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Research Article

Synthesis, characterization and biological activities of *N*-Mannich bases and amide compounds developed from heterocyclic triazol-5-one derivatives: 3-alkyl(aryl)-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones

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Abstract

Among heterocyclic compounds, 1,2,4-triazoles are valuable chemical structures known for their broad spectrum of biological activities and unique roles in medicinal chemistry applications, especially in drug development processes. In this study, eight 3-alkyl(aryl)-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives were synthesized and reacted with 3-nitrobenzoyl chloride to obtain *N*-[3-alkyl(aryl)-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl]-3-nitrobenzamide derivatives. Subsequently, the amide derivatives underwent Mannich reactions with formaldehyde and morpholine, resulting in four *N*-Mannich bases. The structures of a total of 12 synthesized compounds were elucidated using IR, ^{1}H-NMR, and ^{13}C-NMR spectroscopic techniques. The biological activities of the compounds were evaluated in vitro. Antioxidant properties were assessed using reducing power, free radical scavenging, and metal chelating activity assays. Additionally, antimicrobial activities were tested against five microorganisms (*Bacillus cereus, Klebsiella pneumoniae, Staphylococcus aureus, Serratia marcescens,* and *Candida albicans*) using the agar well diffusion method. The results indicated that the compounds exhibited weak antioxidant activity, while some demonstrated notable antimicrobial effects.

Keywords: 4,5-Dihydro-1*H*-1,2,4-triazol-5-one, Amide, Mannich Base, Antioxidant Activity, Antimicrobial Activity

Heterohalkalı triazol-5-on türevlerinden geliştirilen *N*-Mannich bazı ve amid bileşiklerinin sentezi, karakterizasyonu ve biyolojik aktivitelerinin araştırılması: 3-alkil(aril)-4-amino-4,5-dihidro-1*H*-1,2,4-triazol-5-on

Öz

Heterosiklik bileşiklerden biri olan 1,2,4-triazoller, ilaç geliştirme sürecinde ve tıbbi kimyada sahip oldukları geniş biyolojik aktivite yelpazesi sayesinde önemli bir kimyasal sınıf oluşturmaktadır. Bu çalışmada, 8 adet 3-alkil(aril)-4-amino-4,5-dihidro-1*H*-1,2,4-triazol-5-on türevi bileşik, 3-nitrobenzoil klorür ile reaksiyona sokularak *N*-[3-alkil(aril)-4,5-dihidro-1*H*-1,2,4-triazol-5-on-4-il]-3-nitrobenzamid türevleri elde edilmiştir. Sonrasında, bu amid türevleri, Mannich reaksiyonuna tabi tutularak formaldehit ve morfolin varlığında dört adet *N*-Mannich türevi bileşik sentezlenmiştir. Toplamda 12 farklı bileşiğin yapısal özellikleri IR, ^{1}H-NMR ve ^{13}C-NMR spektroskopik yöntemlerle detaylı şekilde incelenmiştir.

Elde edilen bileşiklerin biyolojik aktiviteleri in vitro yöntemlerle değerlendirilmiştir. Antioksidan aktiviteleri indirgeme gücü ve serbest radikal temizleme kapasiteleri üzerinden ölçülmüş, ayrıca metal şelatlama testleriyle desteklenmiştir. Antimikrobiyal aktiviteler ise *Bacillus cereus, Klebsiella pneumoniae, Staphylococcus aureus, Serratia marcescens ve Candida albicans* gibi mikroorganizmalara karşı agar kuyucuk yöntemi kullanılarak test edilmiştir. Sonuçlar, bileşiklerin antioksidan özelliklerinin zayıf olduğunu, ancak belirli bileşiklerin antimikrobiyal etkiler sergilediğini ortaya koymuştur.

Anahtar Kelimeler: 4,5-Dihidro-1*H*-1,2,4-triazol-5-on, Amid, Mannich Bazı, Antioksidan Aktivite, Antimikrobiyal Aktivite

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1. Introduction

Antimicrobial agents have played a central role in combating infections caused by various microorganisms for many decades. These therapeutic agents, which are specifically designed to prevent and treat bacterial, viral, fungal, and parasitic infections, remain one of the most essential tools in modern medicine [1]. Their significance lies not only in treating acute infections but also in preventing complications in surgeries, managing chronic infections, and safeguarding immunocompromised patients. Despite their indispensable role, the overuse and inappropriate prescription of antibiotics in clinical settings, agriculture, and even over-the-counter products have significantly contributed to the development of antibiotic resistance. The emergence of multidrug-resistant strains of bacteria, fungi, and even parasites has created a major obstacle to the effective management of infectious diseases. This escalating resistance crisis not only diminishes the effectiveness of existing antimicrobial agents but also accelerates the global spread of drug-resistant pathogens, leaving fewer treatment options available [2, 3]. Consequently, this phenomenon has intensified the urgency to discover new antimicrobial compounds that can overcome resistance mechanisms, as well as to explore alternative therapeutic strategies that focus on disrupting microbial pathways in innovative ways [4, 5].

Parallel to the need for novel antimicrobial agents, there has been a growing emphasis on the development of antioxidants, primarily due to their critical role in protecting cellular structures against damage caused by oxidative stress. Oxidative stress occurs when there is an imbalance between reactive oxygen species (ROS) and the body's ability to neutralize them through its antioxidant defense systems. These ROS molecules, which are highly reactive byproducts of metabolic processes, can damage vital cellular components such as DNA, proteins, and lipids. This damage contributes to the onset and progression of various degenerative diseases, including cancer, diabetes, cardiovascular diseases, neurodegenerative disorders like Alzheimer’s, and aging-related conditions [6–8]. By targeting the cascade of oxidative reactions, antioxidant compounds aim to mitigate these detrimental effects, thus providing a therapeutic benefit in both the prevention and treatment of oxidative stress-related diseases. In recent years, researchers have turned their attention to the synthesis of novel antioxidants capable of interrupting these damaging chain reactions at multiple levels, underscoring their importance in both therapeutic and preventive medicine [9, 10].

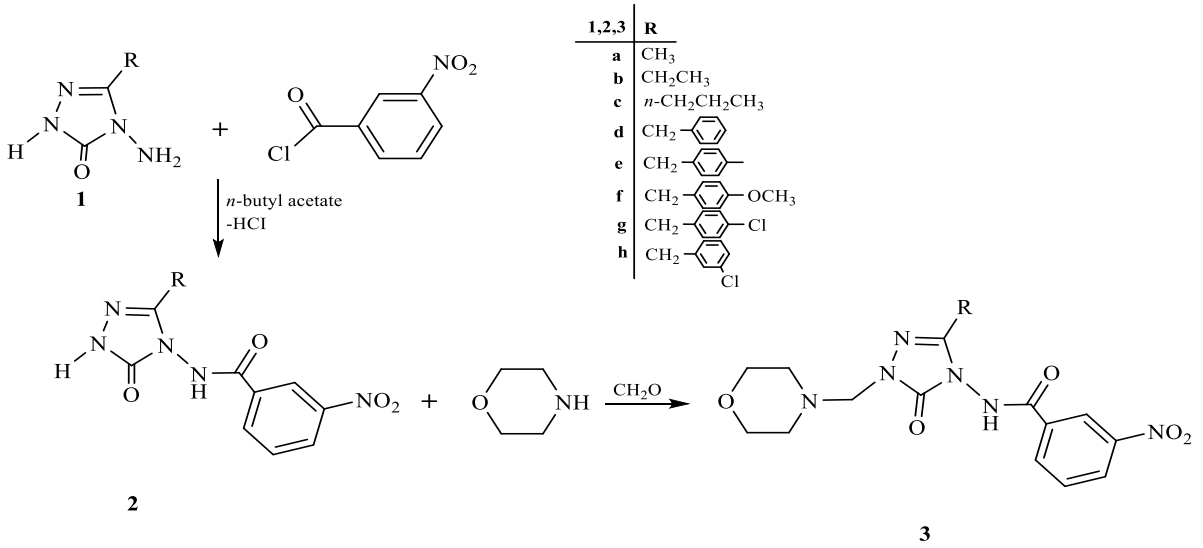
In light of these challenges, heterocyclic compounds, particularly those containing the triazole ring, have emerged as promising candidates in the design of antimicrobial and antioxidant drugs. Triazole derivatives are recognized for their versatile biological properties and have been shown to exhibit potent inhibitory effects against a wide range of bacterial and fungal pathogens [11, 12]. Their pharmacological relevance extends beyond antimicrobial activity; these compounds are also effective in scavenging reactive oxygen species and preventing oxidative damage, making them highly valuable for addressing the dual challenges of microbial resistance and oxidative stress [13, 14]. Structural modifications to triazole derivatives, such as the incorporation of functional groups like amides, have further amplified their biological activities. Amide groups contribute to the stability and efficacy of triazole compounds by facilitating strong and specific interactions with biological targets through the formation of hydrogen

bonds. This enhances their binding affinity and specificity, broadening their therapeutic applications in medicinal chemistry [15].

Among these functionalized derivatives, N-Mannich bases have attracted particular attention due to their chemical stability and dual biological activities. These compounds, synthesized through the Mannich reaction, are known for their ability to exhibit antibacterial and antioxidant properties simultaneously. Their unique structural features not only enhance their efficacy against a variety of microbial strains but also provide significant protection against oxidative stress-induced cellular damage. This dual functionality makes Mannich bases highly appealing for therapeutic applications, particularly in diseases where microbial infections and oxidative damage coexist [16]. Recent studies have highlighted their potential in targeting both infectious diseases and chronic conditions linked to oxidative stress. Consequently, the structural optimization of triazole derivatives and their Mannich bases has become a critical area of focus in the development of innovative drugs aimed at addressing the global health challenges posed by infectious diseases and oxidative stress-related disorders [17].

Among these heterocyclic frameworks, the 4,5-dihydro-1*H*-1,2,4-triazol-5-one ring has attracted considerable attention for its wide-ranging pharmacological and biological activities. Compounds featuring this structural motif are known to exhibit a variety of biological effects, including anticarcinogenic, antimicrobial, antifungal, and antioxidant properties [18–21]. These characteristics position 4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives as promising candidates in drug discovery. Their mechanisms of action and binding properties are both diverse and effective, underscoring their potential in treating a wide array of diseases [22, 23].

This study emphasizes the synthesis and biological evaluation of 1,2,4-triazole derivatives as promising antimicrobial and antioxidant candidates. In particular, two types of *N*-[3-alkyl(aryl)-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl]-3-nitrobenzamide compounds were synthesized through the reaction of 3-nitrobenzoyl chloride with eight different **1**-type compounds. The primary goal of this work is to contribute to the discovery of new therapeutic agents by investigating the pharmacological potential of these heterocyclic structures.



Scheme 1. Synthesis methods of compounds **1–3** (Only **3a**, **3d**, **3e**, and **3g** were obtained)

In the subsequent phase of this study, Mannich reactions were performed on the synthesized compounds **2a, 2d, 2e,** and **2g** using formaldehyde and morpholine. This process led to the formation of four **3**-type compounds: *N*-[1-(morpholine-4-yl-methyl)-3-alkyl(aryl)-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl]-3-nitrobenzamide derivatives (**3a, 3d, 3e, 3g**).

2. Material and Method

The chemicals utilized in this study were sourced from Aldrich, Merck, and Fluka. The solvents used during the experiments were obtained from both local and international suppliers. The melting points of the synthesized compounds were determined using a WRS-2A Microprocessor Melting Point Device. For structural characterization, IR spectra were initially recorded using an ALPHA-P BRUKER FT-IR spectrometer. Additionally, ^{1}H-NMR and ^{13}C-NMR spectra were acquired at the Central Research and Application Laboratory of Mersin University, employing a Bruker 400 MHz NMR device.

To evaluate the antioxidant properties, various methods were employed, including power reduction, free radical scavenging, and metal chelation. These experiments were conducted in the Organic Chemistry Research Laboratory, Department of Chemistry, Faculty of Arts and Sciences, Kafkas University, utilizing a PG Instruments Ltd T80 UV/VIS Spectrometer. Additionally, the antimicrobial activities of the synthesized compounds were thoroughly examined in the Microbiology Research Laboratory of the Faculty of Education at Kafkas University.

2.1. Synthesis and Characterization

In a round-bottom flask, 3-(alkyl/aryl)-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-one (0.01 mol) was dissolved in 20 mL of n-butyl acetate, followed by the addition of 3-nitrobenzoyl chloride (0.01 mol). The reaction mixture was heated under reflux for 5–6 hours using a reflux condenser. After completion, the resulting crude precipitate was isolated through filtration, dried under vacuum over $CaCl_2$ in a desiccator, and subsequently crystallized from ethanol. To enhance purity, the crystals underwent repeated recrystallization in ethanol, and after vacuum drying, they were characterized as type **2** compounds.

In the subsequent phase of the study, compounds **2a, 2d, 2g** (0.01 mol each) were dissolved in 100 mL of ethanol in a round-bottom flask. Morpholine (0.015 mol) and formaldehyde (0.02 mol) were added to the solution, and the mixture was refluxed for 3 hours. Following the reflux, the reaction mixture was allowed to stand overnight at room temperature, facilitating the precipitation of the solid product. The obtained solid was isolated by filtration, further purified through repeated recrystallization in ethanol, and vacuum-dried to yield type **3** compounds (**3a, 3d, 3e, 3g**).

2.1.1. Characterization of compounds 2a, 2b, 2c, 2d, 2e, 2f, 2g and 2h

***N*-(3-methyl-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-4-yl)-3-nitrobenzamide (Compound 2a): IR (KBr, υ, cm-¹)**: 3316 (NH), 1725, 1681 (C=O), 1605 (C=N), 1525 and 1348 (NO_2), 875 and

797 (1,3-disubstituted aromatic ring). **^{1}H NMR (DMSO-d$_6$): δ/ppm** 2.10 (s, CH_3), 7.90 (t, 1H, ArH; *J*=8.00 Hz), 8.38-8.40 (m, 1H, ArH), 8.51-8.54 (m, 1H, ArH), 8.78 (t, 1H, *J*=1.60 Hz, ArH), 11.74 (s, NH), 11.82 (s, NH). **^{13}C-NMR (DMSO-d$_6$): δ/ppm** 164.0 (C=O), 152.5 (Triazole C_5), 144.9 (Triazole C_3), [147.9 (C), 134.1 (CH), 132.2 (C), 130.7 (CH), 127.3 (CH), 122.4 (CH)] (Aromatic C), [10.5 (CH_3)] (Aliphatic C). **Yield: 73.83 %.**

***N*-(3-ethyl-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-4-yl)-3-nitrobenzamide (Compound 2b): IR (KBr, υ, cm^{-1})**: 3200 (NH), 1720, 1687 (C=O), 1590 (C=N), 1522 and 1352 (NO_2), 875 and 786 (1,3-disubstituted aromatic ring). **^{1}H NMR (DMSO-d$_6$): δ/ppm** 1.14 (t, *J* =7.60 Hz, CH_3), 2.43 (q, *J*=7.20 Hz, CH_2), 7.90 (t, 1H, *J*=8.00 Hz, ArH), 8.37-8.39 (m, 1H, ArH), 8.51-8.54 (m, 1H, ArH), 8.77 (t, 1H, *J*=2.00 Hz, ArH), 11.75 (s, NH), 11.80 (s, NH). **^{13}C-NMR (DMSO-d$_6$): δ/ppm** 164.0 (C=O), 152.9 (Triazole C_5), 148.7 (Triazole C_3), [147.9 (C), 134.0 (CH), 132.2 (C), 130.7 (CH), 127.3 (CH), 122.4 (CH)] (Aromatic C), [18.0 ($\underline{CH_2}CH_3$), 9.8 ($CH_2\underline{CH_3}$)] (Aliphatic C). **Yield: 75.54 %.**

***N*-(3-(n-propyl)-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-4-yl)-3-nitrobenzamide (Compound 2c): IR (KBr, υ, cm-^{1})**: 3200 (NH), 1720, 1683 (C=O), 1616, 1588 (C=N), 1517 and 1350 (NO_2), 873 and 773 (1,3-disubstituted aromatic ring. **^{1}H NMR (DMSO-d$_6$): δ/ppm** 0.91 (t, *J*=7.20 Hz, CH_3), 2.38 (t, *J*=7.20 Hz, CH_2), 1.59 (sext, *J*=7.20 Hz, CH_2), 7.90 (t, 1H, *J* =8.00 Hz, ArH), 8.38 (d, 1H, *J*=7.60 Hz, ArH), 8.52 (dd, 1H, *J*= 8.00, 1.60 Hz, ArH), 8.77 (t, 1H, *J*=1.60 Hz, ArH), 11.73 (s, NH), 11.77 (s, NH). **^{13}C-NMR (DMSO-d$_6$): δ/ppm** 164.0 (C=O), 152.6 (Triazole C_5), 147.6 (Triazole C_3), [148.0 (C), 134.1 (CH), 132.2 (C), 130.7 (CH), 127.3 (CH), 122.4 (CH)] (Aromatic C), [26.2 ($\underline{CH_2}CH_2CH_3$), 18.7 ($CH_2\underline{CH_2}CH_3$), 13.3 ($CH_2CH_2\underline{CH_3}$)] (Aliphatic C). **Yield: 77.25 %.**

***N*-(3-benzyl-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-4-yl)-3-nitrobenzamide (Compound 2d): IR (KBr, υ, cm-^{1})**: 3202 (NH), 1727, 1689 (C=O), 1619, 1587 (C=N), 1518 and 1350 (NO_2), 919 and 764 (1,3-disubstituted aromatic ring), 764 and 714 (monosubstituted aromatic ring). **^{1}H NMR (DMSO-d$_6$): δ/ppm** 3.82 (s, CH_2Ph), 7.85-7.89 (m, 5H, ArH), 7.87 (t, 1H, *J*=8.00 Hz, ArH), 8.27 (dt, 1H, *J*=8.00, 1.20 Hz, ArH), 8.50 (dq, 1H, *J*=8.00, 0.80 Hz, ArH), 8.65 (t, 1H, *J*=1.60 Hz, ArH), 11.70 (s, NH), 11.85 (s, NH). **^{13}C-NMR (DMSO-d$_6$): δ/ppm** 164.0 (C=O), 152.6 (Triazole C_5), 146.8 (Triazole C_3), [147.8 (C), 134.0 (CH), 132.4 (C), 130.6 (CH), 127.2 (CH), 122.5 (CH)] (Aromatic C), [134.9 (C), 128.8 (2CH), 128.4 (2CH), 126.8 (CH)] (C-3 Aromatic C), [30.8 (CH_2Ph)] (Aliphatic C). **Yield: 78.82 %.**

***N*-(3-(p-methylbenzyl)-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-4-yl)-3-nitrobenzamide (Compound 2e): IR (KBr, υ, cm-^{1})**: 3135 (NH), 1720, 1681 (C=O), 1615, 1586 (C=N), 1513 and 1349 (NO_2), 875 and 791 (1,3-disubstituted aromatic ring), 815 (1,4-disubstituted aromatic ring). **^{1}H NMR (DMSO-d$_6$): δ/ppm** 2.25 (s, CH_3), 3.77 (s, CH_2Ph), 7.08 (d, 2H, *J*= 8.40 Hz, ArH), 7.11 (d, 2H, *J*= 8.40 Hz, ArH), 7.87 (t, 1H, *J*=8.00 Hz, ArH), 8.28 (dt, 1H, *J*=7.60, 1.20 Hz, ArH), 8.50 (dq, 1H, *J*= 8.40, 1.20 Hz, ArH), 8.64 (t, 1H, *J*=1.60 Hz, ArH), 11.69 (s, NH), 11.83 (s, NH). **^{13}C-NMR (DMSO-d$_6$): δ/ppm** 164.0 (C=O), 152.7 (Triazole C_5), 146.9 (Triazole C_3), [147.8 (C), 134.0 (CH), 132.4 (C), 130.6 (CH), 127.2 (CH), 122.5 (CH)] (Aromatic C), [135.9 (C), 131.7 (C), 129.0 (2CH), 128.7 (2CH)] (C-3 Aromatic C), [30.5 (CH_2Ph), 20.6 ($PhCH_3$)] (Aliphatic C). **Yield: 79.92 %.**

***N*-(3-(p-methoxybenzyl)-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-4-yl)-3-nitrobenzamide (Compound 2f): IR (KBr, υ, cm-¹)**: 3211 (NH), 1727, 1693 (C=O), 1612, 1589 (C=N), 1512 and 1353 (NO_2), 875 and 765 (1,3-disubstituted aromatic ring), 803 (1,4-disubstituted aromatic ring). **¹H NMR (DMSO-d₆): δ/ppm** 3.70 (s, CH_3), 3.76 (s, CH_2Ph), 6.84 (d, 2H, *J*= 8.40 Hz, ArH), 7.14 (d, 2H, *J*=8.40 Hz, ArH), 7.88 (t, 1H, *J*=8.00 Hz, ArH), 8.28-8.30 (m, 1H, ArH), 8.50 (dq, 1H, *J*=8.40, 1.20 Hz, ArH), 8.66 (t, 1H, *J*=1.60 Hz, ArH), 11.69 (s, NH), 11.82 (s, NH). **¹³C-NMR (DMSO-d₆): δ/ppm** 164.0 (C=O), 152.6 (Triazole C_5), 147.1 (Triazole C_3), [147.8 (C), 134.0 (CH), 132.4 (C), 130.6 (CH), 127.2 (CH), 122.5 (CH)] (Aromatic C), [158.2 (C), 129.8 (2CH), 126.6 (C), 113.9 (2CH)] (C-3 Aromatic C), [55.0 (OCH_3), 30.1 (CH_2Ph)] (Aliphatic C). **Yield: 77.19 %.**

***N*-(3-(p-chlorobenzyl)-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-4-yl)-3-nitrobenzamide (Compound 2g): IR (KBr, υ, cm⁻¹)**: 3200 (NH), 1727, 1694 (C=O), 1615, 1594 (C=N), 1513 and 1350 (NO_2), 920 and 797 (1,3-disubstituted aromatic ring), 816 (1,4-disubstituted aromatic ring). **¹H NMR (DMSO-d₆): δ/ppm** 3.85 (s, CH_2Ph), 7.26 (d, 2H, *J*=8.80 Hz, ArH), 7.33 (d, 2H, *J*=8.80 Hz, ArH), 7.88 (t, 1H, *J*=8.00 Hz, ArH), 8.28 (dt, 1H, *J*=8.00, 1.20 Hz, ArH), 8.50 (dq, 1H, *J*=8.40, 1.20 Hz, ArH), 8.66 (t, 1H, *J*=1.60 Hz, ArH), 11.70 (s, NH), 11.89 (s, NH). **¹³C-NMR (DMSO-d₆): δ/ppm** 164.0 (C=O), 152.6 (Triazole C_5), 146.5 (Triazole C_3), [147.8 (C), 134.0 (CH), 132.3 (C), 130.6 (CH), 127.2 (CH), 122.5 (CH)] (Aromatic C), [133.9 (C), 131.6 (C), 130.7 (2CH), 128.4 (2CH)] (C-3 Aromatic C), [30.1 (CH_2Ph)] (Aliphatic C). **Yield: 79.32 %.**

***N*-(3-(m-chlorobenzyl)-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-4-yl)-3-nitrobenzamide (Compound 2h): IR (KBr, υ, cm⁻¹)**: 3240 (NH), 1728 (C=O), 1616, 1589 (C=N), 1516 and 1353 (NO_2), 902 and 785 (1,3-disubstituted aromatic ring). **¹H NMR (DMSO-d₆): δ/ppm** 3.88 (s, CH_2Ph), 7.19-7.22 (m, 1H, ArH), 7.27-7.34 (m, 3H, ArH), 7.88 (t, 1H, *J*=8.00 Hz, ArH), 8.28 (dt, 1H, *J*=8.00, 1.20 Hz, ArH), 8.50 (dq, 1H, *J*=8.40, 1.20 Hz, ArH), 8.66 (t, 1H, *J*=1.60 Hz, ArH), 11.72 (s, NH), 11.90 (s, NH). **¹³C-NMR (DMSO-d₆): δ/ppm** 164.0 (C=O), 152.5 (Triazole C_5), 146.4 (Triazole C_3), [147.8 (C), 134.0 (CH), 133.0 (C), 130.2 (CH), 128.9 (CH), 127.6 (CH), 126.9 (CH)] (Aromatic C), [137.3 (C), 133.0 (C), 130.2 (CH), 128.9 (CH), 127.6 (CH)] (C-3 Aromatic C), [30.3 (CH_2Ph)] (Aliphatic C). **Yield: 79.26 %.**

2.1.2. Characterization of compounds 3a, 3d, 3e and 3g

***N*-[1-(morpholin-4-ylmethyl)-3-methyl-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-4-yl]-3-nitrobenzamide (Compound 3a): IR (KBr, υ, cm-¹):** 770 and 892 (1,3-disubstituted aromatic ring), 1601, 1618 (C=N), 1702, 1681 (C=O), 3198 (NH). **¹H-NMR (DMSO-d₆): δ/ppm** 2.13 (s, CH_3), 2.57 (t, *J*=4.40 Hz, CH_2NCH_2), 3.57 (t, *J*=4.40 Hz, CH_2OCH_2), 4.55 (s, NCH_2N), 7.91 (t, 1H, *J*=8.00 Hz, ArH); 8.39 (dq, 1H, *J*=8.00, 1.20 Hz, ArH); 8.53 (dq, 1H, *J*=8.00, 1.20 Hz, ArH); 8.79 (t, 1H, J=2.00 Hz, ArH), 11.93 (s, NH). **¹³C-NMR (DMSO-d₆): δ/ppm** 164.0 (C=O), 151.8 (Triazole C_5), 143.9 (Triazole C_3), [147.9 (C), 134.1 (CH), 132.1 (C), 130.7 (CH), 127.4 (CH), 122.5 (CH)] (Aromatic C), 66.2 (NCH_2N), 66.0 (CH_2OCH_2), 49.9 (CH_2NCH_2), [10.3 (CH_3)] (Aliphatic C). **Yield: 91.12 %.**

***N*-[1-(morpholin-4-ylmethyl)-3-benzyl-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-4-yl]-3-nitrobenzamide (Compound 3d): IR (KBr, υ, cm-¹):** 3283 (NH), 1712, 1686 (C=O), 1616, 1585 (C=N), 861 and 762 (1,3-disubstituted aromatic ring), 762 and 707 (monosubstituted aromatic ring). **¹H-NMR (DMSO-d₆): δ/ppm** 2.58 (t, *J*=4.40 Hz, CH_2NCH_2), 3.57 (t, *J*=4.40 Hz, CH_2OCH_2), 3.88 (s, CH_2Ph), 4.58 (s, NCH_2N), 7.23-7.31 (m, 5H, ArH); 7.88 (t, 1H, *J*=8.00 Hz, ArH); 8.27 (dt, 1H, *J*=8.00, 1.20 Hz, ArH); 8.50 (dq, 1H, *J*=8.40, 1.20 Hz, ArH); 8.66 (t, 1H, *J*=1.60 Hz, ArH), 11.82 (s, NH). **¹³C-NMR (DMSO-d₆): δ/ppm** 164.0 (C=O), 151.9 (Triazole C_5), 145.8 (Triazole C_3), [147.8 (C), 134.1 (CH), 132.2 (C), 130.6 (CH), 127.3 (CH), 122.5 (CH)] (Aromatic C), [134.7 (C), 128.9 (2CH), 128.5 (2CH), 126.9 (CH)] (C-3 Aromatic C), 66.3 (NCH_2N), 66.1 (CH_2OCH_2), 49.9 (CH_2NCH_2), [30.6 (CH_2Ph)] (Aliphatic C). **Yield: 92.16 %.**

***N*-[1-(morpholin-4-ylmethyl)-3-(p-methylbenzyl)-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-4-yl]-3-nitrobenzamide (Compound 3e): IR (KBr, υ, cm-¹): IR (KBr, υ, cm-¹):** 3158 (NH), 1708, 1685 (C=O), 1616, 1583 (C=N), 927 and 765 (1,3-disubstituted aromatic ring), 816 (1,4-disubstituted aromatic ring). **¹H-NMR (DMSO-d₆): δ/ppm** 2.25 (s, CH_3), 2.59 (t, *J*=4.40 Hz, CH_2NCH_2), 3.58 (t, *J*=4.40 Hz, CH_2OCH_2), 3.82 (s, CH_2Ph), 4.58 (s, NCH_2N), 7.09 (d, 2H, *J*=8.40 Hz, ArH); 7.12 (d, 2H, *J*=8.40 Hz, ArH); 7.88 (t, 1H, *J*=8.00 Hz, ArH); 8.29 (dt, 1H, *J*=8.00, 1.20 Hz, ArH); 8.50 (dq, 1H, *J*=8.40, 1.20 Hz, ArH); 8.65 (t, 1H, *J*=2.00 Hz, ArH), 11.83 (s, NH). **¹³C-NMR (DMSO-d₆): δ/ppm** 164.0 (C=O), 152.0 (Triazole C_5), 145.9 (Triazole C_3), [147.8 (C), 134.0 (CH), 132.3 (C), 130.6 (CH), 127.2 (CH), 122.5 (CH)] (Aromatic C), [136.0 (C), 131.6 (C), 129.1 (2CH), 128.6 (2CH)] (C-3 Aromatic C), 66.2 (NCH_2N), 66.1 (CH_2OCH_2), 49.9 (CH_2NCH_2), [30.5 (CH_2Ph), 20.6 ($PhCH_3$)] (Aliphatic C). **Yield: 90.18 %.**

***N*-[1-(morpholin-4-ylmethyl)-3-(p-chlorobenzyl)-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-4-yl]-3-nitrobenzamide (Compound 3g): IR (KBr, υ, cm-¹):** 3154 (NH), 1714, 1684 (C=O), 1618, 1594 (C=N), 895 and 775 (1,3-disubstituted aromatic ring), 816 (1,4-disubstituted aromatic ring). **¹H-NMR (DMSO-d₆): δ/ppm** 2.59 (t, *J*=4.40 Hz, CH_2NCH_2), 3.58 (t, *J*=4.40 Hz, CH_2OCH_2), 3.91 (s, CH_2Ph), 4.59 (s, NCH_2N), 7.27 (d, 2H, *J*=8.40 Hz, ArH); 7.36 (d, 2H, *J*=8.40 Hz, ArH); 7.88 (t, 1H, J=8.00 Hz, ArH); 8.29 (dt, 1H, *J*=8.00, 1.20 Hz, ArH); 8.51 (dq, 1H, *J*=8.40, 1.20 Hz, ArH); 8.66 (t, 1H, *J*=1.60 Hz, ArH), 11.81 (s, NH). **¹³C-NMR (DMSO-d₆): δ/ppm** 164.0 (C=O), 151.9 (Triazole C_5), 145.5 (Triazole C_3), [147.8 (C), 134.0 (CH), 132.2 (C), 130.6 (CH), 127.3 (CH), 122.5 (CH)] (Aromatic C), [133.7 (C), 131.7 (C), 130.7 (2CH), 128.4 (2CH)] (C-3 Aromatic C), 66.1 (NCH_2N), 66.1 (CH_2OCH_2), 50.0 (CH_2NCH_2), [29.0 (CH_2Ph)] (Aliphatic C). **Yield: 88.91 %.**

2.2. Biological Properties

The antioxidant capacities of the synthesized type **2** and type **3** compounds were comprehensively examined using well-established and widely recognized methods referenced in earlier studies [24, 25]. These evaluations aimed to determine the compounds' ability to mitigate oxidative stress through multiple mechanisms, such as reducing power, free radical scavenging activity, and metal chelation. To assess the reducing power, the Oyaizu protocol was employed [26]. This method measures the electron-donating ability of the compounds,

which is a key indicator of their potential to neutralize reactive oxygen species by reducing oxidized intermediates. The reducing power test provides insight into how effectively these compounds can contribute to antioxidant defense mechanisms.

The free radical scavenging activity, another important parameter in antioxidant evaluation, was determined using Blois' method [27]. This assay focuses on the compounds' capacity to stabilize free radicals, thereby preventing oxidative damage to cellular components. Free radical scavenging is particularly critical in mitigating the harmful effects of reactive oxygen species on lipids, proteins, and DNA. In addition, the metal chelating potential of the synthesized compounds was analyzed following the procedure described by Dinis et al. [28]. This method evaluates the ability of the compounds to bind and neutralize transition metal ions, such as Fe^{2+} and Cu^{2+}, which can catalyze the generation of free radicals through Fenton or Haber-Weiss reactions. By chelating these metal ions, the compounds can effectively interrupt oxidative chain reactions, thereby reducing cellular damage.

Together, these assays provide a comprehensive assessment of the antioxidant properties of the synthesized type **2** and type **3** compounds, highlighting their potential mechanisms of action in combating oxidative stress. The results obtained contribute valuable insights into the compounds' efficacy as antioxidants while also paving the way for further structural optimization to enhance their biological activity.

The antimicrobial activity of the synthesized compounds was evaluated using the Agar Well Diffusion Method, a widely utilized and reliable technique for assessing the antibacterial and antifungal properties of chemical agents [29]. This method involves creating wells in an agar medium inoculated with the target microorganisms, followed by introducing the test compounds into these wells. The compounds then diffuse into the surrounding agar, forming a concentration gradient that allows for the inhibition of microbial growth.

Zones of inhibition around the wells were measured to quantify the antimicrobial efficacy of the synthesized compounds. This straightforward yet effective approach provides a visual and quantitative assessment of the ability of the compounds to inhibit the growth of bacterial and fungal strains. The size of the inhibition zones correlates directly with the antimicrobial potency of the test agents, offering a comparative framework against standard antibiotics and antifungal drugs [30].

By employing this method, both Gram-positive and Gram-negative bacterial strains, as well as fungal strains, were tested to determine the spectrum of activity exhibited by the synthesized compounds. The results obtained through the Agar Well Diffusion Method contribute valuable insights into the potential of these compounds as novel antimicrobial agents, particularly in addressing infections caused by drug-resistant microorganisms. Furthermore, the method's sensitivity and reproducibility ensure that the observed antimicrobial effects are reliable and can serve as a basis for further pharmacological investigations.

For the antimicrobial evaluation, bacterial strains were provided by the Microbiological Environmental Protection Laboratory located in France. The experiments included two Gram-

positive bacteria (*Bacillus cereus* ATCC11778 and *Staphylococcus aureus* ATCC6538), two Gram-negative bacteria (*Klebsiella pneumoniae* ATCC4352 and *Serratia marcescens* ATCC13880), and a fungal strain (*Candida albicans* ATCC10231).

As a reference, Ampicillin, Streptomycin, and Neomycin were selected as standards for antibacterial activity, while Fluconazole was used as the benchmark for antifungal efficacy. These comparisons facilitated the assessment of the synthesized compounds' potential as antimicrobial agents.

3. Results and Discussion

The antioxidant properties of the synthesized compounds were thoroughly investigated, focusing on their reducing power, free radical scavenging ability, and metal chelating activity. These analyses were carried out across three different concentrations to evaluate the dose-dependent behavior of the compounds. For comparison, standard antioxidants, including butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and α-tocopherol were employed as reference substances to establish a baseline for antioxidant efficacy.

Reducing power, which indicates the electron-donating ability of a compound and its potential to neutralize oxidative agents, was measured at a wavelength of 700 nm. The assay results provided insights into the capacity of the synthesized compounds to act as electron donors in reducing reactions. Similarly, the free radical scavenging activity, a critical measure of a compound's ability to stabilize free radicals and prevent oxidative damage, was assessed using a UV-visible spectrophotometer at 517 nm. This test was essential in understanding how the compounds interact with and neutralize reactive oxygen species.

In addition, metal chelating activity, which evaluates the ability of the compounds to bind and deactivate transition metal ions that catalyze oxidative processes, was determined at 562 nm. The use of a UV-visible spectrophotometer ensured precise and reproducible measurements across all antioxidant assays. The results from these analyses provided a comprehensive evaluation of the antioxidant potential of the synthesized compounds and highlighted their performance in comparison to standard antioxidants.

3.1. Investigation of Antioxidant Properties

Measurements of reducing power at 700 nm showed that the absorbance values of the synthesized compounds of types **2** and **3** were close to the control but considerably lower compared to standard antioxidants. This revealed that these compounds did not have a significant reducing property (Figure 1).

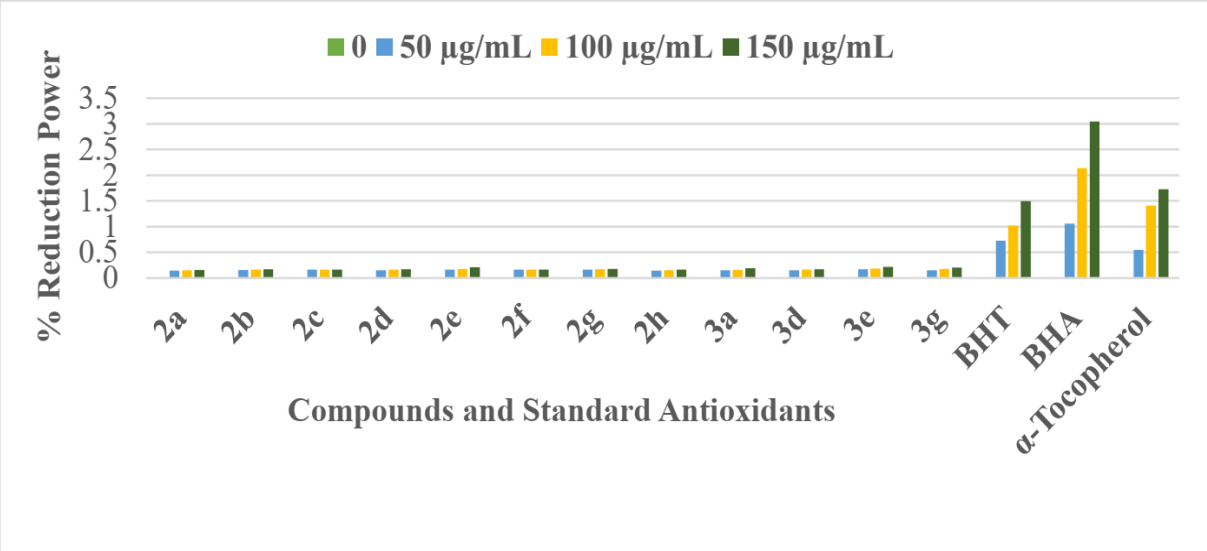


Figure 1. % Reducing power of type **2** and **3** compounds

The findings from the free radical scavenging activity tests for the synthesized compounds **2** and **3** are summarized in Figure 2 and Figure 3. These figures depict the ability of the compounds to neutralize free radicals at a wavelength of 517 nm, measured across varying concentrations. The results are expressed in terms of percentage inhibition, which indicates the extent to which the compounds were able to reduce the activity of free radicals.

Despite being evaluated under multiple conditions, the synthesized compounds demonstrated limited efficacy in scavenging free radicals. The observed values did not reach levels considered significant when compared to those of standard antioxidants, such as BHA, BHT, or α-tocopherol, which are known for their strong radical scavenging properties. This suggests that compounds **2** and **3** may lack the structural features required to interact effectively with and neutralize free radicals.

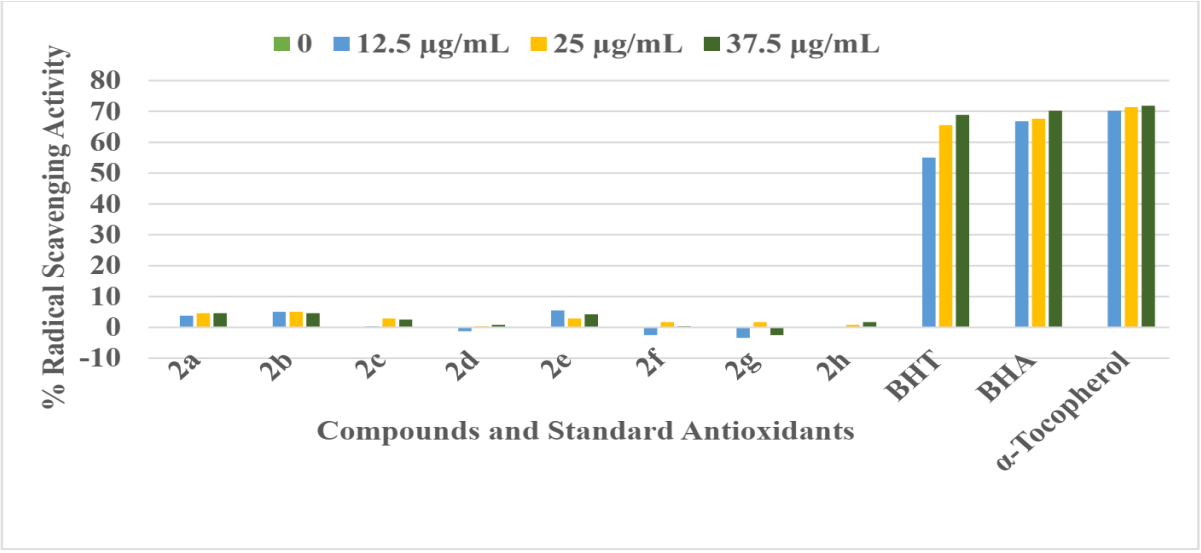


Figure 2. % Radical scavenging activites of type **2** compounds

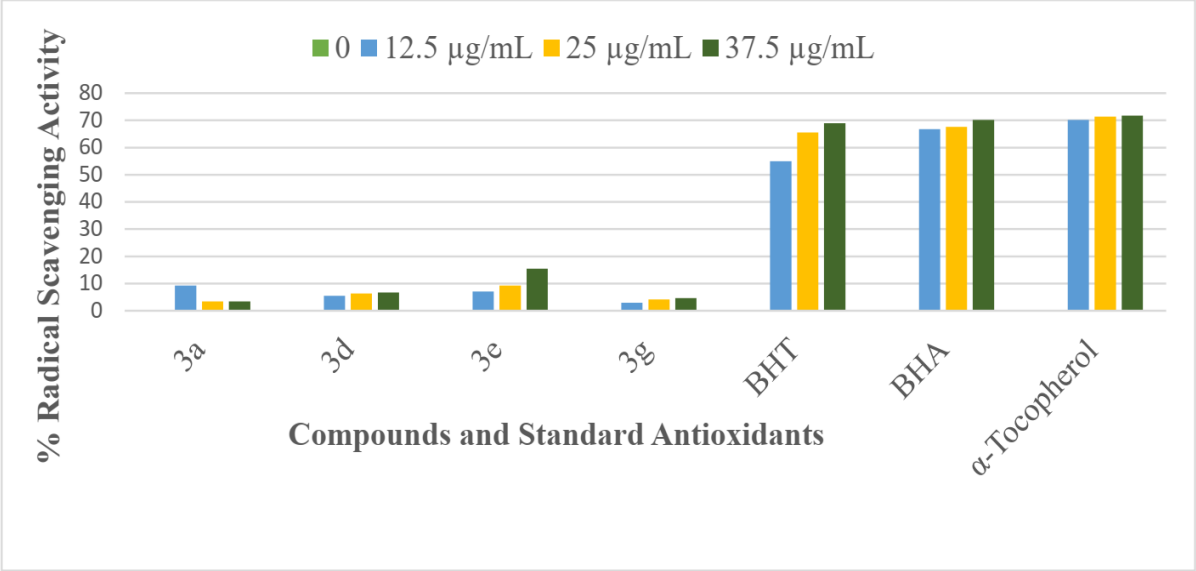


Figure 3. % Radical scavenging activities of type **3** compounds

The lack of notable activity also implies that the antioxidant potential of these compounds is not directly linked to free radical scavenging. Instead, their antioxidant capacity might be associated with alternative mechanisms, such as metal chelate activities, which warrants further investigation. Overall, these results highlight the need for structural modifications to enhance the radical scavenging abilities of these compounds.

The metal chelating potential of the synthesized compounds was assessed across three distinct concentrations to evaluate their effectiveness in binding and neutralizing transition metal ions. These ions are known to catalyze oxidative reactions, and their chelation is critical in mitigating oxidative stress. Absorbance measurements were conducted at 562 nm using a UV-visible spectrophotometer, ensuring precise and reliable quantification of the compounds' chelating activities.

The results, presented in Figure 4 and Figure 5, illustrate the percentage of metal ions chelated by the synthesized compounds at varying concentrations. Compounds belonging to both type **2** and type **3** displayed significant metal chelation activity, demonstrating their ability to interact strongly with metal ions. Among the tested compounds, those of type **3** showed particularly pronounced chelating properties, due to the structural features conferred by the Mannich reaction products.

These findings suggest that the synthesized compounds, especially type **3**, could contribute indirectly to antioxidant defense systems by inhibiting metal ion-catalyzed oxidative damage. The strong chelating properties observed underline their potential utility in applications where the regulation of metal ions is critical, such as in the prevention of oxidative stress-related disorders.

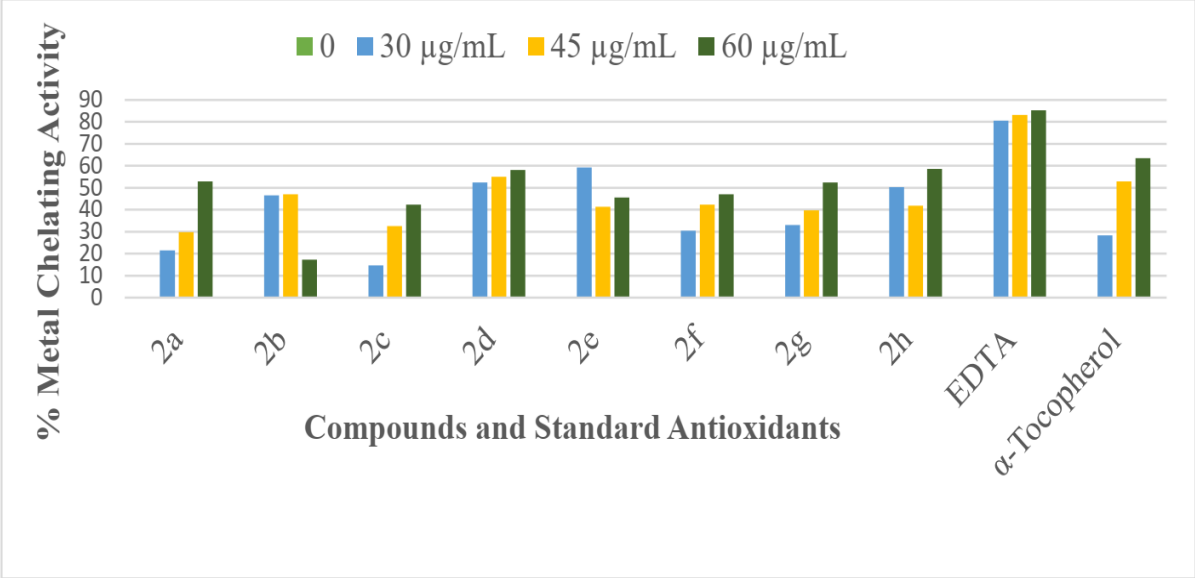


Figure 4. % Metal chelate activities of type **2** compounds

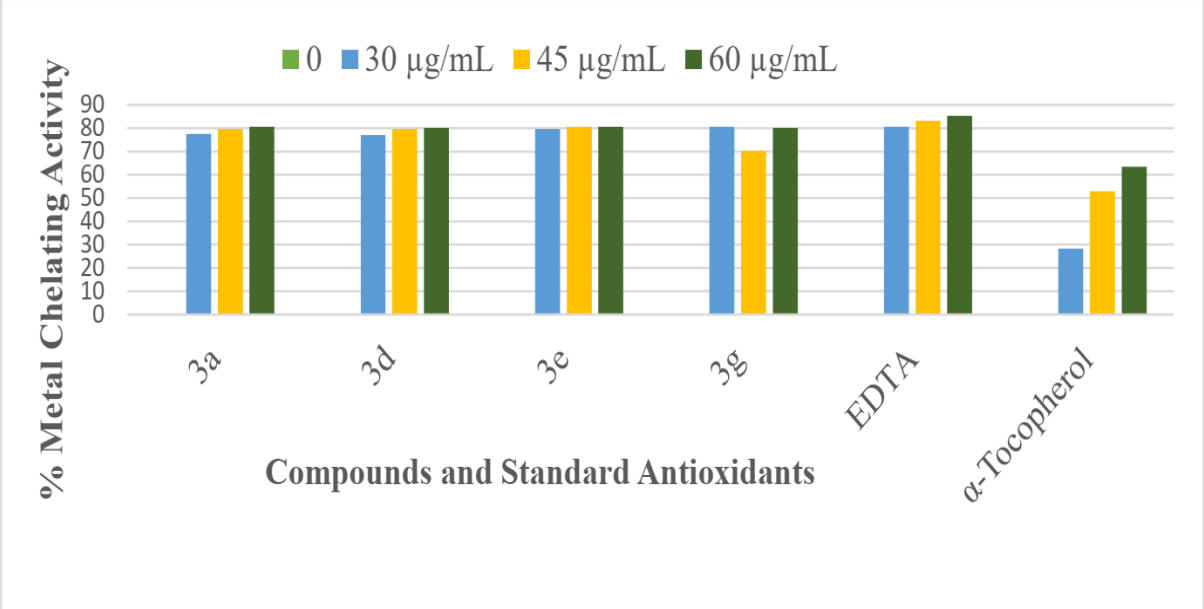


Figure 5. % Metal chelate activities of type **3** compounds

When comparing the chelating activities of type **2** compounds and type **3** compounds, it becomes evident that the Mannich base derivatives (type **3**) exhibit superior chelating capabilities. This enhanced activity is likely due to the structural modifications introduced during the Mannich reaction, which improve the compounds' ability to interact with metal ions.

Type **2** compounds, such as **2a, 2c, 2d, 2f,** and **2g**, demonstrated chelating activities that were remarkably close to that of α-tocopherol, a well-known antioxidant. Meanwhile, the type **3** compounds (**3a–3e**) exhibited chelating activities comparable to EDTA, a highly effective and widely used metal chelator. This suggests that the incorporation of the Mannich base structure

significantly enhances the compounds' affinity for metal ions, further validating their potential as efficient chelating agents.

These observations underscore the value of the structural modifications introduced in the synthesis of type **3** compounds, highlighting their strong potential in mitigating oxidative damage mediated by metal ion-catalyzed reactions. Such results pave the way for exploring these compounds in antioxidant-related therapeutic applications.

Table 1. Antimicrobial activity results of type **2** and **3** compounds

Compound No	Microorganisms and zone diameter values* (mm)				
	Gram positive		Gram negative		Mushroom
	BC **ATCC11778**	***SA*** **ATCC6538**	***KP*** **ATCC4352**	***SM*** **ATCC13880**	***CA*** **ATCC10231**
2a	12 (++)	10 (+)	7 (+)	10 (+)	(-)
2b	**22 (+++)**	8 (+)	10 (+)	12 (++)	9 (+)
2c	15 (++)	(-)	(-)	12 (++)	(-)
2d	**17 (+++)**	(-)	9 (+)	10 (+)	(-)
2e	14 (++)	(-)	(-)	7 (+)	9 (+)
2f	13 (++)	(-)	(-)	10 (+)	9 (+)
2g	14 (++)	(-)	10 (+)	7 (+)	9 (+)
2h	(-)	(-)	(-)	(-)	(-)
3a	**26 (+++)**	13 (++)	**17 (+++)**	**22 (+++)**	15 (++)
3d	**22 (+++)**	13 (++)	13 (++)	**21 (+++)**	13 (++)
3e	**24 (+++)**	13 (++)	14 (++)	**17 (+++)**	14 (++)
3g	**22 (+++)**	13 (++)	13 (++)	**21 (+++)**	16 (++)
A (3261)	**36 (+++)**	**37 (+++)**	**35 (+++)**	15 (++)	(-)
N (3360)	**17 (+++)**	13 (++)	16 (++)	13 (++)	(-)
S (3385)	12 (++)	**21 (+++)**	11 (++)	12 (++)	(-)
F (FCA-25)	(-)	(-)	(-)	(-)	**25 (+++)**

* In the evaluation according to the inhibition diameter: <5.5 mm negative effect (-); 5.5-10 mm low effect (+); 11-16 mm moderate effect (++); ≥17 mm high effect (+++) [31].

** Abbreviations: *Bacillus Cereus (BC); Staphylacoccus Aureus (SA); Klebsiella Pneumoniae (KP); Serratia Mascercens (SM); Candida Albicans (CA)*; Ampicillin (A); Neomycin (N); Steptomycin (S); Fluconazole (F)

In Table 1, where Ampicillin (3261), Steptomycin (3385), Neomycin (3360) antibiotics and Fluconazole (FCA-25) antifungal drug were used as standard, the type **2** and **3** compounds synthesized within the scope of the study were tested with five different microorganisms. In this table, the zone diameters of the antimicrobial activities are presented.

The results of the effect evaluations demonstrated that compounds 2a-2g exhibited antibacterial activity against Gram-positive *B. cereus*. This effect was moderate for compounds **2a, 2c, 2e-2g** and high for compounds **2b** and **2d**. No activity against *B. cereus* was observed for compound **2h**. It is an important result that compounds **2a-2g** showed antibacterial activity close

to Neomycin and Streptomycin. Against *S. aureus*, another Gram-positive bacterium, low activity was observed in compounds **2a** and **2b**, while no activity was observed in compounds **2c-2h.**

When Gram-negative bacteria are taken into consideration, low effect was observed for *K. pneumonia* for compounds **2a, 2b, 2d** and **2g** while no effect value was detected for compounds **2c, 2e, 2f** and **2h**. However, the data obtained for Gram-negative *S. mascercens* are more promising. Low effect was observed for compounds **2a, 2d-2g** while moderate effect was obtained for compounds **2b** and **2c**. For compound **2h**, the effect value could not be obtained as in other strains. It was determined that Gram-negative bacteria had a close effect value especially with Neomycin and Streptomycin standard antibiotics.

The antifungal effects of the synthesized compounds **2a-2h** were also studied on *C. albicans*. Low effect was obtained in compounds **2b, 2e-2g,** and no antifungal effect was found in other compounds. These values obtained are not at a level to compete with standard antifungal drugs.

The new compounds considered as the second group are derivatives of 1,2,4-triazol-5-one compounds containing the morpholine group. It was observed that the antibacterial effect increased significantly with the inclusion of the morpholine group in the structure. All of the synthesized compounds **3a, 3d-3g** showed a high level of activity against *B. cereus*. The fact that this effect was higher than Neomycin and Streptomycin is a very valuable result. For Gram-positive *S. aureus*, a moderate level of activity was found. This value is close to the standard antibiotic Neomycin.

Increased antibacterial effects of morpholine derivatives were also observed for gram-negative *K. pneumonia* and *S. mascercens*. Especially the effect values obtained for *S. mascercens* were high. In *K. pneumoniae*, compound **3a** gave a high effect value, while compounds **3d-3g** gave a moderate effect. These values are competitive with the standard antibiotics Neomycin and Streptomycin.

The antifungal effects of the synthesized morpholine derivatives are also significant. Although the obtained moderate antifungal effect cannot compete with the standard antifungal drug, the result represents a significant effect.

4. Conclusion

The biological evaluation of the synthesized type **2** and type **3** compounds revealed several significant findings. Both compound types exhibited limited reducing power and free radical scavenging activities, indicating relatively modest direct antioxidant effects. However, the strong metal chelating properties observed, particularly in type **3** compounds, suggest potential indirect antioxidant mechanisms through the neutralization of toxic metal ions and the mitigation of oxidative stress. This highlights their capacity to contribute to antioxidant defense systems via metal ion regulation.

Among their biological activities, type **3** compounds demonstrated remarkable antibacterial effects, showing activity against both Gram-positive and Gram-negative bacteria. These effects

were comparable to standard antibiotics such as Neomycin and Streptomycin, underscoring the role of the morpholine group in enhancing antibacterial potential. This positions type **3** compounds as promising candidates for the development of novel antibiotics. Additionally, the moderate antifungal activity observed against *Candida albicans* indicates that while these compounds show promise, further structural modifications could enhance their antifungal efficacy, as they currently remain less potent than standard antifungal agents.

In conclusion, the synthesized type **3** compounds stand out for their strong antibacterial activity and excellent metal chelating properties, offering a solid foundation for further clinical development and optimization. These findings not only underscore the therapeutic potential of triazole-based agents but also pave the way for innovative approaches in the development of antimicrobial and antioxidant drugs.

Ethics in Publishing

The authors report no ethical conflicts.

Author Contributions

Drafting the manuscript, data collection: Songül Ulufer Bulut; Designing the study and evaluating the results: Haydar Yüksek

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