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Article

Preparation, Characterization, Evaluation of Biological Activity, and Study of Molecular Docking of Azetidine Derivatives

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Abstract: In this work, equal moles of hydrazone base derivatives and chloroacetyl chloride react to create quaternary rings of azetidine derivatives. Proton nuclear magnetic resonance spectroscopy, infrared spectroscopy, and physical and spectroscopic techniques were used to confirm the complex compositions. Furthermore, purity and melting points were established, and thin-layer chromatography (TLC) was used to track the development of the reaction. Researchers examined how several produced chemicals affected the development of two bacterial isolates: Escherichia coli, a gram-negative, and Staphylococcus aureus, a gram-positive. Using the MOE software (2009), molecular docking investigations were carried out for the compounds (AB12, AB13) against Escherichia coli. These molecules' most stable conformation (lowest energy barrier) was attained using the energy minimization procedure.

Keywords: Azetidine, Hydrazone , Hydrazide , Biological activity, Molecular docking.

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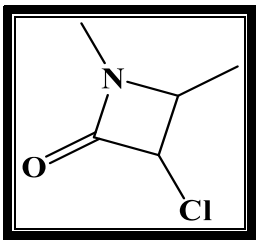
1. Introduction

The quaternary ring azetidine has a carbonyl group and a nitrogen atom at the position. Another name for it is a β-lactam compound. It is regarded as one of the substances with biological activity. Research has demonstrated its efficacy against bacteria, microorganisms, and malignant growths. An enzyme inhibitor is another classification for it[1]. Most researchers focused on creating quaternary rings because of their significance in medicine, and one of the most well-known ways to do it is by reacting chloroacetyl chloride with Schiff bases [2].



Antibiotics like cephalosporin and penicillin contain azetidine molecules, which are physiologically significant cyclic compounds. Its anti-inflammatory [3], anti-bacterial [4], anti-fungal [5], and anti-cancer [6] properties have all been reported. Furthermore, it has demonstrated encouraging efficacy as an anti-malarial [7].

2. Materials and Methods

2.1. Material

Without any additional purification, all of the compounds utilized in this investigation were acquired from BDH, Fluka, and Aldrich.

2.2. Devices Used

A thermoelectric melter 9300 was used to determine melting points. Using KBr disk at a scale of (400–4000) cm-1, Shimadzu FT-IR 8400S spectrophotometer; 1H-NMR and 13C-NMR spectra using Bruker apparatus operating at 400 MHz. Thicknessed at 0.2 mm, Fluka silica gel plates were employed in thin-layer chromatography (TLC). The plates were activated with fluorescent silica gel G, and visibility was achieved by UV light.

2.3. Preparation Of Azetidine Derivatives (AB32-AB37) [8], [9]

I dissolve (0.01 mol) of the prepared hydrazone in (20 ml) of 1,4-dioxane, add to (0.02 mol, 2 ml) of tri ethylamine, and place the mixture in an ice bath (0-5)◦C. With continuous stirring, add (0.01 mol, 0.8 ml) of acetyl chloride gradually for (10 minutes), then add drops of triethylamine (Et3N). Stir the mixture for (5) hours, add the solution to the ice to form a precipitate, then filter it. The residue was dried and recrystallized from absolute ethanol, as shown in Table 2.

2.4. Biological Activity Study [10], [11]

This investigation employed two harmful microorganisms: the Gram-positive Staphylococcus aureus and the Gram-negative Escherichia coli. Bacteria from the Department of Life Sciences and the College of Education for Pure Sciences were used with the growing medium of Multer Hinton Agar [39]. Dimethyl sulfoxide (DMSO) was used to create chemical solutions of AB12, AB13, and AB14 at concentrations (0.01, 0.001, 0.0001) mg/ml. In doing so, the minimum inhibitory concentration (MIC) is measured and established. The diffusion technique was applied to ascertain the sensitivity of the bacterial isolates used in the study, and Mueller-Hinton agar was utilized as the nutritional medium. The medium was prepared, autoclaved, distributed across plates, and allowed to harden. Then, each plate had four microscopic holes drilled into it. After that, it was incubated for an entire day at 37 0C. Of the employed derivatives. To demonstrate the sensitivity of the employed derivatives. These derivatives depend on the diameter of inhibition seen in the dishes around the holes used as the diameter increases.A generated chemical's biological activity rises when it displays inhibition; this may be likened to the antibiotics' diameter of inhibition [12], [13].

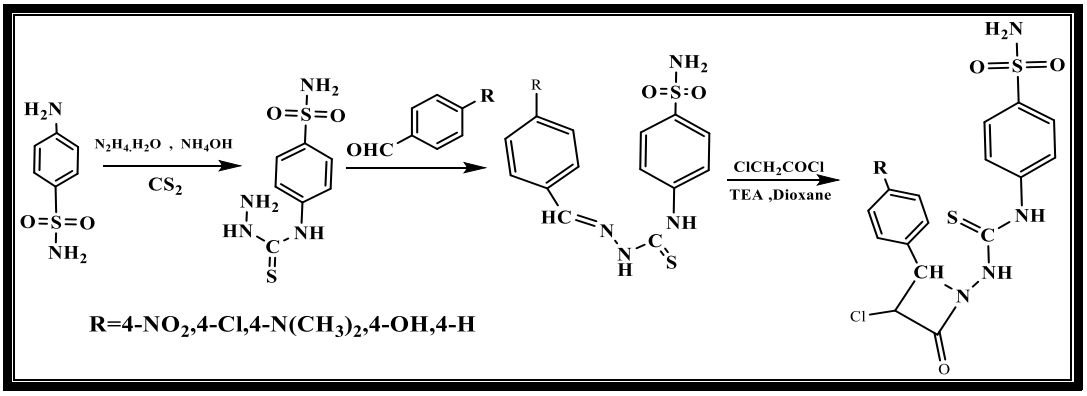
2.5. Molecular Docking Study of Some Prepared Compounds

Using the MOE program (2009), molecular docking investigations were carried out for a few produced compounds (AB12, AB13) against a common bacterial strain, Escherichia coli. The goal was to reduce the energy of the compounds under study (AB12, AB13) in order to achieve the lowest energy barrier, or the most stable conformation. The Escherichia coli protein structure was obtained from the International Protein Bank. High-

performance computing resources were utilized because these programs are very demanding and require sophisticated, multi-core processors for quick and effective computational operations, especially when working with big molecules and complex atom configurations, among other things.

3. Results and Discussion

This study prepared quaternary rings of azetidine derivatives by reacting equal moles of Schiff base derivatives with chloroacetyl chloride in the presence of tri ethylamine as a catalyst and dioxane as a solvent, as shown in Scheme 1.



Scheme 1. Path of the Ready Compounds (AB12-AB16)

3.1. Characterization Of Azetidine Derivatives (AB12-AB16)

Analyzing the azetidine derivatives' infrared (IR) spectra [AB12-AB16], it was noted that the stretching of the ligand (NH) produced absorption bands at the frequency (3162-3188) cm-1, while the stretching of the aromatic (CH) bond produced absorption bands at the frequency (3056-3089) cm-1. Due to the aliphatic (C-H) bond's stretching, absorption bands with a frequency of (2944-2970) cm-1 emerge, Owing to (NH2) stretching, an absorption band at the frequency (3218-3458) cm-1 also appears. Moreover, an absorption band at frequency (1668-1683) cm-1 developed as a result of the carbonyl group (C=O) next to the chlorine group stretching. And because the carbonyl Amide (C=O) bond was stretching, an absorption band developed at the frequency of (1631–1642) cm–1. Additionally, it was noted that two absorption bands resulting from the stretching of the aromatic (C=C) bonds occurred at the frequencies of (1522-1587) cm-1 and (1451-1480) cm-1. At this point, an absorption band emerged at a frequency (725-746) cm-1. It results from the bond (C-Cl) stretching. [14], as shown in Table (2) and Figures (1, 2).

Examining the compound's [AB13] proton NMR spectra. It was noted that just one signal, which was assigned to the proton of the NH group, occurred in the range of (11.33-9.59) ppm, while several signals, which were attributed to the protons of the ring, appeared in the region of (8.10-7.02) ppm. Aromaticity and the emergence of two signals in the quaternary azetidine ring, one in the range of (5.54 - 5.57) ppm assigned to the protonation of the (CH-Cl) group and the other in the range of (4.42 - 4.44) ppm attributed to the protonation of the (CH-N) group. The proton of the (NH2) group is responsible for the signal that appears in the region of 6.30 ppm in the quaternary azetidine ring. Additionally, there is a signal at the chemical shift (2.35). Protons in the solvent are responsible for parts per million (DMSO-d6) [15], as in Figure (3).

When studying the carbon NMR spectrum of the compound [AB13], The quaternary azetidine ring's carbon of the (C-Cl) group was found to have a signal appearing in the range of 66.16 ppm. The carbon of the group (C-N) in the quaternary azetidine ring was found to have a signal occurring in the range of (74.30) ppm. The aromatic benzene ring's carbons were found to have a signal appear in the range of (104.32–153.69) ppm, and it was discovered that a signal for the carbon of the carbonyl group (C=O) appeared in the range of (160.71) ppm. For the carbonyl group (C=S), a signal in the range (189.53) ppm is assigned. Furthermore, many signals associated with the solvent's carbonate were detected at the location (39.03-40.70) [16], as shown in Figure (3).

3.2. Evaluation of the Biological Activity of Prepared Compounds

Compounds with non-homogeneous rings have distinct biological activity against both Gram-positive and Gram-negative bacteria. Escherichia coli and Staphylococcus aureus were the two types of bacteria employed in this dissertation to examine the biological activity of the compounds generated. These bacteria were chosen since they are known to cause various disorders. Furthermore, these microorganisms' antibiotic resistance patterns vary [18]. By measuring the inhibition zone width, Utilizing the agar well diffusion method, the biological activity of the compounds generated was assessed[19], [20]. The results indicate that the compounds that were synthesized had varying degrees of ability to hinder the growth of bacteria, both Gram-positive and Gram-negative. The substances showed significant inhibition activity against Escherichia coli and extraordinary inhibitory effect against Staphylococcus aureus[21], [22]. A concentration of 0.01 milligrams per millilitre increased inhibition percentages in a dose-dependent connection between concentration and inhibition. as presented in Table (3).

3.3. Results of Molecular Docking Study for Some Prepared Compounds

The quantity and kinds of bonds formed with amino acid residues at the active site were identified by molecular docking analysis of the produced chemical derivatives. The investigation results demonstrated that compound AB12 forms two bonds with the remaining amino acid residues at the active site. Generates three hydrogen bonds and two other bonds in response to interactions with amino acid residues in the active site. The first links the aromatic ring, The oxygen atom of the nitro group, two hydrogen bonds, and the amino acid residue ARG173 that is present in the active site. It connects the electronic pairs of the sulfur atom to the amino acid residues GLN 193 and ASP 192, which are found in the enzyme's active site, and a Pi-Alkyl bond connects the aromatic ring's electronic pairs to the amino acid residues GLY 378, which are also found in the active site.

According to the study, the molecule (AB13) forms two different types of bonds—five hydrogen bonds—in response to amino acid residues found in the active site. Firstly, a hydrogen bond links the amino acid residue GLN 348 in the active site to the electron pair of the oxygen atom group (S=O), and the amino acid residue ASP 192 in the active site to the amine group and the electronic pair of the nitrogen atom. and three hydrogen bonds linking the amino acid residues GLN 193, ASP 192 VAL 179, which are located in the active site of the enzyme with the electron pair of the sulfur atom, A Pi-Alkyl bond connects the amino residue PHE 205, which is located in the active site, with the electronic pairs of the aromatic ring.

Table 1. Some Physical Properties Of Azetidine Derivatives.

Comp. No.	R	Molecular formula	m.p. °C	Yield%	Color
AB12	4-NO_2	$C_{16}H_{14}ClN_5O_5S_2$	189-191	76	Yellow
AB113	4-Cl	$C_{16}H_{14}Cl_2N_4O_3S_2$	196-198	79	Orange
AB14	4- $N(CH_3)_2$	$C_{18}H_{20}ClN_5O_3S_2$	184-186	81	Yellow
AB15	4-OH	$C_{16}H_{15}ClN_4O_4S_2$	202-204	84	Brown
AB16	4-H	$C_{16}H_{14}BrN_4O_3S_2$	217-219	82	Brown

Table 2. FT-IR Absorption Results For Azetidine Derivatives (M32-M37)

Comp. No.	R	ν(N-H)	ν(C-H) Aliph. Arom	ν(NH_2)	ν(C=O)	ν(C=C) Arom.	ν(C-Cl)	Others
AB12	NO_2	3166	2950,3063	3360,3458	1683	1522,1465	721	ν (N-O)1317
AB13	Cl	3184	2970,3083	3340,3438	1676	1562,1475	744	--
AB14	$N(CH_3)_2$	3162	2944,3059	3318,3368	1671	1532,1451	732	ν (C-N)1232
AB15	OH	3175	2967,3056	3218,3282	1668	1538,1480	725	(OH) 3346
AB16	H	3188	2964,3089	3336,3394	1681	1587,1475	746	--

Table 3. Biological Efficacy Of Produced Substances And Control Methods (Measured In Millimetres Of Inhibition).

Comp. No.	E. Coil Conc. mg/ml			Staph. Aureus Conc. mg/ml		
	0.01	0.001	0.0001	0.01	0.001	0.0001
AB12	28	28	18	25	21	18
AB13	21	21	10	20	12	10
AB14	26	23	17	15	10	5
Amoxicillin	12	10	10	21	20	10

Table 4. Values Of The Produced Compounds' Binding Energies

Comp. No.	RMSD	Docking Score
AB12	1.89542	-6.7
AB13	2.051467	-7.01

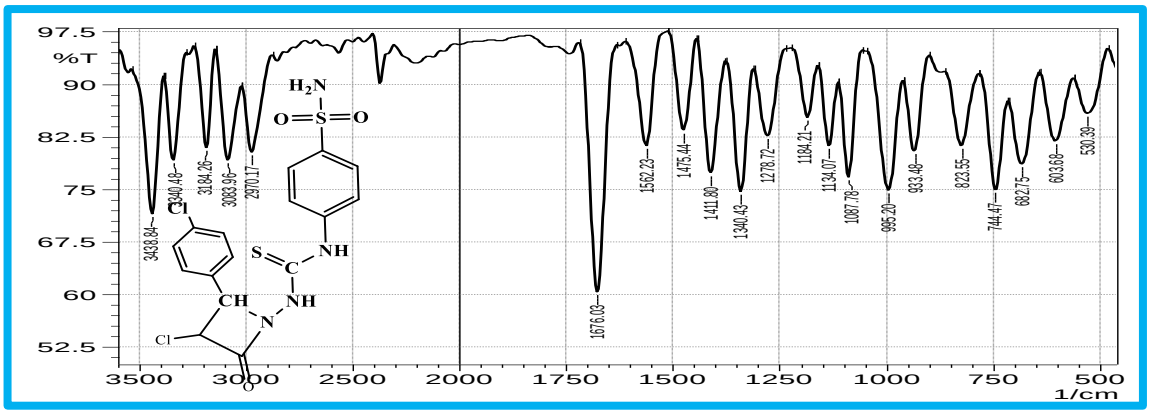


Figure 1. The compound's FT-IR spectra (AB13).

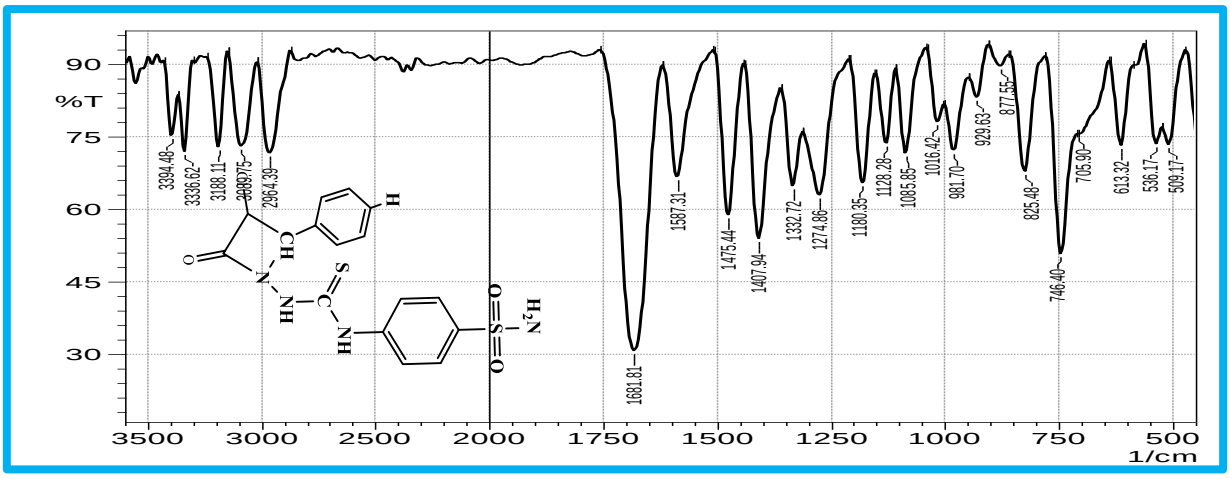


Figure 2. The compound's FT-IR spectra (AB16).

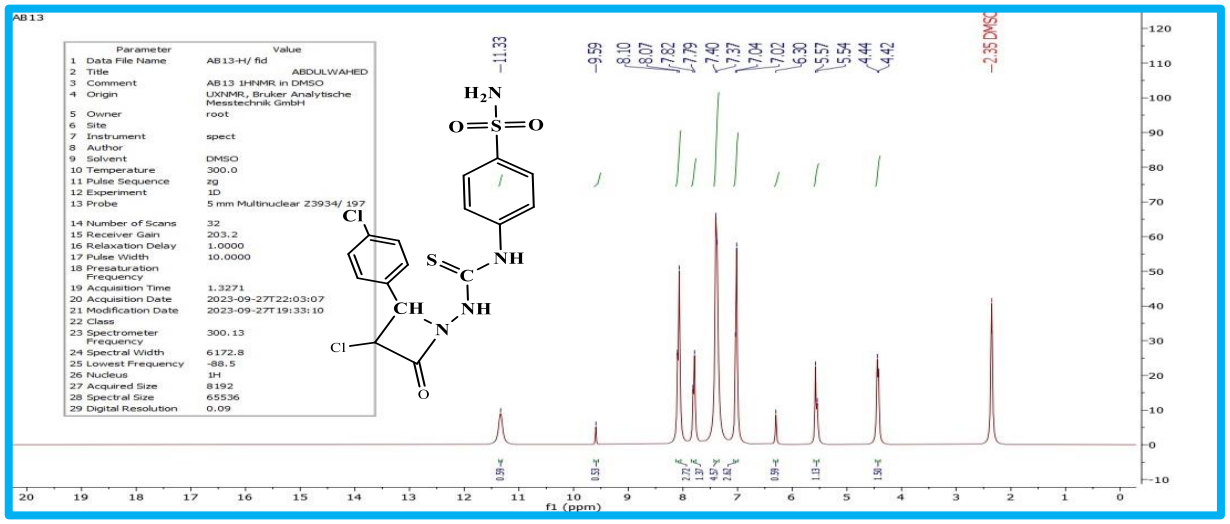


Figure 2.[1]H NMR spectra of the substance (AB13).

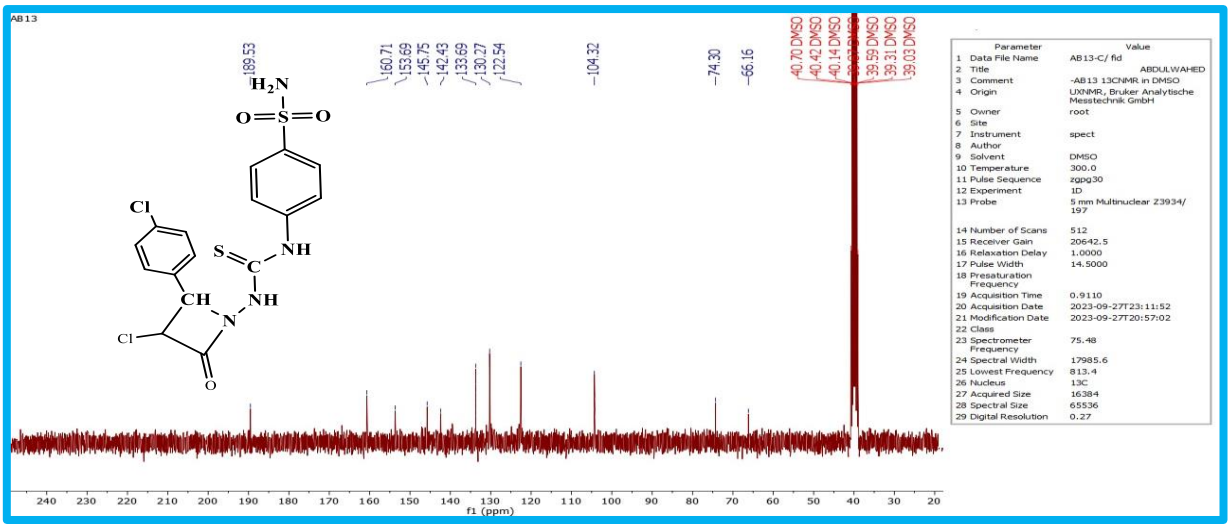


Figure 4. [13]C-NMR spectrum of the compound (AB13).

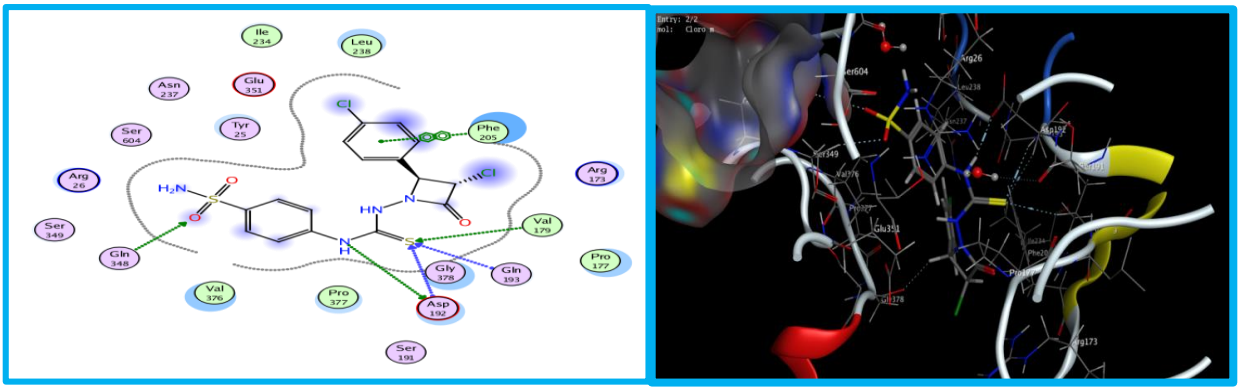


Figure 5. Compound (AB12) interactions in two and three dimensions.

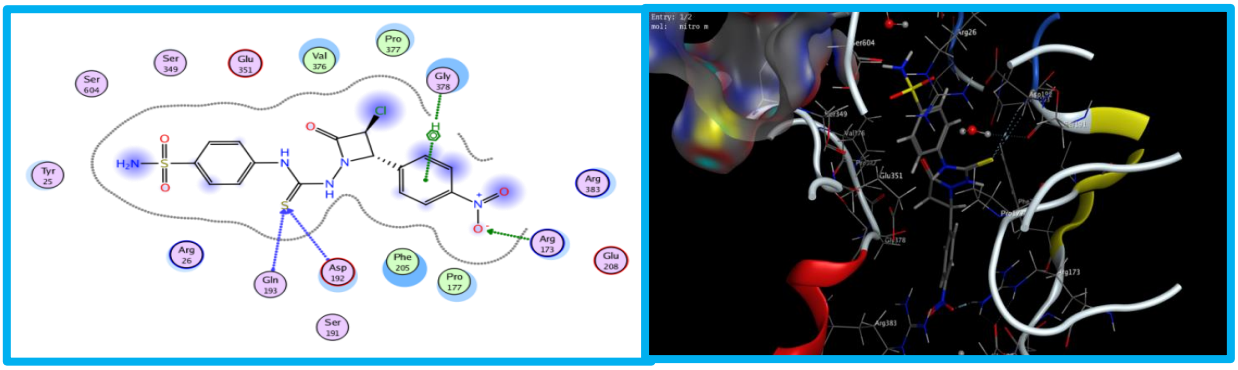


Figure 6. Compound (AB13) interactions in two and three dimensions.

4. Conclusion

The reaction of hydrazide derivatives with compounds containing suitable functional groups often results in heterocyclic quaternary rings. Bioassay results indicate that most prepared compounds showed antibacterial activity and could inhibit bacterial growth. Some of these compounds showed higher biological effectiveness than the antibiotics used as control samples. Physical and spectroscopic measurements demonstrated the prepared compounds' structures' accuracy.

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