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Article

Synthesis, Characterization and Study of Antibacterial Activity and Laser Efficacy of New Isoxazoline Derivatives

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Abstract: This study involves the preparation of heterocyclic pentameric rings derived from isoxazoline compounds using chalcones as nuclei by reacting previously prepared chalcones with hydroxylamine hydrochloride using ethanol as solvent. Spectroscopic measurements confirmed the activity of the prepared compounds. Examples include infrared spectroscopy, proton nuclear magnetic resonance (NMR) and quantitative elemental analysis (C.H.N). Physical measurements such as melting point, color and product percentage were also performed. Bioavailability was evaluated using two known antibiotic resistant bacterial isolates (Gram-negative Escherichia coli (Gram-ve) and Gram-positive Staphylococcus aureus (Gram+ve) and compared with controls. The antibiotic comparison reagent (control) was amoxicillin. The stability of the prepared compounds against a helium-neon laser was tested over periods of 15-60 s by shining the laser on the compounds and then observing the color change and melting point

Keywords: Heterocyclic, Isoxazoline, biological activity, laser.

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1. Introduction

Heterocyclic compounds are composed of atoms containing at least one heteroatom. The most common additional heteroatoms are nitrogen, oxygen, and sulfur, but other heterocycles containing further information, such as phosphorus, iron, magnesium, and selenium, are also common. Other fungal types are also common [1]. Heterocycles are the most essential traditional division in organic chemistry. They are increasingly attractive due to their medicinal, antibiotic, and industrial properties [2,3]. Isoxazoline It is a five-membered heterocyclic ring containing N and O atoms close together. The hydroxylated version of isoxazoles is also called isoxazoles, and the compound containing N function (-C = N-O-) undergoes a cycloaddition reaction [4]. The formation of a ring of α-β unsaturated compounds with hydroxylamines always results in their formation. Because these compounds contain electrons on the nitrogen and oxygen atoms, isoxazoline derivatives have played a crucial role in the theoretical development of heterocyclic

chemistry and have been widely used in organic synthesis. In recent years, the synthesis of isoxazoline derivatives as new antibacterial agents has attracted increasing attention[5]. The synthesis of new isoxazoline derivatives remains a primary focus of medical research. Scientists in the field of medicine have paid attention to their synthesis. They have proven their biological efficacy against various diseases, including anticancer and anti-inflammatory [6]. They have also shown their antibacterial efficacy[7]. Laser The basis of the laser process is absorption and emission, as the stimulated and spontaneous absorption and emission processes become very important in the physical basis of laser production [8]. As we all know, atoms, ions and molecules can exist in specific states, and each state has a specific energy called an energy level, and the energy level smaller than it is called the ground state energy levels, noting that the farther the energy levels are from the ground level, the more active they are [9].In conclusion, the current study aims to prepare new rings derived from chalcone as a nucleus and evaluate their biological activity against two types of bacteria and test the stability of these compounds against helium-neon laser.

2. Materials and Methods

2.1.Chemicals used: Chemicals prepared by Aldrich, BDH Thomas, Fluka, and Merck were used.

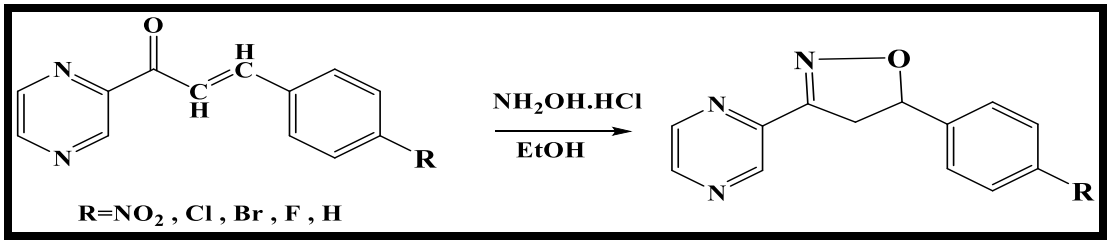
2.3. Preparation of Isoxazoline derivatives (MH_6-Mh_{10}).[10]

Equimolar amounts (0.00 1 mol) of the prepared chalcones were mixed with hydroxylamine hydrochloride in (40 ml) of absolute ethanol in a round-bottomed flask, and then (10 ml) (10% NaOH) was added to it. The mixture was heated for (10 hrs). The solution was then cooled and neutralized by adding crushed ice (10% HCl), and the precipitate was filtered, dried, and recrystallized with absolute ethanol.

Table (1): Some physical properties of for Prepared compounds (Mh6-Mh10).

Comp. No.	R	Molecular formula	m.p. °C	Yield%	Color
MH6	4-Cl	$C_{13}H_{10}N_4O_3$	243-245	73	Whit
MH7	4-NO2	$C_{13}H_{10}ClN_3O$	231-233	70	Dark Brown
MH8	4-CH3	$C_{13}H_{10}BrN_3O$	222-224	76	Light Orange
MH9	4-Br	$C_{13}H_{10}FN_3O$	254-246	75	Yellow
MH10	4-H	$C_{13}H_{11}N_3O$	248-250	71	Light Yellow

Table (1): Some physical properties of for Prepared compounds (Mh6-Mh10).



Scheme (1): Path of the Ready Compounds (MH6-MH10)

2.3. Biological activity study

To prepare Mueller-Hinton agar, dissolve it in 1 liter of distilled water, bring it to a boil by stirring using a magnetic stirrer, and autoclave it at 121 °C and 1.5 bar[11-16]. It is

then placed on a Petri dish, cooled to 50 °C for two hours, and frozen at room temperature. Two types of bacteria were examined: Gram-positive Staphylococcus aureus [Gr+ve] and Gram-negative Escherichia coli. Using heat-sterilized racks, transfer two pure bacterial isolates of Gram-positive and Gram-negative bacteria from the solid culture media to test tubes containing five ml of distilled water[17-22]. After 16–20 h of incubation at 30 °C (37 °C), the tubes were diluted with physiological saline until the turbidity reached standard levels, resulting in a cell count of about 1.5 × 108 cells/ml. Chemical solutions of the compounds were prepared using dimethyl sulfoxide (DMSO) solvent at concentrations of 0.01, 0.001, and 0.0001 mg/mL of each substance (for each solid derivative)[23-27].

2.4. Measurement of laser effectiveness of compounds[28]

The laser efficiency of the prepared compounds (MH6, MH7, MH8, MH9, MH10) was measured using a He-Ne laser (visible laser). The prepared compounds were irradiated at four time intervals (15, 30, 45, 60). Seconds for each compound, as well as the distance between the beam source and the sample (10 cm), power (1 mW), and wavelength (808 nm). The measurements were taken at the University of Tikrit - College of Science - Department of Physics Laser Laboratory. After irradiating the prepared compounds, the physical properties were studied again and the changes in the shape of the prepared compounds were observed.

3. Results

3.1. Characterization of Isoxazoline derivatives (MH6-MH10)

The infrared spectra of the prepared compounds showed a band at (1608-1595) cm-1, two bands at (2971-2930 & 2922-2868) cm-1 due to aliphatic (CH), two bands at (1566-1537 & 1506-1473) cm-1 due to aromatic (C=C), a band at (3082-3023) cm-1 due to aromatic (CH), a band at (1228-1219) cm-1 due to (C-O), and a band at (1294-1287) cm-1 due to (C-N)[29,30], as in Table 2 and Figures 1 and 2.

Table 1: Physical properties of the prepared compounds

Comp.	νCH Arom.	ν(CH) Aliph.	ν(C-O)	ν(C=N)	ν(C=C) Arom.	ν(C-N)	Others
MH6	3026	2951, 2906	1292	1604	1566, 1473	1222	ν(NO) 1519, 1338
MH7	3023	2971, 2910	1289	1601	1542, 1493	1226	ν(C-Cl) 728
MH8	3055	2934, 2883	1287	1608	1549, 1487	1220	ν(C-Br) 681
MH9	3082	2955, 2922	1294	1595	1541, 1479	1228	ν(C-F) 931
MH10	3030	2930, 2868	1291	1598	1537, 1506	1219	--

The 1H-NMR spectrum of the compound (MH8) showed signals belonging to aromatic rings at (9.05-7.46) ppm, a triplet signal at (5.50-5.46) ppm belonging to (CH), and a doublet signal at (3.02,2.99) ppm belonging to (CH2)[31]. As in Figure 3

The 1H-NMR spectrum of the compound (MH10) showed signals attributed to aromatic rings at (8.59-7.57) ppm, a triple signal attributed to (CH) at (5.13-5.10) ppm, and a double signal attributed to (CH2) at (2.85,2.82) ppm. As in Figure 4

The 13C-NMR spectrum of the compound (MH8) showed a signal attributed to (C=N) at (165.83) ppm, signals attributed to aromatic ring carbons at (149.05-122.61) ppm, a signal attributed to (CH) at (59.06) ppm, and a signal attributed to (CH2) at (34.06) ppm. As in Fig 5

The 13C-NMR spectrum of compound (MH10) showed a signal due to (C=N) at (163.22) ppm, signals due to aromatic ring carbons at (149.31-127.58) ppm, a signal due to (CH) at (54.94) ppm, and a signal due to (CH2) at (43.31) ppm. As in Figure 6

3.2. Elemental Analysis (C.H.N.O.) Measurement [32-33]

Elemental analysis (C.H.N.O) was performed on the synthesized compound to verify the accuracy and precision of its structural composition. The obtained element ratios were either consistent with the calculated values or very close, confirming the validity of the prepared compounds [35,36], as shown in Table No. (3).

Table (3): Results of elemental analysis (C.H.N.O) of manufactured compounds

Comp No.	Molecular Formula	Calculated				Found			
		C%	H%	N%	O%	C%	H%	N%	O%
MH6	$C_{13}H_{10}N_4O_3$	57.78	3.73	20.73	20.73	57.43	3.51	20.35	20.35
MH7	$C_{13}H_{10}ClN_3O$	60.13	3.88	16.18	6.16	59.95	3.74	16.24	6.04
MH8	$C_{13}H_{10}BrN_3O$	51.34	3.31	13.82	5.26	51.13	3.22	13.54	5.17
MH9	$C_{13}H_{10}FN_3O$	64.19	4.14	17.28	6.58	64.26	4.29	17.17	6.41
MH10	$C_{13}H_{11}N_3O$	69.32	4.92	18.67	7.10	69.04	5.02	18.58	7.01

3.3. Evaluation of the Biological Activity of Prepared Compounds

The biological activity of the compounds was tested in vitro against Gram-negative bacteria, Escherichia coli, and Gram-positive Staphylococcus aureus by agar diffusion test [34-39]; dip a sterile cotton swab in the prepared suspension and wipe its surface homogeneously on a Mueller-Hinton agar plate. Make three wells of 7 mm diameter on the agar gel at 20 mm intervals and add 100 µl of the prepared dilution concentrations (0.001, 0.01, 0.1) to each well[40-44]. Dimethyl sulfoxide was used as a solvent. One of the wells was filled with dimethyl sulfoxide or ethanol to observe the effect of the solvent. The plates were incubated for 24 h at 37 °C (without transfer), growth was observed, and growth inhibition was measured in cm[45-47]. as shown in Table 3 Scheme 2 and Figures 7&8

Table (3): Antibacterial activity of the synthesized compounds (inhibition zone in cm).

Comp. No.	E. Coil Conc. mg/ml			Staph. Aureus Conc. mg/ml		
	0.001	0.01	0.1	0.001	0.01	0.1
MH6	1	1.7	2.2	1	2.3	3.3
MH7	1	1.4	2	0.5	2.1	2.7
MH8	0.7	1.5	1.9	0.4	1.5	2
MH9	1	3.5	3.5	0	1.2	2.8
MH10	2	2	2.5	0.3	1	1.5
Amoxicillin	2.1	3.4	4.5	1.4	2.2	3

3.4.Results of measuring the laser activity of the prepared compounds

The study showed that the physical properties of the compounds did not change during the time periods (15, 30, 45) seconds because the compounds maintained their structural shape and physical properties during these time periods and were not affected by the laser beams. However, during this period (60 seconds), a change in the physical properties of all the studied compounds was observed, with a clear decrease in the melting point and a slight change in color. These changes may indicate the presence of a chemical substance. Some of the existing bonds in the compound are broken, and new compounds may be produced by continuous exposure to high energy (laser beams) for a long period (60 seconds), resulting in the breaking and formation of new bonds[48], as shown in Table (4)

Table (4): the effect of laser beams on some prepared compounds (MH6-MH10)

	15 S		30 S		45 S		60 S	
Comp No.	**Color**	**M.P (^{0}C)**	**Color**	**M.P (^{0}C)**	**Color**	**M.P (^{0}C)**	**Color**	**M.P (^{0}C)**
MH6	Whit	243-245	Whit	243-245	Whit	243-245	Light Yellow	229-231
MH7	Dark Brown	231-233	Dark Brown	231-233	Dark Brown	231-233	Brown	221-223
MH8	Light Orange	222-224	Light Orange	222-224	Light Orange	222-224	Orange	216-219
MH9	Yellow	254-246	Yellow	254-246	Yellow	254-246	Light Yellow	233-235
MH10	Light Yellow	248-250	Light Yellow	248-250	Light Yellow	248-250	Dark Yellow	240-242

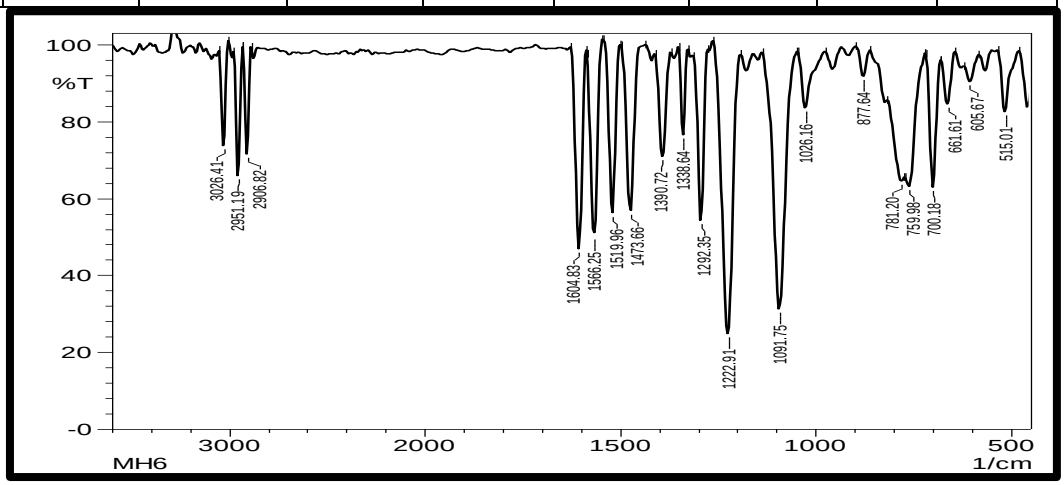


Figure (1): The compound's FT-IR spectra (MH6).

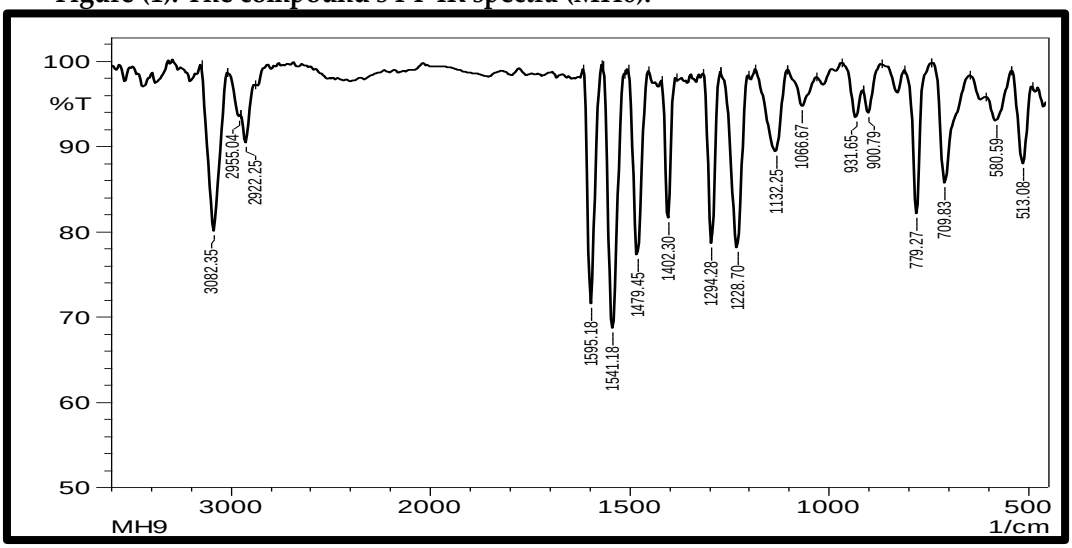


Figure (2): The compound's FT-IR spectra (MH9).

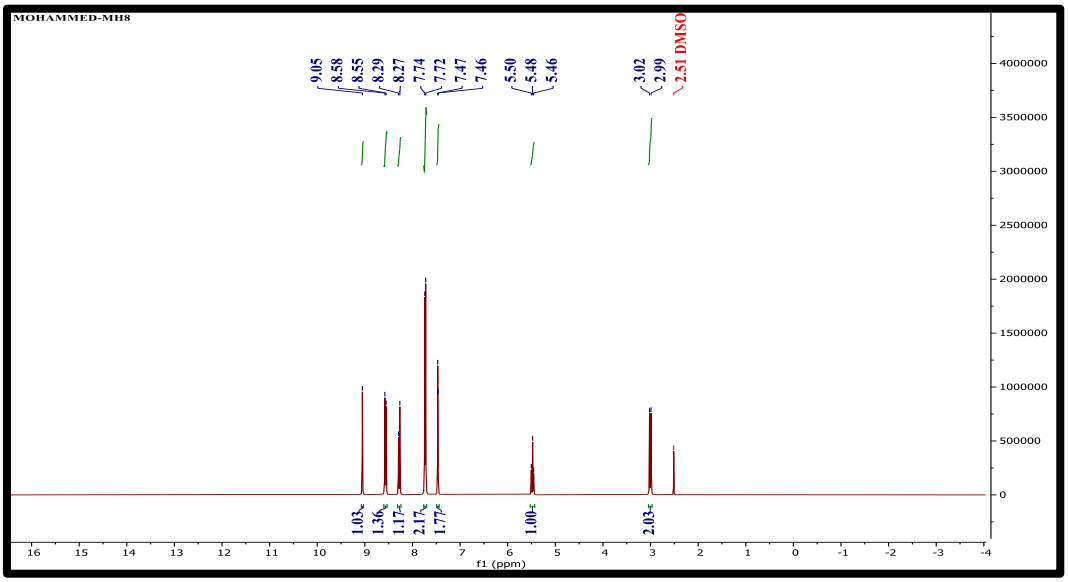


Figure (3): 1-H NMR spectra of the substance (MH8).

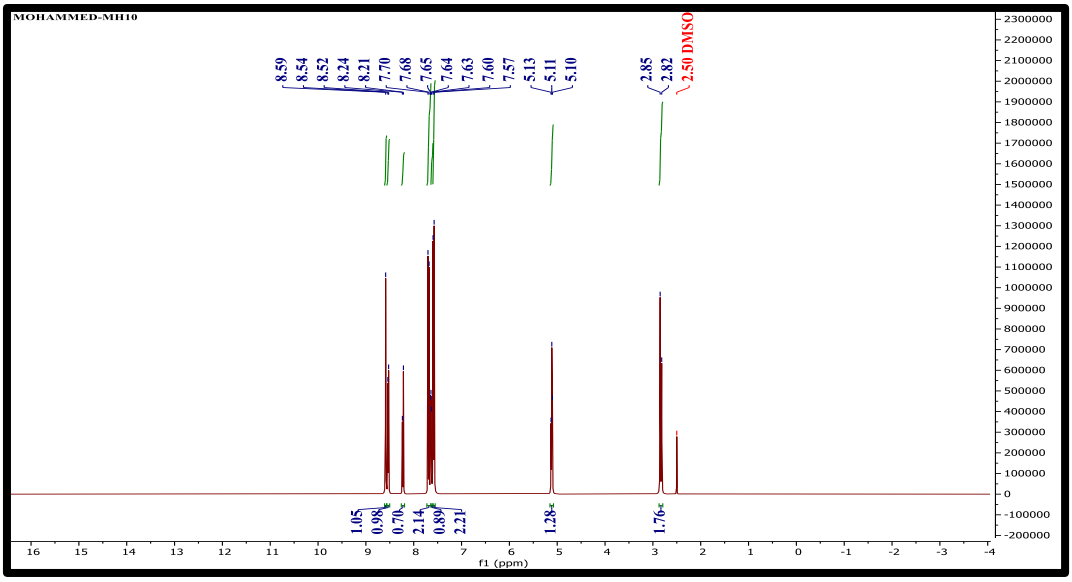


Figure (4): 1-H NMR spectra of the substance (MH10).

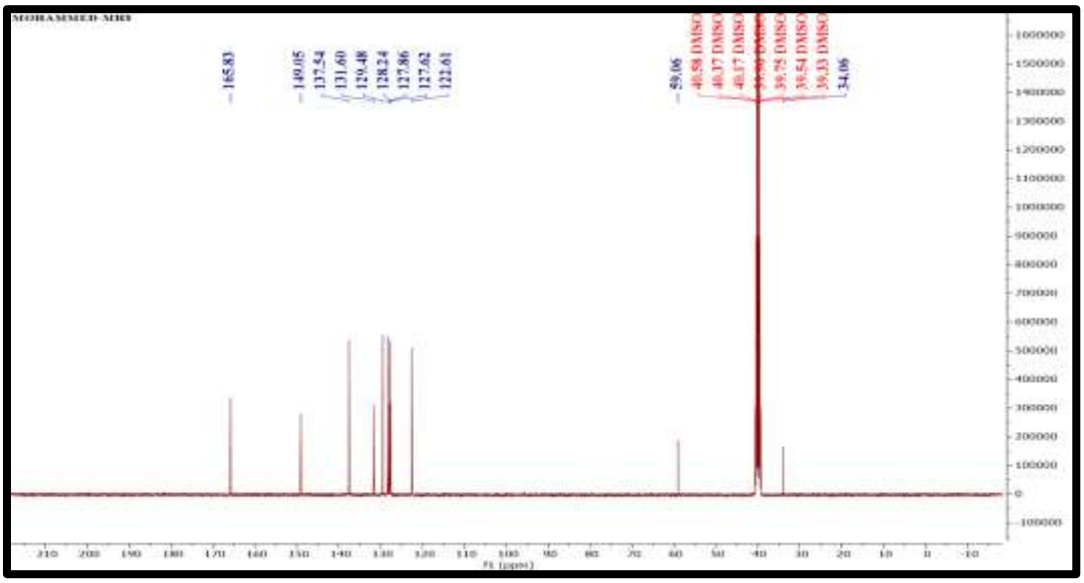


Figure (5): 13C-NMR spectra of the substance (MH8).

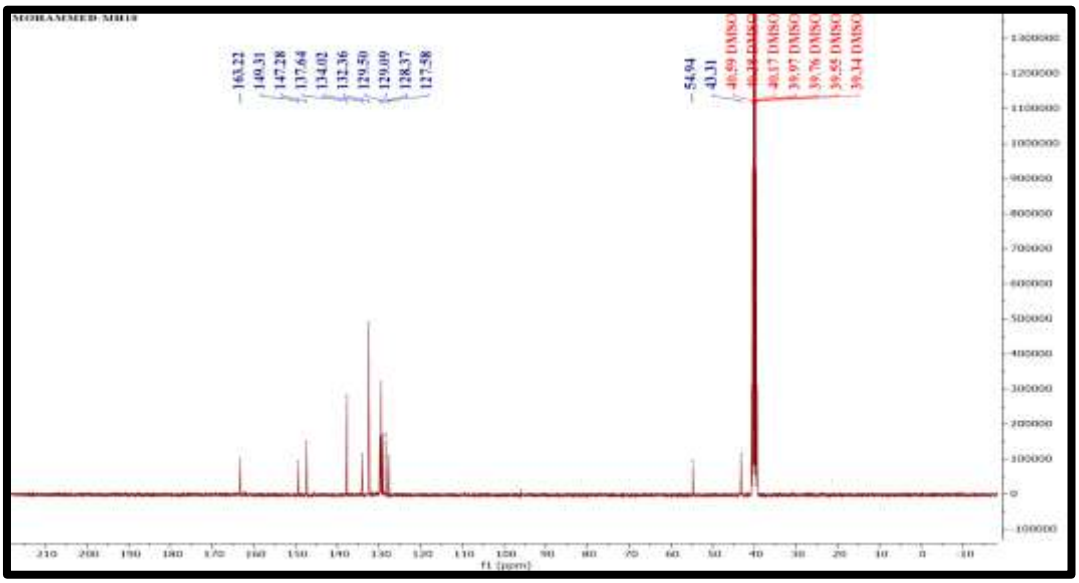
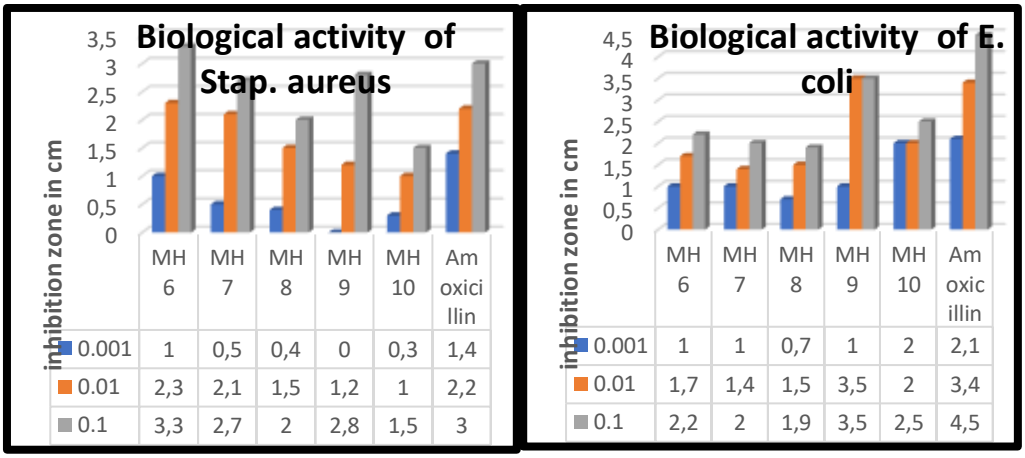


Figure (6): 13C-NMR spectra of the substance (MH10).



Scheme (2): Inhibitory activity of (MH6-MH10) for Staph. aureus & E.Coli

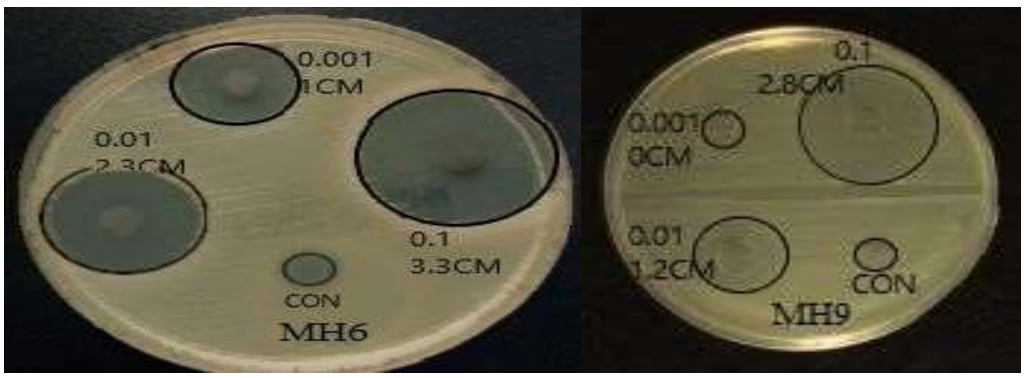


Figure 7: Efficacy of MH6 and MH9 against Staph.aureus bacteria.

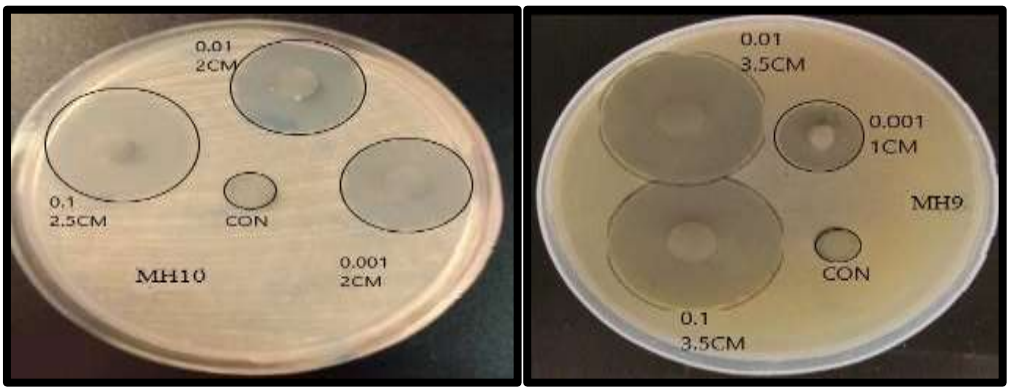


Figure 8: Efficacy of MH10 and MH9 against E.Coli bacteria.

4. Discussion

The study focuses on synthesizing novel isoxazoline derivatives using chalcones as nuclei, followed by a detailed analysis of their antibacterial activity and laser stability. The compounds were characterized through spectroscopic methods (FT-IR, 1H-NMR, and 13C-NMR) and elemental analysis, confirming their structural accuracy and purity.

Biological Activity: The synthesized derivatives demonstrated varying levels of antibacterial efficacy against Escherichia coli (Gram-negative) and Staphylococcus aureus (Gram-positive). Notably, compound MH9 exhibited the highest inhibition against E. coli (3.5 cm), while MH6 showed significant inhibition against S. aureus (3.3 cm). These results are comparable to the standard antibiotic amoxicillin, underscoring the potential of these compounds as alternatives in combating antibiotic-resistant bacterial strains.

Laser Stability: The compounds maintained their physical and structural integrity when exposed to helium-neon laser irradiation for up to 45 seconds. However, prolonged exposure (60 seconds) caused changes in melting points and color, indicating bond disruption and potential new compound formation. This highlights the compounds' resilience under controlled laser exposure, with implications for their use in environments involving high-energy irradiation.

Spectroscopic Characterization: The FT-IR and NMR data validated the successful synthesis of isoxazoline rings, showcasing characteristic bands and signals corresponding to functional groups like C=N, C-O, and aromatic rings. The close alignment of experimental elemental analysis with theoretical values further supports the compounds' precise formulation.

5. Conclusion

The reactions of chalcones with amine hydroxide revealed five-membered rings derived from isoxazoline. The prepared compounds showed high purity and accuracy in the nuclei as shown by the elemental analysis (C.H.N.O) and high purity in spectroscopic measurements such as FT-IR and 1H&13C NMR. These compounds also showed high efficacy against the two types of bacteria used in the study, as compound (MH6) showed the highest efficacy against Staphylococcus aureus with an inhibition diameter of 3.3 cm, and compound (MH9) showed the highest inhibition against Escherichia coli with an inhibition diameter of 3.5 cm, which is a high efficacy comparable to the antibiotic amoxicillin used in the study. These compounds also showed high stability towards the helium-neon laser during the period from 15 to 45 seconds and a change in melting points and color at 60 seconds, indicating their tolerance to laboratory conditions.

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