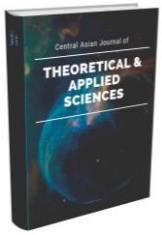

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*Article*

# Synthesis, Characterization, and Validation Of Novel Thiazine Derivatives For Their Biological And Laser Activity

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**Abstract:** The research involves the use of chalcones as intermediates, the synthesis of new hexagonal rings from thiazine precursors by reacting previously prepared chalcones with thiourea, and minor modifications to the usual working methods to show that they generate these new rings. The activity of the prepared compounds was proven by physical and spectroscopic techniques such as FT-IR and (1H, 13C-NMR) spectroscopy, as well as the determination of melting point and purity and monitoring the progress of the reaction by thin layer chromatography. The TLC was evaluated using two bacterial isolates known to be resistant to antibiotics, namely Gram-negative Escherichia coli (Gram-ve) and Gram-positive Staphylococcus aureus (Gram+ve). They were compared with the antibiotic amoxicillin as a control agent, and the results showed good inhibitory activity against both types of bacteria used, with high efficacy and selectivity. A helium-neon laser (visible laser) was used to evaluate the laser efficiency of some of the synthesized compounds. Each compound was irradiated for four different periods (15, 30, 45, and 60 seconds) and then physically irradiated. The compounds were examined again, and the surface changes of the compounds were observed.

**Keywords:** Heterocyclic, Thiazine, Laser, Biological Activity

## 1. Introduction

Ring configurations with distinct atoms, such as nitrogen, sulfur, or oxygen, are found in heterocyclic molecules. These substances are abundant in the natural world. They are vital and utilized in a variety of domains, including industry and medicine. These substances are involved in the creation of proteins, enzymes, nucleic acids, and Carbohydrates and their compounds [1]. Heterocyclic compounds may contain several heteroatoms and are classified according to the kind and number of atoms in the ring.[2]. Chalcone and thiourea combine to generate thiazine, a hexagonal ring with four carbon atoms, one nitrogen atom, and one sulfur atom. [3]. These compounds have attracted the attention of the medical community, especially in the pharmaceutical field, as they have shown antibacterial efficacy [4], anticancer efficacy [5], and good anti-inflammatory efficacy [6]. Lasers either increase light by causing radiation to be released, or they can increase microwaves by causing radiation to be released. Because the geometries of these beams are so similar, the term "laser" is an acronym for "light amplification by stimulated emission of radiation." thin lines. Light. Because lasers emit beams of many wavelengths, they are a unique source of light. Red light is more extended than blue light, for instance—a little glimmer of brightness. Numerous industries and technologies have made use of lasers.[6]. This study aimed to prepare heterocyclic compounds derived from chalcone

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and test their bioactivity against Gram-resistant bacteria in addition to the effectiveness of lasers.

## 2. Materials and Methods

### 1) Chemical Used

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

### 2) Instuments Used

Melting points were measured with a Shimadzu 9300, FT-IR 8400S spectrometer using a KBr at 400-4000sm-1 : 1H and 13C-NMR spectra at 400 MHz on Bruker operating equipment. Thin layer chromatography (TLC) was analysed using 0.2 mm thick Fluka silica gel plates.

### 3) Preparation of Thiazine derivatives ($K_6$-$K_{10}$).[8]

10 mL of 10% sodium ethoxide was added after equal moles (0.001 mol) of the generated Chalcone and thiourea were combined in 10 mL of ethanol. The mixture was heated for six hours and then cooled. After adding the solution to crushed ice, the mixture was refrigerated for two days. Ten percent hydrochloric acid was used to neutralize the medium. As indicated in Table 1, the precipitate was filtered, dried, and recrystallized.

**Table 1.** Some physical properties of for Prepared compounds (K6-K10)

| Comp. No. | R | Molecular formula | m.p. °C | Yield% | Color |
|---|---|---|---|---|---|
| **K6** | 4-NO2 | $C_{14}H_{11}N_5O_2S$ | 191-102 | 75 | Brown |
| **K7** | 4-Cl | $C_{14}H_{11}ClN_4S$ | 212-214 | 73 | White |
| **K8** | 4-F | $C_{14}H_{11}FN_4S$ | 216-218 | 64 | Orange |
| **K9** | 4-Br | $C_{14}H_{11}BrN_4S$ | 231-233 | 71 | light yellow |
| **K10** | 4-H | $C_{14}H_{12}N_4S$ | 187-189 | 67 | Yellow |

### 4) Biological activity study

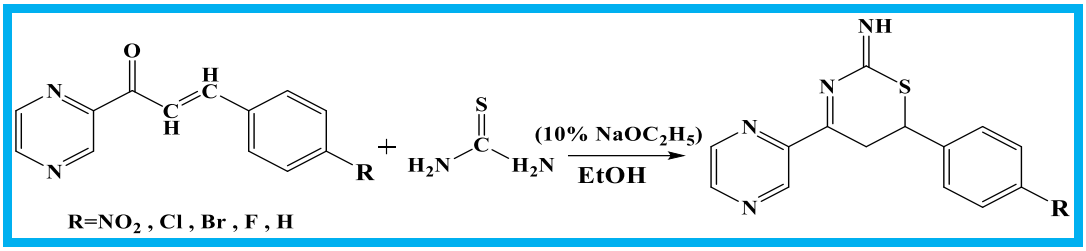
Gram-positive Staphylococcus aureus and Gram-negative Escherichia coli. The pathogen used in this research. In the laboratory of the Department of Life Sciences at Tikrit University. Dimethyl sulfoxide (DMSO) was used to create chemical solutions of K6, K7, K8, K9, and K10 at concentrations of (0.01, 0.001, and 0.0001) mg/ml [9, 10]. Measurement and tracking of minimum inhibitory concentration (MIC). The diffusion method was used to check the viability of the bacterial strains under study, while Mueller Hinton agar was used as a medium for nutrient absorption. The culture media were prepared, sterilized, and then placed in plates for solidification. Each plate was subsequently punctured with four small holes. After that, they underwent a full day of incubation at 37 °C. Derivatives. This shows the sensitivity of the derivatives. These compounds are derived from the damping properties of the plate surrounding the hole. As the diameter increases, this plate also increases. Similar to the inhibitory effects of antibiotics, the biological activity of a chemical increases when it exhibits an inhibitory effect.[12,13].

### 5) Measurement of laser effectiveness of some compounds

A helium-neon laser device (visible laser) was used to assess the laser efficacy of some of the produced compounds (k6, k7, k8, k9, and k10). Each compound was exposed to radiation for four different durations (15, 30, 45, and 60 seconds). At Tikrit University's College of Science, measurements were made of the beam source's distance (10 cm), power (1 mW), and wavelength (808 nm) from the sample. - The Laser Laboratory's Physics Department reexamined the synthesized compounds' physical characteristics following irradiation and noted any alterations [14].

## 3. Result and Discussion

The following plan was followed in the preparation of the compounds' precursors.




Scheme 1. Path of the Ready Compounds (K6-K10)

### 1) Characterization of Thiazine derivatives (K6-K10)

The FT-IR spectra revealed an absorption band in the (1621-1639) cm-1 region of the thiazine ring that was recognized as a member of the (C=N) group and an absorption in the (3016-3073)Cm-1band related to the aromatic band (CH), and the absorption band attributed to (NH) is in the region (3268-3228) cm-1. It also showed two absorption bands for aliphatic reflux (CH) in the periods (2952-2918) cm-1 and (2900-2843) cm-1. In addition, the expansion of the aromatic ring (C=C) is responsible for the two absorption bands in the range (1560-1512) cm-1 and (1467-1486) cm-1. The range in the range (773-751) cm-1 is attributed to the aperture (C-S)[15]. As shown in Table 2 and Figures 1 and 2

**Table 2.** FT-IR Absorption results for prepared compounds (K6-K10)

| Comp. No. | R | ν(C-H) Arom. | ν(C-H) Aliph. | ν(N-H) ν(C-S) | ν(C=N) | ν(C=C) Arom. | Others |
|---|---|---|---|---|---|---|---|
| **K6** | 4-NO2 | 3061 | 2945,2865 | 3268<br>751 | 1621<br>1603 | 1560,1486 | ν(N-O) as sy1521.<br>Sy1323 |
| **K7** | 4-Cl | 3024 | 2950,2843 | 3261<br>764 | 1636<br>1614 | 1531,1481 | ν (C-Cl)716 |
| **K8** | 4-F | 3073 | 2952,2900 | 3252<br>759 | 1633<br>1610 | 1558,1479 | ν (C-F)919 |
| **K9** | 4-Br | 3063 | 2931,2872 | 3265<br>773 | 1637<br>1604 | 1512,1467 | ν (C-Br) 557 |
| **K10** | 4-H | 3016 | 2918,2875 | 3228<br>756 | 1639<br>1618 | 1562,1477 | -- |

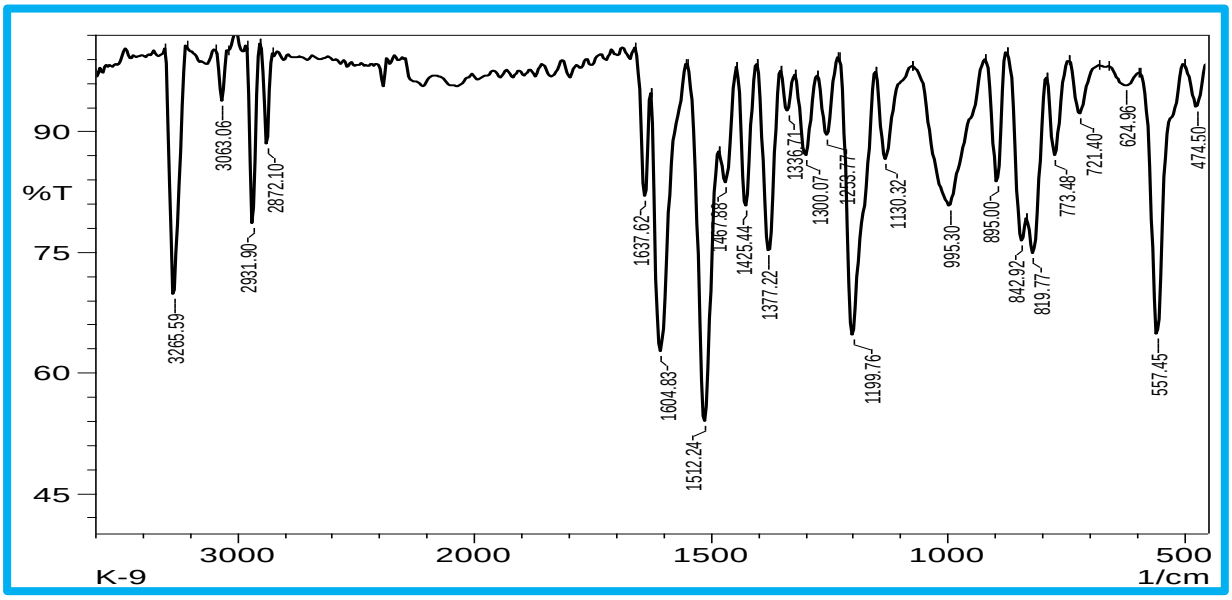



**Figure 1.** The compound's FT-IR spectra (K9)

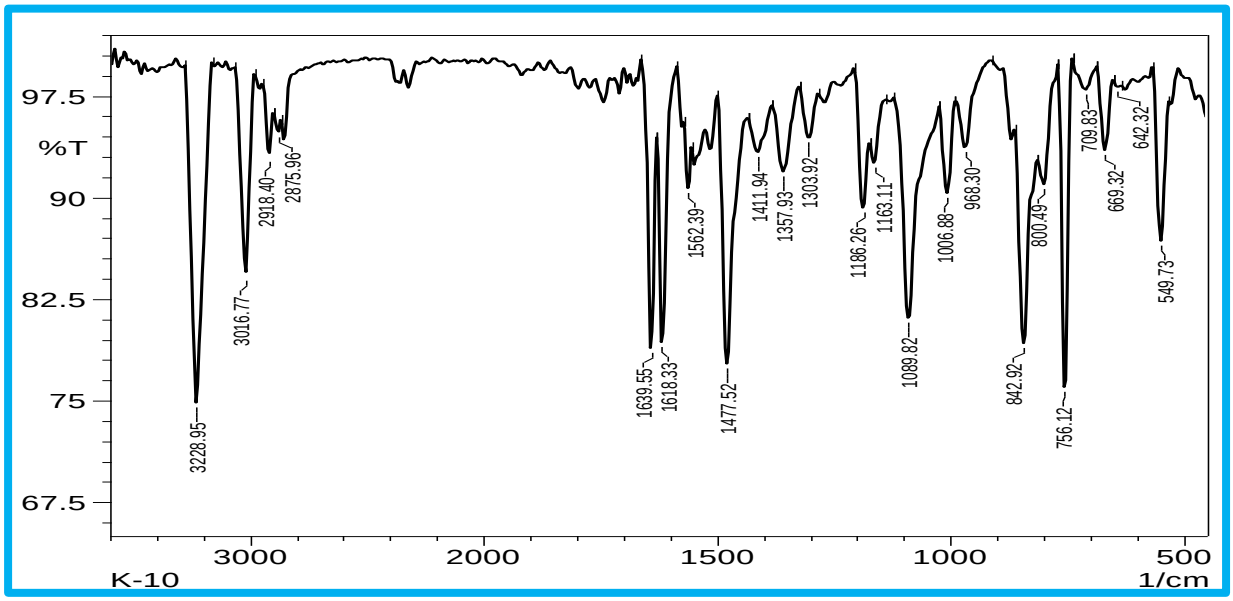



**Figure 2.** The compound's FT-IR spectra (K10)

The 1H-NMR spectrum of K8 showed a signal at (8.99) ppm for (NH) and multiple signals for aromatic rings in the range (7.22-8.63) ppm. The triplet signal is at (3.97-4.02) ppm for the (CH) thiazine ring, and the doublet signal is at (3.00, 3.02) ppm for the ($CH_2$) same ring. The position (2.51) ppm usually refers to DMSO solvent. As shown in Fig. 3

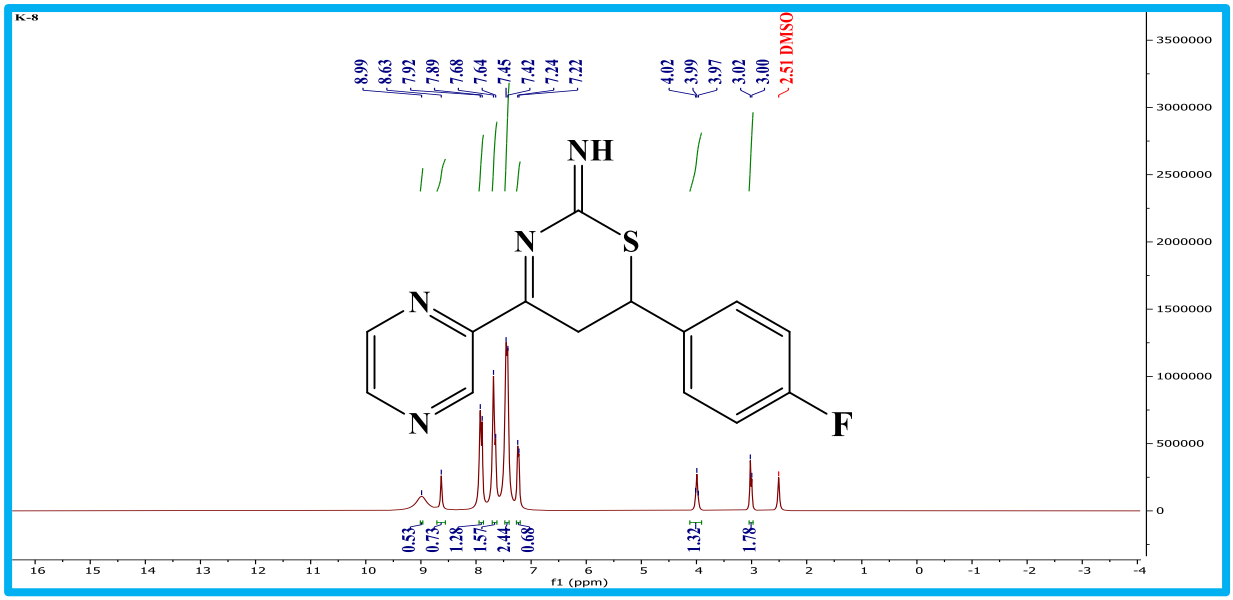



**Figure 3.** 1-H NMR spectra of the substance (K8)

The 1H-NMR spectrum of K10 showed a signal at (9.60) ppm for (NH), and the aromatic ring signals are multiple in the range of (7.03-8.69) ppm. The triplet signal is at (3.60-3.69) ppm for the (CH) thiazine ring, and the doublet signal is at (2.77,2.79) ppm for ($CH_2$) the same ring. The position (2.51) ppm usually refers to DMSO solvent. As shown in Fig. 4

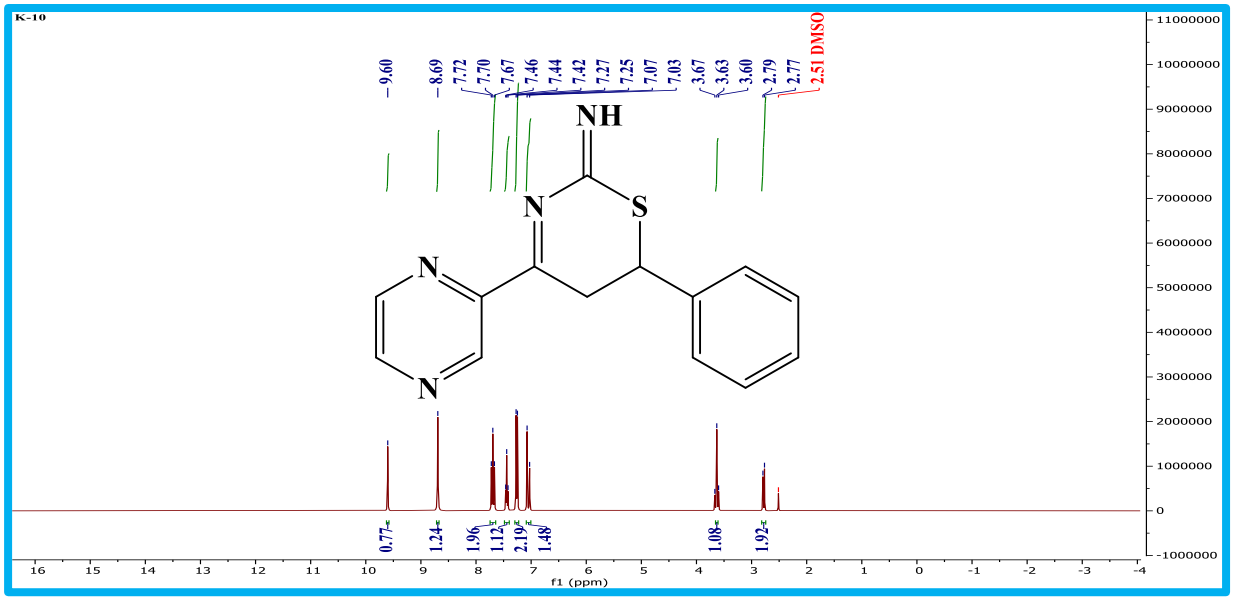



**Figure 4.** 1-H NMR spectra of the substance (K10)

When examining chemical K10 $^{13}$C-NMR spectra, The resultant ring's carbon (CH2) was recognized as the signal, which was recorded at (26.68) ppm, and a signal (60.49) ppm is often linked to the ring's resultant carbon (CH), and signals in the range (128.68-141.35) ppm to the carbons of the aromatic ring and the carbon (C=N) of the resultant ring is typically represented by the signal at position (167.44) The carbon (C=NH) is responsible for the signal at (171.05). And a signal to DMSO at position ( 39.30-40.56) ppm. As in Figure 5

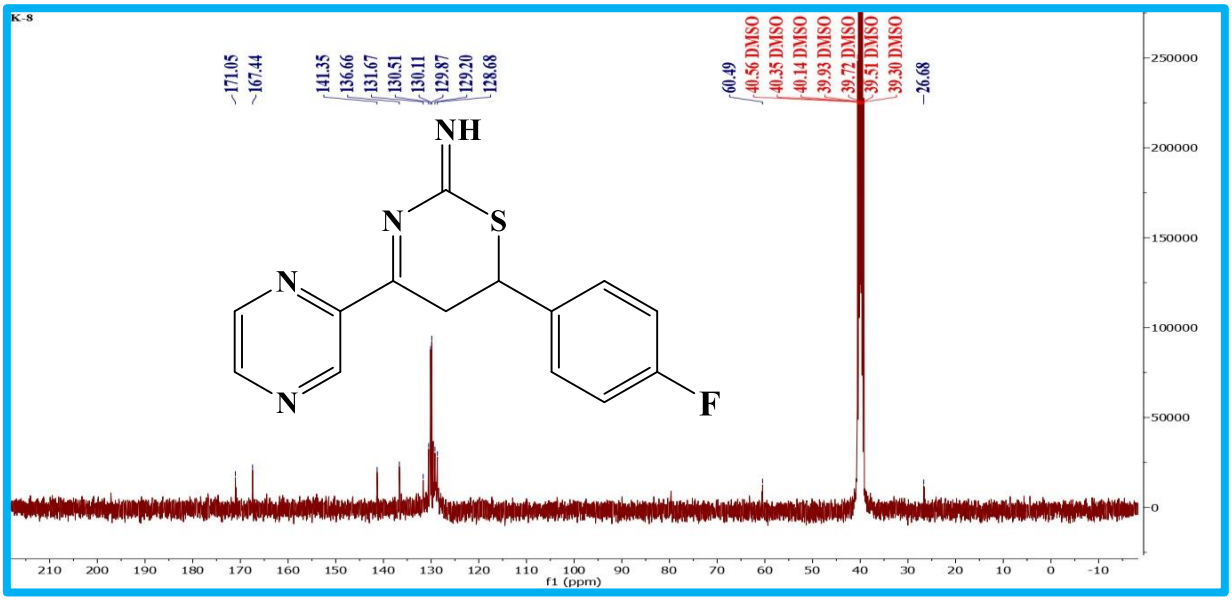



**Figure 5.** $^{13}$C-NMR Spectra of the substance (K8)

When examining chemical K10 $^{13}$C-NMR spectra, The resultant ring's carbon ($CH_2$) was recognized as the signal, which was recorded at (29.06) ppm, and a signal (65.04) ppm is often linked to the ring's resultant carbon (CH), and signals in the range (128.07-142.71) ppm to the carbons of the aromatic ring and the carbon (C=N) of the resultant ring is typically represented by the signal at position (163.71) The carbon (C=NH) is responsible for the signal at (168.75). And a signal to DMSO at position ( 39.23-40.48) ppm. As in Figure 6

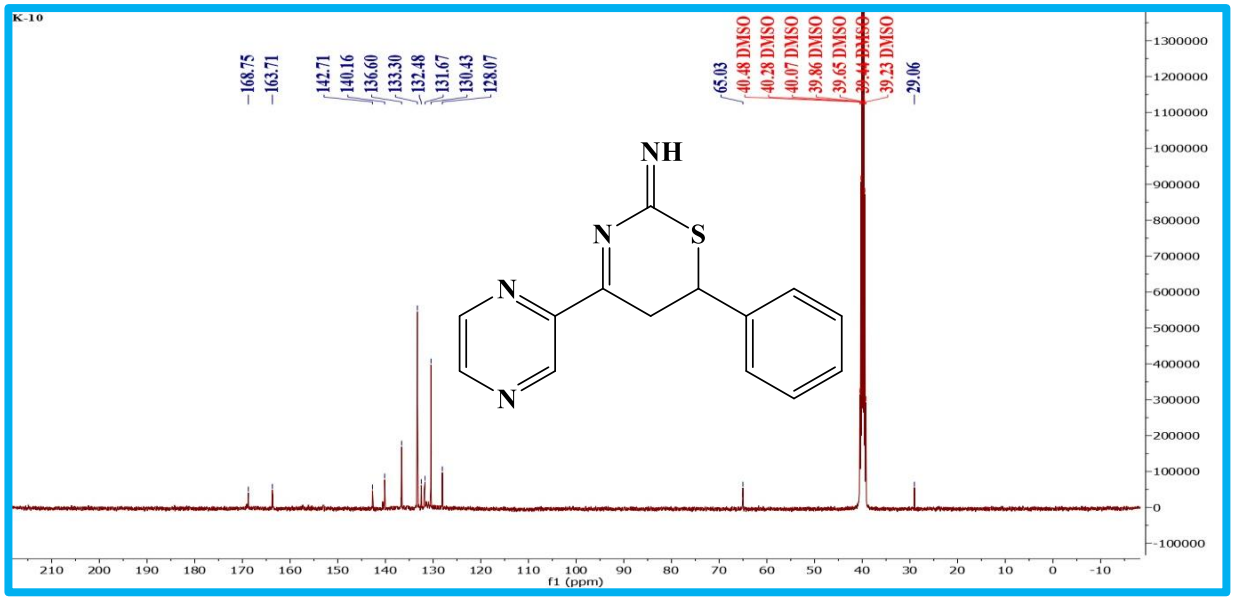
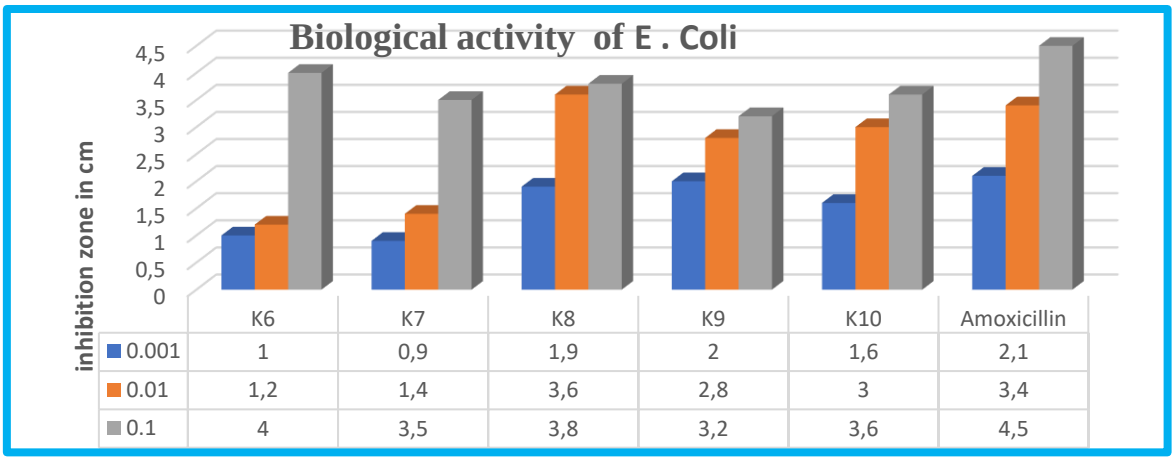



**Figure 6.** $^{13}$C-NMR Spectra of the substance (K10)

**2) Evaluation of the Biological Activity of Prepared Compounds**

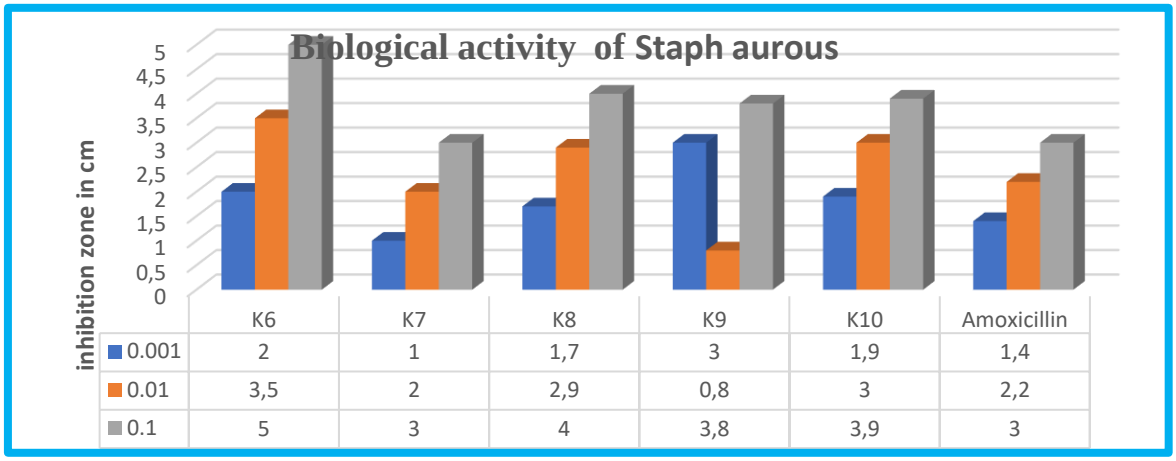
Several compounds were created for this project, and these compounds were tested against two different types of bacteria: Gram-positive Staphylococcus aureus and Gram-negative Escherichia coli. The experiment was conducted on Petri dishes covered with the diffusion technique. Mueller-Hinton broth was used to measure the size of the inhibitory zone (in centimeters) for a few compounds created at a dose of 0.1, 0.01, and 0.001 ml. The results were compared to those achieved using conventional antibiotics. The effects of some compounds were compared to those of traditional antibiotics [18-23]. Compared to the first type of bacteria, many of these chemicals were synthetic and had an effect on the first type. However, based on their impact on bacteria, some of the substances had a more significant effect on the first type of bacteria than on the second type[24-29]. They have a significant impact on germs, including the second type of bacteria. The strange type [30-36]. As can be seen in Figure 7.8 and Scheme 2.3

**3) The effect of laser beams on some prepared compounds**




| | K6 | K7 | K8 | K9 | K10 | Amoxicillin |
|---|---|---|---|---|---|---|
| 0.001 | 1 | 0,9 | 1,9 | 2 | 1,6 | 2,1 |
| 0.01 | 1,2 | 1,4 | 3,6 | 2,8 | 3 | 3,4 |
| 0.1 | 4 | 3,5 | 3,8 | 3,2 | 3,6 | 4,5 |

**Scheme 2.** Inhibitory activity of (K6-K10) for E.Coli




| | K6 | K7 | K8 | K9 | K10 | Amoxicillin |
|---|---|---|---|---|---|---|
| 0.001 | 2 | 1 | 1,7 | 3 | 1,9 | 1,4 |
| 0.01 | 3,5 | 2 | 2,9 | 0,8 | 3 | 2,2 |
| 0.1 | 5 | 3 | 4 | 3,8 | 3,9 | 3 |

**Scheme 3.** Inhibitory activity of (K6-K10) for Staph. aurous

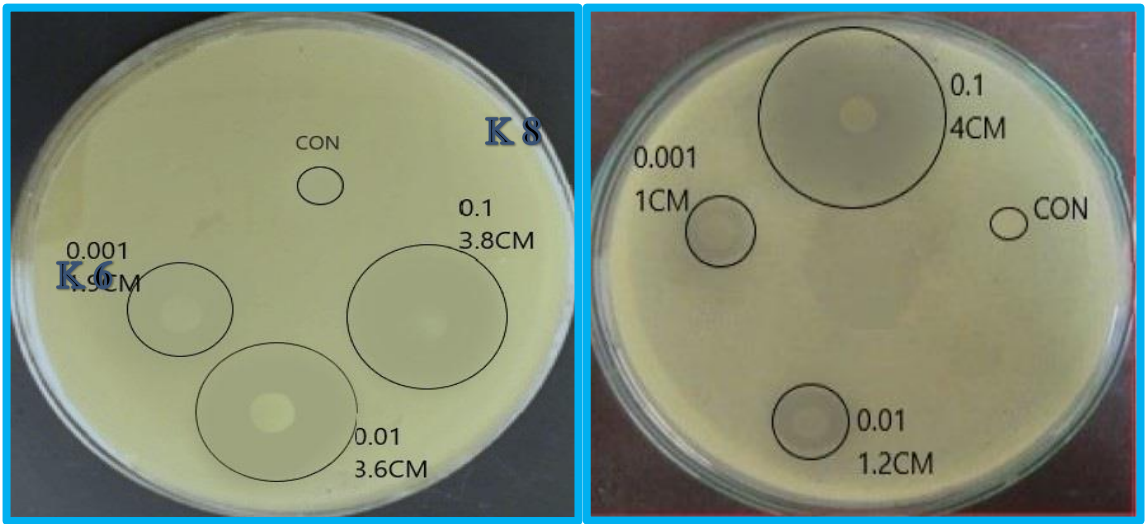



**Figure 7.** Biological effectiveness of the compound K6, K8 against becteral Staph au

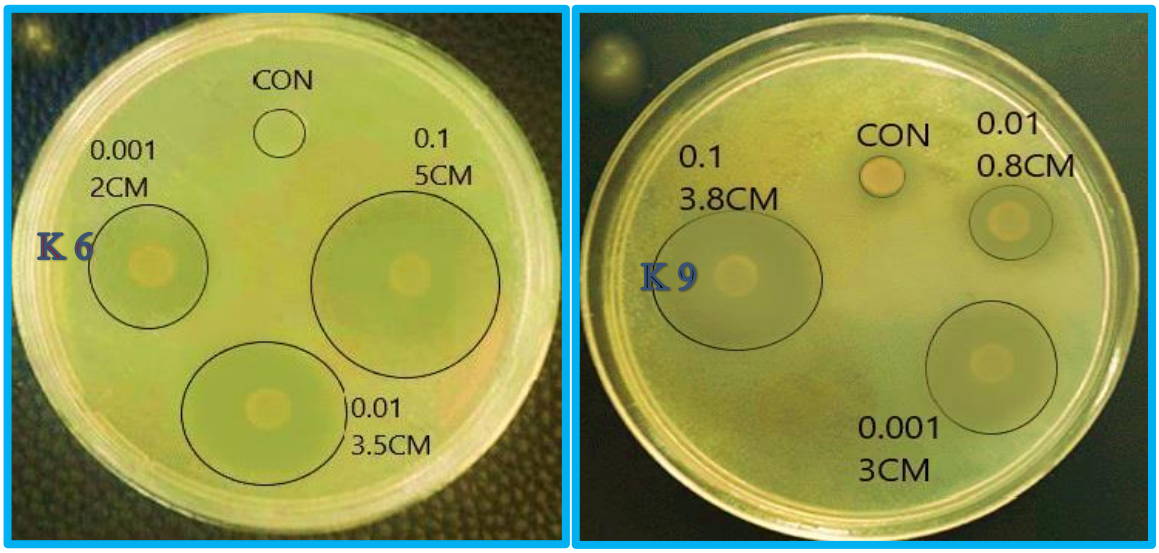



**Figure 7.** Biological effectiveness of the compound K6, K9 against becteral E.Coli

Due to the compounds' ability to maintain their physical properties and structural integrity over time and were not impacted by laser beams, the study demonstrated that the compounds' physical characteristics did not change during the intervals of 15, 30, 45, or more seconds. However, throughout these 60 seconds, they saw alterations in the physical characteristics of every chemical they looked at, including a notable drop in melting point and perhaps minor color changes. An extended period of continuous exposure to high energy (laser) can cause specific bonds in compounds to break, allowing new bonds to form[37]. According to Table 4

**Table 3.** The effect of laser beams on some prepared compounds (K6-K10)

| | 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)** |
| **K6** | Brown | 191-102 | Brown | 191-102 | Brown | 191-102 | Dark brown | 171-173 |
| **K7** | White | 212-214 | White | 212-214 | White | 212-214 | Light yellow | 202-204 |
| **K8** | Orange | 216-218 | Orange | 216-218 | Orange | 216-218 | Yellow | 199-201 |
| **K9** | Light Yellow | 231-233 | Light Yellow | 231-233 | Light Yellow | 231-233 | Orange | 212-214 |
| **K10** | Yellow | 187-189 | Yellow | 187-189 | Yellow | 187-189 | red | 178-180 |

## 4. Conclusion

The compounds demonstrated great purity and a satisfactory product ratio when the formulations were characterized using FT-IR and 1H-13C-NMR spectroscopy. Additionally, it showed excellent efficacy against the employed bacteria—nearly as effective as the antibiotics. The chemical K6's efficient electronic pairings may be the reason it was the most successful of the two microorganisms utilized. The compounds were stabilized for one to forty-five seconds against the helium-neon laser. The colors and melting points altered when the duration was extended to 60 seconds. This occurs as a result of extended exposure to laser radiation.

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