SYNTHESIS, CHARACTERIZATION, AND EVALUATION OF BIOLOGICAL AND LASER ACTIVITIES OF SOME NOVEL AZETIDINONE DERIVATIVES

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Abstract: *Azetidinone rings [M_{11}-M_{15}] were produced as part of this research by reacting pre-synthesized Schiff bases with chloroacetyl chloride in the presence of dioxane as a solvent using conventional methods and sublimation for 9-10 hours. Using spectroscopic techniques, including infrared spectroscopy and proton nuclear magnetic resonance spectroscopy, the structures of the resulting compounds were verified. Physical properties such as color, melting point, and product yield were evaluated using two bacterial isolates known to be resistant to antibiotics (Gram-negative Escherichia coli and Gram-positive Staphylococcus aureus), with amoxicillin as the control antibiotic. Comparison with the results of the control antibiotics showed that they were effective. They had good inhibitory activity against both bacteria and were highly potent and selective. A helium-neon laser (visible laser) was used to test the laser activity of the produced compounds. Each compound was irradiated for four different time periods (15, 30, 45, and 60 seconds), after which its physical properties were evaluated. The preparation was examined again, and the changes in the compounds were recorded.*
Keywords: *azetidinones, biological activity, synthesis, laser characterization.*

1. Introduction

For a long time, chemical synthesis and medicinal chemistry studies have focused on nitrogen-containing heterocycles. Because of their many chemical characteristics and possible uses, these compounds have continuously caught the interest of scientists throughout the years [1]. Because of their wide range of biological characteristics, nitrogen heterocycles have a significant place in the pharmaceutical industry. Nitrogen heterocycles are included in over 75% of authorized medications, and this percentage will rise over the next several decades [2]. Their distinct structural characteristics render them indispensable in the creation of novel materials and molecules [3]. With the discovery of penicillin, four-membered nitrogen heterocycles—small heterocyclic compounds—became extremely important in the area of medicine. Because of their strain ring characteristics and reactivity, azetidine-containing heterocyclic compounds have become increasingly useful in synthetic organic chemistry research and medical development due to the presence of functional groups such as carbonyl and the electron pair of nitrogen [4]. In the last century, a few classes of antibiotics have been developed and made widely available, yet bacterial resistance remains a worldwide problem. Currently, a few solutions exist [5]. The widespread use and frequent abuse of antibacterial agents in domesticated animals has created a false increase in safe strains, raising the risk that the microorganisms from the animals will begin to contaminate humans, even though bacterial resistance may be considered a natural process [6]. Infectious illnesses brought on by microbes have historically led to a high death rate worldwide. Treating bacterial inspection is challenging due to the rise of multidrug-resistant microbial strains and the development of diseases that are impossible to avoid. The emergence of new and old opponents of bacterial resistance strains in past decades has resulted in great demand for new classes of antibacterial agents despite the abundance of antibacterial experts and chemotherapeutics available on the market [7]. One of the biggest risks to human life is the rise of bacteria that are resistant to drugs, and the effective treatment of microbial illnesses depends on the ongoing development of new antibiotics.

Thus, the goal of the study was to create novel azetidinone derivatives and analyze their infrared spectrum before testing them for antibacterial activity [8].

2. Experimental part

2.1. Materials and Method. *Chemicals used:* Fluka, Merck, Aldrich, and BDH Thomas chemicals were utilized.

*Instruments used***:** A 9300 thermoelectric melting equipment was used to measure melting points. A KBr disc for bromine was used to record spectra at (400–4000) cm^{-1} on a Shimadzu FT-IR 8400S spectrometer. Bruker equipment running at 400 MHz was used to record the ^{1}H-NMR and ^{13}C-NMR spectra. Fluka silica gel plates that were 0.2 mm thick and activated with fluorine G silica gel were used for TLC. UV light was used to see the plates. A steam sterilizer made for the Tikrit University research lab by RAIBBA (Spain) was utilized to disinfect the study's material. At the same facility, a Heraeus D-63450 incubator (Germany) was utilized to incubate the plates used in the microbiological study.

2.2. Preparation of Azetidine-2-one [9]. 0.001 mol of the prepared Schiff base was dissolved in 20 ml of anhydrous dioxane solvent under stirring, and 0.002 mol of acetyl chloride and 0.002 mol of dissolved triethylamine (Et_3N) were gradually added to the mixture. The mixture was heated in 20 ml dry dioxane in a water bath for 9-10 hours at 0-5°C and cooled at room temperature for 2 days. Add it to crushed ice. The precipitate was filtered, dried, and recrystallized with ethanol. TLC confirmed the completion of the reaction, as shown in Table 1.

Table 1. Some physical properties of prepared compounds (M_{11}-M_{15})

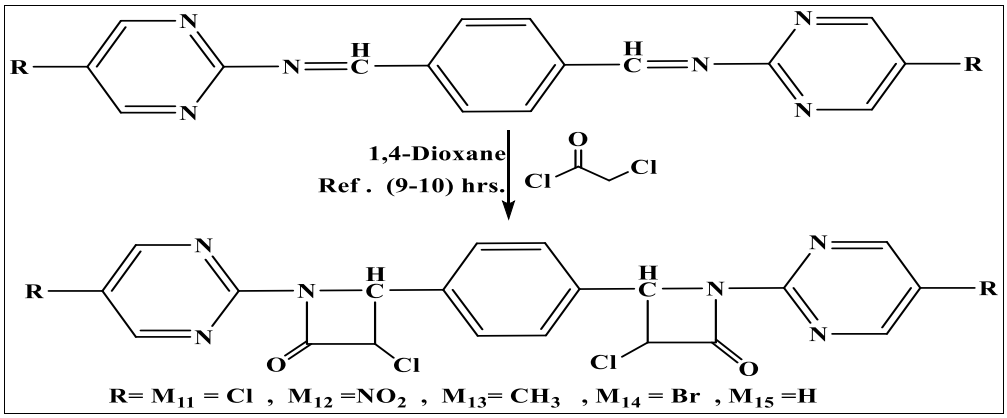
Comp. No	R	Molecular formula	M.p., °C	Yield, %	Color
M_{11}	4-Cl	$C_{20}H_{12}Cl_4N_6O_2$	223-225	76	Light Yellow
M_{12}	4-NO_2	$C_{20}H_{12}Cl_2N_8O_6$	245-247	71	Brown
M_{13}	4-CH_3	$C_{22}H_{18}Cl_2N_6O_2$	232-234	78	Yellow
M_{14}	4-Br	$C_{20}H_{12}Br_2Cl_2N_6O_2$	219-221	69	Red
M_{15}	4-H	$C_{20}H_{14}Cl_2N_6O_2$	247-249	73	Blue

2.3. Study of biological activity. One liter of distilled water dissolved 40 grams of Mueller-Hinton agar. A magnetic stirrer was used to heat and stir the water. After that, the material was sterilized for two hours at 121°C and 1.5 bars in a steam autoclave. After lowering the temperature to 50°C, the mixture was transferred into plates and allowed to cool to room temperature. Two distinct kinds of isolated bacteria were assessed by the Advanced Microbiology Laboratory, Department of Life Sciences, and College of Education for Pure Sciences, Tikrit University: Staphylococcus aureus, a Gram-positive bacterium, and *Escherichia* coli, a Gram-negative bacterium. Chemical solutions were prepared for each component using dimethyl sulfoxide (DMSO) at three distinct concentrations: 0.1, 0.01, and 0.001 mg/ml [10-13]. To achieve a 0.1 mg/ml concentration, 0.1 g of these solid substances was dissolved in 10 ml of dimethyl sulfoxide. To get a concentration of 0.01 mg/ml, 9 ml of dimethyl sulfoxide solvent was added after 1 ml of the 0.1 mg/ml solution was withdrawn [14, 15].

2.4. Measurement of laser effectiveness of some compounds [16]. Some compounds produced (M_{11}, M_{12}, M_{13}, M_{14}, and M_{15}) were tested for laser efficiency using a helium-neon (visible laser) laser. Each of the four radiation exposure times (15, 30, 45, and 60 seconds) was applied to the produced compounds. In the Laser Laboratory of the College of Science, Tikrit University's Department of Physics, measurements were made of the distance (10 cm), power (1 MW), and wavelength (808 nm) between the beam source and the sample. Following radiation exposure, the physical properties of the generated compounds were reexamined, and the changes that occurred were recorded.

3. Results and discussions

The following Scheme shows the preparation of azetidinones derivatives.



Scheme 1. Path of the ready compounds (M_{11}-M_{15})

3.1. Characterization of Azetidine-2-one. *The FT-IR spectrum* showed a band at 1666-1655 cm^{-1} for (C=O), a band at 1231-1222 cm^{-1} for (C-N), two bands at 2970-2932 cm^{-1} and 2912-2854 cm^{-1} for aliphatic (CH), two bands for aromatic (C=C) at 1551-1527 cm^{-1} and 1489-1458 cm^{-1}, a band for aromatic (CH) at 3051-3021 cm^{-1} [17], as in Table 2 and Fig.s 1 and 2.

The ^{1}H-NMR spectrum of compound M_{12} showed a double signal at 3.97 and 4.00 ppm due to (CH-N), a double signal due to (CH-Cl) at 4.36 and 4.39 ppm, two signals due to the aromatic ring at 7.30 and 8.08 ppm, and a signal for the solvent DMSO-d^6 at 2.51 ppm, as in Fig. 3.

The ^{1}H-NMR spectrum of M_{13} showed a signal due to (CH_3) at 2.16 ppm, a double signal due to (CH-N) at 4.76 and 4.79 ppm, a double signal due to (CH-Cl) at 5.10-5.13 ppm, and two signals due to aromatic rings at 7.11 and 7.87 ppm. A signal at 2.53 ppm is due to the solvent DMSO-d^6, as in Fig. 4.

The ^{1}H-NMR spectrum of compound M_{15} showed signals at 7.29-8.09 ppm due to aromatic rings, a double signal at 4.32 and 4.29 ppm due to (CH-Cl), and a double signal due to (CH-N) at 4.02 and 4.05 ppm. A signal at 2.47 ppm is due to the solvent DMSO-d^6, as in Fig. 5.

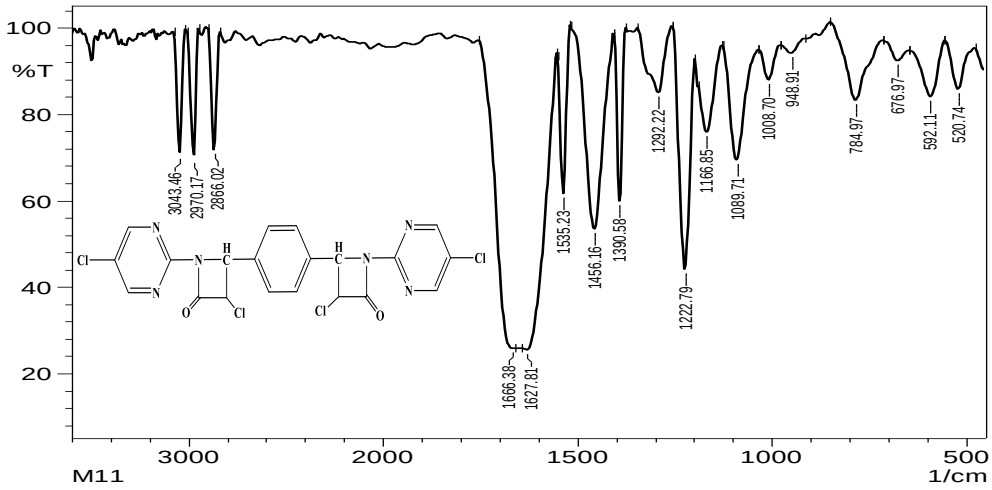


Fig. 1. FT-IR spectra of the (M_{11}) compound

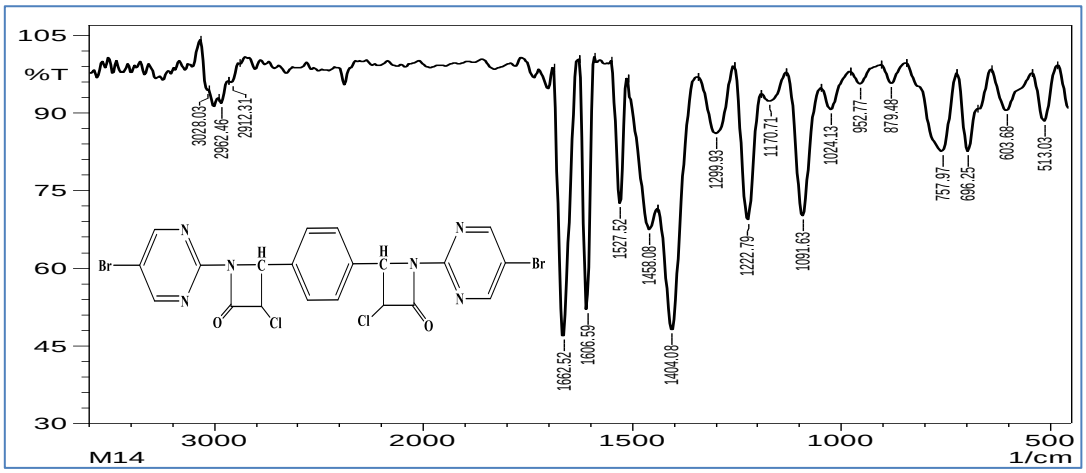


Fig. 2. FT-IR spectra of the (M_{14}) compound

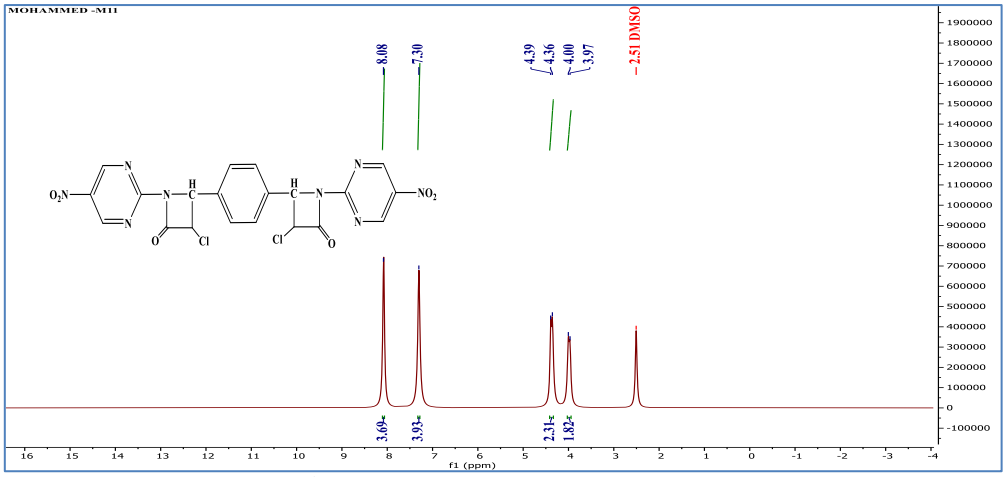


Fig. 3. ^{1}H-NMR spectra for (M_{12}) compound

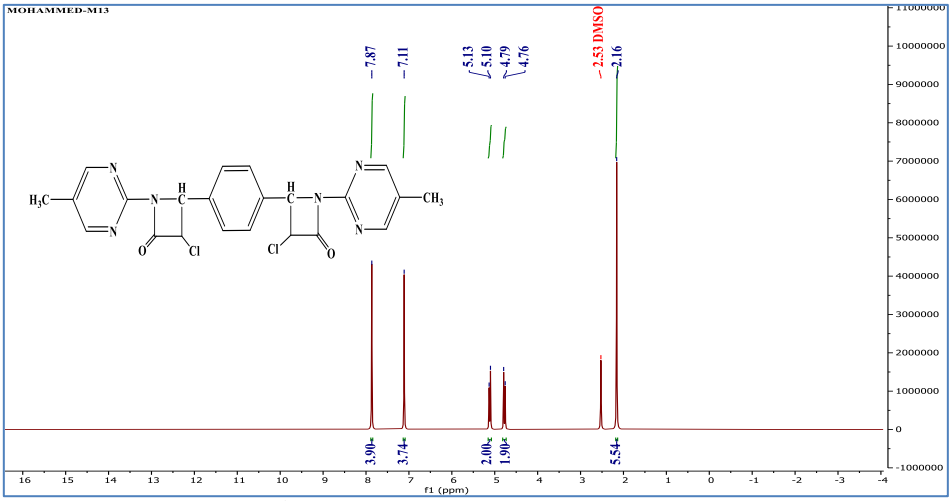


Fig. 4. ^{1}H-NMR spectra for (M_{13}) compound

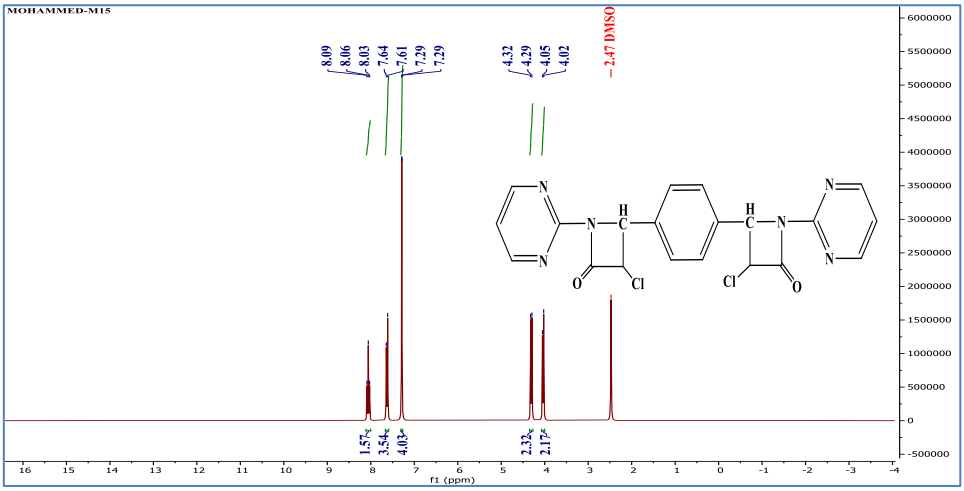
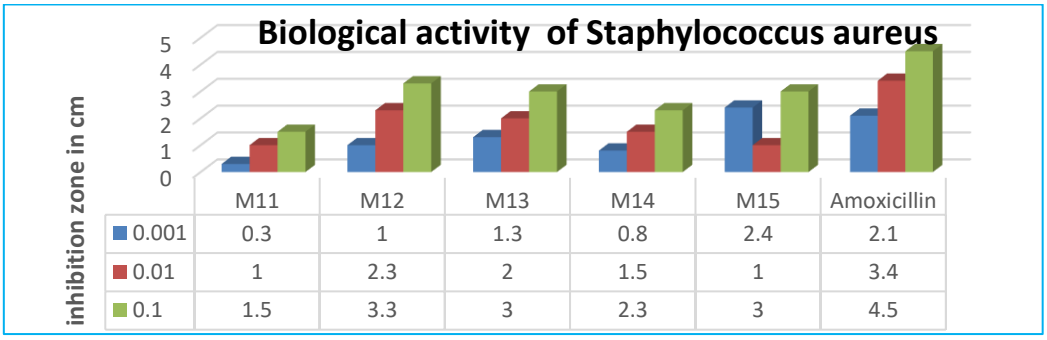


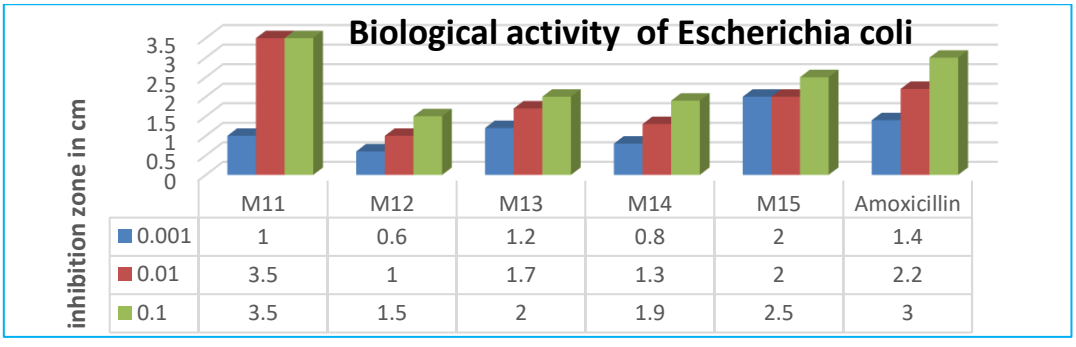
Fig. 5. ^{1}H-NMR spectra (M_{15}) compund

Table 2. FT-IR absorption results for prepared compounds (M_{11}-M_{15})

Comp. No	R	ν(C-H) Arom.	ν(C-H) Aliph.	ν(C=O) ν(C=N)	ν(C-H) ν(C-Cl)	ν(C=C) Arom.	Others
M_{11}	4-Cl	3034	2970,2866	1666 1627	1222 784	1535,1454	ν(N-O) as sy1514. Sy1315
M_{12}	4-NO_2	3051	2932,2875	1658 1613	1225 734	1551,1489	ν(N-O) as sy1526. Sy1312
M_{13}	4-CH_3	3013	2943,2870	1665 1618	1230 764	1541,1476	ν (C-F)912
M_{14}	4-Br	3028	2962,2912	1662 1606	1222 757	1527,1458	ν (C-Br) 513
M_{15}	4--H	3021	2938,2854	1655 1609	1231 742	1534,1467	--



Scheme 2. Inhibitory activity of (M_{11}-M_{15}) for *Staphylococcus Aureus*



Scheme 3. Inhibitory activity of (M_{11}-M_{15}) for *Escherichia Coli*

3.2. Evaluation of the biological activity of prepared compounds. After the dishes were placed in the incubator for a full day, the diameters of inhibition for the concentrations were measured in centimeters, where compound M_{12} showed the highest diameter of inhibition against *Staphylococcus aureus* bacteria with a diameter of approximately 3.3 cm, while compounds M_{13} and M_{15} gave an inhibition estimated at a diameter of 3 cm, which is the highest against gram-positive bacteria (Scheme 2). As for *Escherichia coli* bacteria, compound M_{11} showed the highest inhibition against it with a diameter of approximately 3.5 at concentrations of 0.1 and 0.01 mg/L, which is the highest of the two types, even surpassing the antibiotic used as a control sample, which is Amoxicillin (Scheme 3). Compound M_{15} showed an effectiveness with a diameter of 2.5 cm. This study showed that the constituent compounds were effective against both types used, and their efficacy varied across concentrations, suggesting their potential use as future antibiotics [18-20], as shown in Fig.s 6 and 7, and Table 3.

Table 3. Biological efficacy of produced substances and control methods (measured in millimeters of inhibition)

Comp. No	***E. Coil conc. mg/ml***			***Staph. Aureus conc. mg/ml***		
	0.001	**0.01**	**0.1**	**0.001**	**0.01**	**0.1**
M_{11}	1	3.5	3.5	0.3	1	1.5
M_{12}	0.6	1	1.5	1	2.3	3.3
M_{13}	1.2	1.7	2	1.3	2	3
M_{14}	0.8	1.3	1.9	0.8	1.5	2.3
M_{15}	2	2	2.5	2.4	1	3
Amoxicillin	1.4	2.2	3	2.1	3.4	4.5

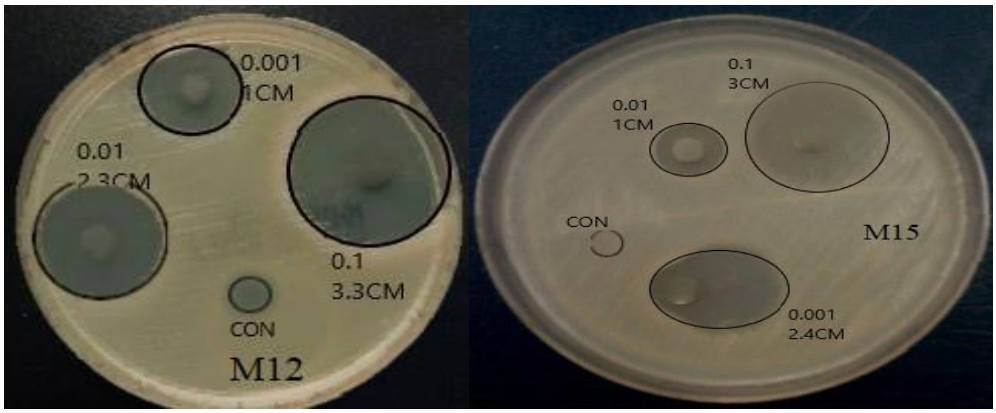


Fig. 6. Biological effectiveness of the compounds M_{12} and M_{15} against bacteria *Staph.Aureus*

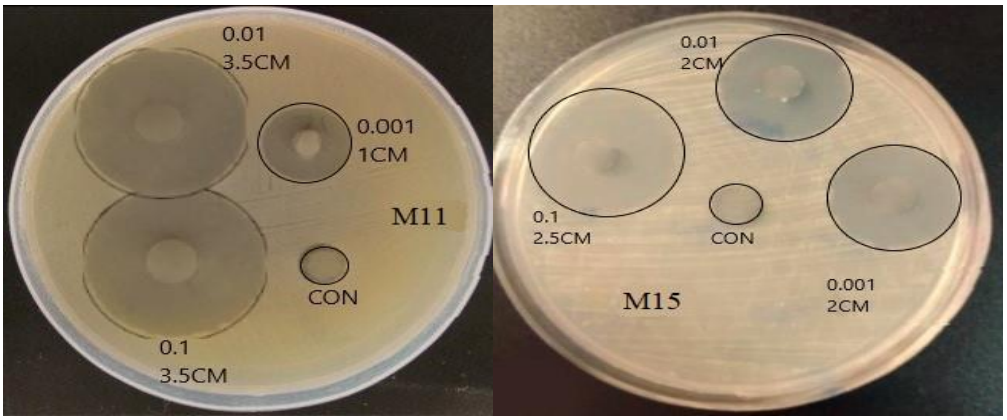


Fig. 7. Biological effectiveness of the compounds M_{12} and M_{15} against *Escherichia Coli*

3.3. Results of measuring the laser activity of some prepared compounds [21, 22]. In this study, the laser effectiveness of each compound was measured by irradiating some of the prepared compounds (M_{11}, M_{12}, M_{13}, M_{14}, M_{15}) for different periods (15, 30, 45, 60 seconds) using a helium-neon laser device, in addition to the beam source and the sample distance between them. Power (1 MW) and wavelength (808 nm) and then studying the physical properties again (color and melting point) and observing the changes that occur in the chemicals that were synthesized. Because the compounds kept their structural structure and physical characteristics throughout the procedure and were unaffected by the laser beams, the investigation demonstrated that the compounds' physical characteristics did not alter throughout the course of 15, 30, and 45 seconds. All of the compounds under study showed a change in their physical characteristics throughout this 60-second period, including a noticeable drop in melting point and a little color shift. These modifications might occur. When compounds are exposed to high energy (laser) for an extended period of time (60 seconds), some of their bonds may break and new compounds may arise as a result, as shown in Table 4.

Table 4. The effect of laser beams on some prepared compounds (M_{11}-M_{15})

	15 s		**30 s**		**45 s**		**60 s**	
Comp No.	**Color**	**M.P (°C)**	**Color**	**M.P (°C)**	**Color**	**M.P (°C)**	**Color**	**M.P (°C)**
M_{11}	Light Yellow	223-225	Light Yellow	223-225	Light Yellow	223-225	Yellow	216-219
M_{12}	Brown	245-247	Brown	245-247	Brown	245-247	Light Brown	231-233
M_{13}	Yellow	232-234	Yellow	232-234	Yellow	232-234	Light Yellow	220-222
M_{14}	Red	219-221	Red	219-221	Red	219-221	Light Red	207-209
M_{15}	Blue	247-249	Blue	247-249	Blue	247-249	Dark Blue	232-234

4. Conclusions

The reaction of the (C=N) group of the hydrazone with chloroacetyl chloride gives four rings called azetidinones [M_{11}-M_{15}]. The validity of the compounds was confirmed using spectroscopic measurements such as infrared and nuclear magnetic resonance spectroscopy to confirm the prepared compounds, as they showed high purity. These compounds also gave high efficacy towards the two types of bacteria used compared to the antibiotic used as a control sample. They also showed high stability towards the helium-neon laser in periods from 1 to 45 seconds, indicating their stability towards normal laboratory conditions and their lack of influence.

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