Synthesis and Biological Evaluation of New 2-Ethoxy-6-[(4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl)iminomethyl]phenyl 4-nitro-benzoate Derivatives

Yeni 2-Etoksi-6-[(4,5-dihidro-1H-1,2,4-triazol-5-on-4-il) iminometil]fenil 4-nitrobenzoat Türevlerinin Sentezi ve Biyolojik Değerlendirmesi

Research Article

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ABSTRACT

In the present study, 3-alkyl(aryl)-4-amino-4,5-dihydro-1H-1,2,4-triazol-5-ones (1) reacted with 3-ethoxy-2-(4-nitrobenzoxy)-benzaldehyde (2) to afford 2-ethoxy-6-[(3-alkyl/aryl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl) iminomethyl]phenyl 4-nitrobenzoates (3). The structures of new nine compounds were established from the elemental analyses, IR, ^{1}H NMR and ^{13}C NMR spectral data. The synthesized compounds were analyzed for their in vitro potential antioxidant activities in three different methods. In addition, these new compounds were screened for their antimicrobial activities.

Key Words
Schiff base, synthesis, acetylation, antioxidant activity, antimicrobial activity.

ÖZ

Bu çalışmada, 3-alkil(aril)-4-amino-4,5-dihidro-1H-1,2,4-triazol-5-on (1) bileşikleri 3-etoksi-2-(4-nitrobenzoksi)-benzaldehid (2) ile reaksiyona sokularak 2-etoksi-6-[(3-alkil/aril-4,5-dihidro- 1H-1,2,4-triazol-5-on-4-il)iminometil]fenil 4-nitrobenzoat (3) bileşikleri elde edildi. Yeni dokuz adet bileşiğin yapısı elementel analiz ve IR, ^{1}H NMR ve ^{13}C NMR spektrum verileri ile aydınlatıldı. Sentezlenen bileşiklerin in vitro potansiyel antioksidan aktiviteleri üç farklı yöntemle analiz edildi. Ayrıca, yeni bileşiklerin antimikrobiyal aktiviteleri incelendi.

Anahtar Kelimeler
Schiff bazı, sentez, asetillendirme, antioksidan aktivite, antimikrobiyal aktivite.

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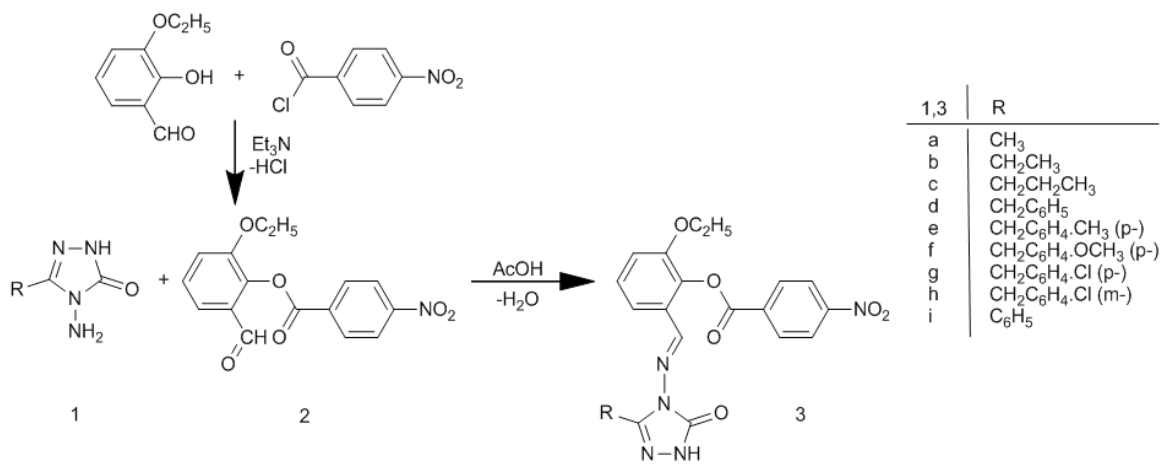
INTRODUCTION

The compounds containing the 1,2,4-triazole moiety have been reported to possess different biological activities such as antiviral, antimicrobial, antifungal, analgesic, anti-inflammatory, antioxidant, hypoglycemic, antihypertensive, diuretic, anticonvulsant, and anticancer activity depending on the substituents in the ring system [1-11]. Moreover several articles reporting the synthesis of some N-arylidenamino-4,5-dihydro-1H-1,2,4-triazol-5-one derivatives have been published [8-11].

In the last two decades there has been an explosive interest in the role of reactive oxygen species (ROS) and of reactive nitrogen species (RNS) in food, drugs, and even living system. Therefore, scientists in various disciplines have become more interested in naturally-occurring antioxidant and also related synthetic derivatives that could provide active components to prevent or reduce the impact of oxidative stress [12]. Exogenous chemicals and endogenous metabolic processes in human body or in food system might produce highly reactive-free radicals, especially oxygen derived radicals, which are capable of oxidizing biomolecules, resulting in cell death and tissue damage. Oxidative damages play a significant pathological role in human diseases. For example, cancer, emphysema, cirrhosis, atherosclerosis and arthritis have all been correlated with oxidative damage. Also, excessive generation of ROS (reactive oxygen species) induced by various stimuli and which exceeds the antioxidant capacity of the organism leads to a variety of pathophysiological processes such as inflammation, diabetes, genotoxicity and cancer [13].

In addition, during the last 50 years, depending on developments in medicine, antimicrobial studies in the field of chemistry have been increased. Especially, many compounds in organic chemistry have been used in drug industry. In particularly, 1,2,4-triazoles and their derivatives are often used in antimicrobial studies. However, many studies including antifungal effects of triazole derivatives have also been studied [1,2,9,11].

In the present study, nine new 2-ethoxy-6-[(3-alkyl/aryl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl) iminomethyl]phenyl 4-nitrobenzoates (3a-i) were synthesized from the reactions of 3-alkyl(aryl)-4-amino-4,5-dihydro-1H-1,2,4-triazol-5-ones (1a-i) with 3-ethoxy-2-(4-nitrobenzoxy)-benzaldehyde (2), which was synthesized by the reaction of 3-ethoxysalicylaldehyde with 4-nitrobenzoyl chloride by using triethylamine (Scheme 1). In addition, due to a wide range of applications to find their possible radical scavenging and antioxidant activity, the newly synthesized compounds were investigated by using different antioxidant methodologies: 1,1-diphenyl-2-picryl-hydrazyl (DPPH.) free-radical scavenging, reducing power and metal chelating activities. Furthermore, the antimicrobial activities of the synthesized compounds were screened agaist seven bacteria such as B*acillus subtilis, Bacillus cereus, Yersinia enterocolitica, Staphylococcus aureu, Escherichia coli, Pasteurella multocida, Klepsiella pneumoniae*.



Scheme1. Synthetic pathway of compounds 3.

EXPERIMENTAL

Materials and Chemicals

Chemical reagents and all solvents used in this study were purchased from Merck AG, Aldrich and Fluka. Melting points were determined in open glass capillaries using a Stuart SMP_30 melting point apparatus and are uncorrected. The IR spectra were obtained on an ALPHA-P BRUKER FT-IR spectrometer. ^1H and ^{13}C NMR spectra were recorded in deuterated dimethyl sulfoxide with TMS as an internal standard using a Varian Mercury spectrometer at 200 MHz and 50 MHz, respectively. Elemental analyses were carried out on a LECO, CHNS-932 for C, H, and N.

Synthesis

Procedure for the synthesis of 3-ethoxy-2-(4-nitrobenzoxy)-benzaldehyde (2)

3-Ethoxysalicylaldehyde (0.01 mol) dissolved in ethyl acetate (100 mL) was treated with 4-nitrobenzoyl chloride (0.01 mol), and to this solution was slowly added triethylamine (0.01 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, yield (3.04g, 96%), m.p. 124 °C. IR (KBr) υ_{max} 2897 and 2791 (CHO), 1739, 1694 (C=O), 1523 and 1348 (NO_2), 1256 (COO), 778 and 707 (1,2,3-trisubstituted benzenoid ring), 842 (1,4-disubstituted benzenoid ring) cm^{-1}. 1H NMR (200 MHz, DMSO-d_6): δ 1.22 (t, 3H, OCH_2CH_3; *J*=6.80 Hz), 4.11 (q, 2H, OCH_2CH_3; *J*=6.80 Hz), 7.52-7.59 (m, 3H, ArH), 8.40-8.46 (m, 4H, ArH), 10.15 (s, 1H, CHO). ^{13}C NMR (50MHz, DMSO-d_6): δ 14.33 (OCH_2CH_3), 64.39 (OCH_2CH_3), [119.82, 122.00, 124.07, 124.38, 127.56, 128.83, 130.62, 131.33, 133.78, 139.87, 150.55, 150.67] (Ar-C), 162.58 (COO), 190.07 (CHO).

General method for the preparation of 2-ethoxy-6-[(3-alkyl/aryl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl)iminomethyl]phenyl 4-nitrobenzoates (3)

The corresponding compound 1 (0.01 mol) was dissolved in acetic acid (15 mL) and treated with 3-ethoxy-2-(4-nitrobenzoxy)-benzaldehyde (2) (0.01 mol). The mixture was refluxed for 1.5 h and then evaporated at 50-55 °C in vacuo. Several recrystallizations of the residue from appropriate solvent gave pure compounds (3a-i) as colorless crystals.

2-Ethoxy-6-[(3-methyl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl)iminomethyl]phenyl 4-nitro-benzoate (3a)

Yield (3.87g, 94%), m.p. 237°C, Anal. calcd. for $C_19H_17N_5O_6$: C, 55.47; H, 4.17; N, 17.02%. Found: C, 55.07; H, 4.23; N, 17.21%. IR (KBr) 3189 (NH), 1744, 1698 (C=O), 1594 (C=N), 1524 and 1351 (NO_2), 1256 (COO), 778 and 713 (1,2,3-trisubstituted benzenoid ring), 845 (1,4-disubstituted benzenoid ring) cm^{-1}. ^1H NMR (200 MHz, DMSO-d_6): δ 1.20 (t, 3H, CH_2CH_3; *J*=6.80 Hz), 2.12 (s, 3H, CH_3), 4.11 (q, 2H, CH_2CH_3; *J*=6.80 Hz), 7.36 (d, 1H, ArH; *J*=8.00 Hz), 7.43 (t, 1H, ArH; *J*=8.00 Hz), 7.58 (1H, ArH; *J*=8.00Hz), 8.43 (q, 4H, ArH), 9.91 (s, 1H, N=CH), 11.79 (s, 1H, NH). ^{13}C NMR (50 MHz, DMSO-d_6): δ 10.85 (CH_3), 14.34 (OCH_2CH_3), 64,39 (OCH_2CH_3), [116.36, 118.30, 124.17 (2C), 126.94, 127.41, 131.29 (2C), 133.44, 138.82, 150.41, 150.78] (Ar-C), 144.05 (triazole C_3), 148.36 (N=CH), 151.08 (triazole C_5), 162.46 (COO).

2-Ethoxy-6-[(3-ethyl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl)iminomethyl]phenyl 4-nitro-benzoate (3b)

Yield (4.11g, 97%), m.p. 229°C, Anal. calcd. for $C_20H_19N_5O_6$: C, 56.47; H, 4.50; N, 16.46%. Found: C, 56.46; H, 4.68; N, 16.22%. IR (KBr) υ_{max} 3188 (NH), 1750, 1699 (C=O), 1588 (C=N), 1525 and 1347 (NO_2), 1252 (COO), 776 and 708 (1,2,3-trisubstituted benzenoid ring), 846 (1,4-disubstituted benzenoid ring) cm^{-1}. ^1H NMR (200 MHz, DMSO-d_6): δ 1.10 (t, 3H, CH_2CH_3; *J*=7.60 Hz) 1.21 (t, 3H, OCH_2CH_3; *J*=6.80 Hz), 2.50 (q, 2H, CH_2CH_3; *J*=7.60 Hz), 4.11 (q, 2H, OCH_2CH_3; *J*=6.80 Hz), 7.36 (d, 1H, ArH; *J*=8.00 Hz), 7.43 (t, 1H, ArH; *J*=8.00 Hz), 7.57 (d, 1H, ArH; *J*=8.00 Hz), 8.43-8.47 (m, 4H, ArH), 9.91 (s, 1H, N=CH), 11.82 (s, 1H, NH). ^{13}C NMR (50 MHz, DMSO-d_6): δ 9.84 (CH_2CH_3), 14.36 (OCH_2CH_3), 18.32 (CH_2CH_3), 64.42 (OCH_2CH_3), [116.36, 118.42, 124.17 (2C), 126.99, 127.44, 131.33 (2C), 133.49, 138.82, 150.45, 150.80] (Ar-C), 147.82 (triazole C_3), 148.46 (N=CH), 151.26 (triazole C_5), 162.48 (COO).

2-Ethoxy-6-[(3-n-propyl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl)iminomethyl]phenyl 4-nitrobenzoate (3c)

Yield (4.10g, 93%), m.p. 219 °C, Anal. calcd. for $C_21H_21N_5O_6$: C, 57.40; H, 4.82; N, 15.94%. Found: C, 57.33; H, 4.96; N, 15.75%. IR (KBr) υ_{max} 3170 (NH), 1751, 1698 (C=O), 1585 (C=N), 15224 and 1347 (NO_2), 1254 (COO), 776 and 708 (1,2,3-trisubstituted benzenoid ring), 846 (1,4-disubstituted benzenoid ring) cm^{-1}. ^1H NMR (200 MHz, DMSO-d_6): δ 0.86 (t, 3H, $CH_2CH_2CH_3$; *J*=7.20 Hz), 1.21 (t, 3H, OCH_2CH_3; *J*=6.80 Hz), 1.59 (sext, 2H, $CH_2CH_2CH_3$; *J*=7.20 Hz), 2.46 (t, 2H, $CH_2CH_2CH_3$; *J*=7.20Hz), 4.11 (q, 2H, OCH_2CH_3; *J*=6.80 Hz), 7.36 (d, 1H, ArH; *J*=8.00 Hz), 7.44 (t, 1H, ArH, *J*=8.00 Hz), 7.56 (d, 1H, ArH; *J*=7.60 Hz), 8.41-8.47 (m, 4H, ArH), 9.92 (s, 1H, N=CH), 11.83 (s, 1H, NH). ^{13}C NMR (50 MHz, DMSO-d_6): δ 13.31 ($CH_2CH_2CH_3$), 14.36 (OCH_2CH_3), 18.64 ($CH_2CH_2CH_3$), 26.49 ($CH_2CH_2CH_3$), 64.43 (OCH_2CH_3), [116.37, 118.46, 124.19 (2C), 126.99, 127.46, 131.33 (2C), 133.52, 138.82, 150.46, 150.80] (Ar-C), 146.68 (triazole C_3), 148,59 (N=CH), 151.22 (triazole C_5), 162.48 (COO).

2-Ethoxy-6-[(3-benzyl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl)iminomethyl]phenyl 4-nitro-benzoate (3d)

Yield (4.68g, 96%), m.p. 235°C, Anal. calcd. for $C_25H_21N_5O_6$: C, 61.60; H, 4.34; N, 14.37%. Found: C, 61.09; H, 4.67; N, 14.16%. IR (KBr) υ_{max} 3158 (NH), 1753, 1699 (C=O), 1582 (C=N), 1521 and 1348 (NO_2), 1266 (COO), 780 and 703 (1,2,3-trisubstituted benzenoid ring), 847 (1,4-disubstituted benzenoid ring) cm^{-1}. ^1H NMR (200 MHz, DMSO-d_6): δ 1.19 (t, 3H, OCH_2CH_3; *J*=6.80 Hz), 3.94 (s, 2H,CH_2Ph), 4.09 (q, 2H, OCH_2CH_3; *J*=6.80Hz), 7.21-7.44 (m, 7H, ArH), 7.54-7.56 (m, 1H, ArH), 8,41 (m, 4H, ArH) 9.91 (s, 1H, (N=CH), 11,96 (s, 1H, NH). ^{13}C NMR (50 MHz, DMSO-d_6): δ 14.36 (OCH_2CH_3), 30.80 (CH_2Ph), 64,43 (OCH_2CH_3), [116.42, 117.93, 124.18 (2C), 126.68, 126.97, 127.47, 128.37 (2C), 128.57 (2C), 131.31 (2C), 133.39, 135.55, 139.04, 150.41, 150.78] (Ar-C), 146.07 (triazole C_3), 148.08 (N=CH), 151.13 (triazole C_5), 162.51 (COO).

2-Ethoxy-6-[(3-p-methylbenzyl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl)iminomethyl] phenyl 4-nitrobenzoate (3e)

Yield (4.75g, 95%), m.p. 236°C, Anal. calcd. for $C_26H_23N_5O_6$: C, 62.27; H, 4.62; N, 13.96%. Found: C, 62.01; H, 4.80; N, 13.98%. IR (KBr) υ_{max} 3163 (NH), 1754, 1699 (C=O), 1579 (C=N), 1520 and 1345 (NO_2), 1260 (COO), 779 and 712 (1,2,3-trisubstituted benzenoid ring), 847 (1,4-disubstituted benzenoid ring) cm^{-1}. ^1H NMR (DMSO-d_6, 200 MHz,): δ 1.19 (t, 3H, OCH_2CH_3; *J*=6.80 Hz), 2.25 (s, 3H, $PhCH_3$), 3.87 (s, 2H, CH_2Ph), 4.09 (q, 2H, OCH_2CH_3; *J*=6.80 Hz), 7.11 (q, 4H, ArH; *J*=8.00 Hz), 7.35 (d, 1H, ArH *J*=8.00 Hz), 7.43 (t, 1H, ArH *J*=8.00 Hz), 7.55 (d, 1H, ArH; *J*=8.00 Hz), 8.37-8.42 (m, 4H, ArH), 9.90 (s, 1H, N=CH), 11.93 (s, 1H, NH). ^{13}C NMR (50 MHz, DMSO-d_6): δ 14.36 (OCH_2CH_3), 20.55 ($PhCH_3$), 30.39 (CH_2Ph), 64.43 (OCH_2CH_3), [116.41, 117.99, 124.18 (2C), 126.97, 127.48, 128.56 (2C), 128.93 (2C), 131.29 (2C), 132.45, 133.39, 135.75, 139.02, 150.42, 150.77] (Ar-C), 146.21 (triazole C_3), 148.09 (N=CH), 151.12 (triazole C_5), 162.51 (COO).

2-Ethoxy-6-[(3-p-methoxybenzyl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl) iminomethyl]-phenyl 4-nitrobenzoate (3f)

Yield (4.95g, 96%), m.p. 226°C, Anal. calcd. for $C_26H_23N_5O_7$: C, 60.35; H, 4.48; N, 13.53%. Found: C, 60.28; H, 4.75; N, 13.15%. IR (KBr) υ_{max} 3177 (NH), 1751, 1691 (C=O), 1590 (C=N), 1516 and 1355 (NO_2), 1246 (COO), 779 and 710 (1,2,3-trisubstituted benzenoid ring), 847 (1,4-disubstituted benzenoid ring) cm^{-1}. ^1H NMR (DMSO-d_6, 200 MHz,): δ 1.19 (t, 3H, OCH_2CH_3; *J*=6.80 Hz), 3.71 (s, 3H, OCH_3), 3.84 (s, 2H, CH_2Ph), 4.10 (q, 2H, OCH_2CH_3; *J*=6.80 Hz), 6.85 (d, 2H, ArH; *J*=8.40 Hz), 7.16 (d, 2H, ArH; *J*=8.40 Hz), 7.36 (d, 1H, ArH; *J*=8.00 Hz), 7.44 (t, 1H, ArH; *J*=8.00 Hz), 7.57 (d, 1H, ArH; *J*=7.60 Hz), 8.37-8.43 (m, 4H, ArH), 9.89 (s, 1H, N=CH), 11.91 (s, 1H, NH). ^{13}C NMR (50 MHz, DMSO-d_6): δ 14.37 (OCH_2CH_3), 29.93 (CH_2Ph), 54.96 (OCH_3), 64.44 (OCH_2CH_3), [113.78 (2C), 116.45, 118.04, 124.20 (2C), 126.97, 127.31, 127.52, 129.76 (2C), 131.31 (2C), 133.38, 139.01, 150.42, 150.79, 158.04] (Ar-C), 146.38 (triazole C_3), 148.16 (N=CH), 151.13 (triazole C_5), 162.52 (COO).

2-Ethoxy-6-[(3-p-chlorobenzyl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl)iminomethyl] phenyl 4-nitrobenzoate (3g)

Yield (5.08g, 97%), m.p. 239°C, Anal. calcd. for $C_25H_20ClN_5O_6$: C, 57.53; H, 3.86; N, 13.42%. Found: C, 57.49; H, 4.11; N, 13.22%. IR (KBr) υ_{max} 3174 (NH), 1755, 1700 (C=O), 1593 (C=N), 1518 and 1349 (NO_2), 1263 (COO), 778 and 714 (1,2,3-trisubstituted

benzenoid ring), 846 (1,4-disubstituted benzenoid ring) cm^{-1}. ^1H NMR (DMSO-d_6, 200 MHz,): δ 1.19 (t, 3H, OCH_2CH_3; *J*=6.80 Hz), 3.93 (s, 2H, CH_2Ph), 4.10 (q, 2H, OCH_2CH_3; *J*=6.80 Hz), 7.27 (d, 2H, ArH; *J*=8.40 Hz), 7.34-7.37 (m, 3H, ArH), 7.43 (t, 1H, ArH; *J*=8.00 Hz), 7.53 (d, 1H, ArH; *J*=8.00 Hz), 8.37-8.43 (m, 4H, ArH), 9.89 (s, 1H, N=CH), 11.96 (s, 1H, NH). ^{13}C NMR (50 MHz, DMSO-d_6): δ 14.38 (OCH_2CH_3), 30.11 (CH_2Ph) 64.45 (OCH_2CH_3), [116.51, 118.03, 124.21 (2C), 126.91, 127.51, 128.31 (2C), 130.63 (2C), 131.32 (2C), 131.41, 133.36, 134.53, 139.02, 150.42, 150.80] (Ar-C), 145.73 (triazole C_3), 148.28 (N=CH), 151.09 (triazole C_5), 162.50 (COO).

2-Ethoxy-6-[(3-m-chlorobenzyl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl)iminomethyl] phenyl 4-nitrobenzoate (3h)

Yield (5.01g, 96%), m.p. 210°C, Anal. calcd. for $C_25H_20OClN_5O_6$: C, 57.53; H, 3.86; N, 13.42%. Found: C, 57.31; H, 4.03; N, 13.26%. IR (KBr) υ_{max} 3187 (NH), 1751, 1689 (C=O), 1613 (C=N), 1520 and 1351 (NO_2), 1253 (COO), 778 and 706 (1,2,3-trisubstituted benzenoid ring), 847 (1,4-disubstituted benzenoid ring), 818 and 706 (1,3-disubstituted benzenoid ring) cm^{-1}. ^1H NMR (DMSO-d_6, 200 MHz,): δ 1.19 (t, 3H, OCH_2CH_3; *J*=7.20 Hz), 3.96 (s, 2H, CH_2Ph), 4.10 (q, 2H, ArH, OCH_2CH_3; *J*=6.80 Hz), 7.21 (d, 1H, ArH; *J*=7.20 Hz), 7.28-7.37 (m, 4H, ArH), 7.42 (t, 1H, ArH; *J*=8.00 Hz), 7.55 (d, 1H, ArH; *J*=7.60 Hz), 8.38-8.43 (m, 4H, ArH), 9.90 (s, 1H, N=CH), 11.98 (s, 1H, NH). ^{13}C NMR (50 MHz, DMSO-d_6): δ 14.37 (OCH_2CH_3), 30.40 (CH_2Ph) 64.44 (OCH_2CH_3), [116.48, 118.04, 124.19 (2C), 126.73, 126.92, 127.46, 127.51, 128.84, 130.18, 131.31 (2C), 132.92, 133.38, 137.91, 139.03, 150.43, 150.78] (Ar-C), 145.55 (triazole C_3), 148.20 (N=CH), 151.10 (triazole C_5), 162.49 (COO).

2-Ethoxy-6-[(3-phenyl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl)iminomethyl]phenyl 4-nitro-benzoate (3i)

Yield (4.62g, 98%), m.p. 234°C, Anal. calcd. for $C_24H_19N_5O_6$: C, 60.89; H, 4.05; N, 14.79%. Found: C, 58.97; H, 4.11; N, 14.36%. IR (KBr) υ_{max} 3169 (NH), 1755, 1716 (C=O), 1617 (C=N), 1526 and 1348 (NO_2), 1264 (COO), 775 and 712 (1,2,3-trisubstituted benzenoid ring), 850 (1,4-disubstituted benzenoid ring) cm^{-1}. 1H NMR (200 MHz, DMSO-d_6): δ 1.20 (t, 3H, OCH_2CH_3; *J*=6.80 Hz), 4.10 (q, 2H, OCH_2CH_3 *J*=6.80 Hz), 7.37 (d, 1H, ArH; *J*=8.00 Hz), 7.43 (t, 1H, ArH; *J*=8.00 Hz), 7.48-7.49 (m, 3H, ArH), 7.53 (d, 1H, ArH; *J*=7.60 Hz), 7.81-7.83 (m, 2H, ArH), 8.31 (d, 2H, ArH; *J*=8.80 Hz), 8.38 (d, 2H, ArH; *J*=8.80 Hz), 9.86 (s, 1H, N=CH), 12.36 (s, 1H, NH). ^{13}C NMR (50 MHz, DMSO-d_6): δ 14.36 (OCH_2CH_3), 64.45 (OCH_2CH_3), [116.62, 118.44, 124.02 (2C), 126.40, 126.84, 127.53, 127.87 (2C), 128.40 (2C), 130.03, 131.21 (2C), 133.53, 139.08, 150.52, 150.62] (Ar-C), 144.55 (triazole C_3), 151.26 (N=CH), 151.33 (triazole C_5), 162.51 (COO).

Antioxidant Activity

Chemicals

The newly synthesized compounds 3a-i were analyzed for their in vitro antioxidant activities in three different methods including reducing power, free radical scavenging and metal chelating activity. Butylated hydroxytoluene (BHT) was purchased from E. Merck. Ferrous chloride, α-tocopherol, 1,1-diphenyl-2-picryl-hydrazyl (DPPH.), 3-(2-pyridyl)-5,6-bis(phenylsulfonic acid)-1,2,4-triazine (ferrozine), butylated hydroxyanisole (BHA), ethylenediaminetetraacetic acid (EDTA) and trichloracetic acid (TCA) were bought from Sigma.

Reducing Power

The reducing power of the synthesized compounds was determined according to the method [14]. 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 50oC for 20 min. after which a portion (2.5 mL) of trichloroacetic acid (10%) was added to the mixture, which was then 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 [15]. 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 stand at room temperature for 30 min. Then the absorbance was measured at 517 nm in a spectrophometer. 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 x DPPH. – 0.0174

The capability to scavenge the DPPH radical was calculated using the following equation:

DPPH. scavenging effect (%) = $(A_0 - A_1/A_0) \times 100$

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 [16]. Briefly, the synthesized compounds (30-90 μg/mL) were added to a 2 mM solution of $FeCl_2.4H_2O$ (0.05 mL). The reaction was initiated by the addition of 5 mM ferrozine (0.2 mL), and the mixture was shaken vigorously and left standing 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. The percentage of inhibition of ferrozine-Fe^{2+} complex formation was given by the formula: % Inhibition = $(A_0 - A_1/A_0) \times 100$, 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

Simple susceptibility screening test using agar-well diffusion method [17] as adapted earlier [18] was used for determination of antimicrobial activities of 3a-i 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) 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. 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.

RESULTS AND DISCUSSION

In this study, the structures of nine new 2-ethoxy-6-[(3-alkyl/aryl-4,5-dihydro-1H-1,2,4-triazol-5-one-4-yl)iminomethyl]phenyl 4-nitrobenzoates (3a-i) were identified using elemental analysis, IR, ^{1}H NMR and ^{13}C NMR spectral data.

Antioxidant Activity

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 [19]. The antioxidant activity of putative antioxidant has been attributed to various mechanisms, among which are prevention chain initiation, binding of transition metal ion catalyst, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging [20]. In this study, all of the concentrations of the compounds of series 3a-i showed lower absorbance than standard antioxidants, but reductive activities were not observed.

DPPH. radical scavenging activity

The model of scavenging the stable DPPH radical model is a widely used method to evaluate antioxidant activities in a relatively short time compared with other methods. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability [21]. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [22]. The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm induced by antioxidants. The absorption maximum of a stable DPPH radical in ethanol was at 517 nm. The decrease in absorbance of DPPH radical was caused by antioxidants because of the reaction between antioxidant molecules and radical, progresses, which resulted in the scavenging of the radical by hydrogen donation. It is visually noticeable as a discoloration from purple to yellow. Hence, DPPH. is usually used as a substrate to evaluate antioxidative activity of antioxidants [23]. In the study, antiradical activities of compounds 3a-i and standard antioxidants such as BHA and α-tocopherol were determined by using DPPH. method. The newly synthesized compounds showed no significant effect as a radical scavenger.

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 color reduction therefore allows estimation of the chelating activity of the coexisting chelator [24]. Transition metals have pivotal role in the generation oxygen free radicals in living organism. The ferric iron (Fe^{3+}) is the relatively biologically inactive form of iron. However, it can be reduced to the active Fe^{2+}, depending on condition, particularly pH [25] 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 [26]. Also, the production of highly active ROS such as O_2.-, H_2O_2 and OH. is also catalyzed by free iron though Haber-Weiss reactions:

$$O._2 + H_2O_2 \rightarrow O_2 + OH^- + OH.$$

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:

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

Fe^{3+} ion also produces radicals from peroxides, even though the rate is tenfold less than that of Fe^{2+} ion, which is the most powerful pro-oxidant among the various types of metal ions [27]. 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 [28].

It was reported that the compounds with structures containing two or more of the functional groups, such as -OH, -SH, -COOH, -PO_3H_2, C=O, -NR_2, -S-, -O- in a favourable structure–function configuration should have chelation activity [29-31]. In this respect, L-carnitine may chelate the ferrous ions with hydroxyl and carboxylate groups [31]. In this study; 3 type compounds contain -O- and C=O groups.

Low absorbance at 562 nm indicates high metal chelating activity. The data acquired from Figure 1 disclose that the metal chelating effect of the compound 3a was significant and concentration-dependent. The metal chelating effect of the compound and references decreased in order of EDTA > α-tocopherol > 3a, which were 87.6, 52.1, 35.2 (%), at the highest concentration, respectively.

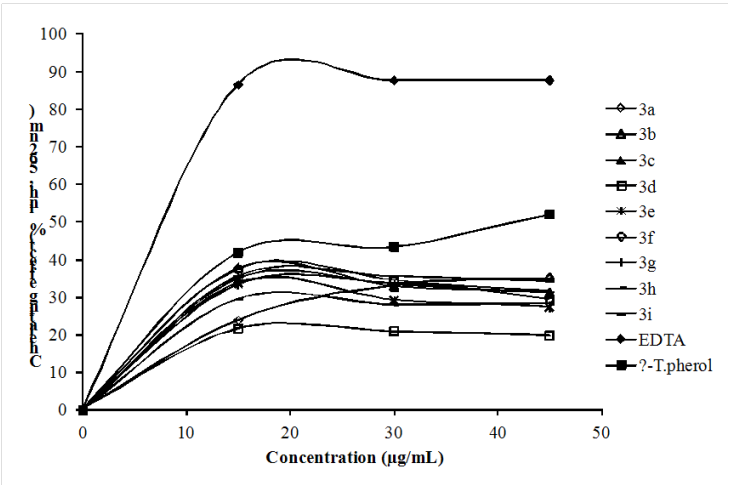


Figure 1. Metal chelating effect of different amount of the compounds 3, EDTA and α-tocopherol on ferrous ions.

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

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

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).

Antimicrobial Activity

The observed data for the antimicrobial activity of 3a-i compounds were given in Table 1. The screening data indicate that all the studied 3 type compounds were found to be highly effective against Escherichia coli (ATCC 25922) strain. Also these compounds showed moderate activity against Staphylococcus aureus (ATCC 6538).

CONCLUSION

In this study, the structures of nine new 4,5-dihydro-1H-1,2,4-triazol-5-one derivatives synthesized from the reactions of 1 type compounds with 3-ethoxy-2-(4-nitrobenzoxy)-benzaldehyde were identified by using elemental analysis, IR, ^{1}H NMR and ^{13}C NMR spectral data, and these obtained spectral values were seen as compatible with literature [10,11]. The newly synthesized compounds were screened for their antimicrobial and antioxidant activities. Considering both the antimicrobial and the antioxidant evaluation, compound 3a exhibited moderate effect.

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