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Synthesis and *In vitro* Antimicrobial Properties of Some New 4-(2-Benzoxy-3-ethoxy)-benzylidenamino-4,5-dihydro-1*H*-1,2,4-triazol-5-one Derivatives

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Authors' contributions

This work was carried out in collaboration between all authors. Authors FK carried out the synthesis and laboratory work. Author OGK managed the antioxidant activity and wrote the first draft of the manuscript. Author MB managed the spectroscopic analyses of the study. Author MA managed the antimicrobial activity. Author HY is a major supervisor, who designed the study and revised the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The present study involved the synthesis of heterocyclic Schiff bases and their biological activity.
Study Design: Synthesizing some new 4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives and determining their chemical structure via IR and NMR spectroscopy. The synthesized compounds were analyzed for their *in vitro* potential antimicrobial and antioxidant activities.
Place and Duration of Study: Department of Chemistry, Kafkas University, Kars, Turkey. Between September 2010 to September 2012.
Methodology: Nine new 3-alkyl(aryl)-4-(2-benzoxy-3-ethoxy-benzylidenamino)-4,5-dihydro-1*H*-

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1,2,4-triazol-5-ones (**3**) were synthesized by the reactions of 3-alkyl(aryl)-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**1**) with 2-benzoxy-3-ethoxy-benzaldehyde (**2**) which had also been synthesized by the reactions of 3-ethoxysalicylaldehyde with benzoyl chloride by using triethylamine.
Results: Schiff bases were synthesized and their structures were determined with spectral methods. Antimicrobial and antioxidant evaluation were carried out, presented and discussed.
Conclusion: Synthesis and structural determination of the new 3-alkyl(aryl)-4-(2-benzoxy-3-ethoxy-benzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**3**) was successful. Considering both the antimicrobial and the antioxidant evaluation, compound **3c** exhibited moderate effect.

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

1. INTRODUCTION

During the last 50 years, depending on developments in medicine, antimicrobial studies in the field of chemistry have increased. Especially, many compounds in organic chemistry have been used in drug industry. One group of these compounds is also triazoles and their derivatives. There are a widespread applications of 1,2,4-triazole derivatives in pharmacology. Some of the drug-containing 1,2,4-triazole derivatives are: alprazolam (tranquilizer), estazolam (hypnotic, sedative, tranquilizer), rilmazafon (hypnotic, anxiolytic, used in the case of neurotic insomnia), benatradin (diuretic), trapidil (hypotensive), trazodon (antidepressant, anxiolytic), etoperidone (antidepressant), nefazodone (antidepressant, 5-HT2 A-antagonist), anastrozole, letrozole, vorozole (antineoplastics, nonsteroidal competitive aromatase inhibitors), ribavirin (the potent antiviral N-nucleoside), fluconazole, itraconazole, terconazole (the powerful azole antifungal agents) [1-3].

1,2,4-triazoles and their derivatives are often used in antimicrobial studies particularly. However, many studies including antifungal effects of triazole derivatives have also been carried out. Changes in antibacterial properties have clearly been determined with the change of substituents [4-7].

While triazole derivatives were investigated for different properties, their Schiff bases also were investigated by working groups. Antibacterial, antifungal, antioxidant, antitumor, analgesic and cytotoxic properties of triazole-derived Schiff bases were investigated in different studies [8-13]. Even biological properties of metal complexes of Schiff Bases obtained triazoles have been studied by several working groups [14,15].

In this study, nine new 3-alkyl(aryl)-4-(2-benzoxy-3-ethoxybenzylidenamino)-4,5-dihidro-1*H*-1,2,4-triazol-5-ones (**3a-i**) were synthesized from the reactions of 3-alkyl(aryl)-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**1a-i**) with 2-benzoxy-3-ethoxy-benzaldehyde (**2**) [16]. In addition, to find their possible antimicrobial and antioxidant activity, the newly synthesized **3** type compounds were investigated by using antimicrobial and antioxidant methodologies due to a wide range of applications.

2. METHODOLOGY

2.1 Chemical Reagents and Apparatus

Chemical reagents used in this study were purchased from Merck AG, Aldrich and Fluka. Melting points were determined in open glass capillaries using a Stuart SMP30 melting point apparatus and are uncorrected. The IR spectra were recorded on an Alpha-P Bruker FT-IR spectrometer. ^{1}H and ^{13}C NMR spectra were recorded in deuterated dimethyl sulfoxide (99%) with TMS (99.5%) as internal standard using a Varian Mercury spectrometer at 400 MHz and 100 MHz, respectively. UV absorption spectra were measured in 10 mm quartz cells between 200 and 400 nm using a PG Instruments Ltd T80 UV/Vis spectrometer. Extinction coefficients (ε) were expressed in $L \cdot mol^{-1} \cdot cm^{-1}$. Elemental analyses were carried out on a LECO, CHNS-932 for C, H, and N.

2.2 Synthesis

2.2.1 Procedure for the synthesis of 2-benzoxy-3-ethoxy-benzaldehyde (2)

3-Ethoxysalicylaldehyde (0.01 mol, 97%) dissolved in ethyl acetate (100 mL, 99.5%) was treated with benzoyl chloride (0.01 mol, 98.5%), and triethylamine (0.02 mol, 99%) with stirring at 0-5 ℃ was slowly added to this solution. 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 (99.8%) to afford compound **2** [16], yield 92%. Mp: 137℃. IR (cm^{-1}) 2889 and 2773 (υ_{CHO}), 1726, 1688 ($\upsilon_{C=O}$), 1250 (υ_{COO}), 771 and 695 ($\upsilon_{monosubstituted\ benzenoid\ ring}$); UV ($C_2H_5OH$) λ_{max} (log ε) 318 (3315), 256 (10337), 222 (17110) nm.

2.2.2 General procedure for the synthesis of compounds 3

The corresponding compound **1** (0.01 mol) was dissolved in acetic acid (15 mL, 100%) and treated with 2-benzoxy-3-ethoxy-benzaldehyde **2** (0.01 mol). The mixture was refluxed for 1.5 h and then evaporated at 50-55℃ *in vacuo*. Several recrystallizations of the residue from appropriate solvent gave pure compounds **3a-i** as colorless crystals.

2.2.2.1 3-Methyl-4-(2-benzoxy-3-ethoxybenzylidenamino)-4,5-dihidro-1H-1,2,4-triazol-5-one (3a)

Yield 94%. Mp: 185ºC. IR (cm^{-1}) 3172 (υ_{NH}), 1738, 1712 ($\upsilon_{C=O}$), 1599 ($\upsilon_{C=N}$), 1259 (υ_{COO}), 760 and 703 ($\upsilon_{monosubstituted\ benzenoid\ ring}$); ^{1}H NMR (200MHz, DMSO-d_6) δ 1.27 (t, 3H, $OCH_2C\underline{H_3}$; *J*=6.95 Hz), 2.15 (s, 3H, CH_3), 4.05 (q, 2H, $OC\underline{H_2}CH_3$; *J*=6.96 Hz), 7.07 (d, 1H, ArH; *J*=8.09 Hz), 7.26 (t, 1H, ArH; *J*=8.08 Hz), 7.48-7.62 (m, 4H, ArH), 8.22-8.24 (m, 2H, ArH), 9.95 (s, 1H, N=CH), 10.31 (s, 1H, NH); ^{13}C NMR (50MHz, DMSO-d_6) δ 11.31 (CH_3), 14.68 ($OCH_2\underline{C}H_3$), 64.82 ($O\underline{C}H_2CH_3$), 116.02, 119.10, 126.61, 127.64, 128.65 (2C), 129.09, 130.37 (2C), 133.67, 140.27, 151.30, (Ar-C), 145.56 (triazole C_3), 150.51 (N=CH), 151.93 (triazole C_5), 164.56 (COO); UV (C_2H_5OH) λ_{max} (log ε) 296 (16783), 260 (16100), 236 (24917), 218 (22375) nm; *Anal.* Calcd. for $C_{19}H_{18}N_4O_4$ (%): C, 62.29; H, 4.95; N, 15.29. Found: C, 62.03; H, 4.84; N, 14.96.

2.2.2.2 3-Ethyl-4-(2-benzoxy-3-ethoxybenzylidenamino)-4,5-dihidro-1H-1,2,4-triazol-5-one (3b)

Yield 93%. Mp: 169ºC. IR (cm^{-1}) 3181 (υ_{NH}), 1746, 1692 ($\upsilon_{C=O}$), 1592 ($\upsilon_{C=N}$), 1252 (υ_{COO}), 760 and 704 ($\upsilon_{monosubstituted\ benzenoid\ ring}$); ^{1}H NMR (200MHz, DMSO-d_6) δ 1.18 (t, 3H, $CH_2C\underline{H_3}$; *J*=7.48 Hz), 1.28 (t, 3H, $OCH_2C\underline{H_3}$; *J*=6.92 Hz), 2.55 (q, 2H, $C\underline{H_2}CH_3$; *J*=7.48 Hz), 4.06 (q, 2H, $OC\underline{H_2}CH_3$; *J*=6.96 Hz), 7.08 (d, 1H, ArH; *J*=8.20 Hz), 7.27 (t, 1H, ArH; *J*=8.04 Hz), 7.49-7.55 (m, 3H, ArH), 7.59-7.63 (m, 1H, ArH), 8.23-8.25 (m, 2H, ArH), 9.96 (s, 1H, N=CH), 10.13 (s, 1H, NH); ^{13}C NMR (50MHz, DMSO-d_6) δ 10.05 ($CH_2\underline{C}H_3$), 14.68 ($OCH_2\underline{C}H_3$), 19.02 ($\underline{C}H_2CH_3$), 64.83 ($O\underline{C}H_2CH_3$), 116.00, 119.11, 126.61, 127.70, 128.65 (2C), 129.11, 130.38 (2C), 133.66, 140.29, 151.31 (Ar-C), 149.53 (triazole C_3), 150.43 (N=CH), 152.11 (triazole C_5), 164.55 (COO); UV (C_2H_5OH) λ_{max} (log ε) 296 (14681), 260 (14543), 236 (24647) nm; *Anal.* Calcd. for $C_{20}H_{20}N_4O_4$ (%): C, 63.15; H, 5.30; N, 14.73. Found: C, 62.76; H, 5.10; N, 14.58.

2.2.2.3 3-Propyl-4-(2-benzoxy-3-ethoxybenzylidenamino)-4,5-dihidro-1H-1,2,4-triazol-5-one (3c)

Yield 98%. Mp: 179ºC. IR (cm^{-1}) 3163 (υ_{NH}), 1746, 1691 ($\upsilon_{C=O}$), 1592 ($\upsilon_{C=N}$), 1242 (υ_{COO}), 765 and 704 ($\upsilon_{monosubstituted\ benzenoid\ ring}$); ^{1}H NMR (200MHz, DMSO-d_6) δ 0.93 (t, 3H, $CH_2CH_2C\underline{H_3}$; *J*=7.44 Hz), 1.29 (t, 3H, $OCH_2C\underline{H_3}$; *J*=6.96 Hz), 1.68 (sext, 2H, $CH_2C\underline{H_2}CH_3$; *J*=7.48 Hz), 2.54 (t, 2H, $C\underline{H_2}CH_2CH_3$; *J*=7.36 Hz), 4.07 (q, 2H, $OC\underline{H_2}CH_3$; *J*=6.92 Hz), 7.09 (d, 1H, ArH; *J*=8.20 Hz), 7.26-7.30 (m, 1H, ArH), 7.50-7.56 (m, 3H, ArH), 7.60-7.62 (m, 1H, ArH), 8.23-8.25 (m, 2H, ArH), 9.92 (s, 1H, NH), 9.97 (s, 1H, N=CH); ^{13}C NMR (50MHz, DMSO-d_6) δ 13.62 ($CH_2CH_2\underline{C}H_3$), 14.68 ($OCH_2\underline{C}H_3$), 19.22 ($CH_2\underline{C}H_2CH_3$), 27.22 ($\underline{C}H_2CH_2CH_3$), 64.84 ($O\underline{C}H_2CH_3$), 115.99, 118.90, 126.61, 127.72, 128.67 (2C), 129.09, 130.38 (2C), 133.66, 140.38, 151.29 (ArC), 148.52 (triazole C_3), 150,36 (N=CH), 152.01 (triazole C_5), 164.56 (COO). UV (C_2H_5OH) λ_{max} (log ε) 296 (13018), 260 (13018), 234 (24929) nm.

2.2.2.4 3-Benzyl-4-(2-benzoxy-3-ethoxybenzylidenamino)-4,5-dihidro-1H-1,2,4-triazol-5-one (3d)

Yield 91%. Mp: 189ºC. IR (cm^{-1}) 3169 (υ_{NH}), 1747, 1709 ($\upsilon_{C=O}$), 1591 ($\upsilon_{C=N}$), 1258 (υ_{COO}), 778 and 698 ($\upsilon_{monosubstituted\ benzenoid\ ring}$); ^{1}H NMR (200MHz, DMSO-d_6) δ 1.28 (t, 3H, $OCH_2C\underline{H_3}$; *J*=6.96 Hz), 3.93 (s, 2H, CH_2Ph), 4.06 (q, 2H, $OC\underline{H_2}CH_3$; *J*=7.00 Hz), 7.08 (d, 1H, ArH; *J*=8.20 Hz), 7.23-7.30 (m, 6H, ArH), 7.45-7.52 (m, 3H, Ar-H), 7.55-7.57 (m, 1H, Ar-H), 8.20-8.22 (m, 2H, Ar-H), 9.91 (s, 1H, NH), 9.94 (s, 1H, N=CH); ^{13}C NMR (50MHz, DMSO-d_6) δ 14.69 ($OCH_2\underline{C}H_3$), 31.70 (CH_2Ph), 64,83 ($O\underline{C}H_2CH_3$), 116.04, 118.50, 126.63, 127.12, 127.66, 128.64 (2C), 128.68 (2C), 129.00, 129.08 (2C), 130.36 (2C), 133.70, 134.94, 140.55, 151.28 (Ar-C), 147.53 (triazole C_3), 150.20 (N=CH), 151.88 (triazole C_5), 164.55 (COO); UV (C_2H_5OH) λ_{max} (log ε) 296 (16711), 262 (16811), 234 (31578) nm; *Anal.* Calcd. for $C_{25}H_{22}N_4O_4$ (%): C, 67.86; H, 5.01; N, 12.66. Found: C, 67.64; H, 5.13; N, 12.64.

2.2.2.5 3-p-Methylbenzyl-4-(2-benzoxy-3-ethoxybenzylidenamino)-4,5-dihidro-1H-1,2,4-triazol-5-one (3e)

Yield 97%. Mp: 218ºC. IR (cm^{-1}) 3165 (υ_{NH}), 1747, 1709 ($\upsilon_{C=O}$), 1592 ($\upsilon_{C=N}$), 1257 (υ_{COO}), 831 ($\upsilon_{1,4\text{-disubstituted benzenoid ring}}$), 760 and 690 ($\upsilon_{monosubstituted\ benzenoid\ ring}$); ^{1}H NMR (200MHz, DMSO-d_6) δ 1.28 (t, 3H, $OCH_2C\underline{H}_3$; *J*=6.96 Hz), 2.29 (s, 3H, $PhCH_3$), 3.88 (s, 2H, CH_2Ph), 4.05 (q, 2H, $OC\underline{H}_2CH_3$; *J*=6.96 Hz), 7.06-7.16 (m, 5H, ArH), 7.27 (t, 1H, ArH; *J*=8.08 Hz), 7.45-7.58 (m, 4H, ArH), 8.20-8.23 (m, 2H, ArH), 9.93 (s, 1H, N=CH), 10.11 (s, 1H, NH); ^{13}C NMR (50MHz, DMSO-d_6) δ 14.70 ($OCH_2\underline{C}H_3$), 21.11 ($PhCH_3$), 31.29 (CH_2Ph), 64.84 ($O\underline{C}H_2CH_3$), 116.02, 118.54, 126.63, 127.71, 128.70 (2C), 129.00 (2C), 129.02, 129.34 (2C), 130.37 (2C), 131.82, 133.69, 136.71, 140.55, 151.29 (Ar-C), 147.70 (triazole C_3), 150.16 (N=CH), 151.97 (triazole C_5), 164.56 (COO); UV (C_2H_5OH) λ_{max} (log ε) 296 (14623), 264 (15009), 234 (25009) nm.

2.2.2.6 3-p-Methoxybenzyl-4-(2-benzoxy-3-ethoxybenzylidenamino)-4,5-dihidro-1H-1,2,4-triazol-5-one (3f)

Yield 99%. Mp: 178ºC. IR (cm^{-1}) 3170 (υ_{NH}), 1741, 1706 ($\upsilon_{C=O}$), 1591 ($\upsilon_{C=N}$), 1246 (υ_{COO}), 846 ($\upsilon_{1,4\text{-disubstituted benzenoid ring}}$), 765 and 695 ($\upsilon_{monosubstituted\ benzenoid\ ring}$); ^{1}H NMR (200MHz, DMSO-d_6) δ 1.28 (t, 3H, $OCH_2C\underline{H}_3$; *J*=6.92 Hz), 3.75 (s, 3H, OCH_3), 3.85 (s, 2H, CH_2Ph), 4.06 (q, 2H, $OC\underline{H}_2CH_3$; *J*=6.96 Hz), 6.82 (d, 2H, ArH; *J*=8.60 Hz), 7.08 (d, 1H, ArH; *J*=8.12 Hz), 7.18 (d, 2H, ArH; *J*=8.56 Hz), 7.24-7.29 (m, 1H, ArH), 7.45-7.58 (m, 4H, Ar-H), 8.20-8.23 (m, 2H, Ar-H), 9.93 (s, 1H, N=CH), 10.13 (s, 1H, NH); ^{13}C NMR (50MHz, DMSO-d_6) δ 14.70 ($OCH_2\underline{C}H_3$), 30.84 (CH_2Ph), 55.28 (OCH_3), 64.84 ($O\underline{C}H_2CH_3$), 114.08 (2C), 116.03, 118.59, 126.65, 126.90, 127.69, 128.70 (2C), 129.02, 130.16 (2C), 130.37 (2C), 133.70, 140.52, 151.30, 158.70 (Ar-C), 147.80 (triazole C_3), 150.23 (N=CH), 151.97 (triazole C_5), 164.57 (COO); UV (C_2H_5OH) λ_{max} (log ε) 284 (14800), 270 (15091), 236 (25973) nm; *Anal.* Calcd. for $C_{26}H_{24}N_4O_5$ (%): C, 66.09; H, 5.12; N, 11.86. Found: C, 65.91; H, 5.21; N, 11.74.

2.2.2.7 3-p-Chlorobenzyl-4-(2-benzoxy-3-ethoxybenzylidenamino)-4,5-dihidro-1H-1,2,4-triazol-5-one (3g)

Yield 99%. Mp: 183ºC. IR (cm^{-1}) 3170 (υ_{NH}), 1750, 1707 ($\upsilon_{C=O}$), 1593 ($\upsilon_{C=N}$), 1258 (υ_{COO}), 822 ($\upsilon_{1,4\text{-disubstituted benzenoid ring}}$), 776 and 699 ($\upsilon_{monosubstituted\ benzenoid\ ring}$); ^{1}H NMR (200MHz, DMSO-d_6) δ 1.27 (t, 3H, $OCH_2C\underline{H}_3$; *J*=6.92 Hz), 3.85 (s, 2H, CH_2Ph), 4.05 (q, 2H, $OC\underline{H}_2CH_3$; *J*=6.96 Hz), 7.08 (d, 1H, ArH; *J*=8.12 Hz), 7.17 (d, 2H, ArH; *J*=8.44 Hz), 7.24-7.28 (m, 3H, ArH), 7.44-7.48 (m, 3H, ArH), 7.54-7.57 (m, 1H, ArH), 8.20-8.22 (m, 2H, Ar-H), 9.92 (s, 1H, N=CH), 10.33 (s, 1H, NH); ^{13}C NMR (50MHz, DMSO-d_6) δ 14.69 ($OCH_2\underline{C}H_3$), 31.01 (CH_2Ph), 64.85 ($O\underline{C}H_2CH_3$), 116.14, 118.64, 126.70, 127.53, 128.70 (2C), 128.78 (2C), 129.00, 130.36 (2C), 130.47 (2C), 133.03, 133.39, 133.71, 140.48, 151.33 (Ar-C), 146.97 (triazole C_3), 150.59 (N=CH), 151.94 (triazole C_5), 164.56 (COO); UV (C_2H_5OH) λ_{max} (log ε) 296 (18859), 262 (18902), 236 (31522) nm; *Anal.* Calcd. for $C_{25}H_{21}ClN_4O_4$ (%): C, 62.96; H, 4.44; N, 11.75. Found: C, 62.58; H, 4.34; N, 11.61.

2.2.2.8 3-m-Chlorobenzyl-4-(2-benzoxy-3-ethoxybenzylidenamino)-4,5-dihidro-1H-1,2,4-triazol-5-one (3h)

Yield 95%. Mp: 175ºC. IR (cm^{-1}) 3167 (υ_{NH}), 1752, 1704 ($\upsilon_{C=O}$), 1593 ($\upsilon_{C=N}$), 1269 (υ_{COO}), 867 and 781 ($\upsilon_{1,3\text{-disubstituted benzenoid ring}}$), 781 and 701 ($\upsilon_{monosubstituted\ benzenoid\ ring}$); ^{1}H NMR (200MHz, DMSO-d_6) δ 1.29 (t, 3H, $OCH_2C\underline{H}_3$; *J*=6.96 Hz), 3.91 (s, 2H, CH_2Ph), 4.07 (q, 2H, $OC\underline{H}_2CH_3$; *J*=6.95 Hz), 7.10 (d, 1H, ArH; *J*=8.05 Hz), 7.14-7.32 (m, 5H, ArH), 7.48-7.52 (m, 3H, ArH), 7.60-7.62 (m, 1H, ArH), 8.21-8.23 (m, 2H, ArH), 8.90 (s, 1H, NH), 9.94 (s, 1H, N=CH); ^{13}C NMR (50MHz, DMSO-d_6) δ 14.88 ($OCH_2\underline{C}H_3$), 31.64 (CH_2Ph), 65.01 ($O\underline{C}H_2CH_3$), 116.28, 118.73, 126.92, 127.41, 127.63, 127.69, 128.88 (2C), 129.15, 129.57, 130.09, 130.57 (2C), 133.95, 134.59, 136.90, 140.69, 151.45 (Ar-C), 147.27 (triazole C_3), 150.52 (N=CH), 151.54 (triazole C_5), 164.72 (COO); UV (C_2H_5OH) λ_{max} (log ε) 296 (12890), 262 (12670), 232 (25963) nm. *Anal.* Calcd. for $C_{25}H_{21}ClN_4O_4$ (%): C, 62.96; H, 4.44; N, 11.75. Found: C, 62.98; H, 4.47; N, 11.56.

2.2.2.9 3-Phenyl-4-(2-benzoxy-3-ethoxybenzylidenamino)-4,5-dihidro-1H-1,2,4-triazol-5-one (3i)

Yield 99%. Mp: 198ºC. IR (cm^{-1}) 3163 (υ_{NH}), 1741, 1694 ($\upsilon_{C=O}$), 1597 ($\upsilon_{C=N}$), 1248 (υ_{COO}), 772 and 700 ($\upsilon_{monosubstituted\ benzenoid\ ring}$); ^{1}H NMR (200MHz, DMSO-d_6) δ 1.27 (t, 3H, $OCH_2C\underline{H}_3$; *J*=6.96 Hz), 4.05 (q, 2H, $OC\underline{H}_2CH_3$ *J*=6.96 Hz), 7.08 (d, 1H, ArH; *J*=8.24 Hz), 7.24 (t, 1H, ArH; *J*=8.08 Hz), 7.39-7.49 (m, 5H, ArH), 7.55-7.59 (m, 2H, ArH), 7.88-7.92 (m, 2H, ArH), 8.19-8.22

(m, 2H, ArH), 9.97 (s, 1H, N=CH), 10.76 (s, 1H, NH); ^{13}C NMR (50MHz, DMSO-d_6) δ 14.69 ($OCH_2\underline{C}H_3$), 64.85 ($O\underline{C}H_2CH_3$), 116.20, 118.27, 126.51, 126.69, 127.63, 128.39 (2C), 128.47 (2C), 128.66 (2C), 128.95, 130.23, 130.37 (2C), 133.64, 140.86, 151.78 (Ar-C), 145.97 (triazole C_3), 151.27 (N=CH), 152.28 (triazole C_5), 164.64 (COO); UV (C_2H_5OH) λ_{max} (log ε) 266 (20786), 238 (26286), 222 (23688) nm.

2.3 Antimicrobial Activity

Simple susceptibility screening test using agar-well diffusion method [17-19] as adapted earlier [20] was used for determination of antimicrobial activities of 3a and 3c-g compounds. All test microorganisms were obtained from the Microbiologics Environmental Protection Laboratories Company in France and are as follows; E. coli ATCC 259222, P. aeruginosa ATCC 27853, K. pneumoniae ATCC 4352, S. aureus ATCC 6538, B. subtilis ATCC 11774, B. cereus ATCC 11778. All the newly synthesized compounds were weighed and dissolved in dimethylsulphoxide (DMSO, 99.9%) 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℃. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism. Dimethylsulphoxide was used as solved control. Results were interpreted in terms of the diameter of the inhibition zone: (–): <5.5 mm; (+): 5.5–10 mm; (++): 11–16 mm; (+++): ≥17 mm.

2.4 Antioxidant Activity

2.4.1 Chemicals

Butylated hydroxytoluene (BHT, 99%) was obtained from E. Merck. Ferrous chloride (98%), α-tocopherol (97%), DPPH·, 3-(2-pyridyl)-5,6-bis(phenylsulfonic acid)-1,2,4-triazine (ferrozine, 97%), butylated hydroxyanisole (BHA, 99%), ethylenediaminetetraacetic acid (EDTA, 99%) and trichloroacetic acid (TCA, 99-100.5%) were obtained from Sigma.

2.4.2 Reducing power

The reducing power of the synthesized compounds was determined according to the method of Oyaizu [21] as explained in the literature [12,13].

2.4.3 Free radical scavenging activity

Free radical scavenging activity of compounds was measured by DPPH, using Blois method [22] as explained in the literature [12,13].

2.4.4 Metal chelating activity

The chelation of ferrous ions by the synthesized compounds and standards were estimated by the method of Dinis et al. [23] as explained in the literature [12, 13].

3. RESULTS AND DISCUSSION

3.1 Chemistry

In this study, the 3-alkyl(aryl)-4-(2-benzoxy-3-ethoxybenzylidenamino)-4,5-dihidro-1*H*-1,2,4-triazol-5-ones **3a-i** were prepared. The starting compounds **1a-i** were prepared as described in the literature [24,25]. Compounds **3** were synthesized from the reactions of compounds **1** with 2-benzoxy-3-ethoxy-benzaldehyde **2** which were synthesized by the reactions of 3-ethoxysalicylaldehyde with benzoyl chloride by using triethylamine (Scheme 1).

The structures of nine new 3-alkyl(aryl)-4-(2-benzoxy-3-ethoxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones **3a-i** were characterized by using elemental analyses and IR, ^{1}H-NMR, ^{13}C-NMR and UV spectral data.

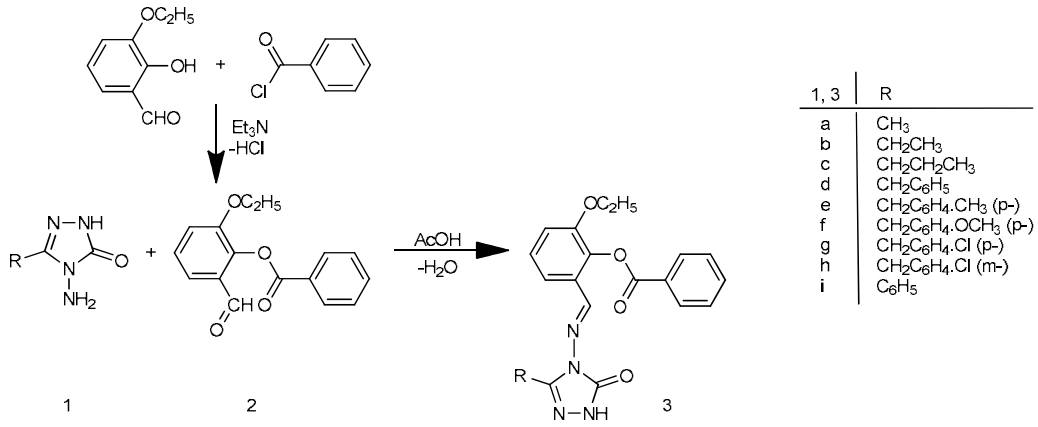
3.2 Antimicrobial Activity

The observed data for the antimicrobial activity of **3a** and **3c-g** compounds were given in Table 1.

The data revealed that the highest zone diameter was obtained from **3a** compound against Bacillus subtilis ATCC 10978 strain and also **3c** compound showed the highest effect on Klepsiella pneumoniae (ATCC 4352).

3.3 Antioxidant Activity

The antioxidant activity of nine new compounds **3a-i** was determined. Several methods have been used to determine antioxidant activities and the methods used in the study are given below.



Scheme 1. Synthetic pathway of compounds 2, 3

Table 1. Screening for antimicrobial activity of the 3 type compounds

Compound	Microorganisms and inhibition zone (mm)						
	Bs	**Bc**	**Ye**	**Sa**	**Ec**	**Pm**	**Kp**
3a	+++	+	-	-	++	++	++
3c	++	+	+	-	-	++	+++
3d	++	-	-	-	+	-	-
3e	++	-	-	-	+	+	++
3f	+	++	-	+	++	-	-
3g	++	++	+	+	++	+	-

Results were interpreted in terms of diameter of the inhibition zone: (-): <5,5 mm; (+): 5,5-10 mm; (++): 11-16 mm; (+++): ≥17 mm.

Bs: Bacillus subtilis ATCC 10978, Bc: Bacillus cereus ATCC 11778, Ye: Yersinia enterocolitica ATCC 27729, Sa: Staphylococcus aureus (ATCC 6538), Ec: Escherichia coli (ATCC 25922), Pm: Pasteurella multocida (ATCC 12945), Kp: Klepsiella pneumoniae (ATCC 4352)

3.3.1 Total reductive capability using the potassium ferricyanide reduction method

The reductive capabilities of compounds were assessed by the extent of conversion of the Fe^{3+} / ferricyanide complex to the Fe^{2+} / ferrous form. The reducing powers of the compounds were observed at different concentrations, and results were compared with BHA, BHT and α-tocopherol. It has been observed that the reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity [26]. The antioxidant activity of putative antioxidant has been attributed to various mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalyst, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging [27].

In this study, all the amount of the compounds showed lower absorbance than those of standard antioxidants. Hence, no activities were observed to reduce metal ions complexes to their lower oxidation state or to take part in any electron transfer reaction. In other words, synthesized compounds did not show reductive activities.

3.3.2 DPPH· radical scavenging activity

The scavenging the stable DPPH radical model is a widely used method to evaluate antioxidant activities in a relatively short time compared to other methods. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability [28]. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [29]. The reduction capability of DPPH radicals was determined by the decreasing of its absorbance at 517 nm induced by antioxidants. The absorption maximum of a stable DPPH radical in ethanol was at 517 nm. The decrease in absorbance of DPPH radical was caused by antioxidants because of reaction between antioxidant molecules and radical, progresses, which resulted in the scavenging of the radical by hydrogen donation. It is visually noticeable as a discoloration from purple to yellow.

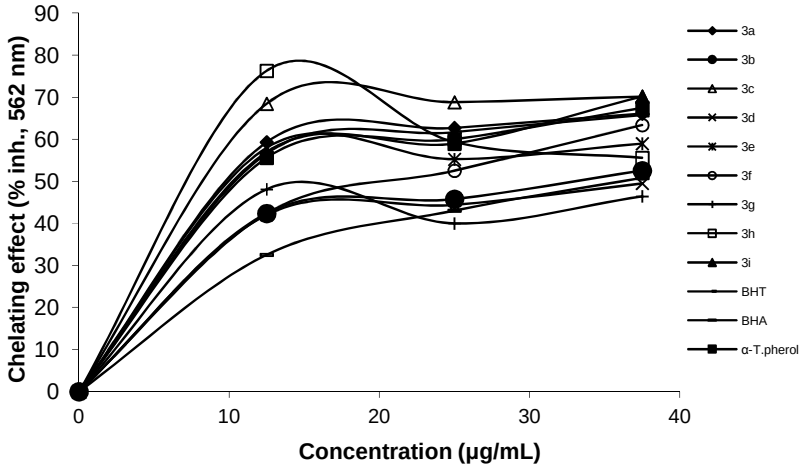


Fig. 1. Metal chelating effect of different amount of the compounds 3a-i, BHT, BHA and α-tocopherol on ferrous ions

Hence, $DPPH^{\cdot}$ is usually used as a substrate to evaluate antioxidative activity of antioxidants [30]. In the study, antiradical activities of compounds and standard antioxidants such as BHA and α-tocopherol were determined by using $DPPH^{\cdot}$ method. The newly synthesized compounds showed no significant effect as a radical scavenger.

3.3.3 Ferrous ion chelating activity

The chelating effect towards ferrous ions by the compounds and standards was determined. Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of chelating agents, the complex formation is disrupted with the result that the red colour of the complex is decreased. Measurement of colour reduction therefore allows estimation of the chelating activity of the coexisting chelator [31]. Transition metals have pivotal role in the generation oxygen free radicals in living organism. The ferric iron (Fe^{3+}) is the relatively biologically inactive form of iron. However, it can be reduced to the active Fe^{2+}, depending on condition, particularly pH [32] and oxidized back through Fenton type reactions with the production of hydroxyl radical or Haber-Weiss reactions with superoxide anions. The production of these radicals may lead to lipid peroxidation, protein modification and DNA damage. Chelating agents may not activate metal ions and potentially inhibit the metal-dependent processes [33]. Also, the production of highly active ROS such as $O_2^{\cdot-}$, H_2O_2 and $OH^{\cdot}$ is also catalyzed by free iron though Haber-Weiss reactions:

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

Among the transition metals, iron is known as the most important lipid oxidation pro-oxidant due to its high reactivity. The ferrous state of iron accelerates lipid oxidation by breaking down the hydrogen and lipid peroxides to reactive free radicals via the Fenton reactions:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^{\cdot}$$

Fe^{3+} ion also produces radicals from peroxides, even though the rate is tenfold less than that of Fe^{2+} ion, which is the most powerful pro-oxidant among the various types of metal ions [34]. Ferrous ion chelating activities of compounds **3** and standard antioxidants are shown in Fig. 1. It is 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 [35].

Low absorbance at 562 nm indicates high metal chelating activity. The data obtained from Fig. 1 reveal that the metal chelating effects of compounds **3a-d**, **3f** and **3i** were concentration-dependent, the other compounds were not. Especially **3c** and **3i** demonstrate a marked capacity for iron binding, suggesting that their action as peroxidation protectors may be related to their iron binding capacity.

4. CONCLUSION

In this study, the structures of 9 new 4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives synthesized from the reactions of **1** type compounds with 2-benzoxy-3-ethoxy-benzaldehyde were identified using elemental analysis, IR, ^{1}H NMR, ^{13}C NMR,

and UV spectral data, and these obtained spectral values were seen as compatible with literature [12,13]. The newly synthesized compounds were screened for their antimicrobial and antioxidant activities. Considering both the antimicrobial and the antioxidant evaluation, compound **3c** exhibited moderate effect.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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