

PREPARATION OF OXAZEPINE DERIVATIVES BY FUSION METHOD AND EVALUATION OF BIOLOGICAL AND LASER ACTIVITY

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Abstract: In this study, some oxazepine-4,7-dione derivatives (SH8-SH13) were prepared from Schiff bases by reacting them with maleic anhydride without using solvents, using the fusion method, which is considered a safe, environmentally friendly, cost-effective, and time-saving method. The compounds were characterized using several spectroscopic techniques, such as FT-IR, UV-Vis, NMR, and GC-Mass. The structural results confirmed the successful synthesis process and the formation of the distinctive seven-membered ring of oxazepine compounds. The biological study showed that the prepared compounds possessed moderate to good activity against the two types of bacteria studied, *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive). Some compounds, such as SH10 and SH12, outperformed the others in terms of the diameter of the inhibition zone. It is worth noting that their activity against negative bacteria was higher than their activity against positive bacteria, likely due to the difference in cell wall composition. The compounds were irradiated with a helium-neon laser for four time periods (15, 30, 45, and 60 seconds), and then their physical properties were studied again. The compounds showed stability in the direction of the periods from 15 to 45 seconds, but at 60 seconds, a difference was found in the color, melting point, and RF.

Keywords: Oxazepine, biological activity, laser.

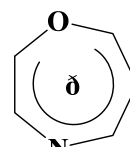
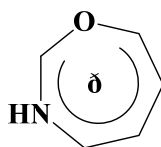
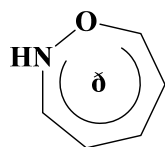
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1. Introduction

These days, the growing resistance of microorganisms to the current antimicrobial medications has led to an expansion of the worldwide health issue. Researchers have focused on creating and modifying novel antimicrobial medications to address this problem, which has prompted them to produce novel antibiotics with low toxicity, affordable prices, and high biological activity [1]. An azomethine ($-C=N-$) linkage found in Schiff bases can join two or more physiologically active aromatic/heterocyclic scaffolds to create a variety of molecular hybrids with intriguing biological characteristics [2]. In the ($-C=N-$) azomethine bond, the nucleophile of nitrogen and the electrophile of carbon provide remarkable binding for joining the electrophilic and nucleophilic functional groups. It has been discovered that if the imine nitrogen atom participates in hydrogen bonding, hydrolytic breakage of the azomethine bond can be avoided in certain Schiff bases, such as the tetraimino diphenol macrocyclic Schiff base [3]. In medical

and pharmaceutical chemistry, Schiff bases are a significant family of extensively used organic compounds with a wide range of biological actions, including antibacterial, analgesic, and anti-inflammatory [4, 5]. Oxazepine compounds have garnered significant interest from scientists and chemists due to their unique structural features and versatile chemical behavior. This subject explores the intriguing nature of oxazepines, focusing on their complex molecular architecture, various synthesis techniques, and wide-ranging applications that contribute to their scientific relevance [6, 7]. They are compounds with a heterogeneous and unsaturated seven-membered ring, most often called oxazepines (85), and they may be saturated and called oxazepanes, containing five carbon atoms and two heterogeneous atoms, which are an oxygen atom and a nitrogen atom (86). There are also three isomers of oxazepines, and these isomers are numbered according to the position of the oxygen and nitrogen atoms in the seven-membered ring, which are: 1,2-, 1,3-, and 1,4-

oxazepines (87), as follows:



Tetrahydro-1,2-oxazepine Tetrahydro-1,3-oxazepine Tetrahydro-1,4-oxazepine

Central to this exploration is their characteristic seven-membered ring structure, which includes one oxygen atom and two nitrogen atoms—an arrangement that imparts distinct chemical properties [11]. Their distinct configuration distinguishes them from other heterocyclic compounds and opens the door to investigating their steric and electronic characteristics [12]. Uncovering the potential

uses of oxazepine molecules requires understanding their synthesis techniques [13]. From conventional approaches to state-of-the-art methodologies, researchers have created a variety of synthetic pathways, each with unique benefits and drawbacks [14]. Investigating these synthesis routes offers essential information on the viability and effectiveness of generating oxazepine molecules in a lab setting [15].

2. Experimental part

2.1. Material: Fluka, Aldrich, and BDH supplied all of the compounds utilized in this investigation, and none required additional purification.

2.2. Devices used: The Electrothermal Melting Apparatus 9300 measured the melting points. FT-IR 8400S Shimadzu spectrophotometer by KBr disc, 400–4000 cm^{-1} scale. Bruker equipment operating at 400 MHz produced ^1H -NMR and ^{13}C -NMR spectra. Tikrit University's Shimadzu UV-1800 spectrophotometer has quartz cells measuring between 200 and 800 nanometers. Mass spectrum of Samarra Hall using Shimadzu GC-MS-QP2010Plus, range 900-300 m/z . Using Fluka silica gel plates that were 0.2 mm thick and activated with fluorescent silica gel G, Thin Layer Chromatography (TLC) was carried out. UV light was used to see the results. The Raypa steam sterilizer (Spain) autoclave at the

University of Tikrit's Advanced Microbiology Research Laboratory was utilized to sterilize the microbiological medium used in the study. Petri dishes utilized for the microbiological investigation were incubated in the same lab using a Heraeus D-63450 incubator (Germany).

2.3. Preparation of oxazepine derivatives (SH₈-SH₁₃) [16]. In an appropriate heat-resistant glass beaker, combine 0.004 moles of Schiff base derivatives with 0.004 moles (0.39 g) of maleic anhydride without a solvent. Stir and thoroughly mix the mixture for 5 to 27 minutes, or until the reactants change color and consistency, then heat it gradually until it melts. After that, the product was extracted from dioxane and recrystallized. Table 1 displays the physical characteristics, percentage, reaction time, and R_f of the derivatives of oxazepine-7,4-diones (SH₈-SH₁₃).

Table 1. Physical properties of oxazepine-7,4-dione derivatives (SH₈-SH₁₃)

Comp. No.	R	Molecular Formula M.Wt g/mol	Color	M.P (°C)	R.T mint	R_f	Yield (%)
SH ₈	4-NO ₂	C ₂₈ H ₁₈ N ₆ O ₅ 518.49	Light Brown	168-170	5	0.81	62
SH ₉	4-F	C ₂₈ H ₁₈ FN ₅ O ₃ 491.48	Brown	155-158	7	0.82	64
SH ₁₀	4-OH	C ₂₈ H ₁₉ N ₅ O ₄ 489.49	Brown	132-135	6	0.86	59
SH ₁₁	4-OCH ₃	C ₂₉ H ₂₁ N ₅ O ₄ 503.52	Light yellow	141-143	7	0.72	70

SH ₁₂	4-N(CH ₃) ₂	C ₃₀ H ₂₄ N ₆ O ₃	516.56	Red	145-150	23	0.76	65
SH ₁₃	3,4-di Cl	C ₂₈ H ₁₇ Cl ₂ N ₅ O ₃	542.38	Dark Red	173-176	27	0.84	68

2.4. Biological activity study. Two types of bacteria were taken from the Department of Life Sciences, Tikrit University, to study the effectiveness of the prepared compounds: Gram-negative *E. coli* and Gram-positive *Staphylococcus aureus*. The compounds were diluted to three concentrations (0.1, 0.01, and 0.001 mg/ml) using dimethyl sulfoxide as a solvent, poured into a culture dish, and wiped with the dried bacterial solution from three directions to ensure even distribution [17, 18]. A 6 mm diameter cork piercing tool was then used to make three holes in the culture dish, and the solution was poured into them. The culture dish was then placed in a special container at 37°C for one day, and the results were read with a centimeter ruler. The two antibiotics,

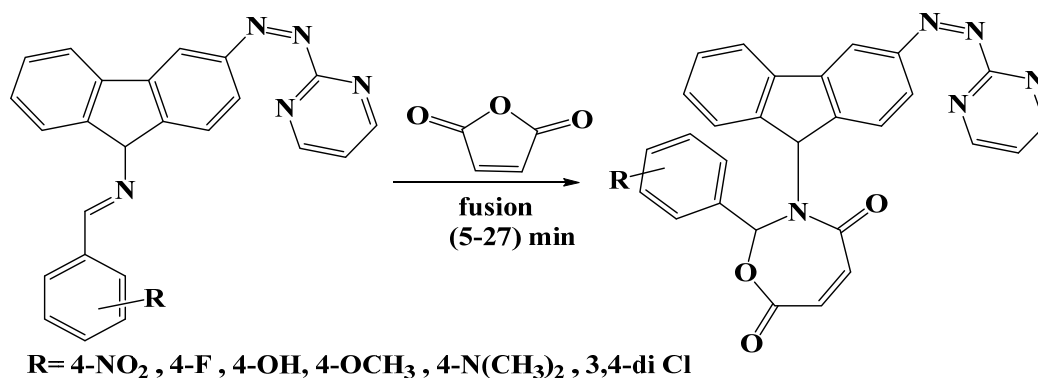
ciprofloxacin and amikacin, were used as control samples [19, 20].

2.5. Laser efficacy of some of the prepared compounds. To study the efficacy of the prepared compounds, the compounds were diluted to three concentrations (0.1, 0.01, and 0.001 mg/ml) using DMSO as solvent, poured into the culture dish, and wiped with the dried bacterial solution from three directions to ensure even distribution. Then, a 6 mm diameter cork borer is used to make three holes in the culture dish, and the solution is poured into them. Then, place the culture dish in a special container at 37°C for one day, and read the results with a millimetre ruler. The antibiotics Ciprofloxacin and Amikacin were used as controls [21, 22].

3. Results and discussion

1,3-oxazepine-4,7-dione derivatives [SH₈-SH₁₃] are prepared by reacting equal amounts of Schiff base derivatives [SH₂-SH₇] with maleic

anhydride without using a solvent, as shown in the following formula:



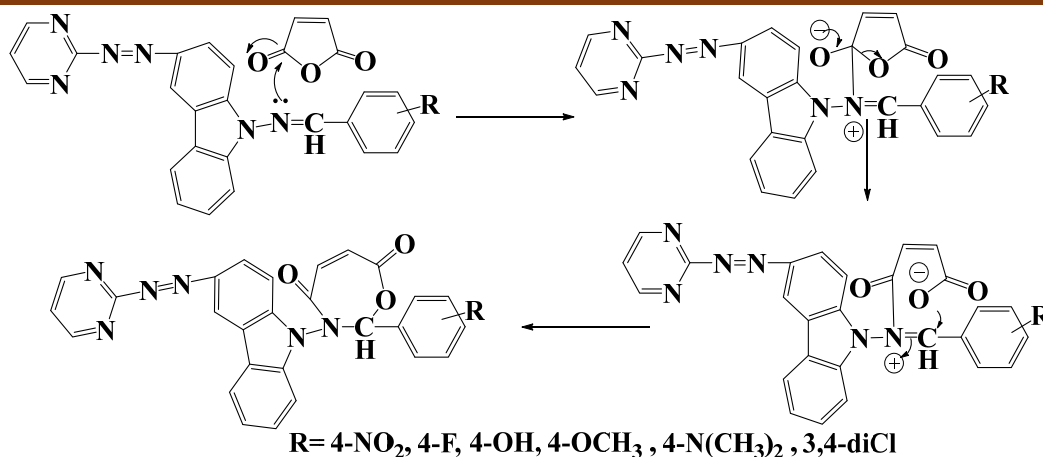
Scheme 1. Route of prepared compounds (SH₈-SH₁₃)

Scheme 2 shows the mechanism of formation of oxazepines upon reaction of Schiff bases with anhydride in detail.

3.1. Characterisation of oxazepine derivatives (SH₈-SH₁₃). Using anhydrous ethanol as a solvent, and when the concentration range of the prepared compounds was 10⁻⁵-10⁻⁴ molar, the UV-Vis spectra of the prepared compounds [SH₈-SH₁₃] were studied, and it was found that the short wavelength (λ_{1max}) appeared at 221-267 nm, which was caused by

the ($\pi \leftarrow \pi$) type electronic transition, and the long wavelength (λ_{2max}) was in the range of 312-324 nm, which was caused by the ($\pi \leftarrow n$) type electronic transition [23], as shown in Table 2.

The infrared (IR) spectra of the investigated compounds reveal the absence of the azomethine stretching band typically at 1629-1632 cm⁻¹, which corresponds to the observed (C=N) group located outside the ring in the synthesized Schiff base compounds [SH₂-SH₇].



Scheme 2. Mechanism of formation of oxazepines

This observation, based on the IR analysis of compounds [SH₈–SH₁₃], confirms structural modifications. Instead, a medium-intensity band appears within the range of 3130–3165 cm⁻¹, attributable to the olefinic (C–H) stretching vibrations, alongside a distinct absorption band in the 3046–3076 cm⁻¹ range, associated with aromatic (C–H) stretching. Additionally, aliphatic (C–H) stretching is also observed. Two prominent absorption bands at 1707–1712 cm⁻¹ and 1668–1677 cm⁻¹ are indicative of carbonyl (C=O) stretching in lactone and lactam groups, respectively. The presence of a medium-intensity band within 1619–1626 cm⁻¹ corresponds to

(C=C) stretching within the seven-membered ring, while a band in the 1593–1602 cm⁻¹ range is attributed to (C=N) stretching within the pyrimidine ring. Moreover, bands appearing at 1549–1563 cm⁻¹ and 1467–1489 cm⁻¹ are associated with aromatic (C=C) stretching vibrations. The spectra also display intermediate bands between 1419 and 1452 cm⁻¹, which are linked to the (N=N) group. Additional bands detected in the 1209–1244 cm⁻¹ range are assigned to (C–N) stretching vibrations [24]. These spectral data are detailed in Table 2 and illustrated in Figs. 1 and 2.

Table 2. Infrared and UV spectrum of the prepared compounds (SH₈–SH₁₃)

Comp. No.	λ max ₁ λ max ₂ EtOH	R	IR (KBr) cm ⁻¹						Others
			C-H Arom. Oliph.	C-H Aliph.	ν C=O Lactone Lactam	ν C=N ν C=C Oliph.	ν C=C Arom.	ν N=N ν C-N	
SH ₈	221 316	4-NO ₂	3046 3143	2908	1711 1671	1621 1598	1563 1467	1423 1231	ν (NO ₂) <i>asy.</i> (1507) <i>sym.</i> (1321)
SH ₉	267 314	4-F	3076 3153	2894	1712 1669	1623 1599	1556 1474	1435 1209	ν (C-F) 822
SH ₁₀	249 324	4-OH	3051 3153	2951	1709 1677	1625 1602	1560 1489	1440 1244	ν (OH) 3394
SH ₁₁	256 312	4-OCH ₃	3053 3165	2935 2867	1707 1673	1623 1601	1558 1481	1427 1217	ν (C-O-C) <i>asy.</i> (1362) <i>sym.</i> (1271)
SH ₁₂	243 314	4-N(CH ₃) ₂	3072 3148	2941 2873	1708 1668	1619 1601	1549 1476	1419 1224	ν (C-H) <i>asy.</i> (2941) <i>sym.</i> (2873)
SH ₁₃	246 323	3,4-diCl	3055 3130	2943	1708 1668	1626 1593	1558 1487	1452 1213	ν (C-Cl) 694

The $^1\text{H-NMR}$ spectrum of compound [SH₁₀], recorded in DMSO-*d*₆, reveals distinct proton environments corresponding to the functional groups within the molecule. A singlet observed at $\delta = 9.68$ ppm is attributed to the hydroxyl (OH) proton. A singlet at $\delta = 8.60$ ppm corresponds to the proton of the methine (CH) group within the oxazepine ring. Aromatic protons produced a

multiplet within the chemical shift range of $\delta = 6.71$ – 8.02 ppm. Additionally, the olefinic proton (=CH) adjacent to the lactam moiety in the oxazepine ring appeared as a doublet at $\delta = 6.53$ and 6.55 ppm, while the olefinic proton next to the lactone group appeared as a doublet at $\delta = 6.45$ and 6.47 ppm.

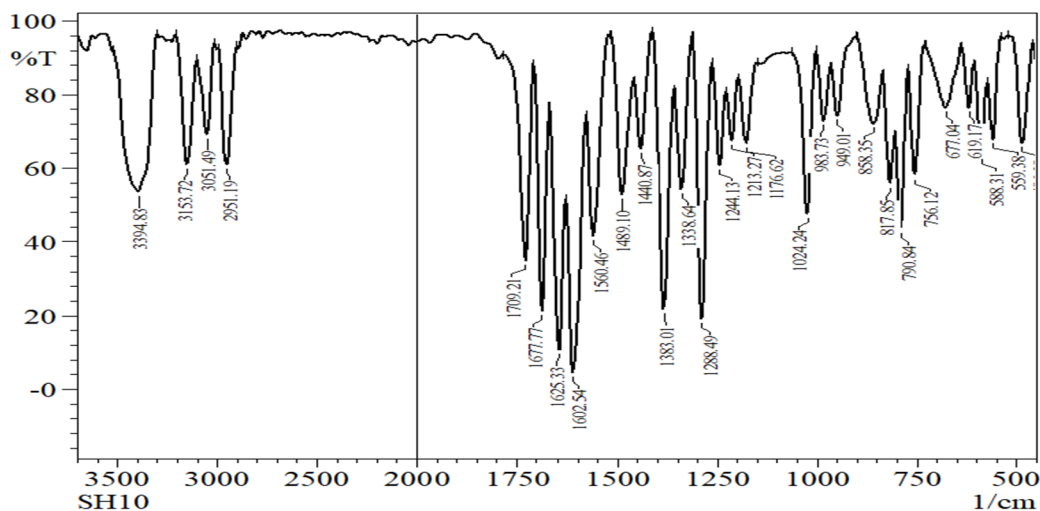


Fig. 1. FT-IR spectrum of the compounds (SH₁₀)

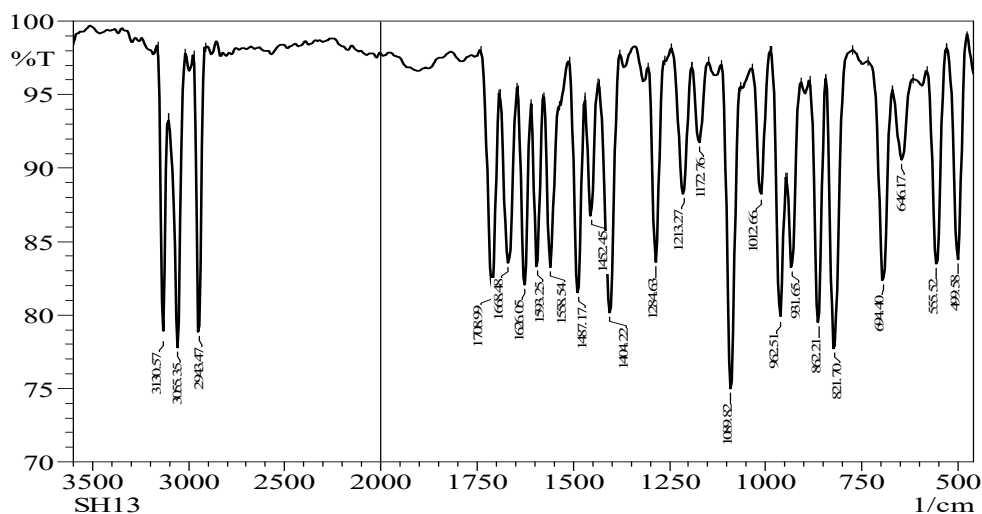


Fig. 2. FT-IR spectrum of the compounds (SH₁₃)

A signal at $\delta = 2.48$ ppm is associated with the residual protons of the DMSO-*d*₆ solvent, as shown in Fig. 3.

The $^{13}\text{C-NMR}$ spectrum of [SH₁₀], also recorded in DMSO-*d*₆, confirms the presence of carbonyl carbons from both lactam and lactone groups, exhibiting signals at $\delta = 163.79$ and 167.25 ppm, respectively. The aromatic carbons show a broad range of signals between $\delta = 114.22$ and 173.87 ppm. The sp^2 hybridized carbons of the (CH=CH) moiety give rise to signals at $\delta = 121.98$ and 134.30 ppm. An additional signal at

$\delta = 99.15$ ppm corresponds to another (CH=CH) carbon environment. The methine carbon (CH) within the oxazepine ring appears as a signal within the range of $\delta = 39.30$ – 40.55 ppm. These assignments are supported by the data illustrated in Fig. 4.

The mass spectrum of [SH₁₀], as shown in Fig. 5, showed a distinct peak at $m/z = 490$, which matches the compound's predicted molecular weight, providing strong evidence for the prepared compound's correct structural formula. A base peak was also observed at $m/z = 121$,

which represents the most stable and relatively abundant fragment, further supporting the compound's hypothesized structure. These spectral data reinforce the other structural results and confirm the success of the preparation process.

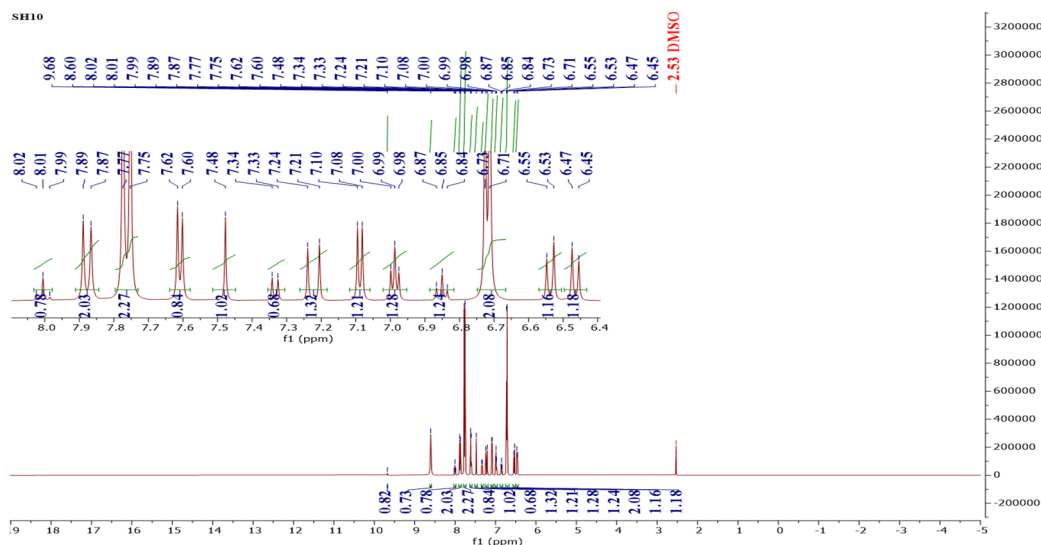


Fig. 3. ¹H-NMR spectrum of the compounds (SH₁₀)

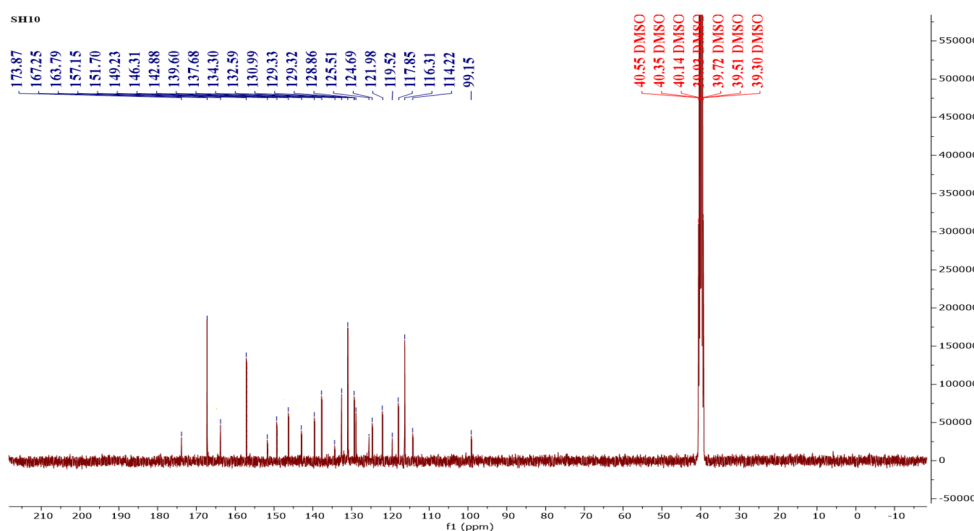


Fig. 4. ¹³C-NMR spectrum of the compounds (SH₁₀)

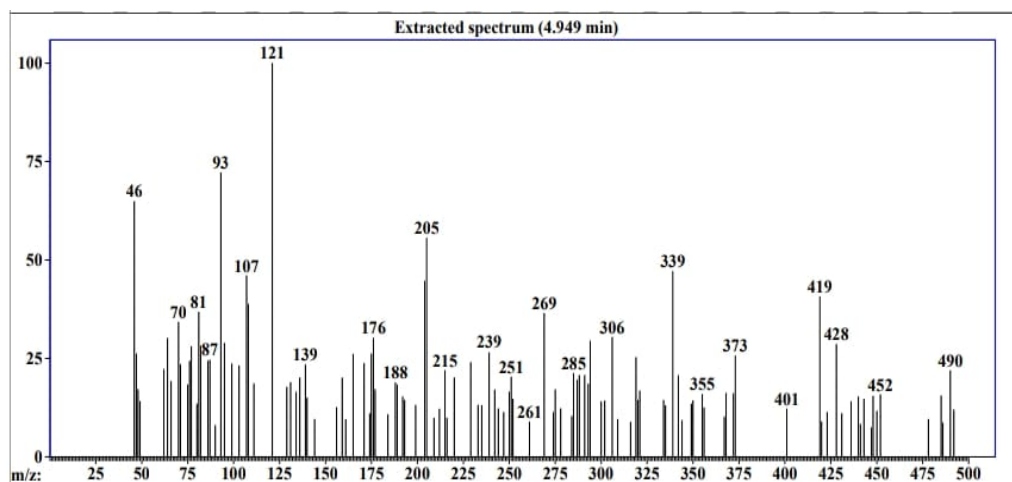


Fig. 5. Mass spectrum of the compounds (SH₁₀)

3.2. Evaluation of the Biological Activity of prepared Compounds. The prepared compounds showed varying effectiveness against the two types of bacteria used, due to the nature of the membrane of these bacteria, as the compounds were more effective against negative bacteria, as compound SH₁₀ recorded the highest effectiveness against negative bacteria with a diameter of 1.5 cm at high concentrations of 0.1 mg/ml, followed by compound SH₁₂ with an inhibition diameter of 1.2 cm, compared to the

antibiotics, which showed an inhibition diameter of 1.9 cm for *Ciprofloxacin* and 1.4 cm for *Amikacin* [25-29]. As for the effectiveness of the compounds against positive bacteria, their effect was less than against negative bacteria, as none of the compounds exceeded 1 cm. Their inhibition is due to the resistance of bacteria to these compounds, while the antibiotics showed an effectiveness against them, reaching 1.5 cm against Ciprofloxacin and 1.3 cm for Amikacin [30-33], as shown in Table 3 and Fig. s 6 and 7.

Table 3. Biological effectiveness of prepared compounds and control treatments (inhibition in cm)

Comp. No.	<i>Escherichia coli</i>			<i>Staphylococcus aureus</i>		
	0.1	0.01	0.001	0.1	0.01	0.001
Conc. mg/ml	0.1	0.01	0.001	0.1	0.01	0.001
SH ₈	0.6	0.9	0.5	0.6	0.3	0.4
SH ₉	0.8	0.2	0.7	0.5	0.3	0.3
SH ₁₀	1.5	0.5	0.5	0.7	0.5	0.8
SH ₁₁	1	0.4	0.4	0.8	0.4	0.2
SH ₁₂	1.2	0.6	0.3	0.5	0.5	0.5
SH ₁₃	0.9	0.6	0.6	0.5	0.3	0
Ciprofloxacin	1.9	1.5	1	1.5	1.3	1
Amikacin	1.4	1	0.8	1.3	1.1	1

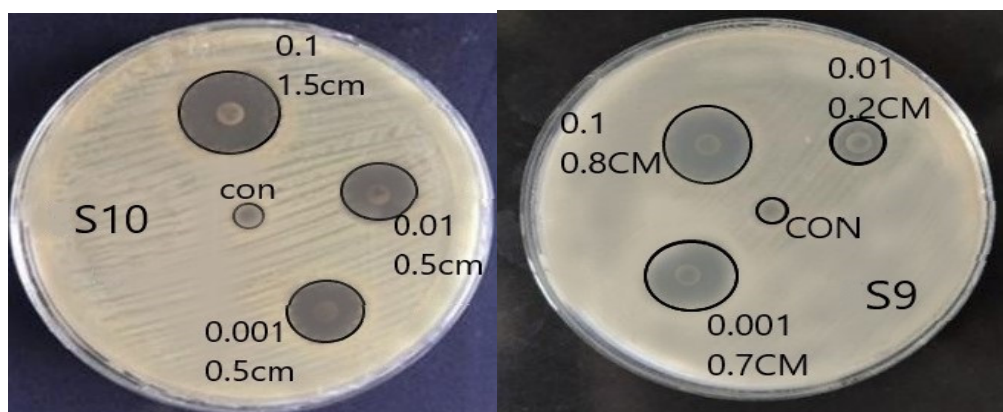


Fig. 6. Efficacy of compounds (SH₉, SH₁₀) against *Escherichia coli* bacteria

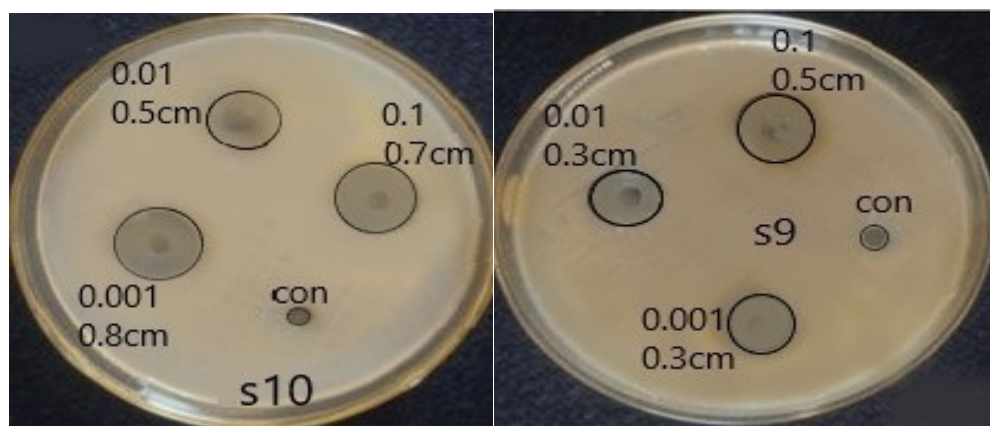


Fig. 7. Efficacy of compounds (SH₉, SH₁₀) against *Staphylococcus aureus* bacteria

3.3. Results of measuring the laser activity of the prepared compounds. The compounds' structural morphology and physical characteristics were unaffected by the laser beam for 15, 30, and 45 seconds, indicating that their physical characteristics stayed constant. The physical characteristics of each molecule under study, however, varied throughout the course of the 60 seconds. They showed a modest change in color, a shift in the thin-layer chromatography (TLC) flow rate (Rf) value, and a notable drop in melting point. These changes may be due to the

breaking of some bonds in the compounds or continuous exposure to high energy (laser beam) for a long period (60 seconds), leading to the breaking of bonds and the formation of new compounds. These changes may be due to the dissociation of some chemical bonds in the compounds or the formation of new bonds due to the absorption of laser energy, indicating the onset of decomposition or structural rearrangement within the molecules [34, 35], as shown in Table 4.

Table 4. Laser irradiation results of the prepared compounds (SH₈-SH₁₃)

Comp No.	(15)Second			(30)Second			(45)Second			(60)Second		
	Color	M.P (°C)	Rf	Color	M.P (°C)	Rf	Color	M.P (°C)	Rf	Color	M.P (°C)	Rf
SH ₈	Light Brown	168-170	0.81	Light Brown	168-170	0.81	Light Brown	168-170	0.81	Brown	156-158	0.76
SH ₉	Brown	155-158	0.82	Brown	155-158	0.82	Brown	155-158	0.82	Light Brown	149-151	0.75
SH ₁₀	Brown	132-135	0.86	Brown	132-135	0.86	Brown	132-135	0.86	Orange	121-123	0.81
SH ₁₁	Light yellow	141-143	0.72	Light yellow	141-143	0.72	Light yellow	141-143	0.72	yellow	131-133	0.65
SH ₁₂	Red	145-150	0.76	Red	145-150	0.76	Red	145-150	0.76	Orange	163-165	0.80
SH ₁₃	Dark Red	173-176	0.84	Dark Red	173-176	0.84	Dark Red	173-176	0.84	Red	166-169	0.79

4. Conclusions

The fusion method represents an efficient and economical approach for the synthesis of heterocyclic compounds, offering advantages in both cost and time while providing high yields. Spectrophotometric analyses confirmed the validity and accuracy of the synthesized products, which exhibited high purity as evidenced by their infrared (IR), proton (¹H) and carbon (¹³C) NMR, and mass spectra. The

compounds demonstrated notable antibacterial activity against two bacterial strains, despite the latter's high resistance to conventional antibiotics. Furthermore, the synthesized compounds showed remarkable stability under laser irradiation over extended periods, indicating strong resistance to environmental and laboratory conditions.

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