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# Synthesis and Investigations of Antimicrobial, Antioxidant Activities of Novel Di-[2-(3-alkyl/aryl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl)-azomethinephenyl] Isophtalates and Mannich Base Derivatives

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## ABSTRACT

In this study, the synthesis of di-[2-(3-alkyl/aryl-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl)-azomethinephenyl] isophtalates (**2a-g**) from the reactions of 3-alkyl/aryl-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**1a-g**) with di-(2-formylphenyl) isophtalate is described. Then, the compounds **2** were treated with morpholine in the presence of formaldehyde to synthesize di-{2-[1-(morpholine-4-yl-methyl)-3-alkyl(aryl)-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl]-azomethinephenyl} isophtalates (**3a-g**). The newly synthesized compounds were characterized using IR, $^{1}$H-NMR and $^{13}$C-NMR spectral data. In addition, the compounds synthesized were screened for their antimicrobial activities. Furthermore, the antioxidant properties of the newly synthesized compounds were analysed for their in-vitro potential antioxidant activities in three different methods (reducing power, free radical scavenging and metal chelating activity). These antioxidant activities were compared to those from standard antioxidants, such as BHA, BHT, EDTA and α-tocopherol.

**Keywords:** Schiff base, Mannich base, Antimicrobial activity, Antioxidant activity.

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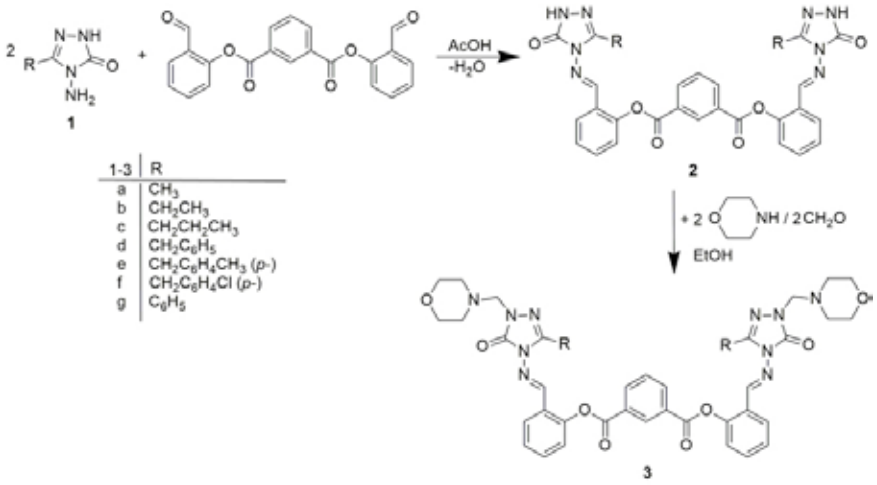
## INTRODUCTION

A large number of heterocyclic compounds containing the 1,2,4-triazole ring, are associated with diverse biological properties such as antioxidant, anticonvulsant, anti inflammatory, antimicrobial and anti-viral activity. Mannich bases have applications the field medicinal chemistry, the product synthetic polymers, the petroleum industry, as products used in water treatment, cosmetics, the dyes industry, etc [1]. In addition, Mannich bases have biological activity such as anticancer [2,3], antibacterial [4–6], antimycobacterial [7–9], anti inflammatory [10–12], analgesic [13,14], antifungal [15,16], antitumor [17,18]namely the 1-aryl-2-dimethylaminomethyl-2-propen-1-one hydrochlorides 1a-e and 1-aryl-3-dimethylamino-2-hydroxymethyl-1-propanone hydrochlorides 2a-e. A number of these compounds possess marked cytotoxic potencies (IC(50, antiviral [19–21], antidepressant [22,23], antiulcer [24], anticonvulsant [25], antimalarial [26,27] and antioxidant activities [28].

Antioxidants were defended organisms and cells from damage induced by oxidative stress. Thus, significant research has been ruled to investigate this feature. Scientists have dealt with the new compounds in recent years. Natural sources provide the effective components forestall or decrease the influence of oxidative stress on cells have been used [29]. Exogenous chemicals and endogenous metabolic steps in human body or in food system might produce highly reactive free radicals, particularly oxygen provided radicals, which are capable of oxidizing biomolecules, resulting in cell death and issue damage. Oxidative damages play a considerable pathological role in human diseases. It has been an important pathological effect of oxidative damage in human disease. For example, cancer, emphysema, cirrhosis, atherosclerosis and arthritis have all been correlated with oxidative injury. In addition to, excessive generation of ROS (reactive oxygen species) induced by various stimuli and which exceeds the antioxidant capacity of the organism leads to a diversity of pathophysiological processes such as inflammation, diabetes, genotoxicity and cancer [30]. In this paper, in order to define antioxidant activity of the synthesized Mannich Bases were researched different antioxidant method; iron binding effect, reducing power and 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging activity [31]. Furthermore, the antimicrobial activities of novel Mannich bases were investigated with agar well diffusion method [32]. In the present paper, the starting materials (**1a-g**) were synthesized from the reactions of the corresponding ester ethoxycarbonylhydrazones with an aqueous solution of hydrazine hydrate [33,34] and di-[2-(3-alkyl/aryl-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl)-azomethinephenyl] isophtalates (**2a-g**) were obtained by the reactions of compounds (**1a-g**) with di-(2-formylphenyl) isophtalate. Then, the

compounds **2** reacted with formaldehyde and morpholine to afford di-{2-[1-(morpholine-4-yl-methyl)-3-alkyl(aryl)-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl]-azomethinephenyl} isophtalates (**3a-g**) (**Scheme 1**).




**Scheme 1.** The synthetic pathway of the compounds **2** and **3**.

## METHODOLOGY

### Synthesis

*Preparation of Compounds **2a-g**:* 3-Alkyl/Aryl-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-one (**1**) (0.01 mol) was dissolved in acetic acid (20 mL) and treated with di-(2-formylphenyl) isophtalate (0.01 mol). The mixture was refluxed for 1.5 hours and then evaporated at 50-55°C *in vacuo*. Several recrystallizations of the residue from ethanol gave pure compound di-[2-(3-alkyl/aryl-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl)-azomethinephenyl] isophtalate (**2**) as white colour crystals.

*Preparation of Compounds **3a-g**:* Compound **2** (5 mmol) was dissolved absolute ethanol and to this solution were added to formaldehyde (% 37, 10 mmol) and morpholine (6 mmol). The reaction mixture was refluxed for 4 hours and filtered. The crude product was recrystallized from ethanol.

Physical data of the new compounds (**2a-g** and **3a-g**) are presented in Table **1**. IR, $^{1}$H-NMR and $^{13}$C-NMR spectral data are given in Tables **2-6**.

**Table 1.** Physical data of the compounds **2** and **3**

| Compounds | 2a | 2b | 2c | 2d | 2e | 2f | 2g | 3a | 3b | 3c | 3d | 3e | 3f | 3g |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| % Yield | 99 | 98 | 90 | 96 | 97 | 96 | 90 | 92 | 91 | 91 | 94 | 90 | 92 | 90 |
| Melting Point ($^0$C) | 271 | 233 | 226 | 273 | 260 | 243 | 251 | 224 | 183 | 165 | 176 | 158 | 155 | 206 |

**Table 2.** IR data of the compounds **2** and **3** ($cm^{-1}$).

| Compounds | $n_{NH}$ | $n_{C=O}$ | $n_{C=N}$ | $n_{COO}$ | $n_{1,4\text{-disubstituted benzenoid ring}}$ | $n_{1,3\text{-disubstituted benzenoid ring}}$ | $n_{1,2\text{-disubstituted benzenoid ring}}$ | $n_{monosubstituted benzenoid ring}$ |
|---|---|---|---|---|---|---|---|---|
| 2a | 3191 | 1744, 1713 | 1604 | 1208 | - | 871 and 789 | 754 | - |
| 2b | 3188 | 1739, 1705 | 1598 | 1203 | - | 902 and 800 | 761 | - |
| 2c | 3180 | 1710 | 1598 | 1207 | - | 902 and 824 | 752 | - |
| 2d | 3183 | 1709 | 1596 | 1202 | - | 904 and 819 | 752 | 752 and 694 |
| 2e | 3173 | 1745, 1705 | 1598 | 1200 | 829 | 903 and 796 | 755 | - |
| 2f | 3185 | 1740, 1705 | 1598 | 1201 | 821 | 903 and 792 | 749 | - |
| 2g | 3180 | 1705 | 1603 | 1203 | - | 905 and 802 | 756 | - |
| 3a | - | 1742, 1704 | 1597 | 1215 | - | 856 and 768 | 768 | - |
| 3b | - | 1742, 1700 | 1593 | 1204 | - | 897 and 765 | 765 | - |
| 3c | - | 1744, 1700 | 1591 | 1205 | - | 897 and 765 | 757 | - |
| 3d | - | 1742, 1700 | 1590 | 1207 | - | 904 and 762 | 762 | 762 and 709 |
| 3e | - | 1746, 1701 | 1596 | 1206 | 841 | 904 and 790 | 757 | - |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 3f | - | 1744, 1701 | 1598 | 1211 | 820 | 907 and 801 | 745 | - |
| 3g | - | 1740, 1697 | 1604 | 1207 | - | 896 and 800 | 766 | 766 and 687 |

**Table 3.** $^{1}$H-NMR data of the compounds **2** (DMSO-$d_6$, δ/ppm)

| Compounds | $2CH_3$ | $2CH_2$ | $2PhCH_3$ | $2CH_2$ | Aromatic H | 2N=CH | 2NH |
|---|---|---|---|---|---|---|---|
| 2a | 2.10(s) | - | - | - | 7.48-7.53(m,4H), 7.66(td,2H,J=8.00,1.60 Hz), 7.90(t,1H,J=8.80 Hz), 8.04(dd,2H,J=8.00 Hz), 8.54(dd,2H,J=8.00,1.60 Hz),8.84(t,1H,J=1.60 Hz) | 9.93(s) | 11.75(s) |
| 2b | 1.09 (t,J= 7.60Hz) | - | - | 2.47(q, J=7.60 Hz) | 7.48-7.52(m,4H), 7.66(td,2H,J=8.40,1.60 Hz), 7.91(t,1H,J=8.00 Hz), 8.02(d,2H,J=8.00 Hz), 8.55(dd,2H,J=8.00,2.00 Hz), 8.85(s,1H) | 9.93(s) | 11.75(s) |
| 2c | 0.85 (t,J= 7.20Hz) | 1.57(sext, J=7.20 Hz) | - | 2.43(t,J= 7.20 Hz) | 7.49-7.53(m,4H), 7.67(t,2H,J=8.00 Hz), 7.91 (t,1H,J=8.00 Hz), 8.02(dd,2H,J=8.00,1.20 Hz), 8.55(dd,2H,J=8.00,1.60 Hz), 8.85(s,1H) | 9.92(s) | 11.79(s) |
| 2d | - | - | - | 3.92(s) | 7.19-7.31(m,10H), 7.47-7.50(m,4H), 7.65(td,2H,J=8.00,1.60 Hz), 7.86(t,1H,J=8.00 Hz), 7.99(d,2H,J=8.00 Hz), 8.51(dd,2H,J=8.00,1.60 Hz), 8.82(s,1H) | 9.91(s) | 11.91(s) |
| 2e | - | - | 2.23(s) | 3.86(s) | 7.08(d,4H,J=8.00 Hz), 7.14(d,4H,J=8.00 Hz), 7.46-7.51 (m,4H), 7.65(td,2H,J=8.00,1.60 Hz), 7.86(t,1H,J=8.00 Hz), 8.00(dd,2H,J=7.60,1.20 Hz), 8.51(dd,2H,J=8.00,1.60 Hz), 8.83(s,1H) | 9.90(s) | 12.00(s) |
| 2f | - | - | - | 3.93(s) | 7.27(d,4H,J=8.40 Hz), 7.35(d,4H,J=8.40 Hz), 7.47-7.51(m,4H), 7.66(t,2H,J=8.00 Hz), 7.86(t,1H,J=8.00 Hz), 7.99(d,2H,J=7.60 Hz), 8.51(dd,2H,J=8.00,1.60Hz), 8.82(s,1H) | 9.92(s) | 11.93(s) |
| 2g | - | - | - | - | 7.48-7.55(m,10H), 7.67-7.82(m,7H), 7.99(dd,2H,J=8.00,1.20 Hz), 8.40(dd,2H,J=7.60,1.60 Hz), 8.68(s,1H) | 9.88(s) | 12.32(s) |

**Table 4.** $^{13}$C-NMR data of the compounds **2** (DMSO-$d_6$, δ/ppm)

| Comp. | 2COO | 2Triazole $C_5$ | 2N=CH | 2Triazole $C_3$ | Aromatic C | C3-Aromatik C | Aliphatic C |
|---|---|---|---|---|---|---|---|
| 2a | 163.54 | 149.51 | 148.91 | 144.09 | 123.64(2CH), 126.01(2C), 126.95(2CH), 127.70(2CH), 129.42(2C), 130.16(CH), 131.00(CH), 132.54(2CH), 135.22(2CH), 151.16(2C) | - | 10.85($2CH_3$) |
| 2b | 163.54 | 149.46 | 149.09 | 147.86 | 123.66(2CH), 126.03(2C), 126.98(2CH), 127.86(2CH), 129.42(2C), 130.14(CH), 131.01(CH), 132.53(2CH), 135.21(2CH), 151.30(2C) | - | 9.87($2CH_2\underline{CH_3}$), 18.31($2\underline{CH_2}CH_3$) |
| 2c | 163.53 | 149.45 | 149.23 | 146.71 | 123.64(2CH), 126.03(2C), 127.00(2CH), 127.87(2CH), 129.44(2C), 130.19(CH), 131.02(CH), 132.54(2CH), 135.21(2CH), 151.23(2C) | - | 13.31($2CH_2CH_2\underline{CH_3}$), 18.65 ($2CH_2\underline{CH_2}CH_3$), 26.46($2\underline{CH_2}CH_2CH_3$) |
| 2d | 163.51 | 149.68 | 148.46 | 146.09 | 123.54(2CH), 125.97(2C), 126.67(2CH), 127.12(2CH), 129.32(2C), 130.13(CH), 131.03(CH), 132.59(2CH), 135.20(2CH), 151.10(2C) | 126.94(2CH), 128.37(4CH), 128.72(4CH), 135.58(2C) | 30.82($2CH_2$Ph) |
| 2e | 163.50 | 149.66 | 148.43 | 146.23 | 123.52(2CH), 125.98(2C), 126.93(2CH), 127.14(2CH), 129.32(2C), 130.11(CH), 131.03(CH), 132.56(2CH), 135.18(2CH), 151.17(2C) | 128.58(4CH), 128.94(4CH), 132.46(2C), 135.74(2C) | 20.55($2PhCH_3$), 30.43($2CH_2$Ph) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 2f | 163.49 | 149.67 | 148.59 | 145.75 | 123.35(2CH), 125.92(2C),126.96 (2CH), 127.21(2CH), 129.32(2C), 130.13(CH), 131.00(CH), 132.62(2CH), 135.19(2CH), 151.14(2C) | 128.31(4CH), 130.65(4CH), 131.43(2C), 134.53(2C) | 30.14($2CH_2$Ph) |
| 2g | 163.51 | 151.47 | 149.80 | 144.64 | 123.69(2CH), 125.88(2C),127.01 (2CH), 127.44(2CH), 129.24(2C), 129.82(CH), 131.02(CH), 132.78(2CH), 134.95(2CH), 151.29(2C) | 126.42(2C), 127.89(4CH), 128.42(4CH), 130.04(2CH) | - |

**Table 5.** $^1$H-NMR data of the compounds **3** (DMSO-$d_6$, δ/ppm)

| Comp. | $2CH_3$ | $2CH_2$ | $2CH_2$ | $2CH_2NCH_2$ | $2CH_2OCH_2$ | $2NCH_2N$ | Aromatic H | 2N=CH |
|---|---|---|---|---|---|---|---|---|
| 3a | 2.11(s) | - | - | 2.54(m) | 3.48(m) | 4.38(s) | 7.49-7.53(m,4H), 7.67(td,2H, J=8.40,1.60 Hz), 7.91(t,1H, J=8.00 Hz), 8.05(d,2H, J=7.60 Hz), 8.54(dd,2H,J=8.00,1.60 Hz), 8.87(s,1H) | 9.93 (s) |
| 3b | 1.09(t, J=7.60 Hz) | 2.50(q,J= 7.60 Hz) | - | 2.55(m) | 3.49(m) | 4.40(s) | 7.49-7.53(m,4H), 7.67(t,2H,J =8.00 Hz), 7.92(t,1H,J=8.00 Hz), 8.03(d, 2H,J = 7.60 Hz), 8.55 (d,2H, J =7.60 Hz), 8.87(s,1H) | 9.92 (s) |
| 3c | 0.85(t, J=7.20 Hz) | 1.57 (sext,J = 7.20 Hz) | 2.48(t,J = 7.20 Hz) | 2.55(m) | 3.49(t, J = 4.40 Hz) | 4.41(s) | 7.50-7.53(m,4H), 7.65-7.68(m,2H), 7.92(t,1H,J=8.00 Hz), 8.00-8.05(m,2H), 8.54(dd,2H,J=8.00,1.60 Hz), 8.87(d,1H,J=1.60 Hz) | 9.92 (s) |
| 3d | - | - | 3.95(s) | 2.48(t,J = 4.40 Hz) | 3.47 (m) | 4.42(s) | 7.18-7.31(m,10H), 7.47-7.51(m,4H), 7.66(td,2H,J=7.60,1.20 Hz), 7.85(t,1H, J=8.00 Hz), 7.99(d,2H, J=7.60 Hz), 8.49(d,2H,J=7.60,1.60 Hz), 8.83(s,1H) | 9.89 (s) |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 3e | 2.22 (s) | - | 3.90(s) | 2.46(t,J = 4.40 Hz) | 3.47(t,J = 4.40 Hz) | 4.41(s) | 7.07-7.14(m,8H), 7.46-7.51(m,4H), 7.66(td,2H,J =7.60,1.60 Hz), 7.85(t,1H, J =7.60 Hz), 8.00(dd,2H,J=8.00,1.60 Hz), 8.49(dd,2H,J=8.00,1.60 Hz), 8.83(m,1H) | 9.88 (s) |
| 3f | - | - | 3.96(s) | 2.46(t,J = 4.40 Hz) | 3.47(t,J = 4.40 Hz) | 4.41(s) | 7.27(d,4H,J=8.40Hz), 7.35(d,4H, J=8.40 Hz), 7.47-7.49(m,4H), 7.64-7.69(m,2H), 7.85(t,1H,J=8.00 Hz), 7.97-8.01(m,2H), 8.49(dd,2H,J=7.60, 1.60 Hz), 8.83(m,1H) | 9.91 (s) |
| 3g | - | - | - | 2.54(m) | 3.49(t,J = 4.40 Hz) | 4.52(s) | 7.47-7.55(m,12H), 7.70 (td,2H,J =8.40, 1.60 Hz), 7.76-7.82(m,3H), 7.99(dd,2H,J=8.00,1.60 Hz), 8.38(dd,2H,J=8.00,1.60 Hz), 8.71(m,1H) | 9.88(s) |

**Table 6.** $^{13}$C-NMR data of the compounds **3** (DMSO-$d_6$, δ/ppm)

| Comp. | 2COO | [a] | 2N=CH | [b] | Aromatic C | C3-Aromatik C | [c] | [d] | [e] | Aliphatic C |
|---|---|---|---|---|---|---|---|---|---|---|
| 3a | 163.53 | 149.53 | 149.18 | 142.97 | 123.66(2CH), 125.84(2C), 126.97(2CH), 127.90(2CH), 129.48(2C), 130.23(CH), 130.97(CH), 132.67(2CH), 135.29(2CH), 150.15(2C) | - | 65.96 | 65.79 | 49.86 | 10.81 ($2CH_3$) |
| 3b | 163.53 | 149.50 | 149.35 | 146.68 | 123.67(2CH), 125.86(2C), 127.00(2CH), 127.99(2CH), 129.48(2C), 130.21(CH), 130.97(CH), 132.67(2CH), 135.28(2CH), 150.28(2C) | - | 65.96 | 65.82 | 49.88 | 9.88 ($2CH_2CH_3$), 18.20 ($2CH_2CH_3$) |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 3c | 163.52 | 149.48 | 149.40 | 145.47 | 123.66(2CH), 125.85(2C), 127.02(2CH), 127.97(2CH), 129.49(2C), 130.25(CH), 130.98(CH), 132.68(2CH), 135.28(2CH), 150.22(2C) | - | 65.97 | 65.77 | 49.88 | 13.30 ($2CH_2CH_2CH_3$), 18.67($2CH_2CH_2CH_3$), 26.25 ($2CH_2CH_2CH_3$) |
| 3d | 163.50 | 149.71 | 148.75 | 144.81 | 123.56(2CH), 125.81(2C), 126.75(2CH), 127.25(2CH), 129.36(2C), 130.19(CH), 130.96(CH), 132.72(2CH), 135.25(2CH), 150.16(2C) | 126.96(2CH), 128.44(4CH), 128.68(4CH), 135.44(2C) | 65.96 | 65.96 | 49.88 | 30.61 ($2CH_2Ph$) |
| 3e | 163.50 | 149.70 | 148.75 | 144.96 | 123.55(2CH), 125.97(2C), 126.96(2CH), 127.16(2CH), 129.36(2C), 130.18(CH), 130.96(CH), 132.56(2CH), 135.14(2CH), 150.15(2C) | 128.55(4CH), 129.01(4CH), 132.30(2C), 135.83(2C) | 65.95 | 65.89 | 49.88 | 20.55 ($2PhCH_3$), 30.37 ($2CH_2Ph$) |
| 3f | 163.53 | 149.70 | 148.88 | 144.50 | 123.57(2CH), 125.76(2C), 126.97(2CH), 127.34(2CH), 129.36 (2C), 130.18(CH), 130.98(CH), 132.76(2CH), 135.24(2CH), 150.15(2C) | 128.38(4CH), 130.62(4CH), 131.49(2C), 134.39(2C) | 65.95 | 65.95 | 49.85 | 30.09 ($2CH_2Ph$) |
| 3g | 163.54 | 151.72 | 149.84 | 143.31 | 123.69(2CH), 125.69(2C), 127.01(2CH), 127.51(2CH), 129.30(2C), 129.86(CH), 130.99(CH), 132.88(2CH), 134.98(2CH), 150.36(2C) | 125.90(2C), 127.87(4CH), 128.46(4CH), 130.03(2CH) | 66.29 | 65.97 | 49.86 | - |

***a)*** *2Triazole* $C_5$, ***b)*** *2Triazole* $C_3$, ***c)*** $2CH_2OCH_2$, ***d)*** $2NCH_2N$, ***e)*** $2CH_2NCH_2$

## Biological Methods

### Antioxidant Activity

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

### Reducing Power

The reducing power of the compounds **2a-g** and **3a-g** were determined using the method of Oyaizu[35] as explained in the literature [31] and were shown in Table 8.

**Table 7.** The reducing power method

| Reagents | $S_1$ | $S_2$ | $S_3$ | $N_1$ | $N_2$ | $N_3$ | Blank |
|---|---|---|---|---|---|---|---|
| **Compound** | - | - | - | 100μL | 250μL | 500μL | - |
| **Standard** | 100μL | 250μL | 500μL | - | - | - | - |
| **Phosphate Buffer** | 2,4 mL | 2,25 mL | 2,0 mL | 2,4 mL | 2,25 mL | 2,0 mL | - |
| **$K_3Fe(CN)_6$** | 2,5 mL | 2,5 mL | 2,5 mL | 2,5 mL | 2,5 mL | 2,5 mL | - |

### Free Radical Scavenging Activity

Free radical scavenging effect of the compounds **2a-g** and **3a-g** were estimated by DPPH·, by the method of Blois [36] as explained in the literature [31] and were summarized in Table 8.

**Table 8.** The free radical scavenging effect method

| Reagents | $S_1$ | $S_2$ | $S_3$ | $N_1$ | $N_2$ | $N_3$ | Blank | Control |
|---|---|---|---|---|---|---|---|---|
| **Compound** | - | - | - | 50μL | 100μL | 150μL | - | - |
| **Standard** | 50μL | 100μL | 150μL | - | - | - | - | - |
| **Ethyl Alcohol** | 2,95 mL | 2,90 mL | 2,85 mL | 2,95 mL | 2,90 mL | 2,85 mL | - | 3 mL |
| **DPPH** | 1 mL | 1 mL | 1 mL | 1 mL | 1 mL | 1 mL | 4 mL | 1 mL |

### Metal Chelating Activity

The chelating of ferrous ions by the compounds **2a-g** and **3a-g** and references were measured according to the method of Dinis et al. [37] as explained in the literature [31] (Table 9).

**Table 9.** The metal chelating activity method

| Reagents | $S_1$ | $S_2$ | $S_3$ | $N_1$ | $N_2$ | $N_3$ | Blank | Control |
|---|---|---|---|---|---|---|---|---|
| **Compound** | - | - | - | 30μL | 60μL | 90μL | - | - |
| **Standard** | 30μL | 60μL | 90μL | - | - | - | - | - |
| **Ethyl Alcohol** | 3,75 mL | 3,75 mL | 3,75 mL | 3,75 mL | 3,75 mL | 3,75 mL | 3,75 mL | 3,75 mL |
| **$FeCl_2 4H_2O$** | 0,05 mL | 0,05 mL | 0,05 mL | 0,05 mL | 0,05 mL | 0,05 mL | 0,05 mL | 0,05 mL |
| **Ferrozine** | 0,2 mL | 0,2 mL | 0,2 mL | 0,2 mL | 0,2 mL | 0,2 mL | 0,2 mL | 0,2 mL |

### Antimicrobial Activity

Antimicrobial activities of **2a-g** and **3a-g** compounds were investigated simple susceptibility screening test using agar-well diffusion method [32] as adapted earlier [38]. All microorganisms present in the test were provided from the Microbiologic Environmental Protection Laboratories Company in France. These microorganisms: *Klebsiella pneumoniae* ATCC4352, *Pseudomonas aeruginosa* ATCC27853, *Escherichia coli* ATCC259222, *Staphylococcus aureus* ATCC6538, *Bacillus subtilis* ATCC11774, *Bacillus cereus* ATCC11778.

## RESULTS AND DISCUSSION

The synthesized seven new Schiff bases and seven new Mannich bases were identified using IR, $^1$H-NMR, $^{13}$C-NMR spectral data.

### Antioxidant Acivity

*In vitro* antioxidant activities of fourteen new compounds **2a-g** and **3a-g** were investigated. Antioxidant activities were determined by the methods showed below.

### Total reductive capability using the potassium ferricyanide reduction method

The reducing power of the compounds **2a-g** and **3a-g** was determined as described in [39, 40]. All compounds in different amounts showed lower absorption rates in this study than the standard compounds. Consequently, no activity was observed with respect to the reduction of metal ion complexes to their lower oxidation states or their involvement in an electron transfer reaction. In summary, synthesized compounds were not involved in reductive activities as seen in Figures **1** and **2**.

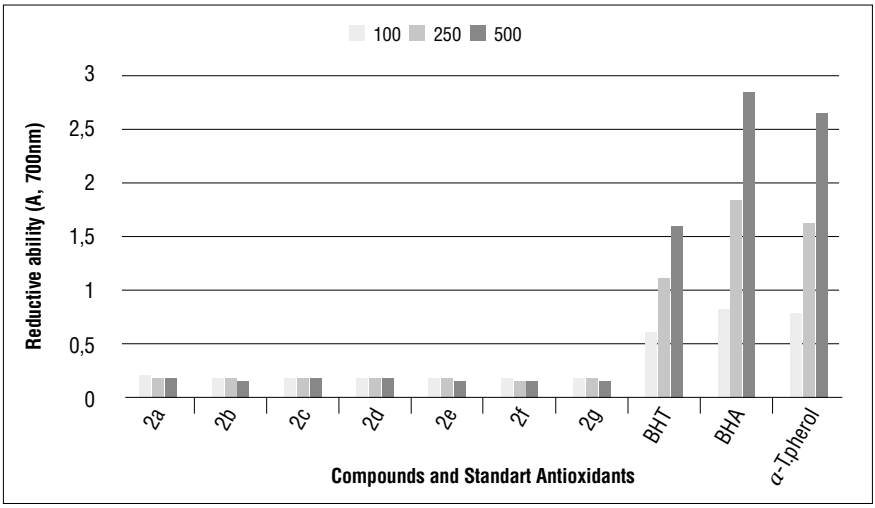



**Figure 1.** Total reductive potential of different concentrations of the compounds **2**, BHT, BHA and α-tocopherol.

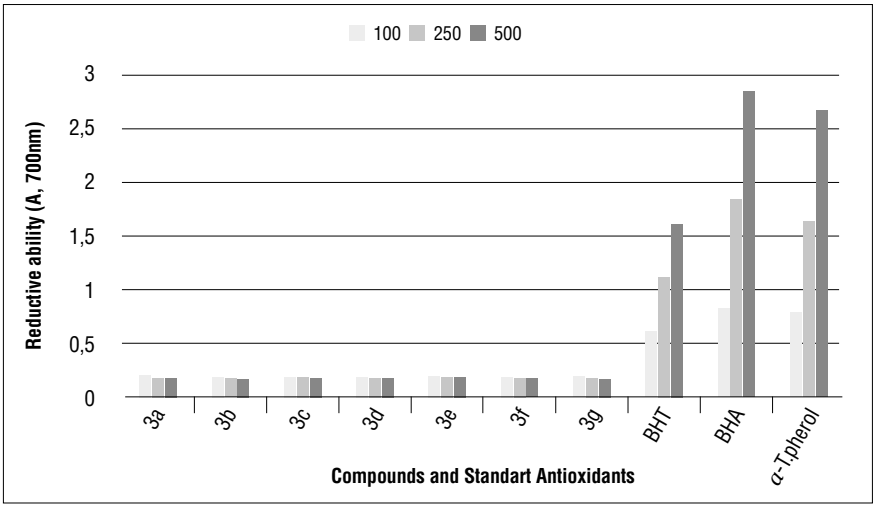



**Figure 2.** Total reductive potential of different concentrations of the compounds **3**, BHT, BHA and α-tocopherol.

### DPPH· radical scavenging activity

The scavenging effect of compounds **2a-g** and **3a-g** was estimated by DPPH as explained in [41-43]. The DPPH method was used to determine the antiradical activity of compounds and standard antioxidants such as BHA, BHT and α-tocopherol in the study. It has been found that recently synthesized compounds have no activity as radical scavengers as shown in Figures **3** and **4**.

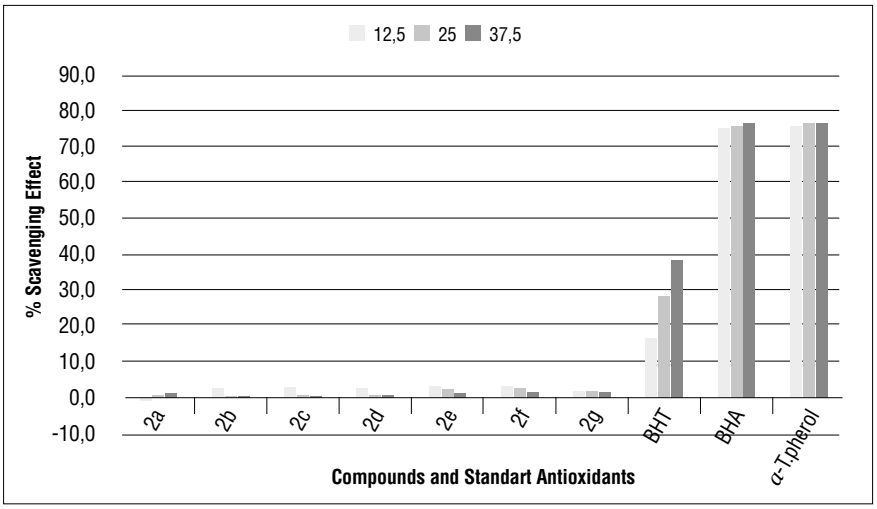



**Figure 3.** Scavenging effect of the compounds **2**, BHT, BHA and α-tocopherol at different concentrations (12.5–25–37.5 μg/mL).

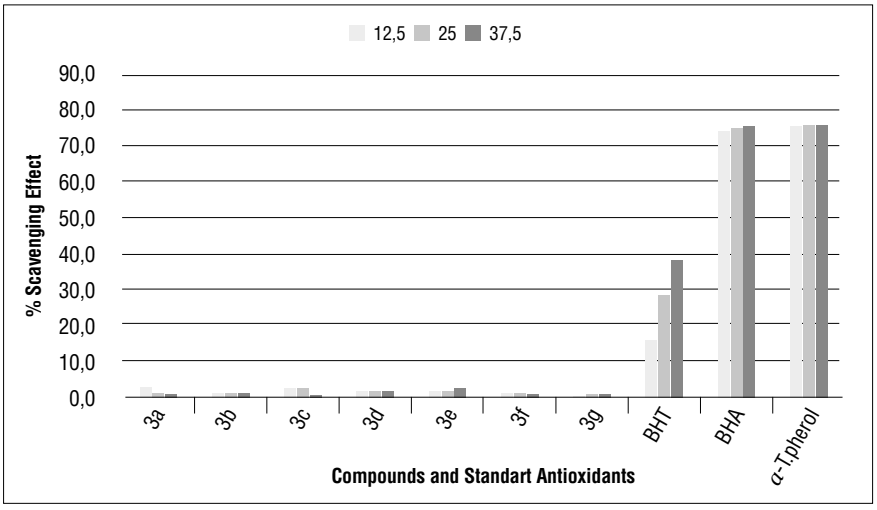



**Figure 4.** Scavenging effect of the compounds **3**, BHT, BHA and α-tocopherol at different concentrations (12.5–25–37.5 μg/mL).

### Ferrous ion chelating activity

The chelation effect against iron ions by the compounds and standards was determined. Ferrozin can form complexes quantitatively with $Fe^{2+}$. In the presence of chelating agents, the complex formation is disturbed, so that the red colour of the complex decreases. The measurement of colour reduction thus allows the estimation of the chelating activity of the coexisting chelator [44]. Iron ion chelating activities of the compounds **2**, **3**, EDTA and α-tocopherol are shown in Figures **5** and **6**, respectively.

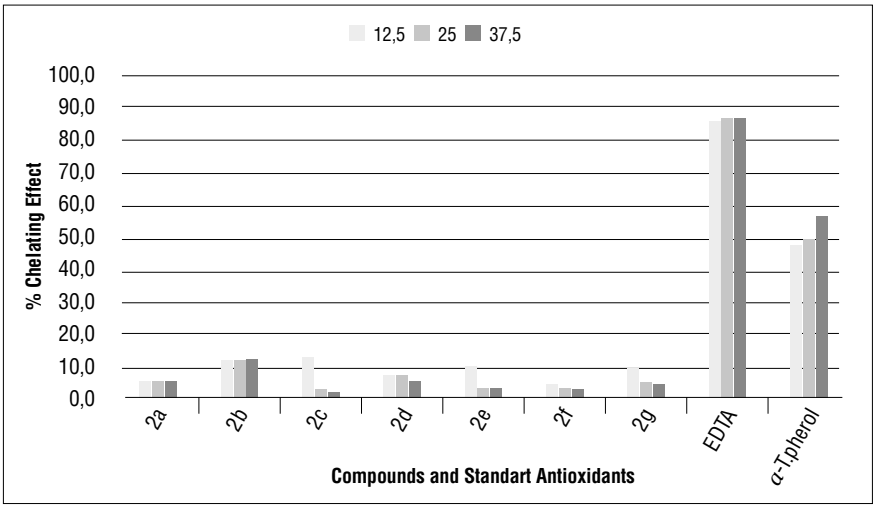



**Figure 5.** Metal chelating effect of different amount of the compounds **2**, EDTA and α-tocopherol on ferrous ions.

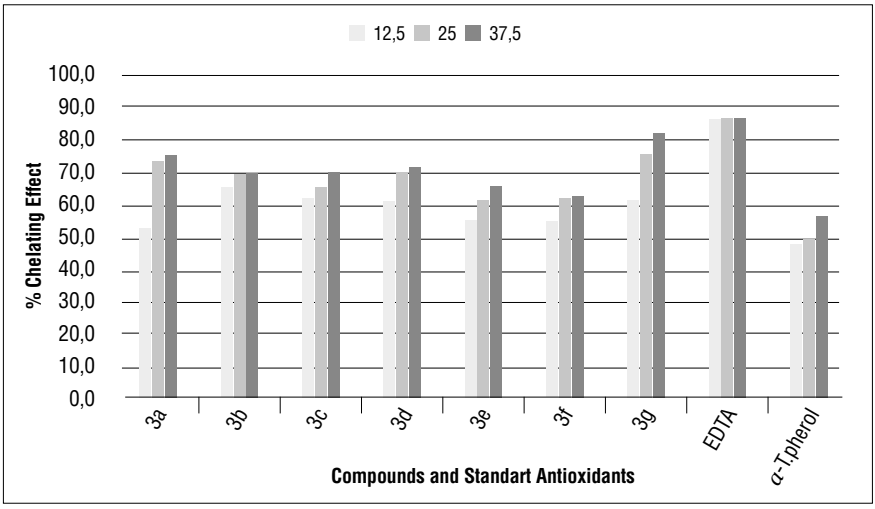



**Figure 6.** Metal chelating effect of different amount of the compounds **3**, EDTA and α-tocopherol on ferrous ions.

The high metal-chelating activity refers to a low absorption level at 562 nm. From the data in Figure 3, it can be deduced that the metal-chelating effects of compounds are concentration-dependent. As a result, compounds with significant iron-binding capacities can prove that their action as peroxidation inhibitors stems from their iron-binding capacities. The order of metal chelation of compounds and standards decreases when EDTA > **3g** > **3a** > **3d** > **3b** ≈ **3c** > **3e** > **3f** > α-tocopherol. Despite the low solubility rate for free iron, chelated iron complexes are known to have higher solubility rates in solutions that can be readily attributed to the ligand. Due to their potential involvement in iron-catalysed reactions, compound iron complexes could also be active.

### Antimicrobial Activity

The antimicrobial activity of the compounds **2** and **3** were investigated. The results are shown in Table **10** and **11**.

**Table 10.** Zone diameters for antimicrobial activity of the **2,3** and Standart compounds

| Compound | Microorganisms and Inhibition Zone (mm) | | | | | |
|---|---|---|---|---|---|---|
| | K.p. | P.a. | E.c. | S.a. | B.s. | B.c. |
| 2a | - | - | - | - | - | - |
| 2b | - | - | - | - | - | - |
| 2c | - | - | - | - | - | - |
| 2d | - | - | - | - | - | - |
| 2e | - | - | - | - | - | - |
| 2f | - | - | - | - | - | - |
| 2g | - | - | - | - | - | - |
| 3a | 12 | 16 | 19 | 10 | - | - |
| 3b | 13 | 17 | 13 | 12 | - | - |
| 3c | 17 | 14 | 17 | 17 | - | - |
| 3d | 15 | 13 | 17 | 15 | - | - |
| 3e | 15 | 12 | 16 | 14 | - | - |
| 3f | 12 | 11 | 15 | 11 | - | - |
| 3g | 23 | 21 | 22 | 24 | - | - |
| Ampicillin (X3261) | 35 | 36 | 34 | 37 | 33 | 36 |
| Neomycin (X3385) | 16 | 17 | 16 | 13 | 17 | 17 |
| Step-tomycin (X3385) | 11 | 12 | 10 | 21 | 12 | 12 |

**Table 11.** Screening for antimicrobial activity of the compounds **2** and **3**.

| Compound | Microorganisms and Inhibition Zone (mm) | | | | | |
|---|---|---|---|---|---|---|
| | K.p. | P.a. | E.c. | S.a. | B.s. | B.c. |
| 2a | - | - | - | - | - | - |
| 2b | - | - | - | - | - | - |
| 2c | - | - | - | - | - | - |
| 2d | - | - | - | - | - | - |
| 2e | - | - | - | - | - | - |
| 2f | - | - | - | - | - | - |
| 2g | - | - | - | - | - | - |
| 3a | ++ | ++ | +++ | + | - | - |
| 3b | ++ | +++ | ++ | ++ | - | - |
| 3c | +++ | ++ | +++ | +++ | - | - |
| 3d | ++ | ++ | +++ | ++ | - | - |
| 3e | ++ | ++ | ++ | ++ | - | - |
| 3f | ++ | ++ | ++ | ++ | - | - |
| 3g | +++ | +++ | +++ | +++ | - | - |

*The inhibition zone: (–): <5.5 mm; (+): 5.5–10 mm; (++): 11–16 mm; (+++): ≥17 mm.*

*K.p.: Klepsiella pneumoniae (ATCC4352), P.a.: Pseudomonas aeruginosa (ATCC27853), E.c.: Escherichia coli (ATCC25922). S.a.: Staphylococcus aureus (ATCC6538), B.s.: Bacillus subtilis (ATCC11774), B.c.: Bacillus cereus (ATCC11778).*

All of the Schiff Bases (**2a-g**) showed no effect against six bacteria. All of the Mannich Bases (**3a-g**) showed no effect against *B. Subtilis* ATCC11774 and B. *cereus* ATCC11778 bacteria strains. The antimicrobial activity of **3a-g** compounds against *K. pneumoniae*, *P. Aeruginos, E. coli*, *S. aureus* is lower than Ampicillin and higher than Neomycin and Streptomycin standards and listed in Table **10**. While compounds **3b**, **3g** showed high activity against *P. Aeruginosa* ATCC27853, compounds **3a, 3c, 3d, 3e** and **3f** showed moderate activity to this strain. On the other hand, different results were obtained from *K. pneumoniae* ATCC4352 strain. While compounds **3c**, **3g** showed high activity against *K. pneumoniae* ATCC4352, *Staphylococcus aureus* (ATCC6538) and *Escherichia coli* (ATCC25922) compounds **3a, 3b, 3d, 3e** and **3f** showed moderate activity to this strain. The compound **3a, 3c, 3d, 3g** showed high activity against *Escherichia coli* (ATCC25922) but, compound **3b, 3e, 3f** showed moderate activity against same bacteria. Compound **3g** showed high activity against first four bacteria and summarized in Table **10**, **11**.

The synthesis and in-vitro antioxidant valuation of fourteen new compounds

are explained. Antioxidant activity (the metal chelate activity) and anti-microbial activity of Mannich bases (**3a-g**) were higher than Schiff base compounds (**2a-g**). Synthesis of these new Mannich Bases can play especially a safety role in modern medicinal chemistry. These results may also ensure some lead for the improving of Mannich Bases curative aim.

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