-MS), m/z : 407(M+1+39), 391(M+1+23), 347, 301, 279, 271, 241, 231, 219, 217, 185, 171, 145, 142, 129(100), 124, 115.

2-[(3-Ethyl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl)azomethine]-phenyl 4-nitrobenzoate, 3b: White solid; yield: 97%; mp 273-275 °C. IR (ATR, cm⁻¹) ν_{max} 3176 (NH), 1739, 1691 (C = O), 1605, 1590 (C = N), 1531 and 1355 (NO₂), 1268 (COO), 845 (1,4-disubstituted benzenoid ring), 774 (1,2-disubstituted benzenoid ring). ^1H NMR (300 MHz, DMSO- d_6) δ : 1.10 (3H, m, CH₂CH₃), 2.60 (2H, m, CH₂CH₃), 7.24-7.67 (3H, m, ArH), 8.01-8.46 (5H, m, ArH), 9.90 (1H, s, N = CH), 11.79 (1H, s, NH) ppm. UV [Ethanol, λ_{max} , nm (ϵ , L.mol⁻¹.cm⁻¹): 304 (11.840), 272 (15.780)]. Mass spectrum (ESI-MS), m/z : 431, 407, 378, 347, 309, 308, 301, 279, 277, 271, 255, 233, 231, 226, 199, 185, 157, 145, 142, 129(100), 124, 115.

2-[(3-(*n*-Propyl)-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl)azomethine]-phenyl 4-nitrobenzoate, 3c: White solid; yield: 99%; mp 234-236 °C. IR (ATR, cm⁻¹) ν_{max} 3176 (NH), 1737, 1710 (C = O), 1606, 1591 (C = N), 1534 and 1353 (NO₂), 1267 (COO), 846 (1,4-disubstituted benzenoid ring), 769 (1,2-disubstituted benzenoid ring). ^1H NMR (300 MHz, DMSO- d_6) δ : 0.86 (3H, t, $J = 7,6$ Hz, CH₂CH₂CH₃), 1.58 (2H, sext, $J = 7,6$ Hz, CH₂CH₂CH₃), 2.42 (2H, t, $J = 7,6$ Hz, CH₂CH₂CH₃), 7.51-7.70 (2H, m, ArH), 8.00-8.05 (1H, m, ArH), 8.16-8.19 (1H, m, ArH), 8.31-8.34 (1H, m, ArH), 8.39-8.43 (3H, m, ArH), 9.90 (1H, s, N = CH), 11.82 (1H, s, NH) ppm. ^{13}C NMR (75 MHz, DMSO- d_6) δ : 13.31 (CH₂CH₂CH₃), 18.64 (CH₂CH₂CH₃), 26.44 (CH₂CH₂CH₃), [123.55, 123.76 (2C), 124.04, 125.86, 127.83, 132.24 (2C), 132.57, 133.95, 149.33, 150.78] (Ar-C), 146.70 (triazole-C₃), 149.18 (triazole-C₅), 151.24 (N = CH), 163.07 (COO) ppm. UV [Ethanol, λ_{max} , nm (ϵ , L.mol⁻¹.cm⁻¹): 304 (8.930), 268 (16.080)]. Mass spectrum (ESI-MS), m/z : 467, 419 (M+1+23), 396 (M+1), 366, 365, 351, 347, 285, 282, 279, 277, 269, 255, 247, 231, 226, 209, 199, 185, 145, 142(100), 120, 102.

2-[(3-Benzyl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl)azomethine]-phenyl 4-nitrobenzoate, 3d: White solid; yield: 98%; mp 238-240 °C. IR (ATR, cm⁻¹) ν_{max}

3170 (NH), 1745, 1706 (C = O), 1607, 1587 (C = N), 1531 and 1349 (NO₂), 1261 (COO), 844 (1,4-disubstituted benzenoid ring), 769 (1,2-disubstituted benzenoid ring), 760 and 711 (monosubstituted benzenoid ring). ^1H NMR (300 MHz, DMSO- d_6) δ : 3.90 (2H, s, CH₂Ph), 7.10-7.33 (6H, m, ArH), 7.48-7.53 (2H, m, ArH), 7.64-7.70 (1H, m, ArH), 8.00 (1H, d, $J = 7,9$ Hz, ArH), 8.38-8.40 (3H, m, ArH), 9.88 (1H, s, N = CH), 11.92 (1H, s, NH) ppm. ^{13}C NMR (75 MHz, DMSO- d_6) δ : 30.78 (CH₂Ph), [122.96, 123.47 (2C), 124.01, 125.81, 128.38, 131.44 (2C), 132.63, 133.84, 149.52, 150.75] (Ar-C), [126.69, 128.43 (2C), 128.71 (2C), 135.56] (Ar-C linked C₃), 146.07 (triazole-C₃), 148.54 (triazole-C₅), 151.15 (N = CH), 162.99 (COO) ppm. UV [Ethanol, λ_{max} , nm (ϵ , L.mol⁻¹.cm⁻¹): 304 (13.670), 268 (20.030)]. Mass spectrum (ESI-MS), m/z : 454(M+1), 440, 407, 395, 365, 351, 337, 318, 317, 301, 295, 279, 277, 239, 231, 226, 209, 199, 185, 171, 145, 142, 129(100), 124, 115.

2-[(3-(*p*-Methylbenzyl)-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl)azomethine]-phenyl 4-nitrobenzoate, 3e: White solid; yield: 97%; mp 253-255 °C. IR (ATR, cm⁻¹) ν_{max} 3164 (NH), 1736, 1705 (C = O), 1608, 1589 (C = N), 1525 and 1348 (NO₂), 1264 (COO), 845 (1,4-disubstituted benzenoid ring), 757 (1,2-disubstituted benzenoid ring). ^1H NMR (300 MHz, DMSO- d_6) δ : 2.25 (3H, s, PhCH₃), 3.86 (2H, s, CH₂Ph), 7.07-7.13 (4H, m, ArH), 7.52 (2H, d, $J = 7,8$ Hz, ArH), 7.65-7.70 (1H, m, ArH), 8.01 (1H, d, $J = 7,4$ Hz, ArH), 8.15 (1H, d, $J = 8,8$ Hz, ArH), 8.40 (3H, s, ArH), 9.90 (1H, s, N = CH), 11.90 (1H, s, NH) ppm. ^{13}C NMR (75 MHz, DMSO- d_6) δ : 20.56 (PhCH₃), 30.36 (CH₂Ph), [123.38, 123.47 (2C), 124.00, 125.81, 127.08, 131.42 (2C), 132.45, 132.81, 149.50, 150.73] (Ar-C), [128.56 (2C), 129.00 (2C), 132.62, 135.75] (Ar-C linked C₃), 146.21 (triazole-C₃), 148.53 (triazole-C₅), 151.15 (N = CH), 162.99 (COO) ppm. UV [Ethanol, λ_{max} , nm (ϵ , L.mol⁻¹.cm⁻¹): 266 (22.570)]. Mass spectrum (ESI-MS), m/z : 481(M+1+23), 454, 438, 409, 365, 351, 331, 310, 309(98), 301, 282, 279, 277, 255, 241, 226, 217, 199, 145, 142, 129(100), 124, 115, 102.

2-[(3-(*p*-Methoxybenzyl)-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl)azomethine]-phenyl 4-nitrobenzoate, 3f: White solid; yield: 93%; mp 259-261 °C. IR (ATR, cm⁻¹) ν_{max} 3167 (NH), 1738, 1710 (C = O), 1608, 1589 (C = N), 1530 and 1348 (NO₂), 1263 (COO), 845 (1,4-disubstituted benzenoid ring), 769 (1,2-disubstituted benzenoid ring). ^1H NMR (300 MHz, DMSO- d_6) δ : 3.70 (3H, s, OCH₃), 3.84 (2H, s,

CH₂Ph), 6.83 (2H, d, *J* = 8,7 Hz, ArH), 7.15 (2H, d, *J* = 8,6 Hz, ArH), 7.53 (2H, d, *J* = 7,8 Hz, ArH), 7.68-7.75 (1H, m, ArH), 8.01-8.04 (1H, m, ArH), 8.41 (4H, s, ArH), 9.90 (1H, s, N = CH), 11.85 (1H, s, NH) ppm. ¹³C NMR (75 MHz, DMSO-d₆) δ: 29.92 (CH₂Ph), 55.01 (OCH₃), [123.48, 124.00 (3C), 125.83, 127.33, 131.43 (2C), 132.62, 132.82, 149.51, 150.74] (Ar-C), [113.89 (2C), 127.30, 129.76 (2C), 158.08] (Ar-C linked C₃), 146.38 (triazole-C₃), 148.55 (triazole-C₅), 151.16 (N = CH), 163.00 (COO) ppm. UV[Ethanol, λ_{max}, nm (ε, L.mol⁻¹.cm⁻¹): 266 (20.350). Mass spectrum (ESI-MS), *m/z*: 496(M+23), 407, 380, 378, 358, 356, 347, 330, 325, 304, 301, 285, 279, 263, 255, 231, 209, 195, 185, 171, 145, 142, 129(100), 124, 120, 115.

2-[[3-(*p*-Chlorobenzyl)-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl]azomethine]-phenyl 4-nitrobenzoate, 3g: White solid; yield: 88%; mp 243-245 °C. IR (ATR, cm⁻¹) ν_{max} 3164 (NH), 1741, 1708 (C = O), 1608, 1588 (C = N), 1523 and 1348 (NO₂), 1262 (COO), 844 (1,4-disubstituted benzenoid ring), 758 (1,2-disubstituted benzenoid ring). ¹HNMR (300 MHz, DMSO-d₆) δ: 3.92 (2H, s, CH₂Ph), 7.20-7.67 (6H, m, ArH), 7.98-8.00 (2H, m, ArH), 8.31-8.39 (4H, m, ArH), 9.90 (1H, s, N = CH), 11.96 (1H, s, NH) ppm. ¹³C NMR (75 MHz, DMSO-d₆) δ: 30.11 (CH₂Ph), [123.49, 123.87 (2C), 124.01, 125.77, 128.01, 131.44 (2C), 132.68, 133.84, 149.54, 150.76] (Ar-C), [128.32 (2C), 130.64 (2C), 131.28, 134.54] (Ar-C linked to C₃), 145.74 (triazole-C₃), 148.66 (triazole-C₅), 151.14 (N = CH), 162.99 (COO) ppm. UV [Ethanol, λ_{max}, nm (ε, L.mol⁻¹.cm⁻¹): 304 (16.235), 270 (21.000). Mass spectrum (ESI-MS), *m/z*: 521, 520, 519, 446, 445(100), 217, 214(98), 199, 115, 111.

2-[[3-(*m*-Chlorobenzyl)-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl]azomethine]-phenyl 4-nitrobenzoate, 3h: White solid; yield: 94%; mp 248-249 °C. IR (ATR, cm⁻¹) ν_{max} 3185 (NH), 1748, 1697 (C = O), 1603, 1588 (C = N), 1524 and 1351 (NO₂), 1260 (COO), 870 and 787 (1,3-disubstituted benzenoid ring), 841 (1,4-disubstituted benzenoid ring), 757 (1,2-disubstituted benzenoid ring). ¹HNMR (300 MHz, DMSO-d₆) δ: 3.92 (2H, s, CH₂Ph), 7.19-7.21 (1H, m, ArH), 7.27-7.33 (3H, m, ArH), 7.47-7.53 (2H, m, ArH), 7.64-7.73 (1H, m, ArH), 8.00 (1H, d, *J* = 7,8 Hz, ArH), 8.40 (4H, m, ArH), 9.90 (1H, s, N = CH), 11.98 (1H, s, NH) ppm. ¹³C NMR (75 MHz, DMSO-d₆) δ: 30.38 (CH₂Ph), [123.43, 123.49 (2C), 124.00, 125.77, 127.51, 131.43 (2C), 132.68, 132.91, 149.50, 150.73] (Ar-C), [126.74, 127.31, 128.82,

130.19, 133.84, 137.92] (Ar-C linked to C₃), 145.55 (triazole-C₃), 148.63 (triazole-C₅), 151.12 (N = CH), 162.97 (COO) ppm. UV [Ethanol, λ_{max}, nm (ε, L.mol⁻¹.cm⁻¹): 266 (18.275). Mass spectrum (ESI-MS), *m/z*: 553.5, 541, 500(M+23), 468, 467, 435, 410, 361, 351, 337, 315, 304, 301, 268, 266, 253, 252, 239(100), 238(96), 226, 225, 224, 207, 193, 179, 160, 147, 142, 129, 115.

2-[[3-Phenyl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl]azomethine]-phenyl 4-nitrobenzoate, 3i: White solid; yield: 89%; mp 275-277 °C. IR (ATR, cm⁻¹) ν_{max} 3154 (NH), 1736, 1712 (C = O), 1607, 1585 (C = N), 1534 and 1352 (NO₂), 1271 (COO), 843 (1,4-disubstituted benzenoid ring), 751 (1,2-disubstituted benzenoid ring), 760 and 683 (monosubstituted benzenoid ring). ¹HNMR (300 MHz, DMSO-d₆) δ: 7.48 (4H, m, ArH), 7.68-7.77 (3H, m, ArH), 7.98 (2H, m, ArH), 8.30-8.41 (4H, m, ArH), 9.90 (1H, s, N = CH), 11.98 (1H, s, NH) ppm. ¹³C NMR (75 MHz, DMSO-d₆) δ: [123.63, 123.83 (2C), 123.98, 125.67, 127.85, 131.45 (2C), 132.80, 133.90, 150.56, 151.26] (Ar-C), [126.38, 127.93 (2C), 128.43, 128.50 (2C)] (Ar-C linked to C₃), 144.61 (triazole-C₃), 149.53 (triazole-C₅), 151.97 (N = CH), 163.03 (COO) ppm. UV[Ethanol, λ_{max}, nm (ε, L.mol⁻¹.cm⁻¹): 304 (12.410), 280 (19.785), 272 (20.190). Mass spectrum (ESI-MS), *m/z*: 408, 407, 379, 365, 347, 303, 301, 279, 277, 255, 231, 226, 199, 185, 171, 145, 142, 129(100), 124, 115.

2-[[3-Cyclopropyl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl]azomethine]-phenyl 4-nitrobenzoate, 3j: White solid; yield: 99%; mp 287-289 °C. IR (ATR, cm⁻¹) ν_{max} 3177 (NH), 1740, 1700 (C = O), 1607, 1588 (C = N), 1528 and 1352 (NO₂), 1267 (COO), 846 (1,4-disubstituted benzenoid ring), 770 (1,2-disubstituted benzenoid ring). ¹HNMR (300 MHz, DMSO-d₆) δ: 0.77-0.82 (4H, m, CH₂CH₂), 1.86-2.02 (1H, m, CH), 7.53 (2H, d, *J* = 7,7 Hz, ArH), 7.65-7.71 (1H, m, ArH), 8.04-8.07 (1H, m, ArH), 8.42 (4H, m, ArH), 9.75 (1H, s, N = CH), 11.75 (1H, s, NH) ppm. ¹³C NMR (75 MHz, DMSO-d₆) δ: 5.34 (CH), 6.45 (CH₂CH₂), [123.49, 123.54 (2C), 124.00, 125.09, 127.79, 131.45 (2C), 132.58, 132.80, 149.65, 151.37] (Ar-C), 148.06 (triazole-C₃), 149.37 (triazole-C₅), 151.67 (N = CH), 162.98 (COO) ppm. UV [Ethanol, λ_{max}, nm (ε, L.mol⁻¹.cm⁻¹): 272 (19.930). Mass spectrum (ESI-MS), *m/z*: 459, 438, 424, 417(M+1+23), 416(95, M+23), 413, 411, 407, 395, 394, 379, 365, 351(100), 337, 323, 288, 279, 277, 267, 255, 245, 231, 226, 209, 195, 185, 157, 145, 142, 141, 125, 120, 115, 113, 107.

General procedure for the synthesis of 2-[[1-acetyl-3-alkyl(aryl)-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl]azomethine]-phenyl 4-nitrobenzoates 4

The corresponding compound **3** (0.01 mol) was refluxed with acetic anhydride (15 mL) for 0.5 h. After addition of absolute ethanol (50 mL), the mixture was refluxed for 1 h. Evaporation of the resulting solution at 40-45 °C *in vacuo* and several recrystallizations of the residue from an appropriate solvent gave pure compounds **4**.

2-[[1-Acetyl-3-methyl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl]azomethine]-phenyl 4-nitrobenzoate, 4a: White solid; yield: 93%; mp 240-242 °C. IR (ATR, cm⁻¹) ν_{\max} 1740, 1717 (C=O), 1625, 1605 (C=N), 1529 and 1355 (NO₂), 1265 (COO), 842 (1,4-disubstituted benzenoid ring), 756 (1,2-disubstituted benzenoid ring). ¹HNMR (300 MHz, DMSO-d₆) δ : 2.18 (3H, s, CH₃), 2.42 (3H, s, COCH₃), 7.49-7.57 (2H, m, ArH), 7.70-7.75 (1H, m, ArH), 8.03-8.10 (1H, m, ArH), 8.40-8.46 (4H, m, ArH), 9.75 (1H, s, N=CH). ¹³C NMR (75 MHz, DMSO-d₆) δ : 11.01 (CH₃), 23.32 (COCH₃), [123.69, 124.12 (2C), 125.43, 127.18, 128.06, 131.47 (2C), 133.17, 133.84, 149.58, 150.90] (Ar-C), 146.34 (triazole-C₃), 149.60 (triazole-C₅), 151.02 (N=CH), 163.00 (COO), 166.30 (COCH₃) ppm. UV [Ethanol, λ_{\max} , nm (ϵ , L.mol⁻¹.cm⁻¹): 296 (21.030), 254 (30.160), 242 (31.860), 216 (29.910). Mass spectrum (ESI-MS), *m/z*: 438, 424, 410(M+1), 409(M), 397, 396, 393, 379, 368, 365, 352, 351(100), 337, 323, 319, 306, 282, 280, 279, 265, 255, 252, 241, 238, 237, 234, 209, 195, 185, 171, 145, 139, 125, 120, 115, 107, 102.

2-[[1-Acetyl-3-ethyl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl]azomethine]-phenyl 4-nitrobenzoate, 4b: White solid; yield: 62%; mp 190-192 °C. IR (ATR, cm⁻¹) ν_{\max} 1745, 1720 (C=O), 1614, 1598 (C=N), 1526 and 1353 (NO₂), 1265 (COO), 846 (1,4-disubstituted benzenoid ring), 759 (1,2-disubstituted benzenoid ring). ¹HNMR (300 MHz, DMSO-d₆) δ : 1.12 (3H, t, *J* = 7,5 Hz, CH₂CH₃), 2.42 (3H, s, COCH₃), 2.60-2.62 (2H, m, CH₂CH₃), 7.51-7.56 (2H, m, ArH), 7.65-7.74 (1H, m, ArH), 8.06 (1H, d, *J* = 8,0 Hz, ArH), 8.41-8.48 (4H, m, ArH), 9.75 (1H, s, N=CH) ppm. ¹³C NMR (75 MHz, DMSO-d₆) δ : 9.33 (CH₂CH₃), 18.40 (CH₂CH₃), 23.33 (COCH₃), [123.71, 123.90 (2C), 124.09, 125.43, 128.11, 131.48 (2C), 133.15, 133.84, 149.84, 150.85] (Ar-C), 147.94 (triazole-C₃), 149.56 (triazole-C₅), 151.13 (N=CH), 162.97 (COO), 166.04 (COCH₃) ppm. UV [Ethanol, λ_{\max} , nm (ϵ , L.mol⁻¹.cm⁻¹): 276 (26.310), 258

(30.720), 242 (29.440), 218 (23.175). Mass spectrum (ESI-MS), *m/z*: 446(M+23), 441, 424 (45, M+1), 397, 393, 391, 382, 365, 351 (100), 337, 323, 309, 284, 281, 280, 279, 268, 257, 255, 252, 241, 238, 234, 217, 209, 195, 177, 163, 145, 139, 125, 120, 115, 107, 102.

2-[[1-Acetyl-3-(*n*-propyl)-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl]azomethine]-phenyl 4-nitrobenzoate, 4c: White solid; yield: 68%; mp 166-168 °C. IR (ATR, cm⁻¹) ν_{\max} 1746, 1718 (C=O), 1607 (C=N), 1532 and 1351 (NO₂), 1260 (COO), 844 (1,4-disubstituted benzenoid ring), 754 (1,2-disubstituted benzenoid ring). ¹HNMR (300 MHz, DMSO-d₆) δ : 0.88 (3H, t, *J* = 7,4 Hz, CH₂CH₂CH₃), 1.60 (2H, sext, *J* = 7,4 Hz, CH₂CH₂CH₃), 2.41 (3H, s, COCH₃), 2.48 (2H, t, *J* = 7,3 Hz, CH₂CH₂CH₃), 7.51-7.55 (2H, m, ArH), 7.63-7.73 (1H, m, ArH), 7.99-8.06 (1H, m, ArH), 8.39-8.46 (4H, m, ArH), 9.75 (1H, s, N=CH) ppm. ¹³C NMR (75 MHz, DMSO-d₆) δ : 13.76 (CH₂CH₂CH₃), 18.69 (CH₂CH₂CH₃), 23.84 (COCH₃), 26.91 (CH₂CH₂CH₃), [124.04, 124.52 (2C), 126.30, 127.69, 128.61, 131.95 (2C), 133.64, 134.40, 149.99, 151.28] (Ar-C), 148.36 (triazole-C₃), 149.78 (triazole-C₅), 151.71 (N=CH), 163.45 (COO), 166.55 (COCH₃) ppm. UV [Ethanol, λ_{\max} , nm (ϵ , L.mol⁻¹.cm⁻¹): 296 (14.625), 254 (29.880), 214 (28.470). Mass spectrum (ESI-MS), *m/z*: 467, 461 (M+1+23), 460 (80, M+23), 438 (35, M+1), 418, 413, 396, 379, 365, 351, 337 (100), 323, 301, 282, 279, 253, 239, 238 (97), 234, 220, 207, 193, 185, 167, 142, 139, 120, 115, 107, 102.

2-[[1-Acetyl-3-benzyl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl]azomethine]-phenyl 4-nitrobenzoate, 4d: White solid; yield: 89%; mp 198-199 °C. IR (ATR, cm⁻¹) ν_{\max} 1743, 1717 (C=O), 1607 (C=N), 1528 and 1352 (NO₂), 1264 (COO), 845 (1,4-disubstituted benzenoid ring), 763 (1,2-disubstituted benzenoid ring), 763 and 691 (monosubstituted benzenoid ring). ¹HNMR (300 MHz, DMSO-d₆) δ : 2.44 (3H, s, COCH₃), 4.00 (2H, s, CH₂Ph), 7.21-7.34 (5H, m, ArH), 7.49-7.55 (2H, m, ArH), 7.67-7.73 (1H, m, ArH), 7.99-8.02 (1H, m, ArH), 8.40 (4H, m, ArH), 9.75 (1H, s, N=CH) ppm. ¹³C NMR (75 MHz, DMSO-d₆) δ : 23.38 (COCH₃), 30.76 (CH₂Ph), [123.62 (2C), 124.07, 125.39, 127.15, 127.55, 131.44 (2C), 133.17, 133.75, 149.72, 150.49] (Ar-C), [126.94, 128.45 (2C), 128.88 (2C), 134.46] (Ar-C linked to C₃), 147.88 (triazole-C₃), 147.98 (triazole-C₅), 150.80 (N=CH), 162.98 (COO), 166.02 (COCH₃) ppm. UV [Ethanol, λ_{\max} , nm (ϵ , L.mol⁻¹.cm⁻¹): 296 (22.740), 286 (23.700) 256 (26.550), 218 (20.340). Mass spectrum (ESI-MS), *m/z*: 486(M+1), 482, 454, 444, 424, 413, 393, 391, 365, 351 (100), 337,

323, 295, 280, 279, 265, 252, 238, 234, 217, 209, 207, 195, 177, 147, 145, 139, 120, 115, 107, 102.

2-[[1-Acetyl-3-(*p*-methylbenzyl)-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl]azomethine}-phenyl 4-nitrobenzoate, 4e: White solid; yield: 86%; mp 233-235 °C. IR (ATR, cm^{-1}) ν_{max} 1740, 1717 (C = O), 1608 (C = N), 1527 and 1353 (NO_2), 1264 (COO), 845 (1,4-disubstituted benzenoid ring), 761 (1,2-disubstituted benzenoid ring). ^1H NMR (300 MHz, DMSO-d_6) δ : 2.24 (3H, s, PhCH_3), 2.41 (3H, s, COCH_3), 4.07 (2H, s, CH_2Ph), 7.07-7.16 (4H, m, ArH), 7.50-7.54 (2H, m, ArH), 7.66-7.72 (1H, m, ArH), 8.01-8.06 (1H, m, ArH), 8.39-8.42 (4H, s, ArH), 9.89 (1H, s, N = CH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ : 20.58 (PhCH_3), 23.39 (COCH_3), 30.35 (CH_2Ph), [123.57, 123.65 (2C), 124.02, 124.08, 127.09, 131.47 (2C), 132.80, 133.18, 149.70, 150.81] (Ar-C), [128.57 (2C), 129.01 (2C), 132.60, 136.06] (Ar-C linked to C_3), 147.00 (triazole- C_3), 149.38 (triazole- C_5), 151.20 (N = CH), 162.99 (COO), 166.20 (COCH_3) ppm. UV [Ethanol, λ_{max} , nm (ϵ , $\text{L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$): 254 (26.450), 218 (24.790), 212 (23.545). Mass spectrum (ESI-MS), m/z : 558, 541, 493, 468, 438, 437, 426, 397, 395, 373, 353, 322, 331 (100), 310, 309, 289, 254, 251, 196, 193, 183, 182, 168, 151, 137, 134.

2-[[1-Acetyl-3-(*p*-methoxybenzyl)-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl]azomethine}-phenyl 4-nitrobenzoate, 4f: White solid; yield: 62%; mp 221-223 °C. IR (ATR, cm^{-1}) ν_{max} 1741, 1717 (C = O), 1607 (C = N), 1527 and 1353 (NO_2), 1251 (COO), 845 (1,4-disubstituted benzenoid ring), 758 (1,2-disubstituted benzenoid ring). ^1H NMR (300 MHz, DMSO-d_6) δ : 2.40 (3H, s, COCH_3), 3.72 (3H, s, OCH_3), 3.90 (2H, s, CH_2Ph), 6.85 (2H, d, $J = 8.5$ Hz, ArH), 7.18 (2H, d, $J = 8.5$ Hz, ArH), 7.51-7.55 (2H, m, ArH), 7.68-7.73 (1H, m, ArH), 8.03-8.05 (1H, m, ArH), 8.40 (4H, s, ArH), 9.72 (1H, s, N = CH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ : 23.39 (COCH_3), 29.91 (CH_2Ph), 55.04 (OCH_3), [123.65, 124.08 (3C), 125.41, 127.69, 131.44 (2C), 133.18, 133.77, 149.70, 150.63] (Ar-C), [113.89 (2C), 127.18, 129.99 (2C), 158.26] (Ar-C linked to C_3), 147.89 (triazole- C_3), 148.28 (triazole- C_5), 150.80 (N = CH), 163.00 (COO), 166.02 (COCH_3) ppm. UV [Ethanol, λ_{max} , nm (ϵ , $\text{L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$): 254 (28.490), 242 (28.710), 222 (26.455), 210 (23.240). Mass spectrum (ESI-MS), m/z : 531, 500, 444, 443, 442, 437, 415, 412, 411, 381, 348, 347, 325 (100), 308, 280, 255, 251, 235, 213, 196, 182, 165, 151, 136, 134.

2-[[1-Acetyl-3-(*p*-chlorobenzyl)-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl]azomethine}-phenyl 4-

nitrobenzoate, 4g: White solid; yield: 89%; mp 201-203 °C. IR (ATR, cm^{-1}) ν_{max} 1748, 1720 (C = O), 1607 (C = N), 1527 and 1353 (NO_2), 1264 (COO), 846 (1,4-disubstituted benzenoid ring), 760 (1,2-disubstituted benzenoid ring). ^1H NMR (300 MHz, DMSO-d_6) δ : 2.40 (3H, s, COCH_3), 4.00 (2H, s, CH_2Ph), 7.30-7.38 (4H, m, ArH), 7.50-7.54 (2H, m, ArH), 7.68-7.72 (1H, m, ArH), 1.99-8.01 (1H, m, ArH), 8.38 (4H, m, ArH), 9.70 (1H, s, N = CH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ : 23.37 (COCH_3), 30.08 (CH_2Ph), [123.63, 123.86 (2C), 124.07, 125.36, 127.56, 131.43 (2C), 131.70, 133.20, 149.72, 150.52] (Ar-C), [128.37 (2C), 130.81 (2C), 131.27, 133.24] (Ar-C linked to C_3), 147.68 (triazole- C_3), 147.87 (triazole- C_5), 150.80 (N = CH), 162.97 (COO), 166.00 (COCH_3) ppm. UV [Ethanol, λ_{max} , nm (ϵ , $\text{L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$): 294 (23.680), 258 (29.230), 246 (30.250), 228 (28.890). Mass spectrum (ESI-MS), m/z : 542, 528, 520 (M+2), 517, 500, 482, 468, 467, 438, 424, 391, 379, 365, 351 (100), 337, 323, 282, 279, 255, 252, 238, 234, 220, 195, 185, 167, 145, 142, 120, 115, 107, 102.

2-[[1-Acetyl-3-(*m*-chlorobenzyl)-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl]azomethine}-phenyl 4-nitrobenzoate, 4h: White solid; yield: 66%; mp 214-215 °C. IR (ATR, cm^{-1}) ν_{max} 1747, 1719 (C = O), 1607 (C = N), 1530 and 1353 (NO_2), 1266 (COO), 880 and 779 (1,3-disubstituted benzenoid ring), 844 (1,4-disubstituted benzenoid ring), 762 (1,2-disubstituted benzenoid ring). ^1H NMR (300 MHz, DMSO-d_6) δ : 2.42 (3H, s, COCH_3), 4.02 (2H, s, CH_2Ph), 7.24-7.37 (4H, m, ArH), 7.48-7.55 (2H, m, ArH), 7.67-7.74 (1H, m, ArH), 8.00 (1H, d, $J = 7$, 4Hz, ArH), 8.40 (4H, m, ArH), 9.76 (1H, s, N = CH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ : 23.90 (COCH_3), 30.80 (CH_2Ph), [124.00, 124.57 (2C), 125.84, 127.22, 128.06, 131.92 (2C), 133.47, 133.71, 149.50, 150.81] (Ar-C), [127.62, 128.18, 129.40, 130.70, 131.03, 134.19] (Ar-C linked to C_3), 147.98 (triazole- C_3), 150.18 (triazole- C_5), 151.24 (N = CH), 163.46 (COO), 166.49 (COCH_3) ppm. UV [Ethanol, λ_{max} , nm (ϵ , $\text{L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$): 250 (32.940), 220 (30.210), 214 (29.020). Mass spectrum (ESI-MS), m/z : 562, 541 (M+23), 504, 470, 468, 467, 446, 419, 416, 415, 413, 380, 372, 353, 351, 329, 311, 289, 265, 251, 238, 211, 196, 182, 165, 151 (100), 136, 120.

2-[[1-Acetyl-3-phenyl-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl]azomethine]phenyl 4-nitrobenzoate, 4i: White solid; yield: 93%; mp 233-235 °C. IR (ATR, cm^{-1}) ν_{max} 1742, 1716 (C = O), 1606 (C = N), 1526 and 1354 (NO_2), 1264 (COO), 844 (1,4-disubstituted benzenoid ring), 765 (1,2-disubstituted benzenoid ring), 759 and 684 (monosubstituted

benzenoid ring). ¹HNMR (300 MHz, DMSO-d₆) δ: 2.50 (3H, s, COCH₃), 7.55-7.98 (9H, m, ArH), 8.32 (4H, m, ArH), 9.65 (1H, s, N = CH) ppm. ¹³C NMR (75 MHz, DMSO-d₆) δ: 23.45 (COCH₃), [123.88 (3C), 123.95, 125.07, 127.60, 131.33 (2C), 133.37, 133.83, 149.50, 150.60] (Ar-C), [125.21, 127.26 (2C), 128.41, 128.53 (2C)] (Ar-C linked to C₃), 145.94 (triazole-C₃), 148.01 (triazole-C₅), 154.43 (N = CH), 163.04 (COO), 166.33 (COCH₃) ppm. UV [Ethanol, λ_{max}, nm (ε, L.mol⁻¹.cm⁻¹): 280 (32.875), 242 (35.375), 236 (35.340)]. Mass spectrum (ESI-MS), *m/z*: 542, 528, 495 (M+1+23), 494(50, M+23), 472 (M+1), 452, 430, 413, 391, 379, 365, 351, 337, 316, 308, 306, 301, 279, 255, 252 (100), 238, 234, 233, 209, 207, 195, 193, 185, 177, 145, 142, 120, 115, 102.

2-[[1-(4-Acetyl-3-cyclopropyl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl)azomethine]phenyl 4-nitrobenzoate, 4j: White solid; yield: 62%; mp 213-215 °C. IR (ATR, cm⁻¹) ν_{max} 1740, 1721, 1689 (C = O), 1607 (C = N), 1527 and 1352 (NO₂), 1265 (COO), 846 (1,4-disubstituted benzenoid ring), 764 (1,2-disubstituted benzenoid ring). ¹HNMR (300 MHz, DMSO-d₆) δ: 0.79-0.94 (4H, m, CH₂CH₂), 1.94-2.03 (1H, m, CH), 2.38 (3H, s, COCH₃), 7.49-7.56 (2H, m, ArH), 7.65-7.74 (1H, m, ArH), 8.09 (1H, d, *J* = 7,9 Hz, ArH), 8.43 (4H, m, ArH), 9.74 (1H, s, N = CH) ppm. ¹³C NMR (75 MHz, DMSO-d₆) δ: 5.35 (CH), 6.45 (CH₂CH₂), 23.30 (COCH₃), [123.53, 123.59 (2C), 124.00, 125.44, 127.77, 131.45 (2C), 132.57, 133.15, 149.59, 151.25] (Ar-C), 147.94 (triazole-C₃), 149.29 (triazole-C₅), 151.53 (N = CH), 162.97 (COO), 165.89 (COCH₃) ppm. UV [Ethanol, λ_{max}, nm (ε, L.mol⁻¹.cm⁻¹): 250 (23.755), 242 (22.930), 216 (19.940)]. Mass spectrum (ESI-MS), *m/z*: 545, 544, 542, 541, 511, 442, 414, 413, 372, 363, 361, 347, 332, 330 (100), 325, 309, 280, 267, 245, 235, 213, 192, 182, 165, 151, 137, 118.

General procedure for the synthesis of 2-[[1-(4-piperidinecarboxamide-1-yl-methyl)-3-alkyl(aryl)-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl]azomethine}-phenyl 4-nitrobenzoates 5

The corresponding compound **3** (5mmol) was dissolved absolute ethanol and to this solution were added formaldehyde (% 37, 10 mmol) and 4-piperidinecarboxamide (6 mmol). The reaction mixture was refluxed for 4 hours and filtered. The solution was left at room temperature for 1 overnight and after cooling of the mixture in the -18 °C refrigerator. The solid formed was obtained by filtration, washed with cold ethanol. Several

recrystallizations of the crude product from ethanol gave pure compounds **5**.

2-[[1-(4-Piperidinecarboxamide-1-yl-methyl)-3-ethyl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl]-azomethine}-phenyl 4-nitrobenzoate, 5b: White solid; yield: 74%; mp 222-224 °C. IR (ATR, cm⁻¹) ν_{max} 3362 and 3182 (NH₂), 1735, 1694, 1653 (C = O), 1604, 1587 (C = N), 1527 and 1349 (NO₂), 1260 (COO), 843 (1,4-disubstituted benzenoid ring), 797 (1,2-disubstituted benzenoid ring). ¹HNMR (300 MHz, DMSO-d₆) δ: 1.08 (3H, t, *J* = 7,6 Hz, CH₂CH₃), [1.46-1.49 (m), 1.55-1.60 (m), 1.90-1.93 (m), 2.20-2.21 (m), 2.83-2.86 (m)] (piperidine 9H), 2.47 (3H, t, *J* = 7,6 Hz, CH₂CH₃), 4.45 (2H, s, NCH₂N), 6.67 (1H, s, NH), 7.12 (1H, s, NH), 7.52 (2H, d, *J* = 8,0 Hz, ArH), 7.67-7.68 (1H, m, ArH), 8.02-8.04 (1H, m, ArH), 8.42-8.46 (4H, m, ArH), 9.88 (1H, s, N = CH). ¹³C NMR (75 MHz, DMSO-d₆) δ: 9.91 (CH₂CH₃), 18.19 (CH₂CH₃), 28.35 (2CH₂), 41.07 (CH), 49.67 (2CH₂), 66.30 (NCH₂N), [123.61, 124.02 (2C), 124.07, 125.88, 127.79, 131.48 (2C), 132.57, 132.70, 149.37, 150.82] (Ar-C), 146.47 (triazole-C₃), 149.01 (triazole-C₅), 151.30 (N = CH), 162.98 (COO), 176.36 (CONH₂) ppm.

2-[[1-(4-Piperidinecarboxamide-1-yl-methyl)-3-(*n*-propyl)-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl]-azomethine}-phenyl 4-nitrobenzoate, 5c: White solid; yield: 73%; mp 227-229°C. IR (ATR, cm⁻¹) ν_{max} 3348 and 3184 (NH₂), 1735, 1695, 1654 (C = O), 1603, 1582 (C = N), 1523 and 1345 (NO₂), 1259 (COO), 813 (1,4-disubstituted benzenoid ring), 744 (1,2-disubstituted benzenoid ring). ¹HNMR (300 MHz, DMSO-d₆) δ: 0.85 (3H, t, *J* = 7,6 Hz, CH₂CH₂CH₃), 1.57 (2H, sext, *J* = 7,6 Hz, CH₂CH₂CH₃), [1.44-1.48 (m), 1.60-1.64 (m), 1.92-1.96 (m), 2.20-2.23 (m), 2.83-2.86 (m)] (piperidine 9H), 2.44 (2H, t, *J* = 7,2 Hz, CH₂CH₂CH₃), 4.46 (2H, s, NCH₂N), 6.69 (1H, s, NH), 7.14 (1H, s, NH), 7.52 (2H, d, *J* = 8,0 Hz, ArH), 7.66-7.68 (1H, m, ArH), 8.02-8.04 (1H, m, ArH), 8.40-8.46 (4H, m, ArH), 9.88 (1H, s, N = CH). ¹³C NMR (75 MHz, DMSO-d₆) δ: 13.31 (CH₂CH₂CH₃), 18.67 (CH₂CH₂CH₃), 26.25 (CH₂CH₂CH₃), 28.36 (2CH₂), 41.08 (CH), 49.66 (2CH₂), 66.25 (NCH₂N), [123.63, 124.09 (2C), 125.76, 127.14, 128.09, 131.49 (2C), 132.72, 133.92, 149.51, 150.22] (Ar-C), 145.25 (triazole-C₃), 149.35 (triazole-C₅), 150.83 (N = CH), 162.98 (COO), 176.37 (CONH₂) ppm.

2-[[1-(4-Piperidinecarboxamide-1-yl-methyl)-3-benzyl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl]-azomethine}-phenyl 4-nitrobenzoate, 5d: White

solid; yield: 80%; mp 210-212°C. IR (ATR, cm^{-1}) ν_{max} 3380 and 3187 (NH_2), 1739, 1707, 1646 ($\text{C}=\text{O}$), 1603 ($\text{C}=\text{N}$), 1530 and 1347 (NO_2), 1261 (COO), 843 (1,4-disubstituted benzenoid ring), 744 (1,2-disubstituted benzenoid ring), 758 and 708 (monosubstituted benzenoid ring). ^1H NMR (300 MHz, DMSO-d_6) δ : [1.45-1.48 (m), 1.61-1.64 (m), 1.91-1.94 (m), 2.18-2.23 (m), 2.83-2.86 (m)] (piperidine 9H), 3.94 (2H, s, CH_2Ph), 4.47 (2H, s, NCH_2N), 6.70 (1H, s, NH), 7.14 (1H, s, NH), 7.21-7.32 (5H, m, ArH), 7.51 (2H, d, $J=8,4$ Hz, ArH), 7.64-7.68 (1H, m, ArH), 7.99 (1H, d, $J=8,0$ Hz, ArH), 8.37-8.39 (4H, m, ArH), 9.87 (1H, s, $\text{N}=\text{CH}$). ^{13}C NMR (75 MHz, DMSO-d_6) δ : 28.35 (2CH_2), 30.78 (CH_2Ph), 41.07 (CH), 49.66 (2CH_2), 66.38 (NCH_2N), [122.95, 123.47 (2C), 124.01, 125.81, 127.38, 131.44 (2C), 132.63, 133.85, 146.07, 150.78] (Ar-C), [126.69, 127.08 (2C), 128.71 (2C), 135.56] (Ar-C linked to C_3), 144.63 (triazole- C_3), 149.53 (triazole- C_5), 151.15 ($\text{N}=\text{CH}$), 162.99 (COO), 176.94 (CONH_2) ppm.

2-[[1-(4-Piperidinecarboxamide-1-yl-methyl)-3-(*p*-methylbenzyl)-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl]-azomethine}-phenyl 4-nitrobenzoate, 5e: White solid; yield: 69%; mp 219-221°C. IR (ATR, cm^{-1}) ν_{max} 3364 and 3187 (NH_2), 1738, 1703, 1649 ($\text{C}=\text{O}$), 1604, 1584 ($\text{C}=\text{N}$), 1524 and 1348 (NO_2), 1262 (COO), 843, 813 (1,4-disubstituted benzenoid ring), 780 (1,2-disubstituted benzenoid ring). ^1H NMR (300 MHz, DMSO-d_6) δ : 2.24 (3H, s, PhCH_3), [1.44-1.48 (m), 1.61-1.64 (m), 1.92 (m), 2.17-2.20 (m), 2.83-2.86 (m)] (piperidine 9H), 3.88 (2H, s, CH_2Ph), 4.47 (2H, s, NCH_2N), 6.70 (1H, s, NH), 7.08-7.13 (4H, m, ArH), 7.14 (1H, s, NH), 7.66 (2H, d, $J=8,0$ Hz, ArH), 7.67-7.69 (1H, m, ArH), 8.00-8.02 (1H, m, ArH), 8.39-8.42 (4H, m, ArH), 9.86 (1H, s, $\text{N}=\text{CH}$). ^{13}C NMR (75 MHz, DMSO-d_6) δ : 20.56 (PhCH_3), 28.35 (2CH_2), 30.19 (CH_2Ph), 41.08 (CH), 49.66 (2CH_2), 66.37 (NCH_2N), [123.54, 124.06 (2C), 125.72, 127.10, 127.47, 131.43 (2C), 132.34, 132.76, 148.79, 150.16] (Ar-C), [128.47 (2C), 129.01 (2C), 133.81, 135.84] (Ar-C linked to C_3), 144.78 (triazole- C_3), 149.55 (triazole- C_5), 150.79 ($\text{N}=\text{CH}$), 163.00 (COO), 176.34 (CONH_2) ppm.

2-[[1-(4-Piperidinecarboxamide-1-yl-methyl)-3-(*p*-methoxybenzyl)-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl]-azomethine}-phenyl 4-nitrobenzoate, 5f: White solid; yield: 74%; mp 212-214°C. IR (ATR, cm^{-1}) ν_{max} 3367 and 3190 (NH_2), 1739, 1702, 1649 ($\text{C}=\text{O}$), 1607, 1585 ($\text{C}=\text{N}$), 1526 and 1346 (NO_2), 1263 (COO), 842, 820 (1,4-disubstituted benzenoid

ring), 766 (1,2-disubstituted benzenoid ring). ^1H NMR (300 MHz, DMSO-d_6) δ : [1.44-1.50 (m), 1.61-1.64 (m), 1.90-1.93 (m), 2.17-2.23 (m), 2.83-2.86 (m)] (piperidine 9H), 3.71 (3H, s, OCH_3), 3.86 (2H, s, CH_2Ph), 4.46 (2H, s, NCH_2N), 6.70 (1H, s, NH), 6.85 (2H, d, $J=8,4$ Hz, ArH), 7.15 (2H, d, $J=8,4$ Hz, ArH), 7.18 (1H, s, NH), 7.49-7.53 (2H, m, ArH), 7.65-7.69 (1H, m, ArH), 8.02-8.03 (1H, m, ArH), 8.39-8.40 (4H, m, ArH), 9.87 (1H, s, $\text{N}=\text{CH}$). ^{13}C NMR (75 MHz, DMSO-d_6) δ : 28.35 (2CH_2), 29.75 (CH_2Ph), 41.07 (CH), 49.66 (2CH_2), 55.02 (OCH_3), 66.37 (NCH_2N), [123.53, 124.05 (2C), 125.73, 127.11, 127.46, 131.43 (2C), 132.75, 133.81, 148.77, 150.17] (Ar-C), [113.90 (2C), 127.20, 129.67 (2C), 158.13] (Ar-C linked to C_3), 144.94 (triazole- C_3), 148.77 (triazole- C_5), 150.78 ($\text{N}=\text{CH}$), 163.00 (COO), 176.34 (CONH_2) ppm.

2-[[1-(4-Piperidinecarboxamide-1-yl-methyl)-3-(*p*-chlorobenzyl)-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl]-azomethine}-phenyl 4-nitrobenzoate, 5g: White solid; yield: 74%; mp 212-214°C. IR (ATR, cm^{-1}) ν_{max} 3360 and 3184 (NH_2), 1738, 1708, 1647 ($\text{C}=\text{O}$), 1605, 1584 ($\text{C}=\text{N}$), 1523 and 1348 (NO_2), 1263 (COO), 842, 804 (1,4-disubstituted benzenoid ring), 778 (1,2-disubstituted benzenoid ring). ^1H NMR (300 MHz, DMSO-d_6) δ : [1.44-1.50 (m), 1.61-1.64 (m), 1.93 (m), 2.17-2.23 (m), 2.82-2.85 (m)] (piperidine 9H), 3.95 (2H, s, CH_2Ph), 4.47 (2H, s, NCH_2N), 6.70 (1H, s, NH), 7.14 (1H, s, NH), 7.24 (2H, d, $J=8,4$ Hz, ArH), 7.36 (2H, d, $J=8,4$ Hz, ArH), 7.49 (2H, d, $J=8,4$ Hz, ArH), 7.65-7.69 (1H, m, ArH), 7.98-8.00 (1H, m, ArH), 8.37-8.40 (4H, m, ArH), 9.87 (1H, s, $\text{N}=\text{CH}$). ^{13}C NMR (75 MHz, DMSO-d_6) δ : 28.33 (2CH_2), 29.93 (CH_2Ph), 41.04 (CH), 49.62 (2CH_2), 66.41 (NCH_2N), [123.54, 124.06 (2C), 125.67, 127.10, 127.44, 131.44 (2C), 132.80, 133.80, 148.87, 150.15] (Ar-C), [128.39 (2C), 130.52 (2C), 131.49, 134.44] (Ar-C linked to C_3), 144.30 (triazole- C_3), 149.57 (triazole- C_5), 150.79 ($\text{N}=\text{CH}$), 162.98 (COO), 176.34 (CONH_2) ppm.

General procedure for the synthesis of *N,N'*-bis-[3-alkyl(aryl)-4-[2-(4-nitrobenzoxy)-benzylideneamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-one-1-yl-methyl]-piperazines 6

The corresponding compound **3** (5 mmol) was dissolved absolute ethanol and to this solution were added formaldehyde (% 37, 10 mmol) and piperazine (6 mmol). The reaction mixture was refluxed for 4 hours and filtered. The solution was left at room temperature for 1 overnight and after cooling of the mixture in the -18 °C refrigerator. The solid formed was obtained by filtration, washed with cold ethanol.

Several recrystallizations of the crude product from ethanol gave pure compounds **6**.

N,N'*-bis-{3-methyl-4-[2-(4-nitrobenzoxy)-benzylidenamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-on-1-yl-methyl}-piperazine, **6a*: White solid; yield: 75%; mp 245-247 °C. IR (ATR, cm⁻¹) ν_{\max} 1739, 1694 (C=O), 1594 (C=N), 1529 and 1346 (NO₂), 1255 (COO), 843 (1,4-disubstituted benzenoid ring), 791 (1,2-disubstituted benzenoid ring). ¹HNMR (300 MHz, DMSO-d₆) δ : 2.08 (6H, s, 2CH₃), 2.54 (8H, s, 4CH₂), 4.40 (4H, s, 2NCH₂N), 7.48-7.52 (4H, m, ArH), 7.65-7.69 (2H, m, ArH), 8.03 (2H, d, *J* = 8,0 Hz, ArH), 8.38-8.42 (8H, m, ArH), 9.86 (2H, s, N = CH). ¹³C NMR (75 MHz, DMSO-d₆) δ : 11.20 (2CH₃), 49.35 (4CH₂), 65.70 (2NCH₂N), [123.50 (2C), 124.00 (4C), 124.20 (2C), 125.90 (2C), 127.75 (2C), 131.40 (4C), 132.25 (2C), 132.60 (2C), 149.40 (2C), 151.00 (2C)] (Ar-C), 148.10 (2triazole-C₃), 149.50 (2triazole-C₅), 151.40 (2N = CH), 163.00 (2COO) ppm.

N,N'*-bis-{3-benzyl-4-[2-(4-nitrobenzoxy)-benzylidenamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-on-1-yl-methyl}-piperazine, **6d*: White solid; yield: 82%; mp 226-227 °C. IR (ATR, cm⁻¹) ν_{\max} 1738, 1704 (C=O), 1603, 1584 (C=N), 1527 and 1350 (NO₂), 1256 (COO), 813 (1,4-disubstituted benzenoid ring), 769 (1,2-disubstituted benzenoid ring), 769 and 709 (monosubstituted benzenoid ring). ¹HNMR (300 MHz, DMSO-d₆) δ : 2.54 (8H, s, 4CH₂), 3.92 (4H, s, 2CH₂Ph), 4.44 (4H, s, 2NCH₂N), 7.18-7.30 (10H, m, ArH), 7.47-7.51 (4H, m, ArH), 7.64-7.68 (2H, m, ArH), 7.98 (2H, d, *J* = 8,0 Hz, ArH), 8.36-8.40 (8H, m, ArH), 9.85 (2H, s, N = CH). ¹³C NMR (75 MHz, DMSO-d₆) δ : 30.59 (2CH₂Ph), 49.29 (4CH₂), 65.83 (2NCH₂N), [123.53 (2C), 124.03 (4C), 125.68 (2C), 127.04 (2C), 127.44 (2C), 131.43 (4C), 132.77 (2C), 133.80 (2C), 148.90 (2C), 150.10 (2C)] (Ar-C), [126.74 (2C), 128.43 (4C), 128.61 (4C), 135.41 (2C)] (Ar-C linked to C₃), 144.70 (2triazole-C₃), 149.54 (2triazole-C₅), 150.76 (2N = CH), 162.97 (2COO) ppm.

N,N'*-bis-{3-(*p*-chlorobenzyl)-4-[2-(4-nitrobenzoxy)-benzylidenamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-on-1-yl-methyl}-piperazine, **6g*: White solid; yield: 70%; mp 248-250 °C. IR (ATR, cm⁻¹) ν_{\max} 1740, 1709 (C=O), 1603, 1582 (C=N), 1524 and 1348 (NO₂), 1260 (COO), 841 (1,4-disubstituted benzenoid ring), 798 (1,2-disubstituted benzenoid ring). ¹HNMR (300 MHz, DMSO-d₆) δ : 2.50 (8H, s, 4CH₂), 3.92 (4H, s, 2CH₂Ph), 4.46 (4H, s, 2NCH₂N), 7.25-7.36 (8H, m, ArH), 7.48-7.53 (4H, m, ArH), 7.65-7.67 (2H, m, ArH), 7.99 (2H, d, *J* = 8,0 Hz, ArH), 8.37-8.40 (8H, m, ArH),

9.90 (2H, s, N = CH). ¹³C NMR (75 MHz, DMSO-d₆) δ : 30.50 (2CH₂Ph), 50.00 (4CH₂), 66.00 (2NCH₂N), [123.50 (2C), 124.03 (4C), 124.10 (2C), 125.80 (2C), 128.01 (2C), 131.46 (4C), 132.60 (2C), 133.80 (2C), 149.50 (2C), 150.80 (2C)] (Ar-C), [128.33 (4C), 130.65 (4C), 131.30 (2C), 134.60 (2C)] (Ar-C linked to C₃), 147.10 (2triazole-C₃), 148.50 (2triazole-C₅), 151.10 (2N = CH), 163.00 (2COO) ppm.

N,N'*-bis-{3-phenyl-4-[2-(4-nitrobenzoxy)-benzylidenamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-on-1-yl-methyl}-piperazine, **6i*: White solid; yield: 78%; mp 258-260 °C. IR (ATR, cm⁻¹) ν_{\max} 1743, 1698 (C=O), 1602 (C=N), 1523 and 1346 (NO₂), 1254 (COO), 841 (1,4-disubstituted benzenoid ring), 791 (1,2-disubstituted benzenoid ring), 769 and 692 (monosubstituted benzenoid ring). ¹HNMR (300 MHz, DMSO-d₆) δ : 2.59 (8H, s, 4CH₂), 4.58 (4H, s, 2NCH₂N), 7.48-7.97 (18H, m, ArH), 8.25-8.29 (8H, m, ArH), 9.77 (2H, s, N = CH). ¹³C NMR (75 MHz, DMSO-d₆) δ : 50.00 (4CH₂), 65.80 (2NCH₂N), [123.60 (2C), 123.87 (4C), 124.00 (2C), 125.70 (2C), 127.90 (2C), 131.31 (4C), 132.80 (2C), 133.80 (2C), 149.90 (2C), 151.20 (2C)] (Ar-C), [126.40 (2C), 128.05 (4C), 128.40 (2C), 128.55 (4C)] (Ar-C linked to C₃), 146.60 (2triazole-C₃), 150.10 (2triazole-C₅), 152.00 (2N = CH), 163.00 (2COO) ppm.

N,N'*-bis-{3-cyclopropyl-4-[2-(4-nitrobenzoxy)-benzylidenamino]-4,5-dihydro-1*H*-1,2,4-triazol-5-on-1-yl-methyl}-piperazine, **6j*: White solid; yield: 74%; mp 229-231 °C. IR (ATR, cm⁻¹) ν_{\max} 1738, 1691 (C=O), 1604, 1587 (C=N), 1527 and 1346 (NO₂), 1257 (COO), 841 (1,4-disubstituted benzenoid ring), 791 (1,2-disubstituted benzenoid ring). ¹HNMR (300 MHz, DMSO-d₆) δ : 0.80 (8H, d, *J* = 6,8 Hz, 2CH₂CH₂), 1.87-1.91 (2H, m, 2CH), 2.50 (8H, s, 4CH₂), 4.40 (4H, s, 2NCH₂N), 7.49-7.54 (4H, m, ArH), 7.66-7.69 (2H, m, ArH), 8.05 (2H, d, *J* = 7,2 Hz, ArH), 8.42 (8H, m, ArH), 9.91 (2H, s, N = CH). ¹³C NMR (75 MHz, DMSO-d₆) δ : 5.35 (2CH), 6.45 (2CH₂CH₂), 49.30 (4CH₂), 65.65 (2NCH₂N), [123.57 (2C), 124.00 (2C), 124.03 (4C), 127.10 (2C), 127.83 (2C), 131.49 (4C), 132.61 (2C), 133.96 (2C), 148.07 (2C), 149.39 (2C)] (Ar-C), 147.10 (2triazole-C₃), 140.05 (2triazole-C₅), 151.26 (2N = CH), 163.00 (2COO) ppm.

Antimicrobial activity

All bacterial and yeast strains were obtained from the company of Microbiological Environmental Protection Laboratories (France) and were as follows: *Bacillus Substilis*(ATCC 11774), *Bacillus Cereus*

(ATCC 11778), *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^{16,17}. 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

The antioxidant properties of newly synthesized compounds and standard antioxidants butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), α -tocopherol and ethylenediaminetetraacetic acid (EDTA) were studied and evaluated using different antioxidant tests; including reducing power, free radical scavenging and metal chelating activity. The reducing power of the synthesized compounds and standards was determined according to the method¹⁸. Free radical scavenging activity of the synthesized compounds and standards was measured via DPPH (2,2-diphenyl-1-picrylhydrazyl) by using the method¹⁹. The chelation of ferrous ions by the synthesized compounds and standards was estimated by the method²⁰. All the methods have been extensively investigated in the literature⁵.

Conclusion

In conclusion, new 4,5-dihydro-1H-1,2,4-triazol-5-one derivatives were obtained and evaluated for

their *in vitro* antimicrobial and antioxidant capacity. From the screening results, some of the Mannich bases showed good activity against to the tested microorganisms. Besides, all the compounds demonstrate a marked ability for metal chelating activity but Mannich bases were found to be most active when compared to Schiff bases. The data reported about the observed biological activities of the studied compounds could improve the new triazole-based therapeutic target.

References

- Sahu J K, Ganguly S & Kaushik A, *Chin J Nat Med*, 11 (2013) 456.
- Singh R J & Singh D K, *Asian J Chem*, 22 (2010) 2664.
- Chen X, Shi Y M, Huang C, Xia S, Yang L J & Yang X D, *Anticancer Agents Med Chem*, 16 (2016) 377.
- Thakkar S S, Thakor P, Doshi H & Ray A, *Bioorg Med Chem*, 25 (2017) 4064.
- Aktaş-Yokuş Ö, Yüksek H, Manap S, Aytemiz F, Alkan M, Beytur M & Gürsoy-Kol Ö, *Bulg Chem Commun*, 49 (2017) 98.
- Roman G, *Eur J Med Chem*, 89 (2015) 743.
- Tramontini M & Angiolini L, *Mannich Bases: Chemistry and Uses*, (CRC Press, Boca Raton), 1994.
- Al-Abdullah E S, Al-Tuwaijri H M, Hassan H M, Haiba M E, Habib E E & El-Emam A A, *Int J Mol Sci*, 15 (2014) 22995.
- Boren Y, Xinyang L, Huan D & Xiaoping B, *Chin J Org Chem*, 36 (2016) 207.
- Ceylan S, *Med Chem Res*, 25 (2016) 1958.
- Manap S, Gürsoy-Kol Ö, Alkan M & Yüksek H, *Indian J Chem*, 59B (2020) 271.
- Antimicrobials: New and Old Molecules in the Fight Against Multi-Resistant Bacteria*, edited by Marinelli F & Genilloud O (Springer-Verlag, Berlin), 2014.
- Ikizler A A & Un R, *Chim Acta Turc*, 7 (1979) 269.
- Ikizler A A & Yuksek H, *Org Prep Proced Int*, 25 (1993) 99.
- Henderson J A R & Heilbron I M, *J Chem Soc Trans*, 107(1915) 1740.
- Perez C, Pauli M & Bazerque P, *Acta Biol Med Exp*, 15 (1990) 113.
- Ahmad I, Mehmood Z & Mohammed F, *J Ethnopharmacol*, 62 (1998) 183.
- Oyaizu M, *Jpn J Nutr*, 44 (1986) 307.
- Blois M S, *Nature*, 181 (1958) 1199.
- Dinis T C, Madeira V M & Almeida L M, *Arch Biochem Biophys*, 315 (1994) 161.