ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Preparation of Some New Bis-[4-(3-alkyl/aryl-4, 5-dihydro-1*H*-1, 2, 4-triazol-5-on-4-yl)-azomethinphenyl] Phtalate Derivatives with Their Antioxidant and Antimicrobial Activities.

Murat Beytur[1], Sevda Manap[1], Gül Özdemir[1], ÖzlemGürsoy Kol[1]*, Fevzi Aytemiz[1], Muzaffer Alkan[2] and Haydar Yüksek[1]

[1]Kafkas University, Department of Chemistry, 36100 Kars, Turkey.
[2]Kafkas University, Faculty of Education, Primary-Science Education, 36100 Kars, Turkey.

ABSTRACT

In this study, nine novel bis-[4-(3-alkyl/aryl-4,5-dihydro-1*H*-1,2,4-triazol-5-on-4-yl)-azomethinphenyl] phthalates (**3**) were synthesized from the reactions of 3-alkyl(aryl)-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**1**) with bis-(4-formylphenyl) phthalate (**2**). Then, the compounds**3a**, **3b**, **3d**, **3e** and **3g** were treated with morpholine in the presence of formaldehyde to obtain bis-{4-[1-(morpholine-4-yl-methyl)-3-alkyl/aryl-4,5-dihydro-1*H*-1,2,4-triazol-5-on-4-yl]-azomethinphenyl} phthalates (**4**).The newly synthesized compounds were characterized using IR, ^{1}H-NMR and ^{13}C-NMR spectral data. In addition, the newly synthesized 3 and 4 type compounds were screened for their antimicrobial activities. Furthermore, the antioxidant properties of the **3** and **4** type compounds were analyzed for their in vitro potential antioxidant activities in three different methods (reducing power, free radical scavenging and metal chelating activity).

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

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Schiff bases having the azomethine group or CH=N imine bonds are prepared by the condensation between amines and activated carbonyl compounds [1, 2]. Schiff bases have been extensively studied due to their applicability in various areas such as biological [3-6], chemical [7-9], industrial [10] and pharmaceutical applications [11, 12]. Schiff base derivatives have recently increased studies related to corrosion inhibitors [13], optical sensors [14], highly selective polymer membrane electrodes [15], semiconducting [16], therapeutic properties, highly thermal stability, modern technology (nonlinear optical materials) [17], various coordination, homogeneous catalysis [17, 18] and biological probes [19]. As a result of well-synthesized structures, all properties make them and their derivatives useful in an organic structure in electronic and opto-electronic devices, pharmaceutical products or thermo-durable materials [20, 21]. They are widely used in the pharmaceutical industry because of their valuable clinical and pharmacological properties [22]. The azomethine moiety plays a very important role in biological active systems [23]. It has also been shown to exhibit a wide range of biological activities including antibacterial [5], antitumor [24], antiproliferative [25], antimalarial [26], anti-inflammatory [27] and antioxidant [6, 9, 28].

In the present study, new Schiff base derivatives **(3a-i)** and new Mannich base derivatives **(4a, b, d, e, g)** were designed and synthesized. The structures of the newly synthesized compounds were characterized by different spectroscopic methods. The titled compounds were analyzed for their antioxidant activities in three different methods (reducing power, free radical scavenging and metal chelating activity), were drawn their graphs and their results were interpreted. Furthermore, *invitro* antimicrobial properties of novel heterocyclic compounds were investigated and evaluated against six different microorganisms with agar well diffusion method.

MATERIAL AND METHODS

Chemical reagents used in the study were supplied from Sigma (Sigma-Aldrich GmbH, Germany), Fluka (Switzerland) and Merck AG, (Germany). The starting compounds **1a-i** were obtained from the reactions of the corresponding ester ethoxycarbonylhydrazones with hydrazine hydrate (37 %) as indicated in the literature [29, 30]. Melting points were identified using a Stuart SMP30 melting point apparatus with open glass capillaries (United Kingdom). ^{1}H and ^{13}C NMR spectra were recorded in deuterated dimethyl sulfoxide (DMSO-d_6) using a Bruker spectrometer (Germany) at 400 MHz and 100 MHz, respectively.

EXPERIMENTAL SECTION

Chemistry

General Procedure for the synthesis of bis-[4-(3-alkyl/aryl-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl)-azomethinphenyl] phthalates (3)

4-Hydroxybenzaldehyde (0.02 mol) dissolved in ethyl acetate (100 mL) was treated with phthaloyl dichloride (0.01 mol), and to this solution was slowly added triethylamine (0.04 mol) with stirring at 0-5 °C. Stirring was 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** [31].Yield: 86.65; m.p. 173°C; IR (cm^{-1}) υ_{max}: 2863 and 2746 (CHO),1757, 1736, 1688 (C=O),1252 (COO),(1,4-disubstituted benzenoid ring),758 (1,2-disubstituted benzenoid ring). ^{1}H-NMR (400 MHz, DMSO-d_6) (ppm) δ H: 7.53 (d, 4H, ArH, *J* = 8.40 Hz), 7.89-7.92 (m, 2H, ArH), 8.02-8.05 (m, 4H, ArH), 8.14-8.16 (m, 2H, ArH), 10.03 (s, 2H, 2CHO).^{13}C-NMR (100 MHz, DMSO-d_6) (ppm) δ C: 122.49 (4C), 128.33, 129.71, 130.41, 130.69, 131.24 (4C), 132.76 (2C), 134.29 (2C), 154.77 (2C) (Ar-C), 164.86 (2COO), 191.92 (2CHO).Then the corresponding compound 1 (0.02mol) was dissolved in acetic acid (20 mL) and treated with bis-(4-formylphenyl) phthalate (2) (0.01 mol). The mixture was refluxed for 2 h and then evaporated at 50-55 °C *in vacuo*. Several recrystallizations of the residue from ethanol gave pure compounds **3**as colorless crystals.

Bis-[4-(3-methyl-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl)-azomethinphenyl] phtalate (3a)

White solid; yield: 94.93%; m.p. 255°C; IR (cm^{-1})υ_{max}: 3268 (NH), 1748, 1698 (C=O),1601 (C=N), 1260 (COO),814 (1,4-disubstituted benzenoid ring),765 (1,2-disubstituted benzenoid ring). ^{1}H-NMR (400 MHz,

DMSO-d_6) (ppm) δ H: 2.29 (s, 6H, $2CH_3$), 7.42 (d, 4H, ArH, *J*= 8.40 Hz), 7.89-7.91 (m, 2H, ArH), 7.95 (d, 4H, ArH; *J*=8.80 Hz), 8.13-8.15 (m, 2H, ArH), 9.76 (s, 2H, N=CH), 11.83 (s, 2H, NH);^{13}C-NMR (100 MHz, DMSO-d_6) (ppm) δ C: 11.06 ($2CH_3$), [122.49 (4C), 129.13, 129.63 (4C), 130.57, 131.24, 131.72, 132.65 (2C), 134.28 (2C), 144.20 (2C)] (Ar-C), 144.27 (2triazole C_3), 151.22 (2triazole C_5), 152.50 (2N=CH), 165.11 (2COO).

Bis-[4-(3-ethyl-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl)-azomethinphenyl] phtalate (3b)

White solid; yield: 91.21%; m.p. 263°C; IR (cm^{-1}) υ_{max}: 3262 (NH), 1736, 1699 (C=O), 1597 (C=N), 1265 (COO), 838 (1,4-disubstituted benzenoid ring), 783 (1,2-disubstituted benzenoid ring). ^{1}H-NMR (400 MHz, DMSO-d_6) (ppm) δ H: 1.13 (t, 6H, $2CH_2C\underline{H}_3$, *J*=7.60 Hz), 2.69 (q, 4H, $2C\underline{H}_2CH_3$, *J*=7.60 Hz), 7.42 (d, 4H, ArH, *J* = 8.40 Hz), 7.89-7.91 (m, 2H, ArH), 7.94 (d, 4H, ArH; *J* = 8.80 Hz), 8.13-8.15 (m, 2H, ArH), 9.76 (s, 2H, N=CH), 11.85 (s, 2H, NH); ^{13}C-NMR (100 MHz, DMSO-d_6) (ppm) δ C: 10.06 ($2CH_2\underline{C}H_3$), 18.50 ($2\underline{C}H_2CH_3$), [122.48 (4C), 128.97, 129.08 (4C), 129.62, 130.58, 131.23, 131.76 (2C), 132.63 (2C), 152.80 (2C)] (Ar-C), 148.04 (2triazole C_3), 151.36 (2triazole C_5), 152.56 (2N=CH), 165.90 (2COO).

Bis-[4-(3-n-propyl-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl)-azomethinphenyl] phtalate (3c)

White solid; yield: 94.59%; m.p. 255°C; IR (cm^{-1}) υ_{max}: 3240 (NH), 1746, 1712 (C=O), 1595 (C=N), 1253 (COO), 838 (1,4-disubstituted benzenoid ring), 799 (1,2-disubstituted benzenoid ring). ^{1}H-NMR (400 MHz, DMSO-d_6) (ppm) δ H: 0.97 (t, 6H, $2CH_2CH_2C\underline{H}_3$, *J*=7.20 Hz), 1.71 (sext, 4H, $2CH_2C\underline{H}_2CH_3$, *J*=7.20 Hz), 2.66 (t, 4H, $2C\underline{H}_2CH_2CH_3$, *J*=7.20 Hz), 7.43 (d, 4H, ArH, *J* = 8.40 Hz), 7.89-7.91 (m, 2H, ArH), 7.94 (d, 4H, ArH; *J* = 8.80 Hz), 8.13-8.16 (m, 2H, ArH), 9.78 (s, 2H, N=CH), 11.87 (s, 2H, NH); ^{13}C-NMR (100 MHz, DMSO-d_6) (ppm) δ C: 13.44 ($2CH_2CH_2\underline{C}H_3$), 18.92 ($2CH_2\underline{C}H_2CH_3$), 26.70 ($2CH_2CH_2\underline{C}H_3$), [122.46 (4C), 129.05 (4C), 129.60, 130.59, 131.21, 131.75, 132.60 (2C), 134.26 (2C), 151.31 (2C)] (Ar-C), 146.90 (2triazole C_3), 151.25 (2triazole C_5), 152.50 (2N=CH), 165.09 (2COO).

Bis-[4-(3-benzyl-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl)-azomethinphenyl] phtalate (3d)

White solid; yield: 95.58%; m.p. 233°C; IR (cm^{-1}) υ_{max}: 3203 (NH), 1707 (C=O), 1596 (C=N), 1263 (COO), 813 (1,4-disubstituted benzenoid ring), 775 (1,2-disubstituted benzenoid ring). ^{1}H-NMR (400 MHz, DMSO-d_6) (ppm) δ H: 4.09 (s, 4H, $2CH_2Ph$), 7.19-7.23 (m, 2H, Ar-H), 7.28-7.35 (m, 8H, Ar-H), 7.42 (d, 4H, ArH, *J* = 8.40 Hz), 7.89-7.92 (m, 2H, ArH), 7.94 (d, 4H, ArH; *J* = 8.80 Hz), 8.13-8.15 (m, 2H, ArH), 9.73 (s, 2H, N=CH), 12.00 (s, 2H, NH); ^{13}C-NMR (100 MHz, DMSO-d_6) (ppm) δ C: 31.07 ($2CH_2Ph$), [122.28, 122.50 (4C), 129.14 (4C), 129.63, 130.57, 131.25, 131.70 (2C), 132.66 (2C), 152.37 (2C)] (Ar-C), [126.68 (2C), 128.42 (4C), 128.77 (4C), 135.77 (2C)] (Ar-C linked to triazole C_3), 146.23 (2triazole C_3), 151.21 (2triazole C_5), 152.52 (2N=CH), 165.13 (2COO).

Bis-[4-(3-p-methylbenzyl-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl)-azomethin-phenyl] phtalate (3e)

White solid; yield: 95.58%; m.p. 242°C; IR (cm^{-1}) υ_{max}: 3195 (NH), 1701 (C=O), 1595 (C=N), 1262 (COO), 802 (1,4-disubstituted benzenoid ring), 760 (1,2-disubstituted benzenoid ring). ^{1}H-NMR (400 MHz, DMSO-d_6) (ppm) δ H: 2.24 (s, 6H, $2PhCH_3$), 4.02 (s, 4H, $2CH_2Ph$), 7.11 (d, 4H, Ar-H, *J* = 8.00 Hz), 7.22 (d, 4H, Ar-H, *J* = 8.00 Hz), 7.44 (d, 4H, ArH, *J* = 8.40 Hz), 7.90-7.92 (m, 2H, ArH), 7.93 (d, 4H, ArH; *J* = 8.80 Hz), 8.15-8.17 (m, 2H, ArH), 9.74 (s, 2H, N=CH), 12.00 (s, 2H, NH); ^{13}C-NMR (100 MHz, DMSO-d_6) (ppm) δ C: 20.55 ($2PhCH_3$), 30.67 ($2CH_2Ph$), [122.29, 122.50 (4C), 129.14 (4C), 129.63, 130.58, 131.24, 131.72 (2C), 132.60 (2C), 152.33 (2C)] (Ar-C), [128.64 (4C), 128.99 (4C), 132.65 (2C), 135.76 (2C)] (Ar-C linked to triazole C_3), 146.38 (2triazole C_3), 151.22 (2triazole C_5), 152.52 (2N=CH), 165.13 (2COO).

Bis-[4-(3-p-methoxybenzyl-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl)-azomethin-phenyl] phtalate (3f)

White solid; yield: 96.77%; m.p. 165°C; IR (cm^{-1}) υ_{max}: 3190 (NH), 1703 (C=O), 1594 (C=N), 1245 (COO), 810 (1,4-disubstituted benzenoid ring), 763 (1,2-disubstituted benzenoid ring). ^{1}H-NMR (400 MHz, DMSO-d_6) (ppm) δ H: 3.70 (s, 6H, $2PhOCH_3$), 4.00 (s, 4H, $2CH_2Ph$),6.88 (d, 4H, Ar-H, *J*=8.40 Hz), 7.27 (d, 4H, Ar-H, *J*=8.40 Hz), 7.45 (d, 4H, ArH, *J* = 8.40 Hz), 7.89-7.93 (m, 2H, ArH), 7.95 (d, 4H, ArH; *J* = 8.80 Hz), 8.16-8.18 (m, 2H, ArH), 9.73 (s, 2H, N=CH), 11.98 (s, 2H, NH); ^{13}C-NMR (100 MHz, DMSO-d_6) (ppm) δ C: 30.20 ($2CH_2Ph$), 54.99 ($2PhOCH_3$), [122.31, 122.51 (4C), 129.16 (4C), 129.64, 130.58, 131.25, 131.73 (2C), 132.67 (2C), 152.38 (2C)] (Ar-C), [113.88 (4C), 127.54 (2C), 129.84 (4C), 158.10 (2C)] (Ar-C linked to triazole C_3), 146.58 (2triazole C_3), 151.23 (2triazole C_5), 152.53 (2N=CH), 165.14 (2COO).

Bis-[4-(3-p-chlorobenzyl-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl)-azomethin-phenyl] phtalate (3g)

White solid; yield: 96.45%; m.p. 240°C; IR (cm^{-1}) υ_{max}: 3229 (NH), 1745, 1711 (C=O), 1597 (C=N), 1262 (COO), 815 (1,4-disubstituted benzenoid ring), 795 (1,2-disubstituted benzenoid ring). ^{1}H-NMR (400 MHz, DMSO-d_6) (ppm) δ H: 4.07 (s, 4H, $2CH_2Ph$), 7.34-7.36 (m, 8H, ArH), 7.42 (d, 4H, ArH, *J* = 8.40 Hz), 7.90-7.92 (m, 6H, ArH), 8.13-8.16 (m, 2H, ArH), 9.73 (s, 2H, N=CH), 12.02 (s, 2H, NH); ^{13}C-NMR (100 MHz, DMSO-d_6) (ppm) δ C: 30.38 ($2CH_2Ph$), [122.31, 122.52 (4C), 129.18 (4C), 129.64, 130.71, 131.25, 131.66 (2C), 132.68 (2C), 152.48 (2C)] (Ar-C),[128.37 (4C),130.57 (4C),131.44 (2C),134.73 (2C)] (Ar-C linked to triazole C_3), 145.90 (2triazole C_3), 151.19 (2triazole C_5), 152.55 (2N=CH), 165.13 (2COO).

Bis-[4-(3-m-chlorobenzyl-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl)-azomethin-phenyl] phtalate (3h)

White solid; yield: 97.87%; m.p. 226°C; IR (cm^{-1}) υ_{max}: 3178 (NH), 1702 (C=O), 1595 (C=N), 1261 (COO), 812 (1,4-disubstituted benzenoid ring), 790 (1,2-disubstituted benzenoid ring). ^{1}H-NMR (400 MHz, DMSO-d_6) (ppm) δ H: 4.06 (s, 4H, $2CH_2Ph$), 7.22-7.26 (m, 8H, ArH), 7.33 (d, 4H, Ar-H, *J*=8.40 Hz), 7.73-7.77 (m, 2H, ArH), 7.78 (d, 4H, Ar-H, *J*=8.80 Hz), 8.01-8.04 (m, 2H, ArH), 9.79 (s, 2H, N=CH), 12.00 (s, 2H, NH); ^{13}C-NMR (100 MHz, DMSO-d_6) (ppm) δ C: 31.65 ($2CH_2Ph$), [122.13, 122.34 (4C), 129.29 (4C), 129.68, 130.60, 131.44, 131.71 (2C), 132.08 (2C), 153.10 (2C)] (Ar-C),[127.11 (2C), 127.44 (2C),129.91 (2C), 130.10 (2C), 134.47 (2C), 136.91 (2C)] (Ar-C linked to triazole C_3), 146.98 (2triazole C_3), 151.50 (2triazole C_5), 153.33 (2N=CH), 165.10 (2COO).

Bis-[4-(3-phenyl-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl)-azomethin-phenyl] phtalate (3i)

White solid; yield: 93.33%; m.p. 270°C; IR (cm^{-1}) υ_{max}: 3210 (NH), 1735, 1704 (C=O), 1599 (C=N), 1258 (COO), 836 (1,4-disubstituted benzenoid ring), 767 (1,2-disubstituted benzenoid ring). ^{1}H-NMR (400 MHz, DMSO-d_6) (ppm) δ H: 7.43 (d, 4H, Ar-H, *J*=8.80 Hz), 7.52-7.55 (m, 6H, ArH), 7.89-7.94 (m, 10H, ArH), 8.13-8.15 (m, 2H, Ar-H), 9.70 (s, 2H, N=CH), 12.40 (s, 2H, NH); ^{13}C-NMR (100 MHz, DMSO-d_6) (ppm) δ C: [122.15, 122.41 (4C), 129.38, 129.64 (4C), 130.55, 131.52 (3C), 132.68 (2C), 152.71 (2C)] (Ar-C),[126.62 (2C), 127.92 (4C), 128.52 (4C), 130.08 (2C)] (Ar-C linked to triazole C_3), 144.59 (2triazole C_3), 151.34 (2triazole C_5), 155.52 (2N=CH), 165.11 (2COO).

General Procedure for the synthesis of bis-[4-(1-(morpholin-4-yl-methyl)3-alkyl/aryl-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl)-azomethinphenyl] phthalates (4)

The corresponding compound **3** (5 mmol) was dissolved absolute ethanol and to this solution were added formaldehyde (% 37, 20 mmol) and morpholine (12 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 **4**.

Bis-[4-(1-(morpholin-4-yl-methyl)-3-methyl-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl)-azomethinphenyl] phtalate (4a)

White solid; yield: 81.96%; m.p. 160°C; IR (cm^{-1}) υ_{max}: 1737, 1689 (C=O), 1599 (C=N), 1255 (COO), 814 (1,4-disubstituted benzenoid ring), 774 (1,2-disubstituted benzenoid ring). ^{1}H-NMR (400 MHz, DMSO-d_6) (ppm) δ H: 2.38 (s, 6H, $2CH_3$), 2.72 (t, 8H, $2CH_2NCH_2$; *J* = 4.80 Hz), 3.71 (t, 8H, $2CH_2OCH_2$; *J* = 4.80 Hz), 4.62 (s, 4H, $2NCH_2N$), 7.33 (d, 4H, ArH, *J* = 8.80 Hz), 7.73-7.75 (m, 2H, ArH), 7.84 (d, 4H, ArH; *J* = 8.40 Hz), 8.01-8.03 (m, 2H, ArH), 9.85(s, 2H, N=CH); ^{13}C-NMR (100 MHz, DMSO-d_6) (ppm) δ C:11.47 ($2CH_3$), 50.45 ($2CH_2NCH_2$), 66.57 ($2NCH_2N$), 66.79 ($2CH_2OCH_2$), [122.06 (4C), 122.33 129.21 (4C), 129.64, 131.29, 131.42, 131.82 (2C), 132.06 (2C), 151.06 (2C)] (Ar-C), 143.93 (2triazole C_3), 152.94 (2triazole C_5), 153.15 (2N=CH), 165.37 (2COO).

Bis-[4-(1-(morpholin-4-yl-methyl)-3-ethyl-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl)-azomethinphenyl] phthalate (3b)

White solid; yield: 76.59%; m.p. 148°C; IR (cm^{-1}) υ_{max}: 1744, 1693 (C=O), 1592 (C=N), 1248 (COO), 860 (1,4-disubstituted benzenoid ring), 778 (1,2-disubstituted benzenoid ring). ^{1}H-NMR (400 MHz, DMSO-d_6) (ppm) δ H: 1.33 (t, 6H, $2CH_2C\underline{H}_3$, *J*=7.60 Hz), 2.73 (t, 8H, $2CH_2NCH_2$; *J* = 4.80 Hz), 2.78 (q, 4H, $2C\underline{H}_2CH_3$, *J*=7.60 Hz), 3.72

(t, 8H, $2CH_2OCH_2$; *J* = 4.80 Hz), 4.62 (s, 4H, $2NCH_2N$), 7.31 (d, 4H, ArH, *J* = 8.80 Hz), 7.73-7.75 (m, 2H, ArH), 7.83 (d, 4H, ArH; *J* = 8.40 Hz), 8.01-8.03 (m, 2H, ArH), 9.85 (s, 2H, N=CH); ^{13}C-NMR (100 MHz, DMSO-d_6) (ppm) δ C: 10.46 ($2CH_2\underline{C}H_3$), 19.11 ($2\underline{C}H_2CH_3$), 50.45 ($2CH_2NCH_2$), 66.56 ($2NCH_2N$), 66.83 ($2CH_2OCH_2$), [122.07 (4C), 122.33, 129.17 (4C), 129.35, 129.64, 131.43, 131.92 (2C), 132.05 (2C), 151.23 (2C)] (Ar-C), 147.82 (2triazole C_3), 152.79 (2triazole C_5), 152.98 (2N=CH), 165.37 (2COO).

Bis-[4-(1-(morpholin-4-yl-methyl)-3-benzyl-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl)-azomethinphenyl] phtalate (4d)

White solid; yield: 88.89%; m.p. 155°C; IR (cm^{-1}) υ_{max}: 1740, 1695 (C=O), 1592 (C=N), 1252 (COO), 814 (1,4-disubstituted benzenoid ring), 775 (1,2-disubstituted benzenoid ring). ^{1}H-NMR (400 MHz, DMSO-d_6) (ppm) δ H: 2.73 (t, 8H, $2CH_2NCH_2$; *J* = 4.80 Hz), 3.72 (t, 8H, $2CH_2OCH_2$; *J* = 4.88 Hz), 4.09 (s, 4H, $2CH_2Ph$), 4.66 (s, 4H, $2NCH_2N$), 7.21-7.35 (m, 14H, Ar-H), 7.73-7.78 (m, 6H, ArH), 8.01-8.03 (m, 2H, ArH), 9.79 (s, 2H, N=CH); ^{13}C-NMR (100 MHz, DMSO-d_6) (ppm) δ C: 31.80 ($2CH_2Ph$), 50.49 ($2CH_2NCH_2$), 66.69 ($2NCH_2N$), 66.85 ($2CH_2OCH_2$), [122.09 (4C), 122.15, 128.96 (4C), 129.23, 129.65, 131.44, 131.86 (2C), 132.06 (2C), 151.11 (2C)] (Ar-C),[127.11 (2C), 129.69 (4C), 128.80 (4C), 135.26 (2C)] (Ar-C linked to triazole C_3), 145.66 (2triazole C_3), 152.83 (2triazole C_5), 152.99 (2N=CH), 165.39 (2COO).

Bis-[4-(1-(morpholin-4-yl-methyl)-3-(p-methylbenzyl)-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl)-azomethin-phenyl] phtalate (4e)

White solid; yield: 83.92%; m.p. 129°C; IR (cm^{-1}) υ_{max}: 1745, 1694 (C=O), 1592 (C=N), 1247 (COO), 857 (1,4-disubstituted benzenoid ring), 778 (1,2-disubstituted benzenoid ring). ^{1}H-NMR (400 MHz, DMSO-d_6) (ppm) δ H: 2.30 (s, 6H, $2PhCH_3$), 2.73 (t, 8H, $2CH_2NCH_2$; *J* = 4.80 Hz), 3.71 (t, 8H, $2CH_2OCH_2$; *J* = 4.88 Hz), 4.05 (s, 4H, $2CH_2Ph$), 4.65 (s, 4H, $2NCH_2N$), 7.11 (d, 4H, Ar-H, *J* = 8.00 Hz), 7.22 (d, 4H, Ar-H, *J* = 8.00 Hz), 7.33 (d, 4H, ArH, *J* = 8.80 Hz), 7.73-7.80 (m, 6H, ArH), 8.01-8.02 (m, 2H, ArH), 9.80(s, 2H, N=CH); ^{13}C-NMR (100 MHz, DMSO-d_6) (ppm) δ C: 21.03 ($2PhCH_3$), 31.37 ($2CH_2Ph$), 50.47 ($2CH_2NCH_2$), 66.67 ($2NCH_2N$), 66.86 ($2CH_2OCH_2$), [122.09 (4C), 122.16, 129.24 (4C), 129.38, 129.68, 131.46, 131.91 (2C), 132.07 (2C), 151.13 (2C)] (Ar-C),[128.69 (4C), 128.87 (4C), 132.15 (2C), 136.74 (2C)] (Ar-C linked to triazole C_3), 145.87 (2triazole C_3), 152.80 (2triazole C_5), 152.98 (2N=CH), 165.40 (2COO).

Bis-[4-(1-(morpholin-4-yl-methyl)-3-(p-chlorobenzyl)-4,5-dihydro-1H-1,2,4-triazol-5-on-4-yl)-azomethin-phenyl] phtalate (4g)

White solid; yield: 89.79%; m.p. 151°C; IR (cm^{-1}) υ_{max}: 1744, 1694 (C=O), 1592 (C=N), 1247 (COO), 857 (1,4-disubstituted benzenoid ring), 778 (1,2-disubstituted benzenoid ring). ^{1}H-NMR (400 MHz, DMSO-d_6) (ppm) δ H: 2.72 (t, 8H, $2CH_2NCH_2$; *J* = 4.80 Hz), 3.71 (t, 8H, $2CH_2OCH_2$; *J* = 4.88 Hz), 4.06 (s, 4H, $2CH_2Ph$), 4.65 (s, 4H, $2NCH_2N$), 7.27 (m, 8H, ArH), 7.29-7.35 (m, 4H, ArH), 7.73-7.78 (m, 6H, ArH), 8.01-8.03 (m, 2H, ArH), 9.80(s, 2H, N=CH); ^{13}C-NMR (100 MHz, DMSO-d_6) (ppm) δ C: 31.07 ($2CH_2Ph$), 50.46 ($2CH_2NCH_2$), 66.73 ($2NCH_2N$), 66.83 ($2CH_2OCH_2$), [122.16 (4C), 122.25, 129.21 (4C), 129.66, 130.18, 131.72, 132.10 (2C), 133.08 (2C), 151.05 (2C)] (Ar-C),[128.85 (4C), 130.32 (4C), 131.42 (2C), 133.67 (2C)] (Ar-C linked to triazole C_3), 145.18 (2triazole C_3), 153.05 (2triazole C_5), 153.08 (2N=CH), 165.37 (2COO).

Biological protocols

Antioxidant activity: Chemicals

Ferrous chloride, α-tocopherol, 1,1-diphenyl-2-picryl-hydrazyl (DPPH.), 3-(2-pyridyl)-5,6-bis(phenylsulfonic acid)-1,2,4-triazine (ferrozine), butylatedhydroxyanisole (BHA), ethylene diamine tetra acetic acid (EDTA) and trichloroacetic acid (TCA) were obtained from Sigma–Aldrich. Butylatedhydroxytoluene (BHT) was obtained from E. Merck.

Reducing power

The reducing power of the synthesized compounds was determined according to the method of Oyaizu [32]. Different concentrations of the samples (50-250 µg/mL) in DMSO (1 mL) were mixed with phosphate buffer (2.5 mL, 0.2 M, pH = 6.6) and potassium ferricyanide (2.5 mL, 1%). The mixture was

incubated at 50°C for 20 min and afterwards a portion (2.5 mL) of trichloroacetic acid (10%) was added to the mixture, which was centrifuged for 10 min at 1000 x g. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%), and then the absorbance at 700 nm was measured in a spectrophometer. Higher absorbance of the reaction mixture indicated greater reducing power.

Free radical scavenging activity

Free radical scavenging activity of compounds was measured by DPPH., using the method of Blois [33]. Briefly, 0.1 mM solution of DPPH·in ethanol was prepared,and this solution (1 mL) was added to sample solutions in DMSO (3 mL) at different concentrations (50-250 µg/mL). The mixture was shaken vigorously and allowed to remain at the room temperature for 30 min. Then, the absorbance was measured at 517 nm in a spectrophometer. The lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The DPPH·concentration (mM) in the reaction medium was calculated from the following calibration curve and determined by linear regression (R: 0.997): Absorbance = (0.0003 × DPPH·) – 0.0174

The capability to scavenge the DPPH radical was calculated by using the following equation: DPPH· scavenging effect (%) = (A_0 – A_1/A_0) × 100, where A_0 is the absorbance of the control reaction, and A_1 is the absorbance in the presence of the samples or standards.

Metal chelating activity

The chelation of ferrous ions by the synthesized compounds and standards were estimated by the method of Dinis et al. [34]. Shortly, the synthesized compounds (50-250 µgxmL^{-1}) were added to a 2 mM solution of $FeCl_2$ (0.05 mL). The reaction was initiated by the addition of 5 mMferrozine (0.2 mL), and then the mixture was shaken vigorously and left remaining at the room temperature for 10 min. After the mixture had reached equilibrium, the absorbance of the solution was measured at 562 nm in a spectrophotometer. All tests and analyses were carried out in triplicate and averaged. The percentage of inhibition of ferrozine-Fe^{2+} complex formation was given by the formula: Inhibition% = (A_0 - A_1/A_0) x 100, where $A_0$is the absorbance of the control, and A_1 is the absorbance in the presence of the samples or standards. The control did not contain compound or standard.

Antimicrobial activity

The newly synthesized compounds were screened for their antimicrobial activities. 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 [35, 36] as explained in the literature [5]. All the newly synthesized compounds were weighed and dissolved in dimethylsulphoxide (DMSO) to prepare extract stock solution of 1 mg/ml. Ampicillin (X3261), neomycin (X3385) and streptomycin (X3385) were standard antibacterial and antifungal agents, DMSO was used as solved control.

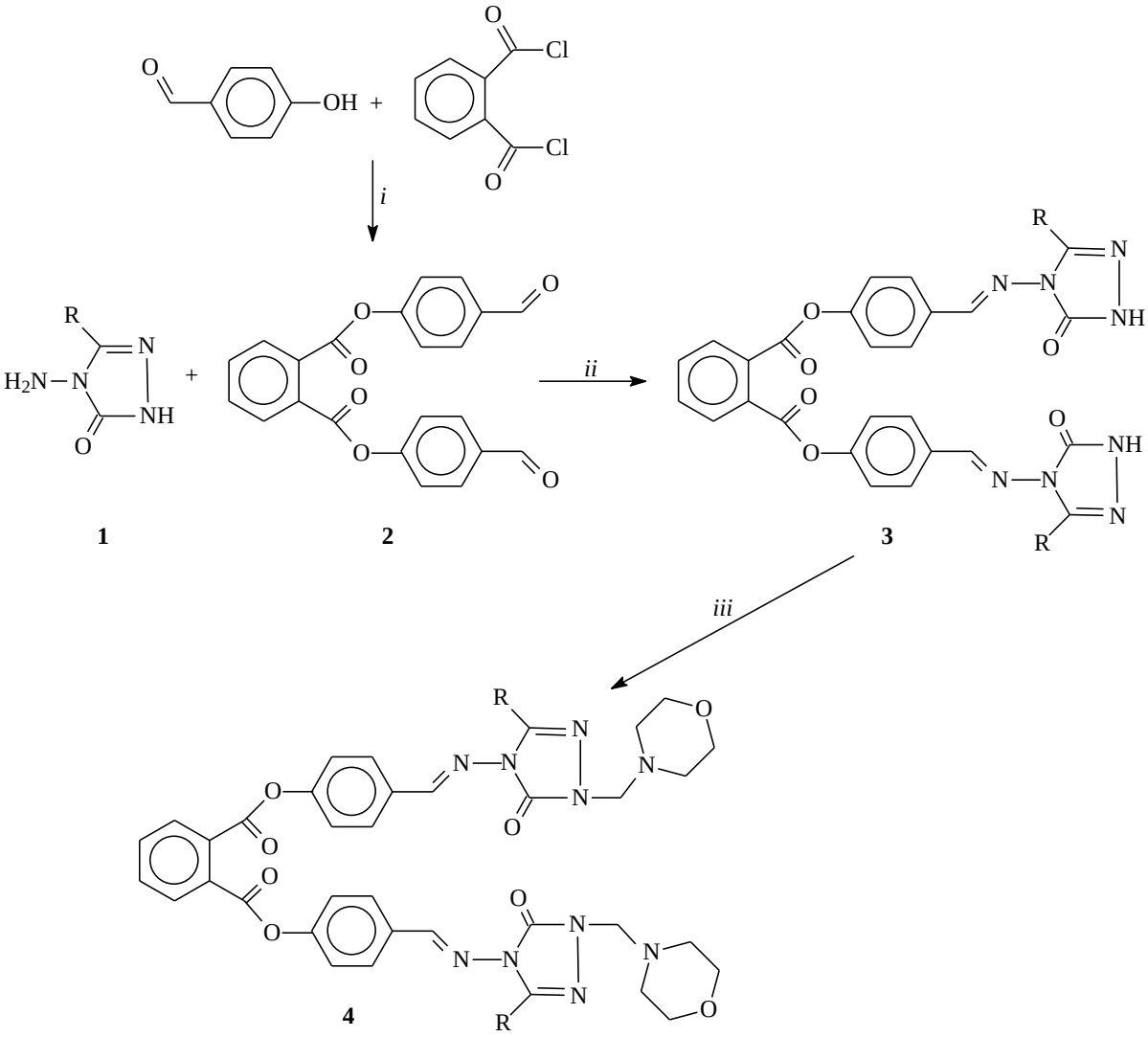
RESULTS AND DISCUSSION

Chemistry

In the present work, bis-[4-(3-alkyl/aryl-4,5-dihydro-1*H*-1,2,4-triazol-5-on-4-yl)-azomethinphenyl] phthalates (**3**) were synthesized from the reactions of 3-alkyl/aryl-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**1**) with bis-(4-formylphenyl) phthalate (**2**) [31], which was synthesized by the reaction of 4-hydroxybenzaldehyde with phthaloyl dichloride by using triethylamine. In addition, the bis-[4-(1-(morpholin-4-yl-methyl)-3-alkyl/aryl-4,5-dihydro-1*H*-1,2,4-triazol-5-on-4-yl)-azomethinphenyl] phthalates (**4**) were obtained from the reactions of bis-[4-(3-alkyl/aryl-4,5-dihydro-1*H*-1,2,4-triazol-5-on-4-yl)-azomethinphenyl] phthalates (**3**) with morpholine in the presence of formaldehyde (Scheme 1).

ISSN: 0975-8585

Scheme 1: Synthetic pathway of compounds 2, 3 and 4



i) Et_3N; *ii*) AcOH, reflux, 1h; *iii*) CH_2O, morpholine, reflux
a) R = CH_3, b) R = CH_2CH_3, c) R = $CH_2CH_2CH_3$, d) R = $CH_2C_6H_5$, e) R = $CH_2C_6H_4CH_3$ (*p*-),
f) R = $CH_2C_6H_4OCH_3$ (*p*-), g) R = $CH_2C_6H_4Cl$ (*p*-), h) R = $CH_2C_6H_4Cl$ (*m*-), i) R = C_6H_5

The structures of nine new bis-[4-(3-alkyl/aryl-4,5-dihydro-1*H*-1,2,4-triazol-5-on-4-yl)-azomethinphenyl] phthalates (**3**) and five newly synthesized Mannich bases**4a**, **4b**, **4d**, **4e** and **4g** compoundswere identified by using IR, ^{1}H NMR and^{13}C NMR spectral data.

Antioxidant activity

Fifteen newly synthesized compounds **3a-i**, **4a**, **4b**, **4d**, **4e** and **4g**were examined for their antioxidant activities. The methods used for the study are given below:

Total reductive capability using the potassium ferricyanide reduction method

In the present study, all of the concentrations of the compounds had a lower absorbance than the reference antioxidants. Hereby, no activity was observed for reducing metal ion complexes to their lower oxidation state or for any electron transfer reaction. Therefore, the compounds did not exhibit any reductive activity.

ISSN: 0975-8585

DPPH· radical scavenging activity

The reduction capability of DPPH radicals was determined by decrease in its absorbance at 517 nm induced by antioxidants. In the study, antiradical activities of compounds and standard antioxidants such as BHT, BHA and α-tocopherol were determined. The newly synthesized compounds showed no activity as a radical scavenger.

Ferrous ion chelating activity

In this study, Metal chelate activities of newly synthesized compounds and standard antioxidants are shown as % inhibition in the graphs of Figures **1** and **2**.The metal chelating capacity was significant since it reduced the concentrations of the catalyzing transition metal. It was reported that chelating agents 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 [37]. It is observed that some of the synthesized Schiff base compounds have a low degree of chelator. Compounds **3a**, **3d** and **3f** were observed to have chelating effect close to α-tocopherol, at the lowest concentration. The metal chelating effect of the**3** type compounds and references decreased in the order of EDTA > α-tocopherol> 3a > 3d ≈ 3f, which were 79.6, 56.0, 42.7, 39.1 (%), at the lowest concentration, respectively. But, it was observed that Mannich some compounds obtained from Schiff bases had a very high degree of chelation. These compounds' activities were found to be higher than α-tocopherol and close to EDTA. The metal chelating effect of these compounds and references decreased in order of EDTA > 4d > 4a > 4g > 4b ≈ 4e > α-tocopherol, which were 80.4, 76.0, 75.6, 75.1, 74.7, 62.7 (%), at the highest concentration, respectively.

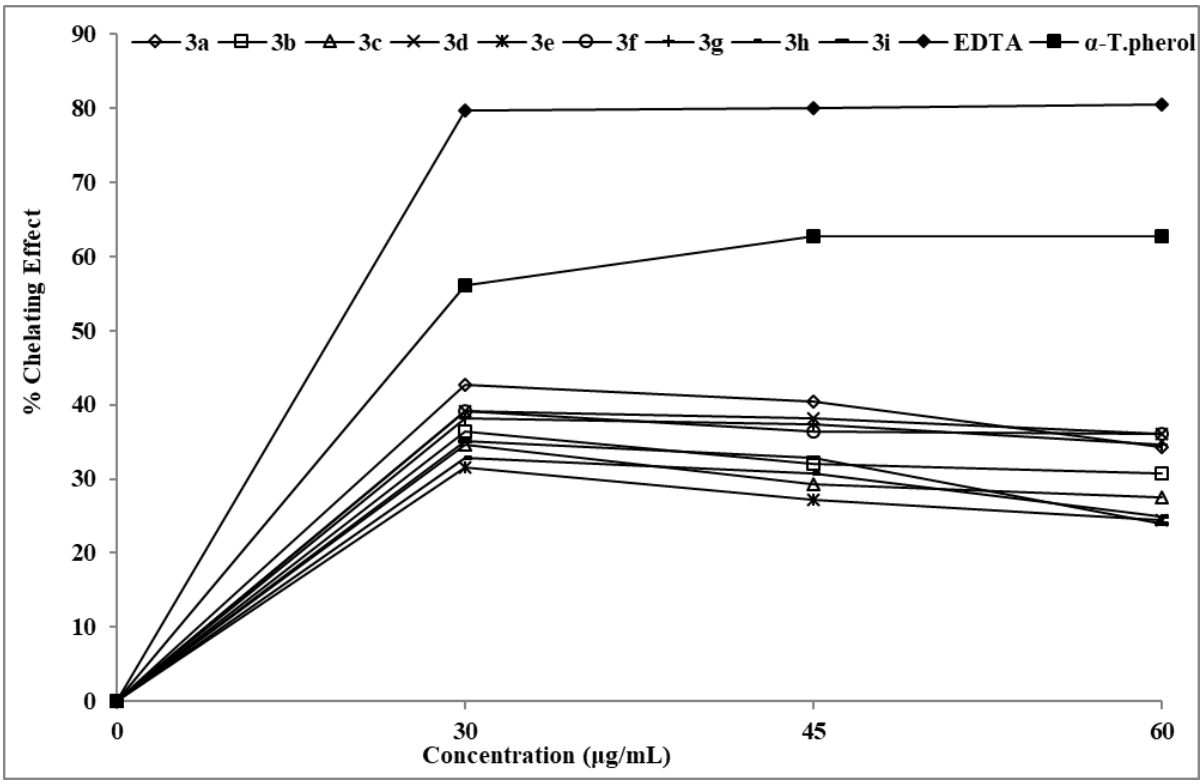


Figure 1: Iron binding effect of diverse amount of the compounds 3, and reference antioxidants.

ISSN: 0975-8585

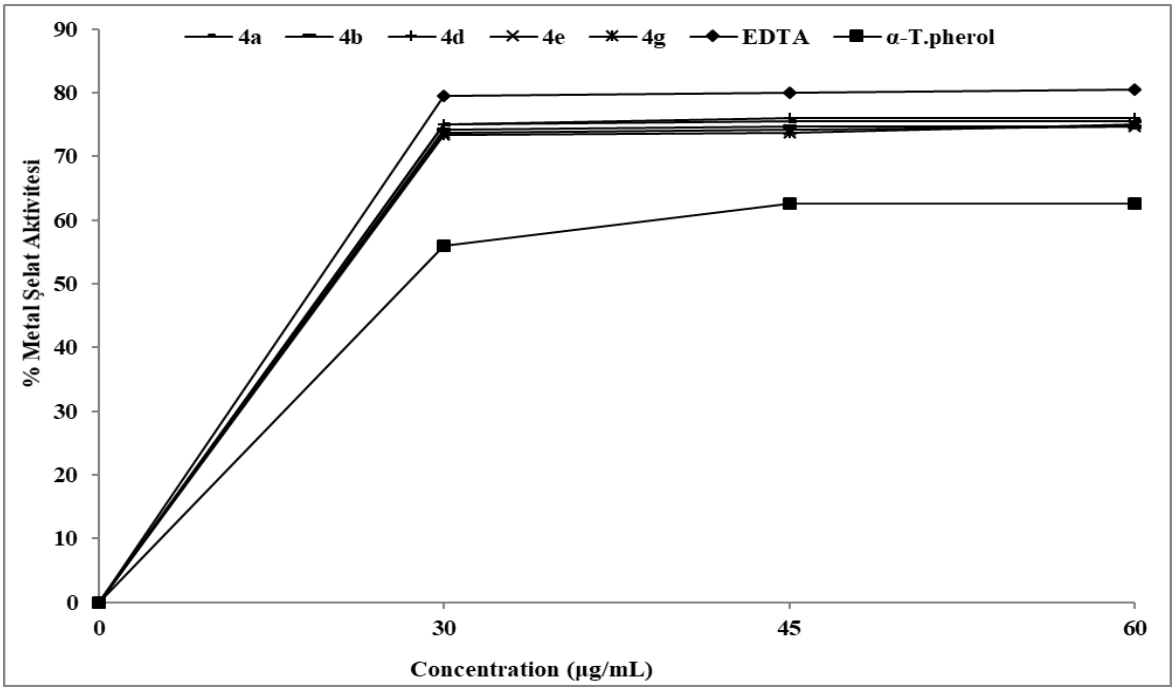


Figure 2: Iron binding effect of diverse amount of the compounds 4, and reference antioxidants.

Antimicrobial activity

The microbiological results are summarized in Table I. The microbiological results of the synthesized compounds showed high antimicrobial activity against tested microorganisms. The antimicrobial activities of these compounds were observed to be higher than the standard compounds, neomycin, and streptomycin. In particular, compounds **3b-e**, **4a**, and **4b** showed antimicrobial activity close to ampicillin (it's one of the oldest and most widely used antibiotics) against *Bacillus subtilis*.

Table I: Antimicrobial activity of the compounds 3 and 4.

Compounds	Microorganisms and inhibition zone (mm)					
	Bs	Bc	Pa	Kp	Sa	Ec
3a	**20**	19	8	13	**20**	**21**
3b	**29**	17	14	**24**	8	**21**
3c	**30**	17	17	**20**	12	18
3d	**28**	18	14	18	**21**	**23**
3e	**28**	15	15	**23**	16	**24**
3f	**24**	14	**20**	**26**	12	**20**
3g	**22**	14	19	**22**	15	**21**
3h	**27**	18	17	14	8	**27**
3i	**26**	17	16	15	**26**	**20**
4a	**29**	**23**	**22**	18	12	**21**
4b	**28**	18	19	**23**	**20**	**27**
4d	**27**	17	**23**	19	17	17
4e	17	17	18	**24**	12	**23**
4g	**23**	**23**	**20**	**21**	11	**20**
Ampicillin	33	36	36	35	37	34
Neomycin	17	17	17	16	13	16
Streptomycin	12	12	12	11	21	10

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

ISSN: 0975-8585

CONCLUSION

In the present study, new Schiff base derivatives (**3a-i**) and Mannich base derivatives (**4a**, **4b**, **4d**, **4e**, **4g**) were designed and synthesized. Their structures were identified using IR, ^{1}H NMR and^{13}C NMR spectral data. The synthesis and *invitro* antioxidant evaluation of new 4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives are described. The newly synthesized Schiff bases showed low metal chelating activity but the novel Mannich bases obtained from these compounds showed high metal chelating activity. In addition, the antimicrobial activities of these compounds were observed to be higher than the standard compounds. Design and synthesis of novel small molecules can play specifically a protective role in biological systems and in modern medicinal chemistry. These results may also provide some guidance for the development of novel triazole-Mannich based therapeutic target.

REFERENCES

[1] Habibi MH,Montazerozohori M, Lalegani A, Harrington RW, Clegg W. J Fluor Chem2006; 127: 769-773.
[2] Abbas SA, Munir M, Fatima A, Naheed S, Ilyas ZE. J Life Sci2010; 1: 37-40.
[3] Cimerman Z,Miljanic SJ. AntolicSpectroscLett1999; 32: 181-196.
[4] Jones RD, Basolo DA. Chem Rev1979; 79: 139-179.
[5] Aktaş-Yokuş Ö, Yüksek H, Manap S, Aytemiz F, Alkan M, Beytur M, Gürsoy-Kol Ö.BulgChemCommun2017; 49(1): 98-106.
[6] Yüksek, H.; Göksu, B.; Manap, S.; Beytur, M.; GürsoyKol, Ö. Chemical Science International Journal, **2018**, 22 (2), 1-29.
[7] Ismail TMA. J CoordChem2005; 58(2): 141-151.
[8] Tarafder MTH, Saravanan N, Crouse KA, Ali AN. Trans Met Chem2001; 26: 613-618.
[9] Bahçeci Ş, Yıldırım N, Manap S, Beytur M, Yüksek H. RasayanJChem2016; 9(3): 494-501.
[10] McAuliffe CA, Parish RV, Abu-El-Wafa SM, Issa RM. InorgChimActa1986; 115: 91-94.
[11] Zolezzi S, Spodine E, Decinti A. Polyhedron 2002; 21: 55-59.
[12] Ambike V, Adsule S, Ahmed F, Wang Z, Afrasiabi Z, Sinn E, Sarkar F, Padhye SJ. InorgBiochem2007; 101: 1517-1524.
[13] Mejeha MI, Nwandu MC, Okeoma KB, Nnanna LA, Chidiebere MA, Eze FC, Oguzie EE. J Mater Sci2012; 47: 2559-2572.
[14] Abdel Aziz AA, J Lumin2013; 143: 663-669.
[15] Al Zoubi W, Al Mohanna ND. SpectrochimActaA2014; 132: 854-870.
[16] Andres S, Guarin P, Dufresne S, Tsang D, Sylla A, Skene WG. J Mater Chem2007; 17: 2801-2811.
[17] Di Bella S, Oliveri IP, Colombo A,Dragonetti C, Righetto S, Roberto D. Dalton T2012; 41: 7013-7016.
[18] Kumar S, Dhar DN, Saxena PN. JSciInt Res2009; 68: 181-187.
[19] Hosnya NM, Hussiena MA, Radwana FM, Nawar N. SpectrochimActaA2014; 132: 121-129.
[20] Sek D, Iwan A, Jarzabek B, Kaczmarczyk B, Kasperczyk J, Mazurak Z, Domanski M, Karon K, Lapkowski M. Macromolecules2008; 41: 6653-6663.
[21] Zabulica A, Balan M, Belei D, Sava M, Simionescu BC, Marin L. DyesPigm2013; 96: 686-698.
[22] Azam F, Singh S,Khokhra SL, Prakash O, J Zhejiang.UnivSci B.2007; 8: 446-452.
[23] Przybylski P, Huczynski A, Pyta K, Brzezinski B, Bartl F. Cur Org Chem2009; 13: 124-148.
[24] Amer S, El-Wakiel N, El-Ghamry H. JMolStruct2013; 1049: 326-335.
[25] Kraicheva I, Tsacheva I, Vodenicharova E,Tashev E,Tosheva T,Kril A,Topashka-Ancheva M,Iliev I,Gerasimova TS,Troev K. Bioorg Med Chem2012; 20: 117-124.
[26] Khanye SD, Gutb J, Rosenthal PJ,Chibale K, Smith SJ. OrganometChem2011; 696: 3296-3300.
[27] Alafeefy AM,Bakht MA, Ganaie MA, Ansarie MN, El-Sayed NN, Awaad AS.Bioorg Med ChemLett2015; 25: 179-183.
[28] Vivekanand B, Raj KM, Mruthyunjayaswamy BHM. J MolStruct2015; 1079: 214-224.
[29] İkizler, A. A., Ün, R.ChimActaTurc1979; 7: 269-290.
[30] İkizler AA, İkizler A, Yüksek H. MagnResonChem1993; 31(12): 1088-1090.
[31] Zalova TV, Borukaev TA. JBalk TribolAssoc2010; 16(2): 279-283
[32] Oyaizu M.Jpn J Nutr1986; 44: 307-315.
[33] Blois, M. S., Nature, **1958**, 181: 1199-1200.
[34] Soares JR, Dinis TCP, Cunha AP, Almedia LM. Free Radical Res1997; 26(5): 469-478.
[35] Perez C, Pauli M, Bazerque P.ActaBiol Med Exp1990; 15: 113-115.
[36] Ahmad I, Mehmood Z, Mohammed F. J. Ethnopharmacol1998; 62: 183-193.

[37] Gordon MH. Food Antioxidants. Elsevier, London, 1990.