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Synthesis, Antimicrobial and Antioxidant Activities of 3-Alkyl(aryl)-4-(3-methoxy-4-hydroxybenzylideneamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one Derivatives

Abstract
8 Pieces 3-alkyl(aryl)-4-(3-methoxy-4-hydroxybenzylideneamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (3) compounds have been obtained from reacting with 3-methoxy-4-hydroxybenzaldehyde (2) of 3-Alkyl(aryl)-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-one (1) compounds. Synthesized compounds have been obtained 8 pieces 1-(morpholin-4-yl-methyl)-3-alkyl(aryl)-4-(3-methoxy-4-hydroxybenzylideneamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (4) compounds from reaciton with morpholine and 7 pieces 1-(4-piperidinecarboxamide-1-yl-methyl)-3-alkyl(aryl)-4-(3-methoxy-4-hydroxybenzylideneamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (5) compounds from reaciton with 4-piperidinecarboxamide ın formaldehyde environment, based on the Mannich reaction. IR, ^{13}C NMR and ^{1}H NMR spectroscopy methods have been utilized to analyze the structures of obtained 15 molecules. Additionally, the *in-vitro* antioxidant properties the obtained potentially biologically active Mannich bases have been examined using, metal chelate activity, reducing power methods and free radical scavenging activity, and the outcomes obtained have been contrasted with the standard antioxidant compounds used. The final section of the study examined the invitro antimicrobial effects of the synthesized molecules in opposition to six distinct microorganisms (*Bacillus cereus, Bacillius Subtilis, Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli, and Staphylococcus aureus*) utilizing the Agar Well Technique. Those findings were assessed.

Keywords Antimicrobial activity · 4,5-dihydro-1*H*-1,2,4-triazol-5-one · Schiff Base · Antioxidant activity · Mannich base

Introduction

There is a lot of research on antioxidants' capacity to defend cells and organisms from harm caused by oxidative stresses. Numerous investigations on different types of antioxidants both man-made and natural have been carried out. Including the 1,2,4-triazole ring, many heterocyclic molecules have been stated to have a range of biological characteristics, like antiviral, antibacterial, anti-inflammatory, and antioxidant properties. Very reactive free radicals, notably those come from oxygen, may be created by the food system, internal bodily metabolic processes, or external agents [1]. These free radicals can harm tissue and kill cells, which oxidize biomolecules. In human diseases, oxidative damage has a significant pathogenic role. Emphysema, cancer, cirrhosis, arthritis, and atherosclerosis have all been related to oxidative damage. Reactive oxygen species (ROS) are also produced too much by many stimuli and exceed the body's antioxidant capability, leading to an array of pathophysiological steps like diabetes, cancer, genotoxicity, and inflammation [2]. Across the globe, antibiotic resistance is acknowledged as one of the main issues pertaining to public health. It requires significant work to find, create, and develop new antibiotics due to the quick rise and spread of microorganisms resistant to antibiotics [3].

The synthesis and design of heterocycles can be crucial in this setting, being the significance of heterocyclic molecules in medicinal chemistry [4]. Heterocyclic compounds with three nitrogen atoms are called triazoles [5]. Estazolam, alprazolam, triazolam (hypnotic), trazodone (anxiolytic, antidepressant), terconazole (antifungal), trapidil (hypotensive), etizolam (amnesic, anticonvulsant, anxiolytic), hexaconazole (antifungal), rilmazafon (anxiolytic, hypnotic), and rizatriptan (antimigratory agent) are some of the contemporary medications that contain the triazole ring [6-13].

1,2,4-Triazole's biological activity and its derivatives have been shown to have a wide spectrum [14]. The typical Mannich reaction, a three-part condensation involving different structural substrates with a minimum of one actor hydrogen atom, an amine reagent, and an aldehyde component, provides a group of compounds that are called Mannich bases [15]. Mannich bases find use in the medicine industry as well as other sectors like the food, beverage, cosmetics, and color industries [16]. The ability of the Mannich reaction to unite two distinct molecules into a single structure is its primary benefit [17]. It has been demonstrated that mannich bases generated from 1,2,4-triazole derivatiands possess biological characteristics like antifungal, antioxidant, antilipase, and antibacterial qualities [18-22].

We looked at how to create 1,2,4-triazoles with morpholine and 4-piperidinecarboxamide rings in order to create new heterogroups by including potentially biologic active structures. We next assessed the antibacterial and antioxidant properties of these compounds.

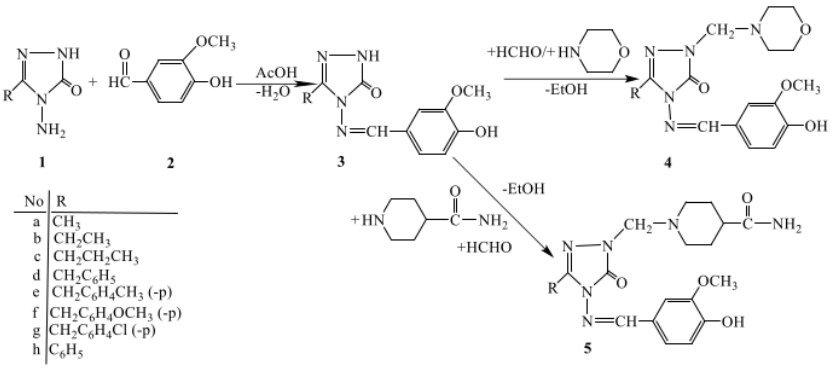
Method and Materıals

Chemical reagents and apparatus

The brands of all chemicals used in the study are Merck, Fluka, and Aldrich, and they were supplied from distributors in Turkey. The melting points of all obtained structures have been analyzed by the Stuart SMP30 brand determination dev28. FT-IR spectra utilized to illuminate the structure of the study have been obtained with a BRUKER brand ALPHA-P model FT-IR spectrometer instrument. ^{13}C NMR and ^{1}H NMR spectra were taken at Mersin University on a Bruker brand Ultrasheld Plus Bıospın model 400 MHz NMR device. Using the PG Devices Ltd T80 UV/VIS Spectrometer, three distinct approaches have been utilized to assess the obtained compounds' antioxidant qualities. Antioxidant activity graphs were obtained using the Microsoft Excel 97-2003 program. The synthesis scheme of the synthesized compounds was obtained using the ChemDraw 22 program.

Synthesis

In this study, 8 pieces 3-type, 8 pieces 4-type and 7 pieces 5-type compounds were obtained. In this context, 8 pieces 3-type compounds have been obtained by reacting with 3-methoxy-4-hydroxybenzaldehyde of 1-type compounds. From the synthesised 3-type compounds' reaction with morpholine in accordance with the Mannich process, 8 pieces 4-type compounds have been obtained (Equation 1). From the synthesised 3-type compounds' reaction with 4-piperidinecarboxamide in accordance with the Mannich process in the continuation of our work, we synthesized 7 pieces 5-type compounds (cf. Scheme 1).



No	R
a	CH_3
b	CH_2CH_3
c	$CH_2CH_2CH_3$
d	$CH_2C_6H_5$
e	$CH_2C_6H_4CH_3$ (-p)
f	$CH_2C_6H_4OCH_3$ (-p)
g	$CH_2C_6H_4Cl$ (-p)
h	C_6H_5

Scheme 1 4 and 5-Type compounds' synthesis

General procedure for synthesis 1-(morpholin-4-yl-methyl)-3-alkyl(aryl)-4-(3-methoxy-4-hydroxybenzylideneamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (4)

In a glass balloon, 0.01 mol 3-type compounds were dissolved in 0.1 L ethanol. Final solution was left to cool in the deep freezer at -18°C on12ight by refluxing for 3-4 hours by adding 0.015 mol morpholine and 37% 0.02 mol formaldehyde solution. The crude product that precipitated was filtered, cleaned with cold alcohol, and put in a desiccator to dry. The obtained crude substance was crystallized with ethanol a few times. These crystals were then dried in vacuum and identified a15type compounds. The physical information of the obtained 4-type compounds is shown in Table 1, IR values in Table 2, ^{1}H NMR values in Table 3, and ^{13}C NMR values in Table 4.

Table 1 4-Type compounds' physical data

Compounds	Yield %	Melting Point °C
4a	91	174
4b	62	164
4c	97	101
4d	89	117
4e	78	133
4f	75	149
4g	67	164
4h	64	149

Table 2 4-Type compounds' FTIR data

Compounds	ν_{OH}	$\nu_{C=O}$	$\nu_{C=N}$	$\nu_{1,2,4}$ trisubstituted benzene ring	$\nu_{1,4}$-disubstituted benzene ring	$\nu_{monosubstitute}$ benzene ring
4a	3185	1687	1595	868 and 779	-	-
4b	3179	1701	1579	890 and 831	-	-
4c	2959	1702	1581	901 and 776	-	-
4d	3030	1702	1588	890 and 808	-	774 and 699
4e	2961	1705	1589	890 and 818	817	-
4f	2952	1702	1587	881 and 813	816	-
4g	2956	1697	1588	858 and 801	801	-
4h	3049	1689	1591	906 and 808	-	761 and 686

Table 3 4-Type compounds' ^{1}H NMR values

No	CH_3	CH_2	CH_2NCH_2	CH_2OCH_2	OCH_3	NCH_2N	Aromatic H	N=CH	OH
4a	2.29(s)	-	2.57 (t; *J*=4.40 Hz)	3.54 (t; *J*=4.40 Hz)	3.84(s)	4.52(s)	6.90 (d, 1H; *J*=8.00 Hz), 7.27 (dd, 1H; *J*=8.00 Hz), 7.41 (d, 1H; *J*=1.60 Hz)	9.50(s)	9.79(s)
4b	1.25 (t; *J*=7.60 Hz)	2.71(q; *J*=7.20 Hz)	2.47 (t; *J*=4.40 Hz)	3.55 (t; *J*=4.40 Hz)	3.84(s)	4.53(s)	6.89 (d, 1H; *J*=8.40 Hz), 7.27 (dd, 1H; *J*=8.00), 7.41 (d, 1H; *J*=2.00 Hz)	9.49(s)	9.80(s)
4c	0.97 (t; *J*=7.60 Hz)	2.65 (t; *J*=7.20 Hz) 1.71 (sext; *J*=7.60 Hz)	2.49 (t; *J*=4.40 Hz)	3.55 (t; *J*=4.40 Hz)	3.84(s)	4.53(s)	6.90 (d, 1H; *J*=8.00 Hz), 7.27 (dd, 1H; *J*=8.00 Hz), 7.40 (d, 1H; *J*=1.60 Hz)	9.48(s)	9.78(s)
4d	-	4.08(s)	2.60 (t; *J*=4.40 Hz)	3.55 (t; *J*=4.40 Hz)	3.84(s)	4.53(s)	6.8[illegible], 1H; *J*=8.40 Hz), 7.17-7.23 (m, 1H), 7.28-7.36 (m, 6H)	9.47(s)	9.80(s)
4e	2.24(s)	4.02(s)	2.48 (t; *J*=4.40 Hz)	3.57 (t; *J*=4.40 Hz)	3.84(s)	4.52(s)	6.87 (d, 1H; *J*=8.00 Hz), 7.12 (d, 2H; *J*=8.00 Hz), 7.19 (dd, 1H; *J*=8.40 Hz), 7.22 (d, 2H; *J*=8.00 Hz), 7.35 (d, 1H; *J*=2.00 Hz)	9.46(s)	9.80(s)
4f	-	4.00(s)	2.59 (t; *J*=4.40 Hz)	3.57 (t; *J*=4.40 Hz)	3.85(s) 3.71(s)	4.53(s)	6.87 (d, 2H; *J*=8.80 Hz), 6.88 (d, 1H; *J*=8.00 Hz), (dd, 1H; *J*=8.00Hz), 7.26 (d, 2H; *J*=8.80 Hz), 7.36 (d, 1H; *J*=1.60 Hz)	9.47(s)	9.80(s)
4g	-	4.10(s)	2.49 (t; *J*=4.40 Hz)	3.55 (t; *J*=4.40 Hz)	3.84(s)	4.52(s)	-6.89 (m, 1H), 7.15-7.16 (m, 1H), 7.25-7.26 (1H), 7.35-7.40 (m, 4H)	9.50(s)	9.70(s)
4h	-	-	2.66 (t; *J*=4.40 Hz)	3.55 (t; *J*=4.40 Hz)	3.81(s)	4.69(s)	6.91 (d, 1H; *J*=8.40 Hz), 7.28 (dd, 1H; *J*=8.40 Hz), 7.40 (d, 1H; *J*=1.60 Hz), 7.53-7.55 (m, 3H), 7.92-7.95 (m, 2H)	9.42(s)	9.70(s)

Table 4 4-Type compounds' ^{13}C NMR values

No	CH_3	CH_2	CH_2NCH_2	OCH_3	NCH_2N	CH_2OCH_2	Aromatic C	Aromatic C (C-3'e Bağlı)	Triazole C3	Triazole C5	N=CH
4a	11.11	-	49.98	55.63	65.87	66.03	110.37 (CH), 115.65 (CH), 122.53 (CH), 124.57 (C), 148.05 (C), 150.35 (C)	-	142.99	150.37	155.38

4b	9.97	18.58	50.01	55.54	65.92	66.04	110.37 (CH), 115.69 (CH), 122.66 (CH), 124.33 (C), 148.06 (C), 150.37(C)	-	146.76	150.52	155.42
4c	13.28	18.90 26.58	50.00	55.60	66.05	66.12	110.42 (CH), 115.71 (CH), 122.62 (CH), 124.61 (C), 148.05 (C), 150.37 (C)	-	145.55	150.45	155.36
4d	-	31.11	50.02	55.68	66.11	66.17	109.59 (CH), 115.54 (CH), 122.90 (CH), 124.59 (C), 148.09 (C), 150.37 (C)	126.72 (CH), 128.38 (2CH), 128.67 (2CH), 135.79 (C)	144.85	150.40	154.57
4e	20.56	30.71	50.02	55.54	65.98	66.04	109.61(CH), 115.54 (CH), 123.13 (CH), 124.61 (C), 148.09 (C), 150.34 (C)	128.56 (2CH), 129.33 (2CH), 132.65 (C), 135.32 (C)	145.00	150.40	154.53
4f	-	30.25	50.02	55.70 55.00	65.98	66.05	109.67 (CH), 115.57 (CH), 123.10 (CH), 124.62 (C), 148.10 (C), 150.36 (C)	113.91 (2CH), 127.72 (C), 129.74 (2CH), 158.11 (C)	145.15	150.41	154.61
4g	-	30.51	50.00	55.71	66.05	66.11	108.69 (CH), 115.57 (CH), 123.17 (CH), 124.54 (C), 147.34 (C), 149.55 (C)	128.41 (2CH), 130.7 (2CH), 131.45 (C), 134.78 (C)	144.50	150.39	154.76
4h	-	-	50.00	55.54	66.07	66.41	110.36 (CH), 115.73 (CH), 123.07 (CH), 124.43 (C), 148.11 (C), 150.60 (C)	126.33 (C), 128.02 (2CH), 128.51 (2CH), 130.25 (CH)	143.08	150.62	158.05

General procedure for synthesis 1-(4-piperidinecarboxamid-1-yl-methyl)-3-alkyl(aryl)-4-(3-methoxy-4-hydroxy-benzylidene-amino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (5)

In a glass balloon, 0.01 mol of 3-type compounds were dissolved in 0.1 L ethanol. Final solution was left to cool in the deep freezer at -18°C one night by refluxing for 3-4 hours by adding 0.015 mol 4-piperidinecarboxamide and 37% 0.02 mol formaldehyde solution. The crude product that precipitated was filtered, cleaned with cold alcohol, and put in a desiccator to dry. The obtained crude substance was crystallized with ethanol a few times. These crystals were then dried in vacuum and identified as 5-type compounds. The physical information of the synthesized 5-type compounds is shown in Table 5, IR values in Table 6, ^{1}H NMR values in Table 7 and ^{13}C NMR values in Table 8.

Table 5 5-Type compounds' physical data

Compounds	Yield %	Melting Point °C
5a	69	174
5b	79	164
5c	85	101
5d	59	117
5e	71	133
5f	79	149
5g	67	164
5h	64	149

Table 6 5-Type compounds' FT-IR data

Compounds	ν_{NH2}	ν_{OH}	$\nu_{C=O}$	$\nu_{C=N}$	$\nu_{1,2,4}$ trisubstituted benzene ring	$\nu_{1,4}$-disubstituted benzene ring	ν monosubstitute benzene ring
5a	3351 and 3186	3186	1706, 1653	1597	867 ve 812	-	-
5b	3351 and 3187	2937	1697, 1654	1588	862 ve 816	-	-
5c	3348 and 3176	3075	1702, 1643	1592	857 ve 812	-	-
5d	3355 and 3187	3064	1702, 1648	1589	922 ve 812	-	756 and 698
5e	3363 and 3185	3004	1706, 1646	1588	901 ve 813	815	-
5f	3416 and 3294	3175	1707, 1678	1583	881 ve 814	811	-
5g	3358 and 3183	3004	1706, 1643	1585	901 ve 824	811	-

Table 7 5-Type compounds' ^{1}H NMR values

No	CH_3	CH_2	OCH_3	Piperidine Ring H	NCH_2N	NH	Aromatic H	N=CH	OH
5a	2.29 (s)	-	3.83(s)	1.44-1.56 (m, 2H, CH_2), 1.66-1.67 (m, 2H, CH_2), 1.93-1.97 (m, 1H, CH), 2.23-2.26 (m, 2H, CH_2), 2.89 (d, 2H; *J*=11.60 Hz)	4.52 (s)	6.71 (s, 1H) 7.15 (s, 1H)	6.78 (d, 1H; *J*=8.40 Hz), 7.26 (dd, 1H; *J*=8.40 Hz), 7.41 (d, 1H; *J*=1.60 Hz)	9.50 (s)	9.66(s)
5b	1.24 (t, *J*=7.20 Hz)	2.70 (q, *J*=7.20 Hz)	3.84(s)	1.64 (m, 2H, CH_2), 1.90 (m, 2H, CH_2), 1.88-2.03 (m, 1H, CH), 2.17 (m, 2H, CH_2), 2.89 (d, 2H; CH_2; *J*=11.60 Hz)	4.53 (s)	6.71 (s, 1H) 7.17 (s, 1H)	6.90 (d, 1H; *J*=8.40 Hz), 7.27 (dd, 1H; *J*=8.40 Hz), 7.40 (d, 1H; *J*=1.60 Hz)	9.50(s)	9.80(s)
5c	2.96 (t, *J*=7.20 Hz)	2.66 (t, *J*=7.20 Hz) 1.65-1.71 (sext)	3.83(s)	1.48-1.52 (m, 2H, CH_2), 1.64-1.73 (m, 2H, CH_2), 1.98-2.02 (m, CH), 2.23-2.26 (m, 2H, CH_2), 2.89 (d, 2H, CH_2; *J*=11.60 Hz)	4.54 (s)	6.89 (s,1H) 7.16 (s,1H)	6.89 (d, 1H; *J*=8.00 Hz), 7.27 (dd, 1H; *J*=8.00, Hz), 7.40 (d, 1H; *J*=1.60 Hz)	9.49 (s)	9.77 (s)
5d	-	4.07(s)	3.83(s)	1.48-1.53 (m, 2H, CH_2), 1.64-1.69 (m, 2H, CH_2), 1.69 (d, 2H, CH_2; *J*=10.40 Hz), 1.97-2.01 (m, 1H, CH), 2.24-2.32 (m, 2H, CH_2), 2.89 (d, 2H, CH_2; *J*=11.60 Hz),	4.56 (s)	6.89 (s,1H) 7.15 (s,1H)	6.86 (d, 1H; *J*=8.00 Hz), 7.18-7.32 (m, 7H),	9.46 (s)	9.80 (s)
5e	2.26(s)	4.01(s)	3.84(s)	1.48-1.55 (m, 2H, CH_2), 1.64-1.69 (m, 2H, CH_2), 1.67 (d, 2H, CH_2; *J*=10.40 Hz) 1.96-2.01 (m, 1H, CH), 2.24-2.29 (m, 2H, CH_2), 2.89 (t, 2H; *J*=11.60 Hz)	4.56 (s)	6.70 (s,1H) 7.16 (s,1H)	6.86 (d, 1H; *J*=8.00 Hz), 7.19 (dd, 1H; *J*=8.00 Hz), 7.34 (d, 1H; *J*=7.60 Hz), 7.18-7.22 (m, 3H), 7.34 (d, 1H; *J*=2.00 Hz)	9.47 (s)	9.80 (s)
5f	-	4.08(s)	3.84(s) 3.70(s)	1.49-1.53 (m, 2H, CH_2), 1.64-1.69 (m, 2H, CH_2), 1.69 (d, 2H, CH_2; *J*=10.40 Hz), 1.96-2.01 (m, 1H, CH), 2.23-2.29 (m, 2H, CH_2), 2.91 (d, 2H, CH_2; *J*=11.60 Hz)	4.56 (s)	6.70 (s,1H) 7.17 (s,1H)	6.86 (d, 1H; *J*=8.00 Hz), 6.87 (d, 2H; *J*=8.00 Hz), 7.19-7.26 (m, 3H), 7.35 (d, 1H; *J*=2.00 Hz)	9.46 (s)	9.80 (s)
5g	-	4.08(s)	3.82(s)	1.49-1.55 (m, 2H, CH_2), 1.67 (d, 2H, CH_2; *J*=10.40 Hz), 1.96-2.02 (m, 1H, CH), 2.19-2.30 (m, 2H, CH_2), 2.89 (d, 2H, CH_2; *J*=11.60 Hz)	4.54 (s)	6.73 (s,1H) 7.17 (s,1H)	6.86 (d, 1H; *J*=8.00 Hz), 7.19 (dd, 1H; *J*=8.40, Hz), 7.33 (d, 1H; *J*=2.00 Hz), 7.33-7.40 (m, 4H)	9.46(s)	9.80 (s)

Table 8 5-Type compounds' ^{13}C NMR values

No	CH_3	CH_2	Piperidine Ring C	OCH_3	NCH_2N	Aromatik C	Aromatic C (C-3'e bonded)	Triazole C3	Triazole C5	N=CH	CONH
5a	11.01	-	28.42 ($2CH_2$), 41.11 (CH), 49.57 ($2CH_2$)	55.66	66.29	110.38 (CH), 115.68 (CH), 123.74 (CH), 124.60 (C), 148.06 (C) 150.36 (C)	-	142.81	150.38	155.35	176.42
5b	10.01	18.43	28.41 ($2CH_2$), 41.51 (CH), 49.75 (2CH)	55.62	66.31	110.38 (CH), 115.69 (CH), 122.64 (CH), 124.56 (C), 148.31 (C) 150.44 (C)	-	146.56	150.50	155.38	176.44
5c	13.40	26.66 18.93	28.43 ($2CH_2$), 41.12 (CH), 49.75 ($2CH_2$)	55.62	66.24	110.47 (CH), 115.71 (CH), 122.54 (CH), 124.63 (C), 148.04 (C) 150.33 (C)	-	145.37	150.43	155.28	176.39
5d	-	31.12	28.43 ($2CH_2$), 41.12 (CH), 49.77 ($2CH_2$)	55.58	66.41	109.63 (CH), 115.55 (CH), 123.12 (CH), 124.62 (C), 148.08 (C) 150.34 (C)	125.69 (CH), 127.49 (2CH), 127.69 (2CH), 134.79 (C)	144.67	150.40	154.48	176.38
5e	20.57	30.89	28.42 ($2CH_2$), 41.12 (CH), 49.76 ($2CH_2$)	55.54	66.37	109.62 (CH), 115.54 (CH), 122.91 (CH), 124.63 (C), 148.08 (C) 150.32 (C)	127.53 (2CH), 129.04 (2CH), 132.70 (C), 135.70 (C)	144.80	150.32	154.60	176.38
5f	-	30.42	28.42 ($2CH_2$), 41.12 (CH), 49.77 ($2CH_2$)	55.58 55.02	66.37	109.69 (CH), 115.56 (CH), 122.87 (CH), 124.64 (C), 148.09 (C) 150.33 (C)	113.92 (2CH), 127.73 (C), 129.72 (2CH), 158.10 (C)	144.96	150.40	154.48	176.39
5g	-	-	28.41 ($2CH_2$), 41.07 (CH), 49.73 ($2CH_2$)	55.57	66.42	109.67 (CH), 115.56 (CH), 123.12 (CH), 124.54 (C), 148.08 (C) 150.37 (C)	128.43 (2CH), 130.64 (2CH), 131.43 (C), 134.85 (C)	144.72	150.37	154.62	176.37

Antioxidant activity

Ferric chloride, α-tocopherol, 3-(2-pyridyl)-5,6-bis(phenylsulfonic acid)-1,2,4-triazine (ferrozine), butylated hydroxyanisole (BHA), trichloroacetic acid (TCA), and 1,1-diphenyl-2-picryl-hydrazyl (DPPH) for Antioxidant Activity have been used.

Reducing power

The Oyaizu method was utilized to find the reduction power of the obtained compounds [23]. A compound's ability to reduce can serve as a crucial predictor of its possible antioxidant action. Reductants, such as antioxidants, induce the Fe^{3+}/ferricyanide complex to be reduced to the iron form in the samples. Consequently, the obtaining of Perl's Prussian blue at 700 nm may be utilized to measure Fe^{2+} [24, 25]. Numerous processes, including the restriction of chain initiation, the binding of transition metal ion catalyst, the dissociation of peroxides, the prevention of continuous hydrogen extraction, reducing capacity, and radical scavenging, have been linked to the putative antioxidant's antioxidant activity [26]. The absorbance values obtained at 700 nm in the UV spectrophotometer have been shown in Table 9 and 10.

Table 9 4-Type compounds' reducing power

Compounds	Reducing Power (μg/mL, 700 nm)		
	50	100	150
4a	0.119	0.122	0.120
4b	0.125	0.128	0.126
4c	0.080	0.084	0.073
4d	0.053	0.057	0.092
4e	0.042	0.045	0.083
4f	0.102	0.116	0.110
4g	0.004	0.061	0.056
4h	0.102	0.103	0.103
BHT	0.301	0.454	0.616
BHA	0.791	1.189	1.300
α-Tocopherol	0.420	0.841	1.209
Absorbance of control reaction: **0.037**			

Table 10 5-Type compounds' reducing power

Compounds	Reducing Power (μg/mL, 700 nm)		
	50	100	150
5a	0.126	0.126	0.126
5b	0.098	0.121	0.120
5c	0.114	0.114	0.098
5d	0.089	0.113	0.110
5e	0.065	0.093	0.110
5f	0.092	0.091	0.098
5g	0.045	0.045	0.042
BHT	0.301	0.454	0.616
BHA	0.791	1.189	1.300
α-Tocopherol	0.420	0.841	1.209
Absorbance of control reaction: **0.037**			

Free radical Scavenging activity

The Blois method has been utilized so as to identify the compounds' free radical scavenging activity by employing DPPH [27]. When compared to other approaches, stable DPPH radical scavenging is a frequently utilized technique to assess quickly antioxidant activity [28, 29]. It has been previously thought that antioxidants' capacity to donate hydrogen was the reason behind their impact on DPPH radical scavenging [30, 31]. To transform from a stable free radical into a consistent diamagnetic compound, DPPH can take on an electron or a hydrogen radical [32]. The reduction in DPPH radical absorbance at 517 nm that antioxidants cause is a measure

of the radicals' reducing ability. DPPH is frequently employed as a substrate to assess antioxidants' levels of antioxidant activity [33, 34]. Equation 1 is utilized in the computation of the reaction medium's DPPH radical scavenging activity. % Free radical scavenging activities have been shown in Table 11 and Table 12.

$$\% \text{ Free radical scavenging activity} = \frac{A_0 - A_1}{A_0} x100 \quad (1)$$

A_0: Control reaction absorbance, **A_1:** Absorbance of the standard or sample

Table 11 4-Type compounds' absorbance measurements and associated percentage of free radical scavenging activities

Compounds	Absorbance and %Free Radical scavenging Activity (μg/mL, %inh, 517 nm)					
	12.5		25		37.5	
	A	% Activity	A	% Activity	A	% Activity
4b	0.115	17.9	0.108	22.9	0.095	32.1
4c	0.130	7.1	0.124	11.4	0.116	17.1
4d	0.118	15.7	0.109	22.1	0.099	29.3
4e	0.113	19.3	0.104	25.7	0.100	28.6
4f	0.105	25.0	0.102	27.1	0.088	37.1
4g	0.114	18.6	0.100	28.6	0.090	35.7
4h	0.115	17.9	0.103	26.4	0.095	32.1
BHT	0.115	17.9	0.102	27.1	0.087	37.9
BHA	0.076	45.7	0.051	63.6	0.032	77.1
α-Tocopherol	0.021	85.0	0.017	87.9	0.015	89.3
	0.015	89.3	0.015	89.3	0.014	90.0
Absorbance of control reaction: **0.140**						

Table 12 5-Type compounds' absorbance measurements and associated percentage of free radical scavenging activities

Compounds	Absorbance and %Free Radical scavenging Activity (μg/mL, %inh, 517 nm)					
	12.5		25		37.5	
	A	% Activity	A	% Activity	A	% Activity
5a	0.117	16.4	0.104	25.7	0.104	25.7
5b	0.110	21.4	0.094	32.9	0.086	38.6
5c	0.113	19.3	0.102	27.1	0.093	33.6
5d	0.110	21.4	0.103	26.4	0.096	31.4
5e	0.114	18.6	0.101	27.9	0.091	35.0
5f	0.113	19.3	0.102	27.1	0.086	38.6
5g	0.115	17.9	0.095	32.1	0.084	40.0
BHT	0.076	45.7	0.051	63.6	0.032	77.1
BHA	0.021	85.0	0.017	87.9	0.015	89.3
α-Tocopherol	0.015	89.3	0.015	89.3	0.014	90.0
Absorbance of control reaction: **0.140**						

Metal chelate activity

The Dinis method has been utilized to determine metal chelate activity [35]. It was identified how the compounds and standards chelated iron ions. Ferrozine and Fe^{2+} can combine to obtain quantiand complexes. The complex loses its red when chelating chemicals are present because they interfere with the formation of the complex [36]. As a result, evaluation of the coexisting chelator's chelating activity is possible through measurement of color decrease [37]. The comparable passive type of iron in the body is called ferric iron (Fe^{3+}) [38]. However, it might be activated by Fe^{2+} and oxidized again via Fenton-type interactions with the generation of the hydroxyl radical or Haber-Weiss interactions with superoxide anions, depending on the circumstances, particularly pH 25. [39]. Chelating substances have the ability to inhibit processes that rely on metals and fail to activate metal ions [40]. Every test and analysis was run three times and checked. Equation 2 provides the ferrozine-Fe2+ complexs' the inhibition percentage. The corresponding of the obtained compounds' % Metal Chelate Activities have been shown in Table 13 and Table 14.

$$\text{Chelate Percentage} = \frac{A_0 - A_1}{A_0} x100 \quad (2)$$

Table 13 4-Type compounds' absorbance measurements and associated percentage of metal chelate activities

Compounds	Absorbance and % Metal Chelate Activity (μg/mL, %inh, 562 nm)					
	12.5		25		37.5	
	A	% Activity	A	% Activity	A	% Activity
4a	0.037	81.1	0.036	81.6	0.035	82.1
4b	0.035	82.1	0.035	82.1	0.032	83.7
4c	0.036	81.6	0.035	82.1	0.034	82.7
4d	0.037	81.1	0.034	82.7	0.033	83.2
4e	0.037	81.1	0.035	82.1	0.034	82.7
4f	0.034	82.7	0.034	82.7	0.033	83.2
4g	0.035	82.1	0.033	83.2	0.032	83.7
4h	0.036	81.6	0.032	83.7	0.031	84.2
α-Tocopherol	0.030	84.7	0.029	85.2	0.029	85.2
EDTA	0.100	49.0	0.097	50.5	0.089	54.6
Absorbance of control reaction: **0.196**						

Tablo 14 5-Type compounds' absorbance measurements and associated percentage of metal chelate activities

Compounds	Absorbance and % Metal Chelate Activity (μg/mL, %inh, 562 nm)					
	12.5		25		37.5	
	A	% Activity	A	% Activity	A	% Activity
5a	0.038	80.6	0.035	82.1	0.034	82.7
5b	0.036	81.6	0.036	81.6	0.034	82.7
5c	0.035	82.1	0.034	82.7	0.033	83.2
5d	0.036	81.6	0.034	82.7	0.032	83.7
5e	0.037	81.1	0.036	81.6	0.035	82.1
5f	0.036	81.6	0.035	82.1	0.035	82.1
5g	0.037	81.1	0.036	81.6	0.032	83.7
α-Tocopherol	0.030	84.7	0.029	85.2	0.029	85.2
EDTA	0.100	49.0	0.097	50.5	0.089	54.6
Absorbance of control reaction: **0.196**						

Antimicrobial activity

The antibacterial characteristics of 4 and 5-type substances identified in our research have been determined using the agar well diffusion procedure [41, 42]. The agar plug diffusion technique is widely utilized to illustrate the hostility between bacteria, much like the disk diffusion method. It entails growing the strain of interest as an agar culture on culture medium that has fine lines across the plate's surface. Because they grow, microbial organisms release compounds which multiply in the agar environment. Once incubated, an agar cylinder or plot is cut with cork borer that is clean and placed on the agar plate of a different disk that has been infected with the experiment bacteria. The inhibition region development surrounding the agar plug is the next step in determining the antibacterial activity of the chemicals released by the microorganisms [43]. Antimicrobial tests of the synthesized compounds were carried out against 6 different bacteria, and the obtained data has been shown in Table 15.

Table 15 Towards bacteria, synthesized 4 and 5-type compounds' ZONE diameter measurements

Compound Code	ZONE diameter values (mm)					
	Bacillus Cereus	*Bacillius Subtilis*	*Pseudomonas aeruginosa*	*Esherichia Coli*	*Staphlacocus aureus*	*Klebsiella pneumoniae*
4a	32	16	11	13	17	14
4b	26	15	12	13	18	15
4c	30	13	12	15	19	16
4d	14	12	8	11	14	12
4e	30	8	-	11	11	12
4f	23	-	8	12	11	11
4g	22	11	8	9	-	8

4h	24	17	8	8	15	12
5a	26	8	9	11	19	14
5b	29	18	9	14	16	15
5c	30	14	9	14	17	15
5d	29	-	-	16	15	16
5e	24	-	-	-	13	13
5f	24	-	-	8	8	12
5g	26	-	-	8	10	10
Ampicillin	35	32	34	33	35	36
Streptomycin	13	13	13	11	22	12
Neomycin	16	16	16	15	13	15

Results and Discussion

Spectral study results

The characteristic peaks appearing in the IR spectra of 15 compounds synthesized in the study were evaluated, used to elucidate the structure of the compounds, and the peak values have been presented in Tables 2 and 6. The IR spectrum exhibited a band between 1581-1597 cm^{-1} because of the azomethine ν(C=N) group and a band between 1643-1707 cm^{-1} for carbonyl ν(C=O). While broad bands are seen in the spectrum of the Schif base ligand in the range of 2952-3186 cm^{-1} due to the stretching vibration of hycroxyl ν(OH), two bands are seen in the range of 3176-3358 cm^{-1} in 4-piperidinecarboxamide.

When we examined the ^{1}H NMR spectra of the compounds synthesized in the study, we observed proton chemical shifts in the range of 4.49-4.69 ppm in NCH_2N in the Mannich group, in the range of 2.47-2.67 ppm in CH_2NCH_2 and in the range of 3.56-3.56 ppm in CH_2OCH_2. Proton chemical shifts in the piperidine ring NH_2 were observed in the range of 6.69-7.17 ppm. We observed proton chemical shifts in our compound N=CH around 9.50 ppm and OH around 9.80 ppm.

When we examined synthesized the compounds' the ^{13}C NMR spectra in the study, carbon chemical shifts were observed in the Mannich group around 50.00 ppm in CH_2NCH_2, in the range of 65.87-66.11 ppm in NCH_2N, and in the range of 66.05-66.41 ppm in CH_2OCH_2. In our compound, we observed carbon chemical shifts in the range of N=CH 154.57-158.05 ppm, OCH_3 in the range of 55.54-55.71 ppm, and $CONH_2$ in the piperidine ring around 176.39 ppm.

Antioxidant activity results

Reducing power

Tables 9 and 10 show the data gathered from the obtained compounds' the reducing power tests. Since almost all of the synthesized compounds were lower than the absorbance of the control in the measurements made at 700 nm, it is possible to state that the compounds lack reducing abilities. However, some of the compounds had significantly higher reducing power compared to others, and the graphs for these compounds have been given in Fig. 1.

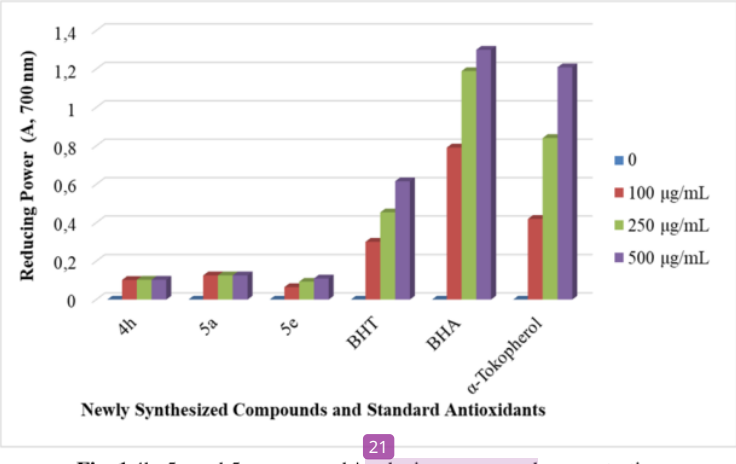


Fig. 1 4h, 5a and 5e compounds' reducing power and concentration

Free Radical Scavenging Activity

The scavenging tests of free radicals of obtained compounds are given in Tables 11 and 12. The free radicals scavenging tests of obtained compounds are displayed as % inhibition in obtained measurements obtained in different amounts at 517 nm in the graphs in Figs. 2 and 3. Upon examining the graphs, one can observe a important rise in the scavenging activities of radicals along with concentrations of each component group.

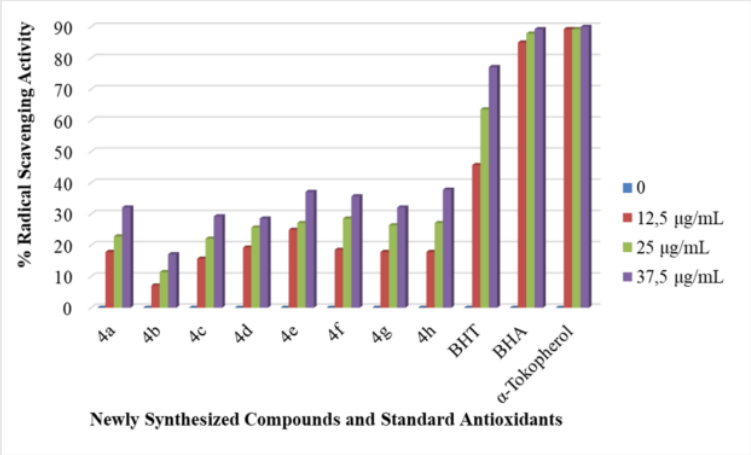


Fig. 2 4-Type compounds' the scavenging activities of % free radicals

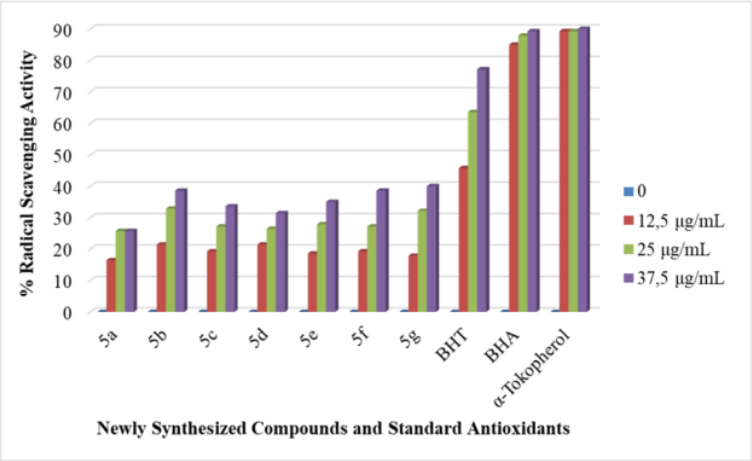


Fig. 3 5-Type compounds' the scavenging activities of % free radical

Metal chelate activity

Metal chelation tests of the obtained compounds have been shown in Table 13 and 14. The metal chelation activities of the obtained compounds and standards are displayed as a percentage of inhibition in the graphs presented in Fig. 4 and 5. Upon examining the graphs, it is seen that all compound groups have metal chelate activity that increases with concentration.

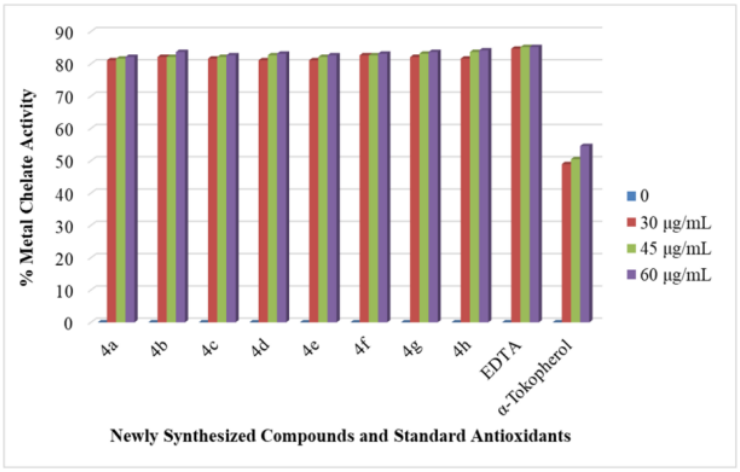


Fig. 4 4-Type compounds' % metal chelate activities

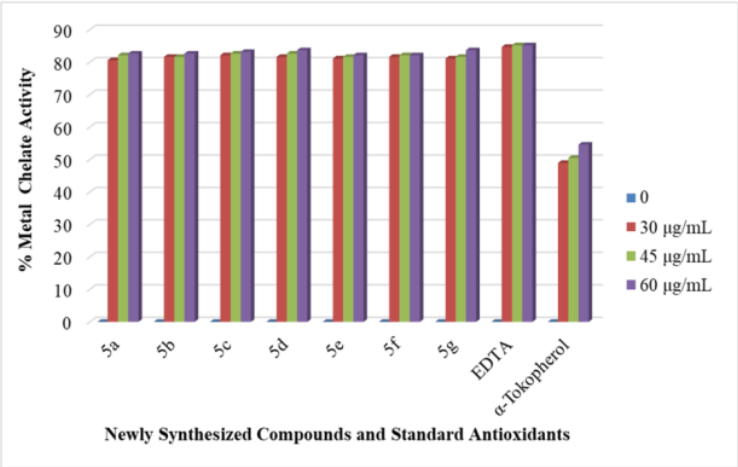


Fig. 5 5-Type compounds' % metal chelate activities

Antimicrobial activity results

The obtained compounds' antibacterial characteristics have been examined by the agar well method, and *Bacillus cereus*, *Bacillus substilis*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* activity against bacteria has been tested. Ampicillin (3261), Neomycin (3385) and Streptomycin (3385) were used as reference standard substances. The obtained chemicals' antimicrobial Activity outcomes have been shown in Table 16.

Table 16 Results of synthesized 4 and 5-types compounds' antimicrobial activity

Compound Code	ZONE diameter values (mm)					
	Bacillus Cereus	*Bacillius Subtilis*	*Pseudomonas aeruginosa*	*Esherichia Coli*	*Staphlacoccus aureus*	*Klebsiella pneumoniae*
4a	++	+++	+++	++	++	+++
4b	+++	++	++	+	+++	++
4c	++	+++	+++	++	++	+
4d	+	++	++	+	+++	++
4e	+++	++	-	++	+++	++
4f	++	-	++	+++	++	+
4g	+++	++	+	+	-	+
4h	++	+++	+	+	+++	+
5a	+++	+	++	+++	++	++
5b	++	+++	+	++	+++	+
5c	+++	++	++	+	++	++
5d	++	+	-	+	+++	+
5e	+++	-	++	-	++	++
5f	++	+	-	++	+	+
5g	+++	+	+	+	+	+
Ampicillin X3261	+++	+++	+++	+++	+++	+++
Neomycin X3385	+++	+++	+++	++	++	++
Streptomycin X3385	++	++	++	+	+++	++

Conclusion

In this study, 8 piece 1-(morpholin-4-yl-methyl)-3-alkyl(aryl)-4-(3-methoxy-4-hydroxybenzylideneamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (4) and 7 piece 1-(4-piperidinecarboxamide-1-yl-methyl)-3-alkyl(aryl)-4-(3-ethoxy-4-hydroxybenzylideneamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (5) compounds have been obtained. IR, ^{1}H NMR, and ^{13}C NMR spectroscopy techniques have been utilized to analyze the obtained compounds' structures. The antioxidant activities of compound structures have been evaluated with three separate techniques (metal chelating activity, free radical scavenging, and reducing power). Graphs have been drawn, and the results have been analyzed. The data obtained indicates a significant capability for antioxidant activity, particularly in

4h and 5e compounds. It can be said that the examined compounds show moderate radical scavenging activity compared to standard antioxidants. Upon examining the graphs, it becomes evident that the metal chelate activity of each chemical group rises with concentration. The synthesized compounds have better activity than the standard antioxidant, α-tocopherol, and are close to EDTA. This is an expected result for these compounds with the Mannich base structure. In addition, antimicrobial tests of the synthesized compounds against six different bacteria were carried out, and the obtained data were listed in the tables. These results show a similar effect to the standard antibiotics ampicillin and neomycin. On the other hand, the fact that compounds 4a, 4b, 4c, 4g, and 5c, showed moderate effects appears to be close to the effect of the streptomycin standard strain. Considering the bacillus cereus bacterial strain, compounds numbered 4a-4h, 5a-5g showed a high level of antibacterial effect, supporting that these compounds are effective against the spore-forming bacteria used. When their antimicrobial values are examined, it is seen that compounds 4h and 5b show high-level antibacterial effects in the Bacillus subtilis bacterial strain.

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Author contributions: S.M. wrote the main draft. H.Y. designed the experiments and analyzed the results obtained from them. K.G. carried out the experiments.

Competing interests The writers say they have no conflicting interests.

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