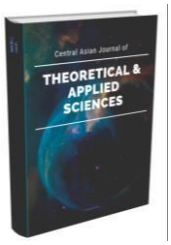

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Article

Preparation and Characterization of New Rings of Oxazine Derivatives and Studying Their Biological and Laser Effectiveness and Molecular Docking

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Abstract: The study included the preparation of new hexagonal rings by reacting the prepared chalcone with urea by sublimation and verifying the validity of the prepared structures using physical measurements such as color and melting point. For example, infrared spectroscopy, proton, and carbon NMR spectroscopy were also tested for bioavailability against two types of Gram-positive and Gram-negative bacteria Using MOE software (2009), the molecular docking of a few produced drugs against E. coli was investigated. The laser activity of some of the prepared compounds was measured using a helium-neon laser device (visible laser), where each compound irradiated the prepared compounds for four times (15, 30, 45, 60) seconds.

Keywords: Heterocyclic, Oxazine, biological activity, Molecular docking, laser

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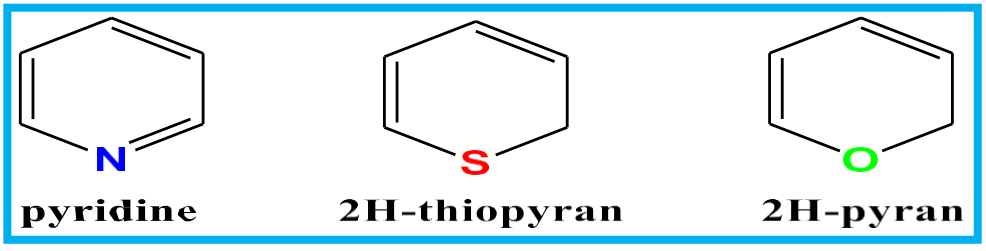
1. Introduction

Heterocyclic are substances that have a ring structure made up of one or more carbon atoms together with additional heterogeneous elements like nitrogen and phosphorus or oxygen and sulfur [1]. The names of these compounds are derived from the Greek word (hetero), which means different, and these compounds are widely spread in nature. It has multiple importance and uses in many fields, including industrial and medical. These compounds are involved in the synthesis of sugars and their derivatives, as well as enzymes, proteins, and nucleic acids [2]. Examples of this include:



Oxazine

They are hexagonal heterocyclic compounds containing a nitrogen atom, an oxygen atom, and four carbon atoms [3]. They are manufactured by condensation of chalcones with urea in the basic medium. It has received wide medical attention, as it is used in the field of medicine as an antibiotic [4], anti-inflammatory [5], and anti-cancer [6], and it has also shown good effectiveness against antifungal [7].

Molecular docking, a key tool in molecular and structural biology and computer-aided drug design and discovery, is the process of attaching two molecules in an orientation and position determined by the geometric shape and physical properties of the two molecules (proteins). Pharmaceutical compounds. Geometry determines how surfaces bond to each other, while physical properties determine the energy strength of the bond between two molecules [8], so the goal of studying molecular docking is to see whether molecules interfere with each other, and if interference occurs, to determine the interference. The position of the highest and lowest energy determines the correct configuration of bonds in the active site of the enzyme. He predicted familiarity between them [9]. Laser The basis of the laser process is absorption and emission, as stimulated and spontaneous absorption and emission processes become very important in the physical basis of laser production [11]. As we all know, atoms, ions, and molecules can exist in specific states, and each energy state has a specific energy level called the energy level, and the energy level smaller than it is called the ground state energy level, noting that the farther the energy levels are from the ground level, the more active they are [12].

2. Materials and Methods

Chemicals used

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

Instruments used

A thermometer 9300, a KBr disk with a 400–4000 cm–1 scale, an FT-IR 8400S Shimadzu spectrophotometer, and 1H– and 13C–NMR spectra from Bruker apparatus running at 400 MHz are used to determine the melting point. Using Fluka silica gel plates with a thickness of 0.2 mm, thin-layer chromatography (TLC) was examined.

Preparation of Oxazine derivatives (F6-F10).[13]

Equal moles (0.001 mol) in 10 ml of ethanol of the prepared Chalcone were mixed with urea and sodium ethoxide (10 ml) was added at a concentration of 10%. The mixture is then heated for (5 hours), then the solution is cooled, added to crushed ice, and left in the refrigerator for two days, which is equivalent to the solution using (10% HCl). The precipitate is filtered, dried, and recrystallized from absolute ethanol. As shown in Table 1.

Table 1. Some physical properties of for Prepared comppounds (F6-F10)

Comp. No.	R	Molecular formula	m.p. °C	Yield%	Color
F6	4-NO2	$C_{14}H_{11}N_5O_3$	195-197	70	Brown
F7	4-Cl	$C_{14}H_{11}ClN_4O$	221-223	68	Yellow
F8	4-F	$C_{14}H_{11}FN_4O$	208-210	73	light yellow
F9	4-Br	$C_{14}H_{11}BrN_4O$	217-219	65	Orange
F10	4-H	$C_{14}H_{12}N_4O$	191-193	76	White

Study of Biological Activity

Two different kinds of pathogenic bacteria were employed in this study: Escherichia coli, a gram-negative bacterium, and Staphylococcus aureus, a gram-positive bacterium. This makes these kinds of bacteria significant in the medical domain. Resistant to the prescribed medications (22), these bacteria were obtained from pharmaceutical laboratories where chemical solutions were prepared using the solvent dimethyl sulfoxide (F6, F7, F8, F9, F10). The concentration of DMSO is (0.1, 0.01, 0.001) mg/mL, as shown below [14]: Dissolve (0.1) g in (10 mL) solvent (DMSO). to obtain the concentration (0.01 mg/ml). To create a new solution at a concentration of 0.01 mg/mL, remove 1 mL of the previous concentration (0.1 mg/mL) and add 9 mL of DMSO solvent. To obtain a 0.001 mg/mL solution [15], remove 1 mL of the final 0.01 mg/mL solution and add 9 mL of solvent (DMSO). After adding pollen to a test tube containing diluted bacterial growth [16, 17], inoculate it into Agar Muller-Hinton (MHA) medium using a well-sterilized cotton swab, removing the excess by pressing the swab against the internal pollen. tube wall. After that, the culture medium is wiped evenly from three directions to distribute the vaccine evenly and left for (15-20) minutes to allow the culture to absorb it, and left until the culture medium dries [18,19].

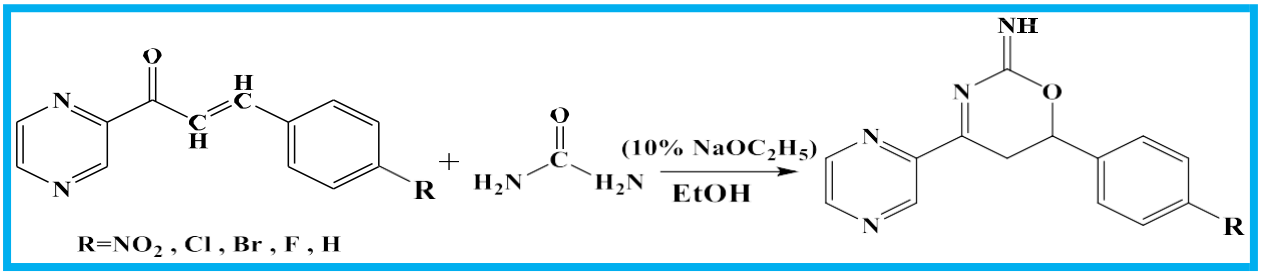
Study of the molecular docking of some prepared compounds

The molecular docking of some of the prepared compounds (F6, F7, F8, F9, F10) on E. coli bacteria was studied using the MOE program (2009), where the energy reduction process was completed for the studied compounds to obtain the most stable ones. Stereo (minimum conserved energy), then download E. coli from the World Protein Bank website and use a high-spec personal calculator, as these programs require advanced computers with fast, multi-core processors. The work can be done quickly and limitations such as molecule size, number of atoms, etc. are circumvented [20, 21].

Study the effect of laser radiation [22]

The laser effectiveness of the prepared composites (F6, F7, F8, F9, F10) was measured using a helium-neon laser (visible laser). The prepared compounds were irradiated for four time periods (15, 30, 45, 60). seconds for each compound, as well as the distance between the beam source and sample (10 cm), power (1 mW), and wavelength (808 nm). Measurements were conducted at the Department of Physics, College of Science, Tikrit University. Laser laboratory. After irradiation of the prepared composites, the changes in the physical properties and the prepared DMVs were studied again.

3. Results and Discussion



Scheme 1. The compounds formed by the reaction of Chalcone with Urea

When studying the FT-IR spectrum of compounds (F6-F10), It was noted that a band developed in the region of (1619–1631) cm-1 as a result of the ensuing ring (C=N) stretching, a band in the (1331-1375) cm-1 range was identified as the result of (C-O-C) stretching The absorption band in the region of (3321-3236) cm-1, which results, is often ascribed to (NH) stretch, an absorption band in the range (3078-3023) cm-1 is attributed to aromatic (C-H) stretch, and two absorption bands at water (2961-2851) (cm-1) is usually

attributed to the aliphatic (C-H) stretch, and two bands are attributed to the aromatic (C=C) stretch in the range (1559-1481) [23]. As in Table 2 and Figure 1 and 2.

Table 2. FT-IR absorption results for prepared compounds (F6-F10)

Comp. No.	R	ν(C-H) Arom.	ν(C-H) Aliph.	ν(N-H) ν(C-O)	ν(C=N)	ν(C=C) Arom.	Others
F6	4-NO2	3078	2933,2875	3296 1375	1631 1597	1550,1504	ν(N-O) as sy1518. Sy1313
F7	4-Cl	3034	2962,2854	3321 1338	1626 1612	1541,1491	ν (C-Cl)736
F8	4-F	3058	2961,2920	3291 1345	1621 1608	1559,1476	ν (C-F)914
F9	4-Br	3031	2948,2861	3236 1331	1619 1599	1544,1487	ν (C-Br) 579
F10	4-H	3023	2939,2851	3221 1342	1623 1602	1537,1481	``--

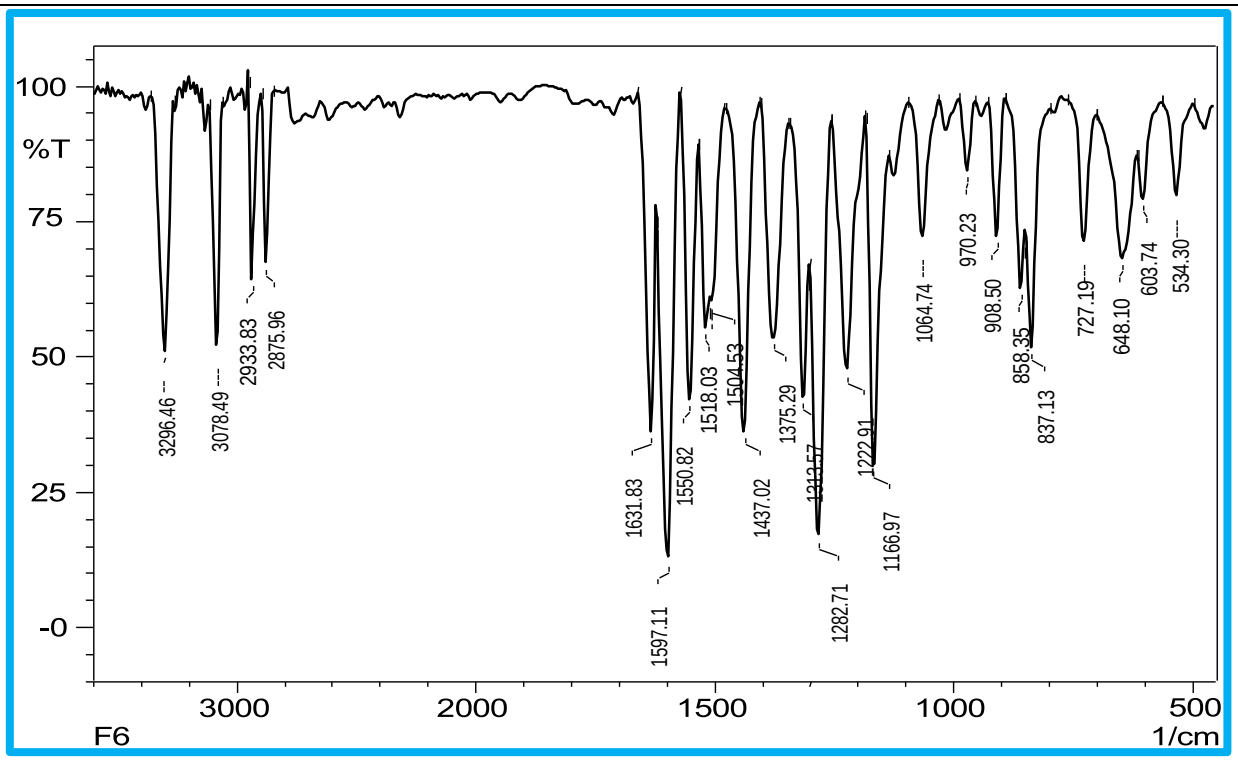


Figure 1. The compound's FT-IR spectra (F6)

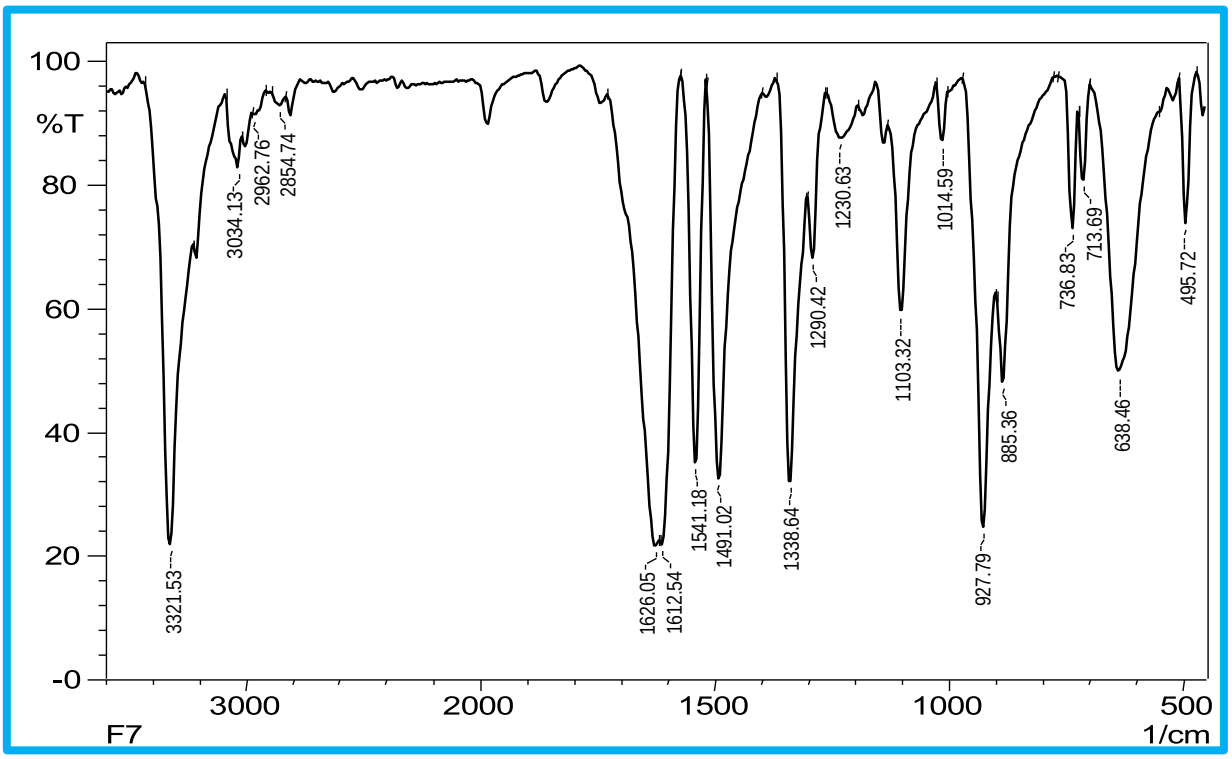


Figure 2. The compound's FT-IR spectra (F7)

When studying the H-NMR spectrum of landfill F6, it was noted that a double signal appeared at the location (3.24-3.26) ppm attributed to the proton (CH) of the resulting ring, a triple signal in the range (3.63-3.67) ppm, usually for a proton (CH2), usually a proton of the resulting ring, and a multiple signal at The range (6.97-8.71) ppm usually refers to the protons of the aromatic ring, and the (NH) proton is responsible for the signal at location (9.81) ppm. The signal for the solvent DMSO is at position (2.51) ppm. As in Figure 3.

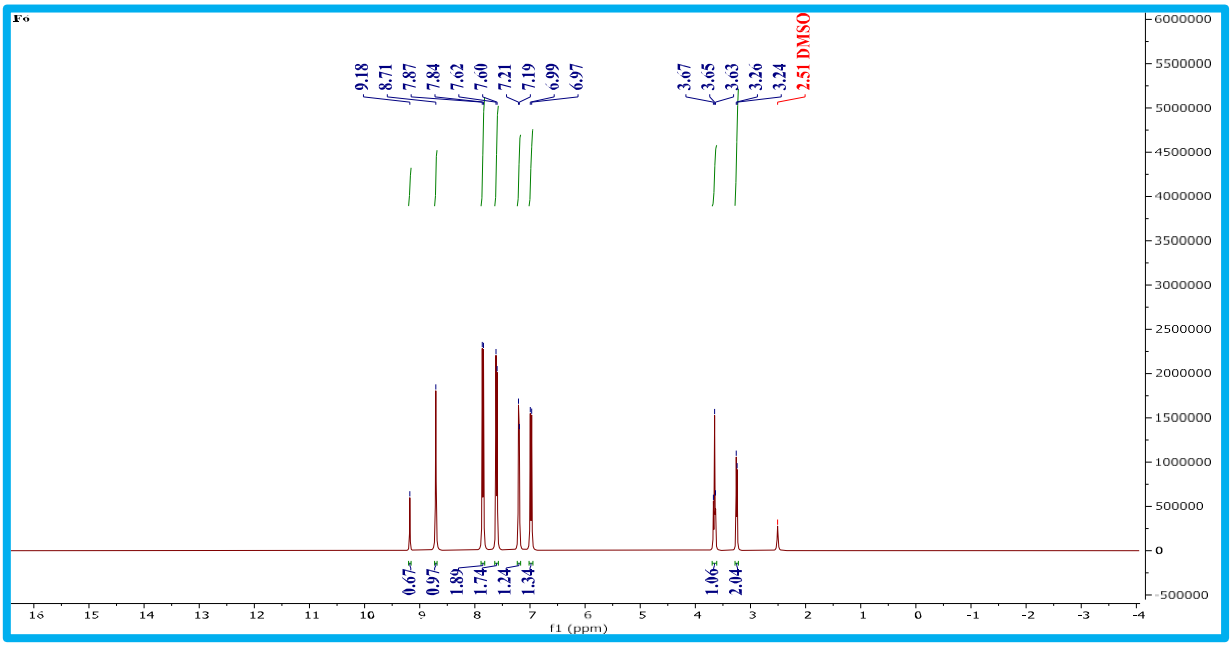


Figure 3. [1]-H NMR spectra of the substance (F6)

When studying the H-NMR spectrum of F7, it was noted that a double signal appeared at the location (2.98,2.99) ppm attributed to the proton (CH) of the resulting ring, a triple signal in the range (3.85-3.92) ppm, usually for a proton (CH2), usually a proton of

the resulting ring, and many signals at Typically, the range (7.16-8.63) ppm relates to the aromatic ring protons. The signal at position (9.58) ppm belongs to the (NH) proton, and the signal for the solvent DMSO is at position (2.51) ppm. As in Figure 4.

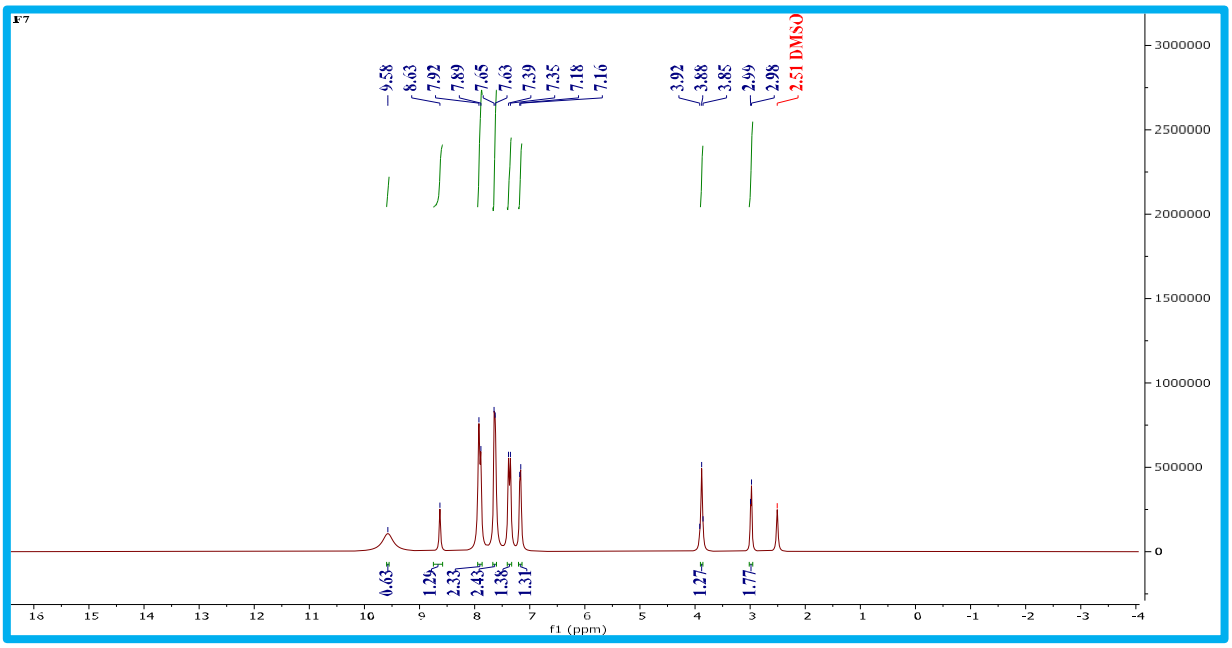


Figure 4. [1]-H NMR spectra of the substance (F7)

When examining chemical F6 13C-NMR spectra, the signal was detected at)34.48(ppm, which was identified as the resulting ring's carbon (CH2), and a signal (49.37) ppm is usually attributed to the carbon (CH) of the resulting ring, and signals in the range (124.33-155.91) ppm usually refers to the carbons of the aromatic ring, and the signal at position (159.95) ppm is usually for the carbon (C=N) of the resulting ring and the signal at (164.08) is attributed to the carbon (C=NH), and the solvent signals are DMSO at position (39.37-40.62) ppm. As in Figure 5.

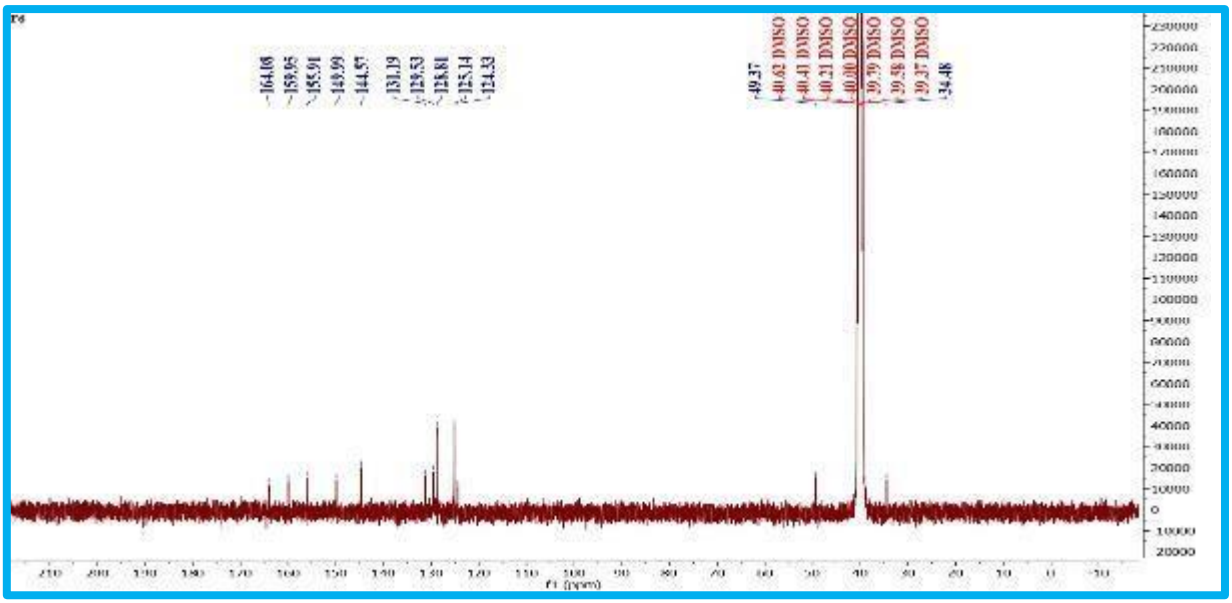


Figure 5. [13]C-NMR spectra of the substance (F6)

When studying the 13C-NMR spectrum of compound F6, A signal was seen at location (28.26) ppm, which was determined to be the carbon (CH2) of the resultant ring,

and a signal (76.18) ppm is usually attributed to the carbon (CH) of the resulting ring, and signals within the 117.09–155.96 range ppm often refers to the aromatic ring's carbons., and the signal at position (166.65) ppm is usually for the carbon (C=N) of the resulting ring and the signal at (168.08) is attributed to the carbon (C=NH), and the solvent signals are DMSO at position (39.18-40.43) ppm. As in Figure 6.

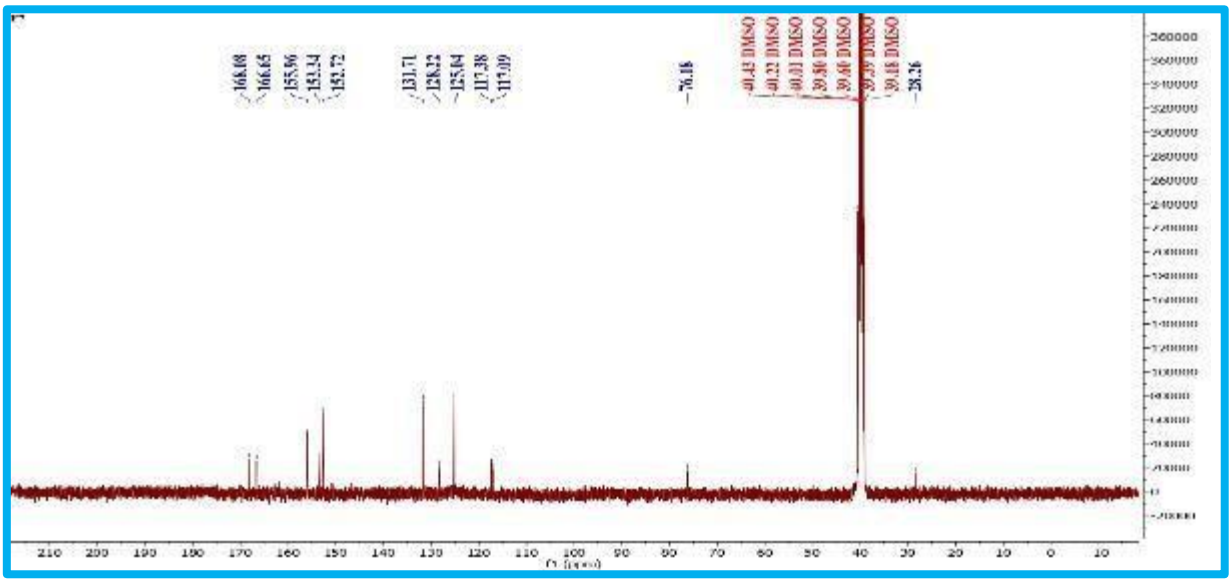
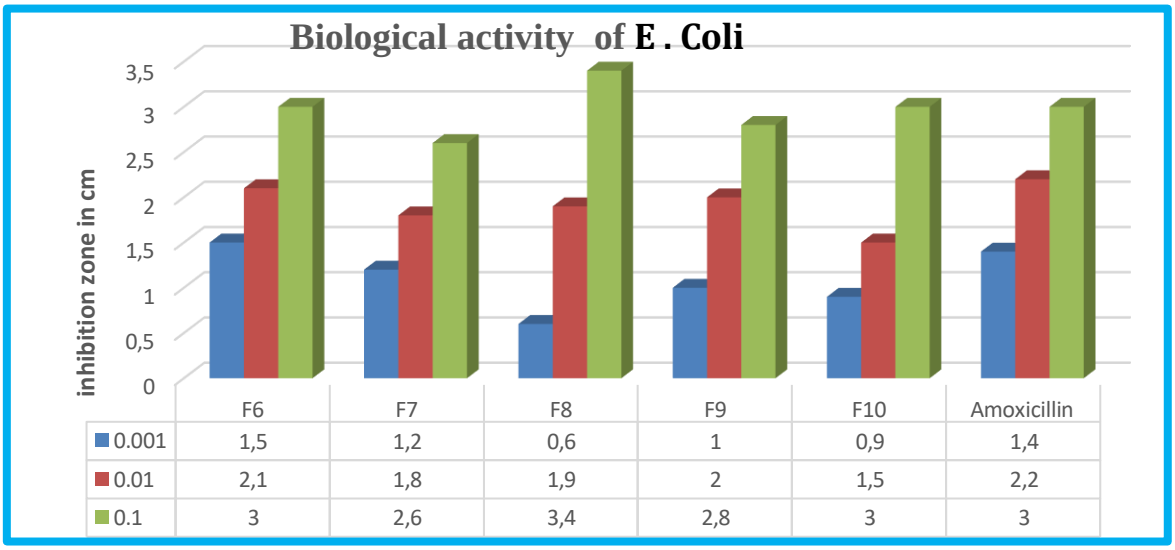


Figure 6. ^{13}C-NMR spectra of the substance (F7)

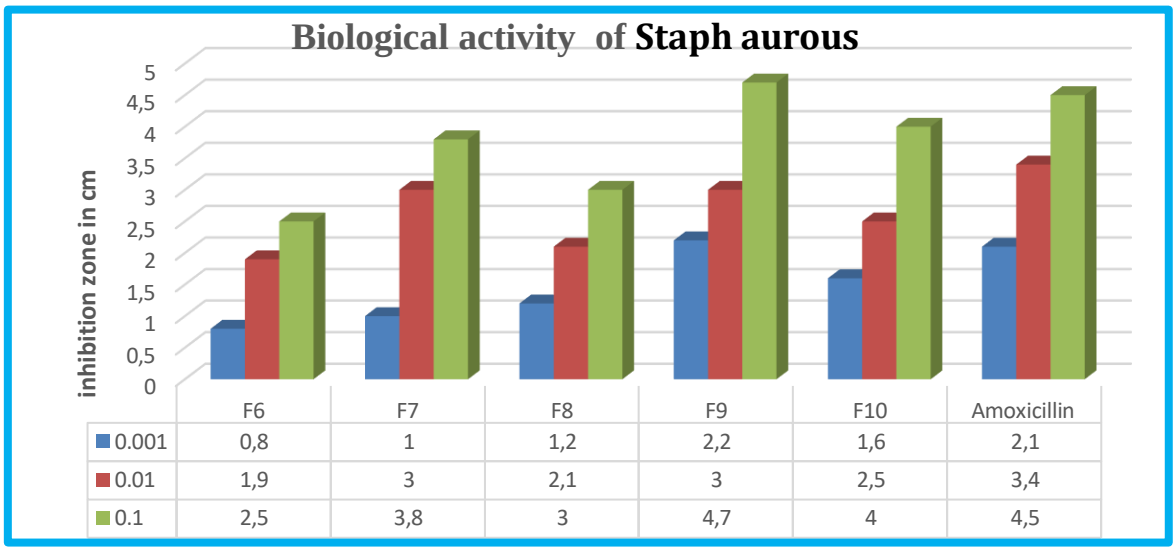
Evaluation of the Biological Activity of Prepared Compounds

Some of the compounds prepared in this study were tested against two types of bacteria: Staphylococcus epidermis (Gram-positive bacteria) and Escherichia coli (Gram-negative bacteria). The test is performed on Petri dishes by the diffusion method [24, 25]. Using Mueller-Hinton medium, the diameter of the inhibition zone (in centimeters) of a few compounds synthesized at doses (0. 1, 0.01, 0.001 mg/mL) was measured. The outcomes were compared with those of conventional antibiotics. From the effects of some of these prepared compounds on bacteria, it was noted that some of them had a clear effect on the first type of bacteria compared to the first type, while some of them had a clear effect on the first type of bacteria compared to the first type of bacteria. To other types of bacteria and has clear effects on type II bacteria. another type [26, 27]. As shown in Figure 7 and 8 and Scheme 2 and 3.



	F6	F7	F8	F9	F10	Amoxicillin
0.001	1,5	1,2	0,6	1	0,9	1,4
0.01	2,1	1,8	1,9	2	1,5	2,2
0.1	3	2,6	3,4	2,8	3	3

Scheme 2. Inhibitory activity of (F6-F10) for E. Coli



	F6	F7	F8	F9	F10	Amoxicillin
0.001	0,8	1	1,2	2,2	1,6	2,1
0.01	1,9	3	2,1	3	2,5	3,4
0.1	2,5	3,8	3	4,7	4	4,5

Scheme 3. Inhibitory activity of (F6-F10) for Staph. Aurous

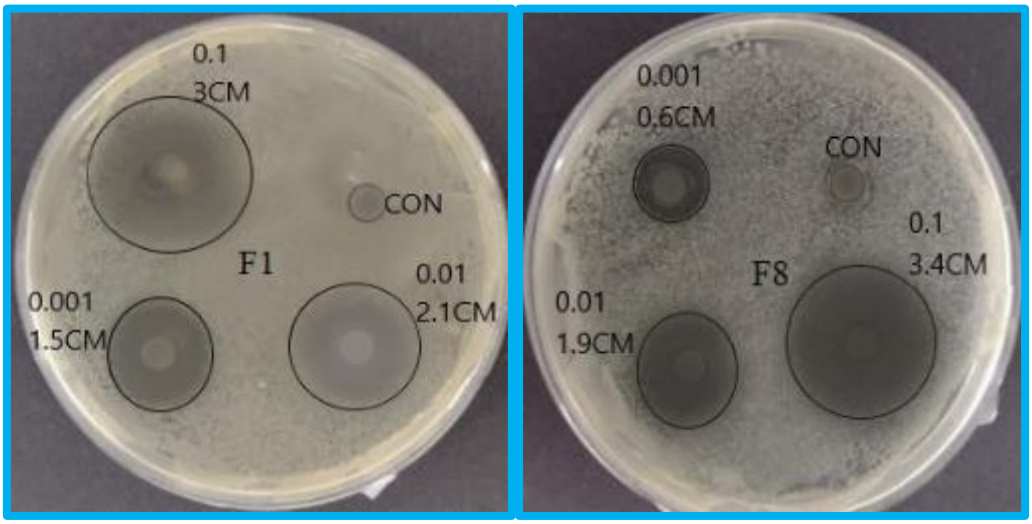


Figure 7. Biological effectiveness of the compound F6, F8 against bacterial E. Coli

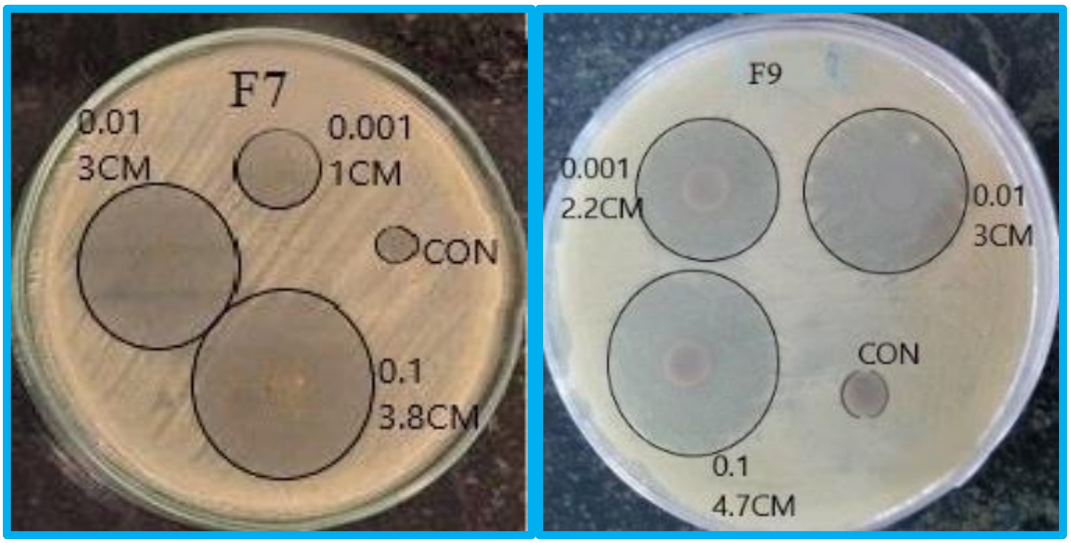


Figure 8. Biological effectiveness of the compound F7, F9 against bacterial Staph. Aurous

Result of a molecular docking study of some prepared compounds

The amount and kind of bonds that these created derivatives engage with the amino acids found in the protein's active site by establishing numerous residual bonds are revealed by analyzing the molecular docking of the prepared organic derivatives. The study showed that the two compounds (F7 and F9) show the same bond, as it interacts with amino acid residues that are present in the active site by forming three types of bonds, which are a hydrogen bond that links the amino acid residue ASP 192 present in the active site with the electronic pair of a nitrogen atom. Attached to the hexagonal ring, two Pi-Alkyl bonds link the amino acid residues Val 376 and Ser 379, which are located in the active site with the electronic pair of the aromatic ring [28]. As shown in Table 3 and Figure 9 and 10.

Table 3. Values of binding energies for the prepared compounds

Comp. No.	S score (kcal/mol)	RMSD
F7	-5.75312	1.995876
F9	-3.2914	1.917693

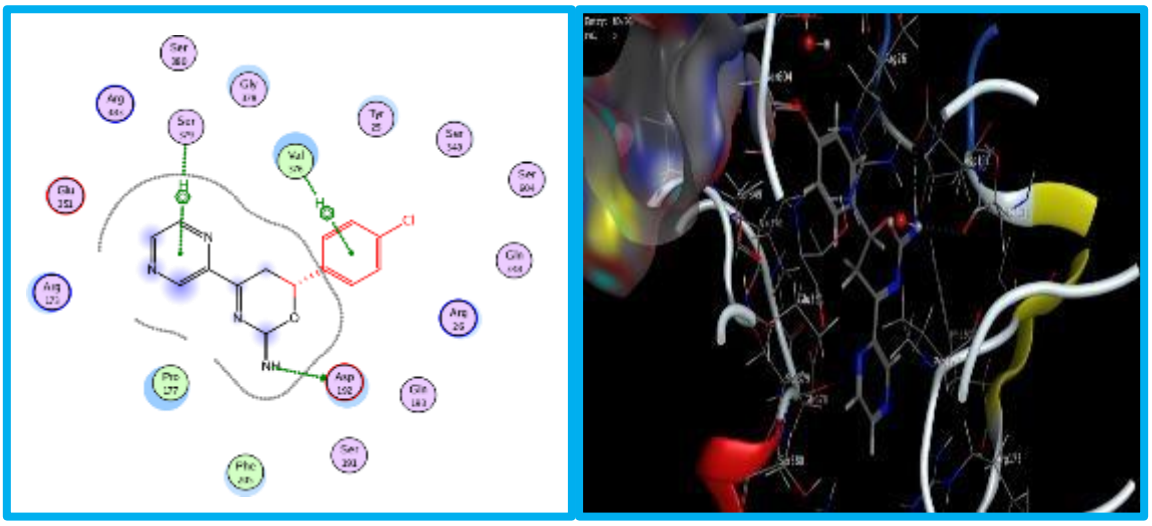


Figure 9. Interactions between compound F7 in 3D

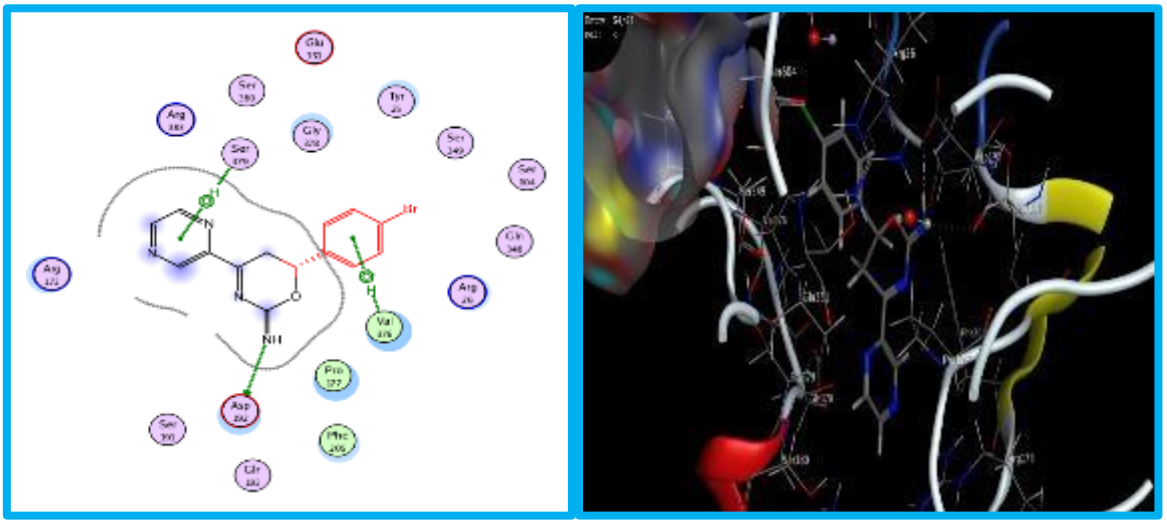


Figure 10. Interactions between compound F9 in 3D

The effect of laser beams on some prepared compounds [29]:

The study showed that the physical properties of the compounds did not change during the periods (15, 30, 45) seconds because the compounds maintained their structural shape and physical properties during these periods and were not affected by laser rays. However, during this time (60 seconds), they observed changes in the physical properties of all the compounds studied, with a significant decrease in the melting point and slight color changes that may have resulted. Breaking of certain bonds in compounds may result from prolonged and continuous exposure to high energy (laser), leading to the breaking and formation of new bonds. As shown in Table 4.

Table 4. The effect of laser beams on some prepared compounds (F6-F10)

	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)**
F6	Brown	195-197	Brown	195-197	Brown	195-197	Light brown	176-178
F7	Yellow	221-223	Yellow	221-223	Yellow	221-223	Light yellow	206-208
F8	Light Yellow	208-210	Light Yellow	208-210	Light Yellow	208-210	Light brown	195-197
F9	Orange	217-219	Orange	217-219	Orange	217-219	Yellow	196-198
F10	White	191-193	White	191-193	White	191-193	Yellow	173-175

4. Conclusion

The validity of the compositions was confirmed by diagnosing them using FT-IR and ^{1}H-^{13}C-NMR spectroscopy, where the compounds showed high purity and good product percentage. They also showed high effectiveness against the bacteria used, reaching close to the effectiveness of the antibiotic used. As for the compounds for which the molecular docking was studied, they were It gave the same correlations and this is due to the atoms of the two compounds being similar. The compounds were given stability against the helium-neon laser from 1 second to 45 seconds. When the time was increased to 60 seconds, the colors and melting points changed. This is due to leaving them exposed to laser rays for a longer period.

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