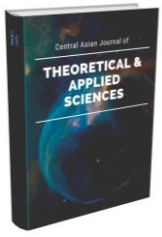

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Article

Synthesis and Characterization of Heterocyclic Compounds from Natural Carboxylic Acids: Evaluation of Their Antioxidant and Biological Activities

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Abstract: Heterocyclic compounds derived from natural carboxylic acids have gained significant attention for their biological activities, yet their potential remains underexplored. This study focuses on the synthesis of benzimidazole derivatives (Z1-Z6) using adipic, citric, salicylic, oleic, tartaric, and hydroxy acetic acids with o-phenylenediamine. Characterization was conducted using FTIR and NMR spectroscopy. Antioxidant activity was evaluated in vitro via the DPPH assay, revealing significant scavenging activity for Z2 and Z4. Antibacterial activity against Staphylococcus aureus was assessed for Z3 and Z6 using the agar diffusion method, showing maximum inhibition zones of 22–24 mm at 800 μg/mL. These findings highlight the potential of these compounds as antioxidants and antibacterial agents, offering promising leads for further pharmaceutical development.

Keywords: Heterocyclic compounds, Benzimidazole derivatives, Antioxidant activity, Antibacterial activity, Natural carboxylic acids

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1. Introduction

One group of cyclic organic compounds is known as heterocyclic compounds· These compounds are distinguished by the presence of at least one heteroatom, which is defined as an atom that is not carbon, inside the structure of the cyclic ring. One of the most common types of heteroatoms is nitrogen (N), followed by oxygen (O), and then sulfur (S). In addition to being present in a wide variety of plant and animal products, heterocyclic compounds are responsible for roughly half of the natural organic chemicals that are now recognized. Alkaloids, natural colors, medicines, proteins, and enzymes are all examples of significant categories of heterocyclic compounds that are found in nature [1,2].

Carboxylic acids are the most important group of organic acids. They are responsible for the pungent odor of butter, the agony that an ant experiences, the taste of vinegar, and the alleviation that is supplied by aspirin or ibuprofen. Adipic acid is a necessary precursor for the reaction that results in the formation of resins·Plasticizers, nylons, and lubricants are all included· [3]: Citric acid, also known as 2-hydroxypropane-1,2,3-tricarboxylic acid, is an essential carboxylic acid for living organisms. It is also found in the tissues of both plants and animals, including blood, bone, and muscle. The Krebs cycle, which involves the oxidation of glucose into carbon dioxide and water, is a crucial component in the production of energy. Citric acid is utilized in various industries,

including the food industry, pharmaceutical industry, chemical industry, and metallurgical industry, due to its non-toxic properties and its ability to chelate and sequester metal ions [4,5]. A molecule known as salicylic acid is an organic compound that has been employed in the chemical industry and in the field of medicine for a considerable of time. The bark of white willow trees, the leaves of birch trees, and several species of the savoy tree are the natural sources from which it is obtained. The first instance of isolation occurred in the year 1828. The major piece of evidence that suggested that salicylic acid may be employed by humans was the dental plaque that was found on the bones of Neanderthals that were discovered in the El Sidron cave.

It has been shown that fossils include remnants of poplar bark, which suggests that it was used for the purpose of alleviating the pain that is linked with periodontal inflammatory illnesses [6,7]. When it comes to monounsaturated fatty acids, oleic acid is a naturally occurring component that may be found in a wide range of foods, notably vegetable oils. As a result of the significant health benefits that it has been shown to possess, it has the potential to be included into processed functional diets [8]. Because of this, it has an effect on cancer.

In addition to being the most abundant organic acid found in grapes, tartaric acid is an essential component of wine that has a significant impact on the wine's overall quality, aroma, and taste. In the family of organic acids known as hydroxyl acids, hydroxy acetic acid is classified as an organic acid. It may be created using chemical or enzymatic methods since it is present in nature and can also be made· Hydroxyl acids are used in the cosmetic industry to treat a wide range of dermatological diseases, including but not limited to acne, psoriasis, pigmentation disorders, and photoaging instances [12].

Benzoimidazole is a chemical that is relevant in the field of medicine since it exhibits a broad variety of biological activities and multiple therapeutic uses· Some of these applications include it having anti-inflammatory, antibacterial, antidepressant, and analgesic outcomes

2. Materials and Methods

The chemicals prepared by the companies indicated next to each of them were used, and the molecular formula and purity were as shown in the table(1).

Table 1. Chemicals, molecular formula, purity and companies producing them

Chemical Materials	Molecular formula	Purity %	Company
Abs· Ethanol	C_2H_6O	99·8	Supelco
Adipic acid	$C_6H_{10}O_4$	98	VWR
Ammonium chloride	NH_4Cl	99·5	BDH
Citric acid	$C_6H_8O_7$	99	VWR
Hydrochloric acid	HCl	37	THOMAS BAKER
Hydroxy acetic acid	$C_2H_4O_3$	98	SIGMA-ALDRICH
Oleic acid	$C_{18}H34O_2$	98	Loba Chemie
o-Phenylene diamine	$C_6H_8N_2$	97	CDH
Salicylic acid	$C_7H_6O_3$	99	VWR
Tartaric acid	$C_4H_6O_6$	98.5	Loba Chemie

Preparation of Benzoimidazole Compounds (Z1-Z6)

Mix the reactants according to table (2).

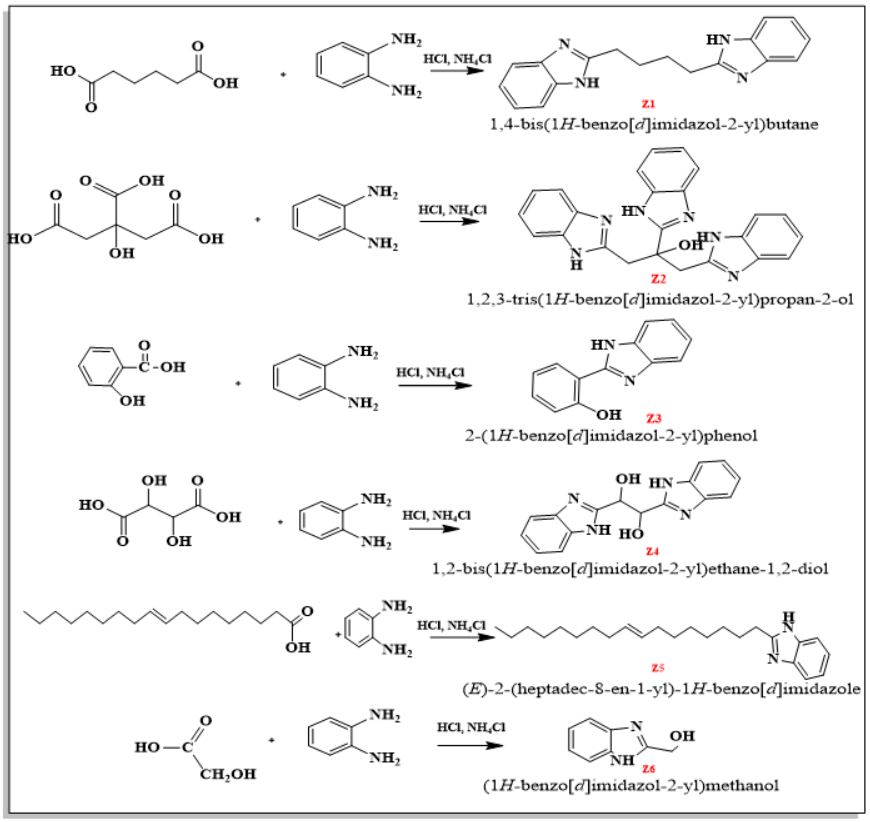
Table 2. Chemicals and the number of Moles needed to form compounds

Comp·	carboxylic acid	o-Phenylene diamine	NH_4Cl
Z1	0·0034 Mole of Adipic acid	0·0068 Mole	0·0068 Mole
Z2	0·0026 Mole of citric acid	0·0078 Mole	0·0078 Mole
Z3	0·0036 Mole of Salicylic acid	0·0036 Mole	0·0036 Mole
Z4	0·0033 Mole of Tartaric acid	0·0066 Mole	0·0066 Mole
Z5	0·002 Mole of Oleic acid	0·002 Mole	0·002 Mole
Z6	0·013 Mole of Hydroxy acetic acid	0·013 Mole	0·013 Mole

The carboxylic acid is placed in a round flask equipped with a magnetic stirrer containing 20 ml of absolute ethanol. Then two drops of concentrated hydrochloric acid were added to it, stirred the mixture, then added the amino derivative with continuous stirring for several minutes, and the mixture rose for 7-8 hours, after which the reaction mixture was cooled and kept for 24 hours, filtered, washed, dried, and re-quenched with absolute ethanol [15]·at shown in this Scheme (1) and Table (3).

Table 3. Physical properties of Compounds Preparation

Comp·no	Chemical Formula	Molecular Weight	Color	M·P °C	Yield %
Z1	$C_{18}H_{18}N_4$	290·37	Black	90-92	76
Z2	$C_{24}H_{20}N_6O$	408·47	Brown	87-88	80
Z3	$C_{13}H_{10}N_2O$	201·24	Light brown	109-111	75
Z4	$C_{16}H_{14}N_4O_2$	294·31	Dark brown	195-197	70
Z5	$C_{24}H_{38}N_2$	354·58	Brown	146-147	68
Z6	$C_8H_8N_2O$	148·17	black	95-97	80



Scheme 1. Prepared compounds

Antioxidant Activity:

Using the agar well diffusion test [17, 18], we were able to determine whether or not the synthesized compounds had the capacity to limit the growth of both Gram-negative and Gram-positive bacteria. Within a sterile environment, twenty milliliters of Muller-Hinton (MH) agar was pipetted onto Petri plates that had been well cleaned· For the purpose of removing the bacterial species from their respective stock cultures, a sterile wire loop was used. After the organisms were cultivated, wells with a diameter of six millimeters were formed in the agar plates by using a sterile tip. Samples ranging from Z6 to Z3 were used in the bored wells, and the amount of these samples varied. Following the cultivation of the test organisms and samples (Z6, Z3) on plates at 37 degrees Celsius for one night, the average widths of the inhibitory zones were measured and reported [20,21].

Preparation of DPPH Stain

The preparation of DPPH (2, 2-diphenyl-1-picrylhydrazyl) stain for antioxidant activity involves a few key steps· Here's a brief overview of the process. Typically, 20 mg of DPPH powder dissolved in 100 ml of ethanol to create a 0·2 mg/ml solution, mixed for 5min on magnetic stirrer. Quantitatively transfer the solution to a volumetric flask, the DPPH solution was storedin the fridge, wrapped in foil to protect it from light and reduce degradation.

Preparation of Ascorbic acid

Dissolved 0·5g of Ascorbic acid in 50ml of deionized water and 50ml of ethanol and mixed for 5min on magnetic stirrer·

Preparation of Samples

1. Prepare of (+ve) add 1000Ml of both DPPH and Ascorbic acid in the tube·
2. Prepare of (-ve) add 1000Ml of DPPH and Ethanol in the tube·

3. Prepare of (samples (Z2, Z4) add 1000Ml of both DPPH and samples (Z2, Z4)· in the tube· Than incubate the tubes for 30min at room temperature, after that we measure the OD of them [16]·

Biological Activity:

Prepare of Mueller Hinton agar

Muller-Hinton (M-H) prepared by adding 38 g of the powder into 1 L distilled water and then heated on a burner with shaking. M-H must be autoclaved for 15 minutes at 121°C to be sterilized. Then it was allowed to cool to 50 °C before pouring into a petri dish and leaving for about 15 minutes for solidification before flipping upside down and storing in the refrigerator at 4 °C.

Antibacterial activity

Using the agar well diffusion test [17, 18], we were able to determine whether or not the synthesized compounds had the capacity to limit the growth of both Gram-negative and Gram-positive bacteria. Within a sterile environment, twenty milliliters of Muller-Hinton (MH) agar was pipetted onto Petri plates that had been well cleaned· For the purpose of removing the bacterial species from their respective stock cultures, a sterile wire loop was used. After the organisms were cultivated, wells with a diameter of six millimeters were formed in the agar plates by using a sterile tip. Samples ranging from Z6 to Z3 were used in the bored wells, and the amount of these samples varied. Following the cultivation of the test organisms and samples (Z6, Z3) on plates at 37 degrees Celsius for one night, the average widths of the inhibitory zones were measured and reported [20,21].

3. Results and Discussion

Characterization of prepared compounds

The prepared compounds were identified through measurements of (FT-IR) spectra (1H-NMR), and it was confirmed that the reaction occurred by observing the changes that occurred in the physical characteristics of the degree of melting and the large change in color. When studying the infrared spectrum of the prepared compounds, and was close to what is found in the literature [22,23],at shown in this table (4) and Figures (1-6) which shows the infrared absorption of the compounds (Z1-Z6).

Table 4. FT - IR Spectral for Compounds Preparation (KBr)cm-1

Comp· No·	υN-H	υC-H Arom·	υ(C-H) Aliph Asym·/sym.	υC=N	υC=C Ring
Z1	3192	3057	2947/2906	1653	1573/1500
Z2	3211	3024	2860/2601	1718	1633/1500
Z3	3360	3242	2900/2866	1656	1637/1600
Z4	3325	3219	3014/2920	1643	1627/1590
Z5	3332	3253	2924/2856	1624	1494/1480
Z6	3352	3278	3037/2929	1651	1589/1500

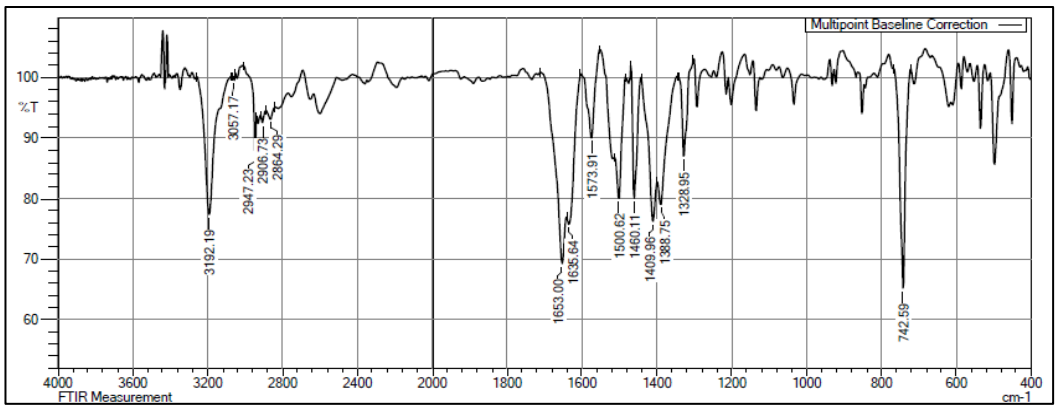


Figure 1. FT-IR spectrum of compound Z1

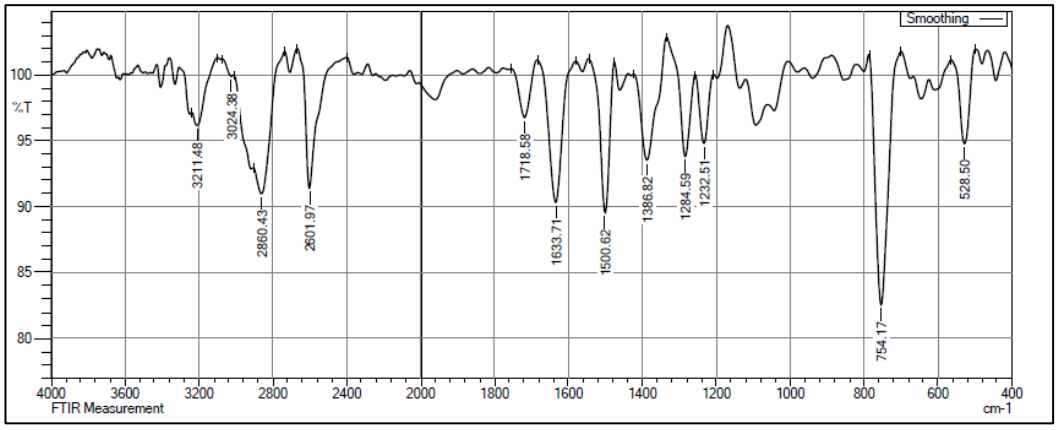


Figure 2. FT-IR spectrum of compound Z2

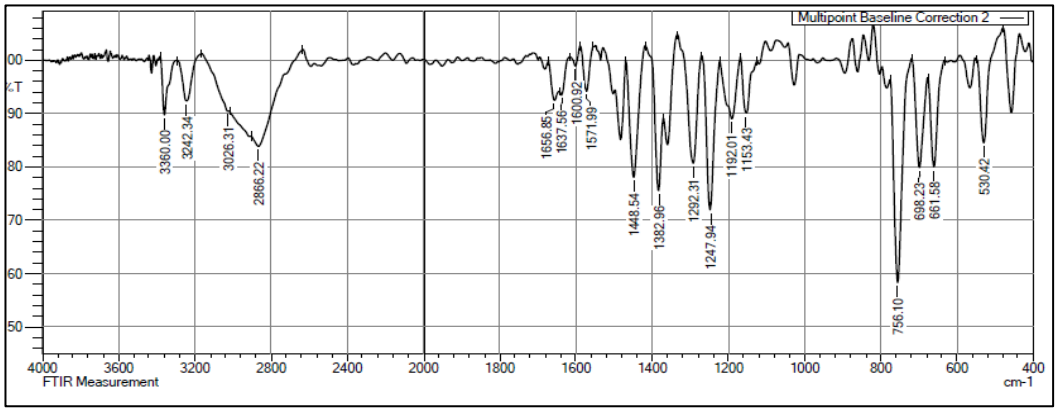


Figure 3. FT-IR spectrum of compound Z3

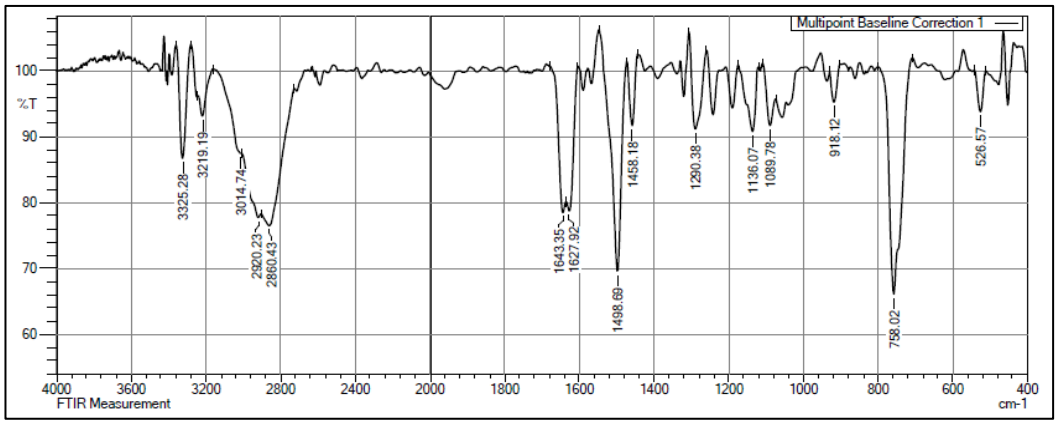


Figure 4. FT-IR spectrum of compound Z4

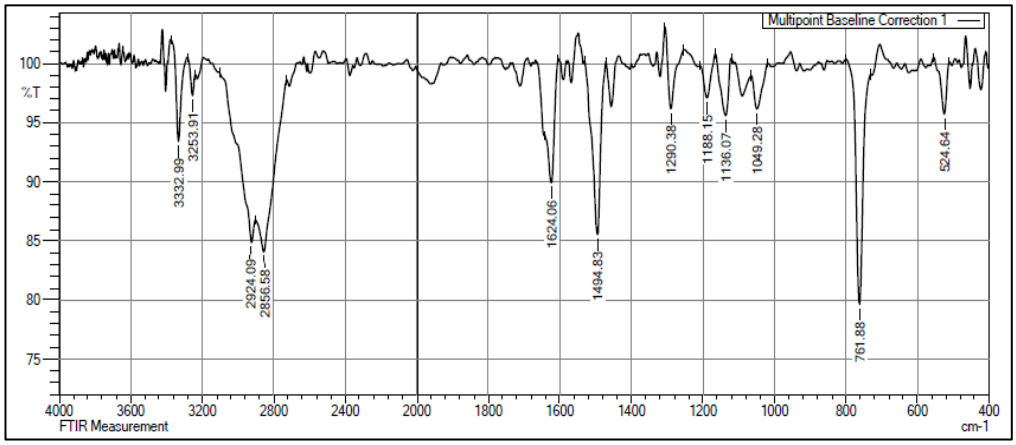


Figure 5. FT-IR spectrum of compound Z5

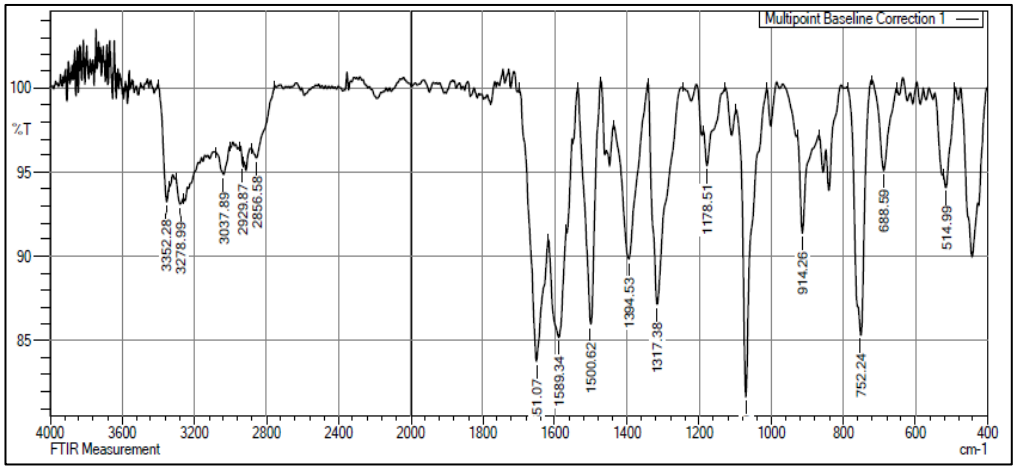


Figure 6. FT-IR spectrum of compound Z6

It was determined via the use of proton nuclear magnetic resonance (1H-NMR) that the preparation of compounds and the use of DMSO-d6 as a solvent were successful. The appearance of a single signal in widespread at the range of (2·5 ppm) is another confirmation of their formation. In addition, the efflorescence of a single signal at (3·3 ppm) due to the water protons used in the solvent, in addition to the appearance of many signs at chemical displacements, is also a confirmation of their formation. According to the multiplicity correlation (n + 1), and depending on the kind of proton and what influences it from the compensated groups, whether they are pushing or pulling electrons, the electrons are pushed or pulled when the multiplicity correlation is applied. There are values of chemical shifts for the compounds that have been created, which are shown in Table 5.

Table 5. Values of chemical shifts for the prepared compounds

Comp· No·	Chemical Shift(ppm)	No· of Protons	Type of single	Group
Z_1	2·24-2·18	4	m	2(-CH_2-)
	2·69-2·72	4	t	2(-CH_2-)
	7·16-7·50	8	m	Aro· Protons
	12·10	2	s	2(-NH)
Z_2	3·83	4	s	2(-CH_2-)
	6·49	1	s	(-OH)
	7·16-7·60	2	m	Aro· Protons

	12·15	3	s	2(-NH)
Z_3	6·95-7·84	8	m	Aro· Protons
	9·76	1	s	(-OH)
	12·56	1	s	(-NH)
Z_4	5·16	2	s	2(-OH)
	5·36	2	s	2(-CH-)
	7·15-7·56	8	m	Aro· Protons
	12·16	2	s	2(-NH)
Z_5	0·87-0·89	3	t	-CH_3))
	1·25-2·00	26	m	13(-CH_2-)
	2·80-2·84	2	t	(-CH_2-)
	5·33	2	s	(CH=CH)
	7·16-7·49	4	m	Aro· Protons
	12·16	1	s	(-NH)
Z_6	4·81	2	s	(-CH_2-)
	5·57	1	s	(-OH)
	7·56-7·15	4	m	Aro· Protons
	12·22	1	s	(-NH)

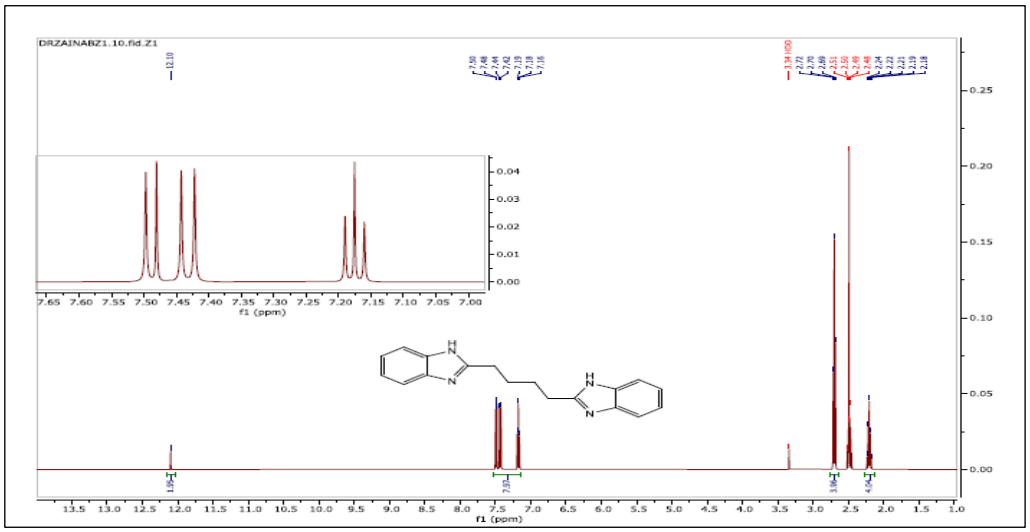


Figure 7. (1H-NMR) spectrum of synthesized compound Z1

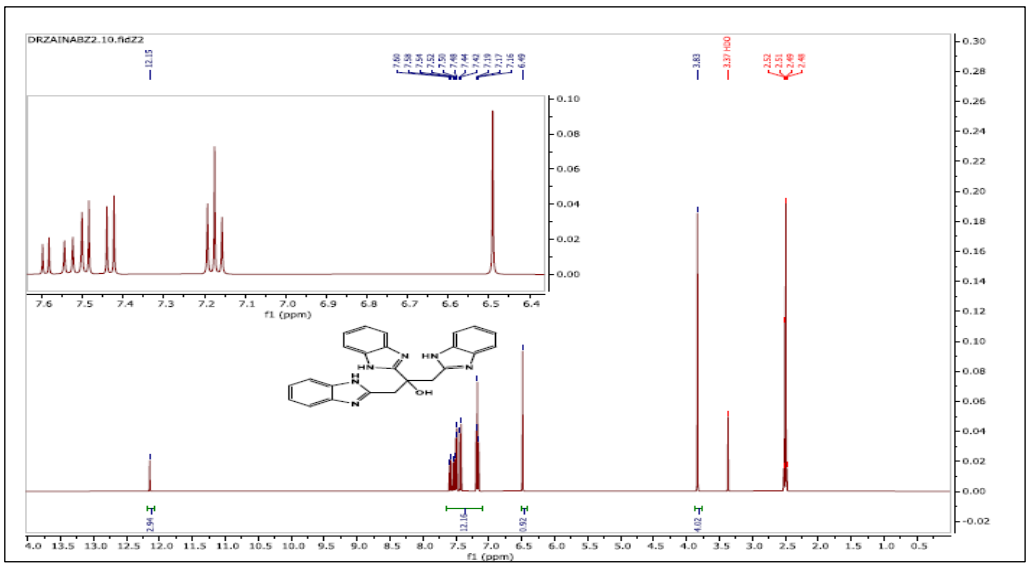


Figure 8. (1H-NMR) spectrum of synthesized compound Z2

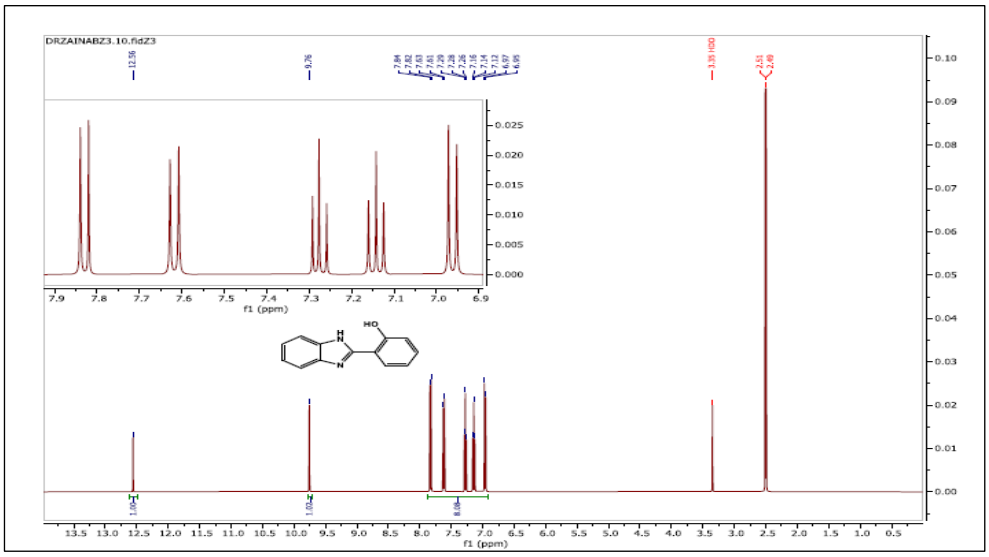


Figure 9. The (1H-NMR) spectra of the chemical Z3 that was synthesized

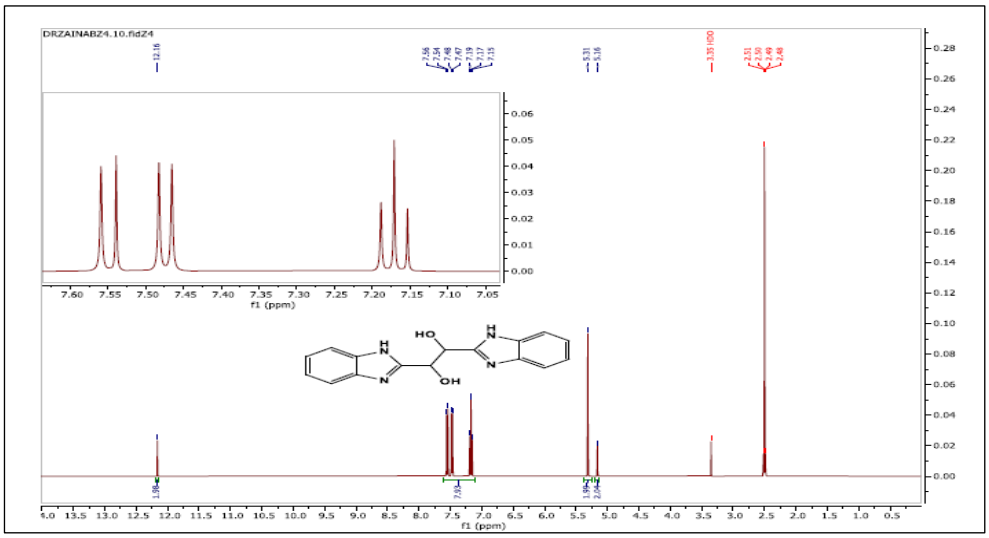


Figure 10. The (1H-NMR) spectrum of the chemical that was synthesized Z4

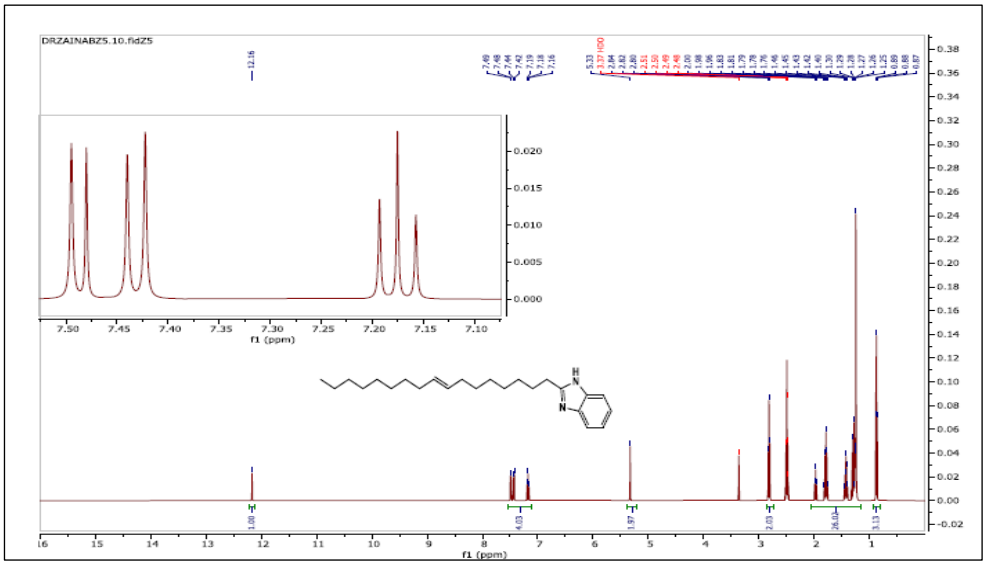


Figure 11. (1H-NMR) spectrum of the chemical that was synthesized a Z5

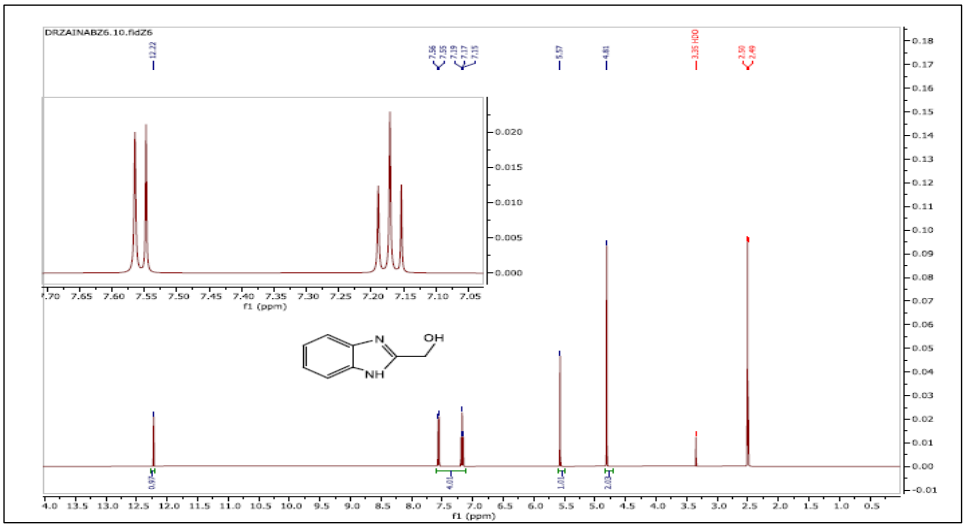


Figure 12. (1H-NMR) spectrum of synthesized compound Z6

Antioxidant Activity (DPPH Assay)

DPPH is a stable free radical that binds with replacement electrons [16]. DPPH may be combined with a substrate capable of donating a hydrogen atom. This may provide a diminished form characterized by a color transition from violet to yellow, and should be shielded from light prior to measuring their optical density (OD) at 517 nm. The sample with the lowest optical density exhibited the greatest DPPH scavenging activity percentage, as determined by Equation (1) [25]. The DPPH radical scavenging activity (%) of samples Z2 and Z4 is shown in Figure 2.

$$DPPH\ Scavenging\ activity\ (\%) = \frac{OD_{Control} - OD_{Sample}}{OD_{Contro;}} \times 100 \qquad \text{-------- Equation(1)}$$

Where: $OD_{Control}$ is Negative

Table 6. The activity of synthesized compounds (Z2,Z4) as antioxidants.

Comp· No·	Concentration	
	0·008	0·01
Z2	86·34146	85·36585
	85·65854	85·85366
	85·95122	84·68293
Z4	88·09756	86·14634
	88·09756	85·95122
	88·01	86·34146

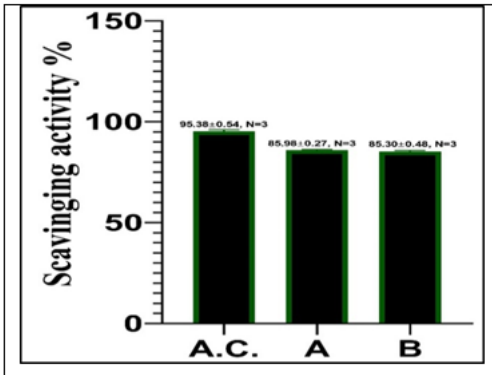


Figure 13. DPPH Assay of compound Z2(A) 0·008, (B) 0·014·

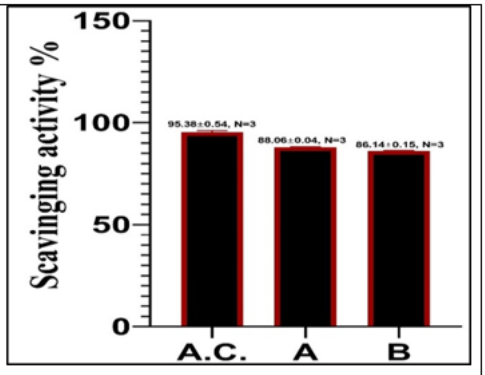


Figure 14. DPPH Assay of compound Z4(A) 0·008, (B) 0·014·

The table (6) and figures (13,14) show that the compounds (Z2,Z4) have a higher antioxidant activity[26] than the standard substance (ascorbic acid) due to the formation of free radicals as shown below.

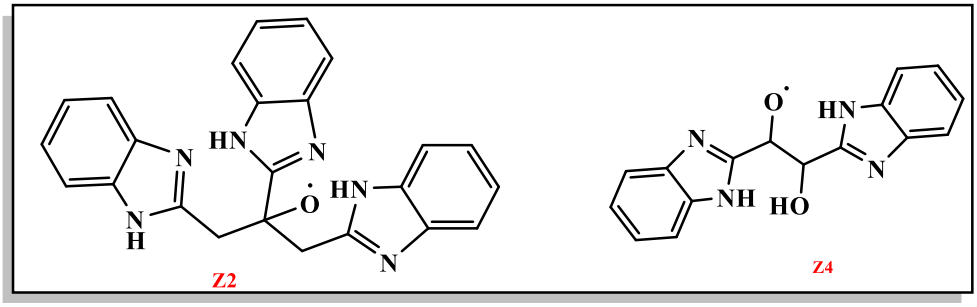


Figure 15. These compounds can be used as food preservatives.

These compounds can be used as food preservatives

Biological Activity:

All result of antibacterial activity [27] with different concentration shown by the figures below all details explained by table (7)

Table 7. Antibacterial analysis(Zone of inhibition (mm)

	Comp· No·	A	B	C	D	E	F
S·aureus	Z6	6	20	6	8	18	24
	Z3	6	18	6	8	17	22

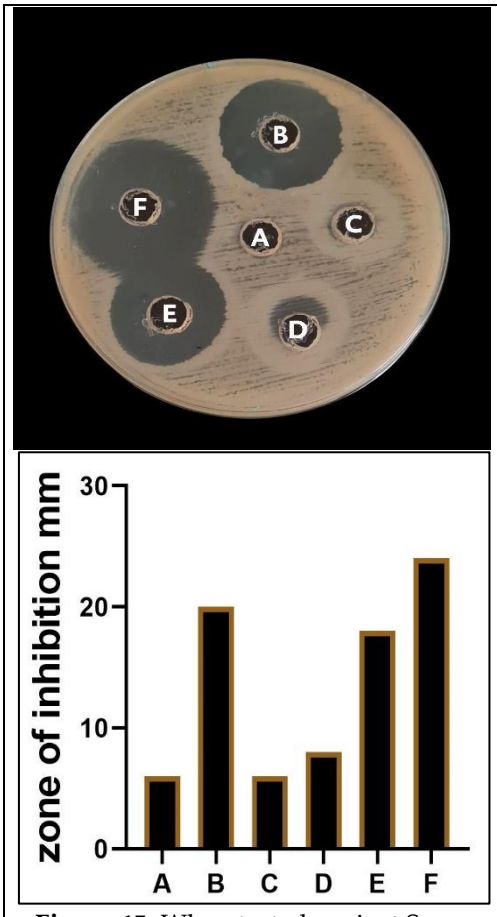


Figure 15. When tested against S· aureus, (Z6) exhibited antibacterial activity· A, Take charge· Control, also known as Ampicillin·

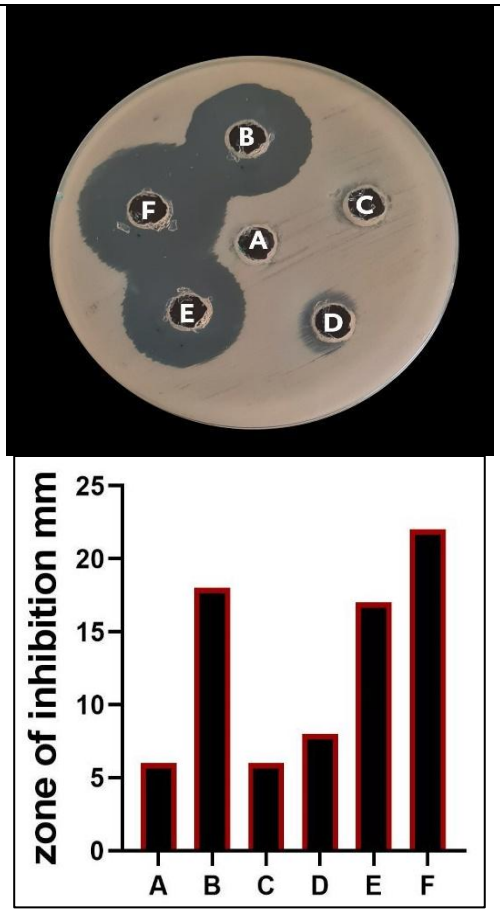


Figure 16. When tested against S· aureus, (Z3) exhibited antibacterial activity· A, Take charge· Control, also known as

200 μg/mL of C· D, measured at 400 μg/mL· 600 μg/mL is the value for E· 800 micrograms per milliliter	Ampicillin· 200 μg/mL of C· D, measured at 400 μg/mL· 600 μg/mL is the value for E· 800 micrograms per milliliter

The table (7) and figures (15,16) show that the compounds (Z3,Z6) at a concentration of (800 μg/mL) have a higher inhibitory [28] effect on bacterial species (S·aureus) than the standard substance (Ampicillin)·These compounds can be used to manufacture medicines for bacterial infections that affect the skin·

4. Conclusion

In this study, good results were obtained for the antioxidant activity of some of the prepared compounds. The compounds (Z2,Z4) showed higher activity than the standard substance, and the compounds (Z3,Z4) also showed high inhibition against bacteria (S·aureus) compared to the standard substance. It can be concluded that heterocyclic compounds derived from natural products such as carboxylic acids can have antioxidant and inhibitory activity on some bacterial species.

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