AMERICAN JOURNAL OF BIOSCIENCE AND CLINICAL INTEGRITY
https://biojournals.us/index.php/AJBCI
ISSN: 2997-7347

Article

Preparation and Study of The Laser and Biological Activity of Some Pyrrolidine-Derived Formazans

Marwan Hasan Ali[1], Maha Mahdi Salih[2], Farah Muthanna Muhammad[3]
1. Teacher, Nineveh Education Directorate, Iraq
2. Teacher, Department of Chemistry, College of Education for Pure Sciences, Kirkuk University, Iraq
3. Teacher, Department of Chemistry, College of Education for Pure Sciences, Tikrit University, Iraq
Email: marwanchem1986@gmail.com[1], mahamahdi@uokirkuk.edu.iq[2], farah.m.mohammed@tu.edu.iq[3]

Citation: Ali, M. H., Salih, M. M., Muhammad, F. M. Preparation and Study of The Laser and Biological Activity of Some Pyrrolidine-Derived Formazans. American Journal Of Bioscience And Clinical Integrity 2026, 3(4), 64-74.

Received: 10th Mar 2026
Revised: 30th Mar 2026
Accepted: 10th Apr 2026
Published: 27th Apr 2026

Abstract: This research involved the preparation of precursors for several heterocyclic compounds using 4-(1-pyrrolidinyl)benzaldehyde as the synthesis nucleus. Imines were reacted with 3-nateroaniline to form pharmazans. Spectroscopic tests, such as proton/carbon nuclear magnetic resonance spectroscopy and infrared spectroscopy, were used to validate the reaction and assess the compounds' activity against two species of bacteria, Staphylococcus aureus and Escherichia coli. The compounds exhibited varying activity levels that increased with increasing concentration, suggesting potential future applications for these precursors in antibiotics.

Keywords: 4-(1-pyrrolidinyl) benzaldehyde, Imines, Pharmazan, Laser, Biological Activity

Introduction

Formazans are considered colored compounds and contain in their composition an active group (-NH -CH -N=N-). These compounds contain electronic transitions which are the cause of the appearance of the color (n*). Formazans are of great importance in analytical chemistry and industrial importance as their bright colors are distinctive dyes. Formazans are considered good antibiotics for infections, allergies, malaria and cancer. The most common methods for preparing formazan include coupling diazonium salt cations with imine hydrazine.The reaction occurs under controlled conditions of pH(6-8) and temperature (5-0°C). Formazan also has important pharmaceutical applications [1].

Experimental part

1. Preparation: carboxylic acid derivative. (HA)

Similar moles (0.025) of four different substances with different weights (4.375 g) of 4-(1-pyrrolidinyl)benzaldehyde, (1 g) of sodium hydroxide, (3.2 g) of alpha-dichloroacetic acid and (1.9 g) of thiourea were collected in ethanol in (15) ml of absolute ethanol and the mixture was heated for (4) hours, then cooled, filtered and crystallized from the ethanol. The precipitate was pink [2], with a melting point of (207-210) and a yield of (64%).

2. Preparation:-1,3,4-thiadiazol-2-amine derivative (HA$_2$)

Equal amounts of HA and thiamacarbide were mixed in a suitable heat-resistant conical glass flask without the use of any solvent. The mixture was then heated slowly and gently for (5-10) minutes, stirring and mixing thoroughly until the color changed, indicating that the reaction was complete. The product was collected and then recrystallized from ethanol. The yield was (91%) and had a melting point of (225-227) °C [3].

3. Preparation of Eminem (HA$_3$-HA$_6$): The following compounds were prepared by dissolving (0.0025) mol of (HA) substitutes in (30) ml of absolute ethanol, to which (0.0025) mol of benzaldehyde substitutes were added with stirring, then (2) drops of glacial acetic acid were added to it and the mixture was raised for a period of (5) hours, the solution was cooled and concentrated, then the precipitate was filtered, dried and recrystallized from ethanol [4] as shown in the equations and physical properties in Table No. (1) according to the following**:**

Table1. The physicochemical characteristics of Eminem derivatives (HA3-HA6)

Comp. No.	R	M.P (°C)	Yield %	Powder Color
HA$_3$	4- Br	215-217	64	Gray
HA$_4$	4- Cl	220-222	67	Light Yellow
HA$_5$	4- NO$_2$	231-233	70	Light brown
HA$_6$	4- OCH$_3$	221-223	69	Yellow

4. Preparation of diazonium salt(HA$_7$): (**a**)- Dissolve 0.015 mol of nitroaniline in (10) ml of a solution containing an acidic solution of (1:1) water concentrated hydrochloric acid (37%), with stirring and maintaining the temperature of the solution between (0-5) °C using an ice bath. (**b**)- In another flask, dissolve 0.015 mol of sodium nitrite in the smallest possible amount of distilled water and add the solution of flask (**a**) to it gradually over 5 minutes with stirring and maintaining the temperature between (5-0) °C for 15 minutes [5].

5. Preparation of formazan(HA$_8$-HA$_{11}$): In 20 milliliters of ethanol, dissolve one mole of each of (HA3-HA6) and one mole of diazonium salt. Heat the mixture for six hours, then add crushed ice, filter the precipitate, and recrystallize it from ethanol [6], [7]. The physical characteristics of derivatives of Schiff bases (HA7-HA10) are displayed in Table No. (2).

Table 2. Physical Properties of Synthesized Formazan Derivatives (HA8–HA11)

Comp. No.	R	M.P (°C)	Yield %	Powder Color
HA$_8$	4- Br	200-202	66	Greenish
HA$_9$	4- Cl	196-198	63	LightYellow
HA$_{10}$	4- NO$_2$	201-203	65	LightYellow
HA$_{11}$	4- OCH$_3$	186-188	58	Yellow

*Identification of the prepared compounds The prepared compounds were identified and were in accordance with the organic diagnostic literature

Table 3. FT-IR Spectral Data of Synthesized Compounds (HA3–HA6)

Comp. No	R	ν Ar-H	ν C=N	ν C=C-Ar	νC-N	Others
HA$_3$	4-Br	3063	1593	1558,1525	1386	ν C-Br 636
HA$_4$	4-Cl	3047	1623	1533,1502	1384	ν C-Cl 808

HA$_5$	4-NO$_2$	3041	1631	1602,1521	1388	νNO$_2$asy1456 sy 1311
HA$_6$	4-OCH$_3$	3053	1623	1531,1512	1385	νCH$_3$ sy 2860 asy 2947

Table 4. FT-IR Spectral Data of Synthesized Formazan Derivatives (HA8–HA11)

Comp. No	R	ν N-H	ν Ar-H	ν C=C-Ar	νN=N	νC-N	Others
HA$_8$	4-Br	3413	3076	1600,1521	1475	1386	ν C-Br 684
HA$_9$	4-Cl	3398	3057	1604,1566	1481	1361	ν C-Cl 731
HA$_{10}$	4-NO$_2$	3401	3067	1602,1531	1432	1319	νNO$_2$asy1483 sy 1352
HA$_{11}$	4-OCH$_3$	3228	3049	1596,1523	1452	1377	νCH$_3$sy2833 asy 2956

In ^{1}H-NMR analysis of compound HA$_3$, two signals were found at 2.00 ppm and 3.27 ppm, which were traced to CH$_2$ in the pyrrolidine ring. Aromatic ring signatures were also found in the 6.53–8.21 ppm range, with a signal at 9.66 ppm that was implied to H-C=N, as shown in Figure 5.

In 1H-NMR analysis of compound HA$_5$, two signals were found at 2.00 ppm and 3.30 ppm, which were traced to CH$_2$ in the pyrrolidine ring. Aromatic ring signatures were also found in the 6.58–7.70 ppm range, with a signal at 8.23 ppm that was implied to H-C=N, as shown in Figure 6.

In analysis (^{1}H-NMR) analysis of compound (HA$_8$) showed the removal of azomethine, leaving two signals at 1.98 ppm and 3.33 ppm, which were traced back to CH$_2$ on the pyrrolidine ring. Aromatic ring signals were also found in the 6.63–8.51 ppm range, with one signal being incorporated into the CH-N=N- group at 3.34 ppm, as shown in Figure 7.

In the (^{1}H-NMR) analysis of compound (HA$_{11}$), the removal of azomethine was shown, leaving two signals at 1.36 ppm and 1.99 ppm, which were traced back to CH$_2$ on the pyrrolidine ring. Aromatic ring signals were also found in the 7.45–8.04 ppm range, with one signal being incorporated into the (CH-N=N-) for the azo group at (3.45 ppm), as shown by the sign of the (OCH$_3$) substituent at (3.19) parts per million as in Figure 8.

When analyzing the ^{13}C-NMR spectrum of compound HA$_3$, two signals were found at 33.64 and 47.73 ppm, which were incorporated into the CH$_2$ subunit of the pyrrolidine ring. Aromatic ring signals were also found at 111.76–175.52 ppm, as shown in Figure 9.

When analyzing the ^{13}C-NMR spectrum of compound HA$_5$, two signals were found at 33.38 and 47.84 ppm, which were incorporated into the CH$_2$ subunit of the pyrrolidine ring. Aromatic ring signals were also found at 111.75–190.17 ppm, as shown in Figure 10.

When discussing the (^{13}C-NMR) spectrum of the compound (HA$_3$), two signals were found in two locations, (33.41, 44.00) ppm , which were included in (CH$_2$) in the pyrrolidine ring. The (OCH$_3$) substituent signal was also found at (48.27) ppm , and a signal was included in (CH-N=N-) at (107.14) parts per million. The aromatic ring signals were also found at (176.56-119.56) ppm, as shown in Figure (11).

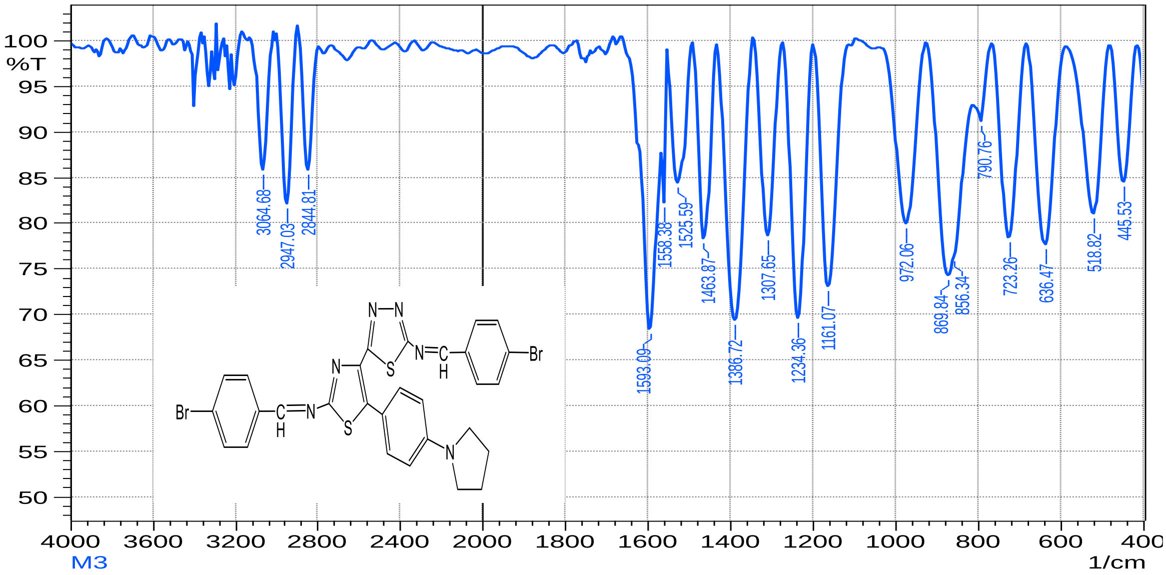


Figure 1. FT-IR of (HA3).

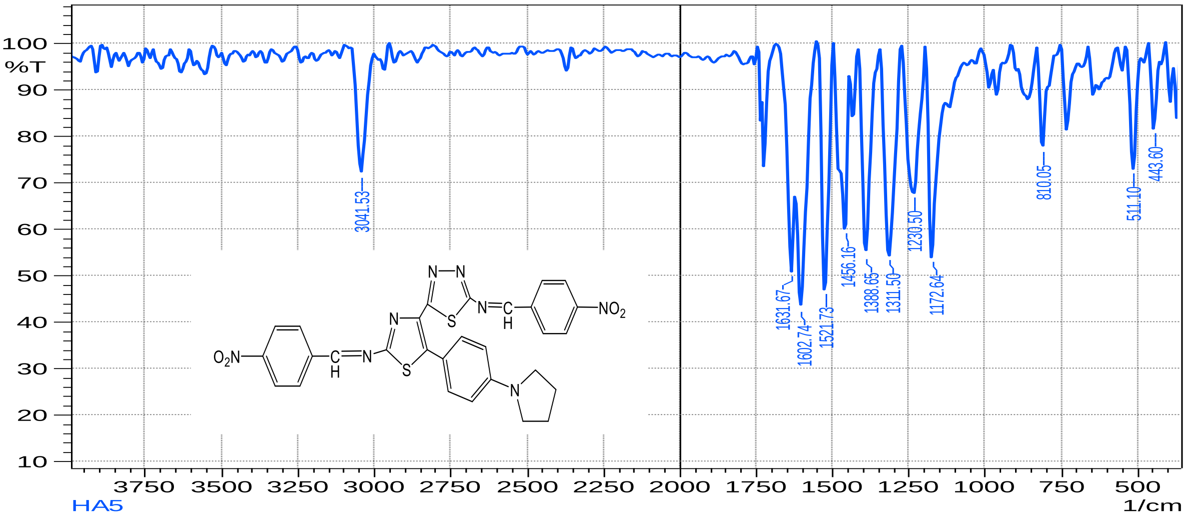


Figure 2. FT-IR of (HA5).

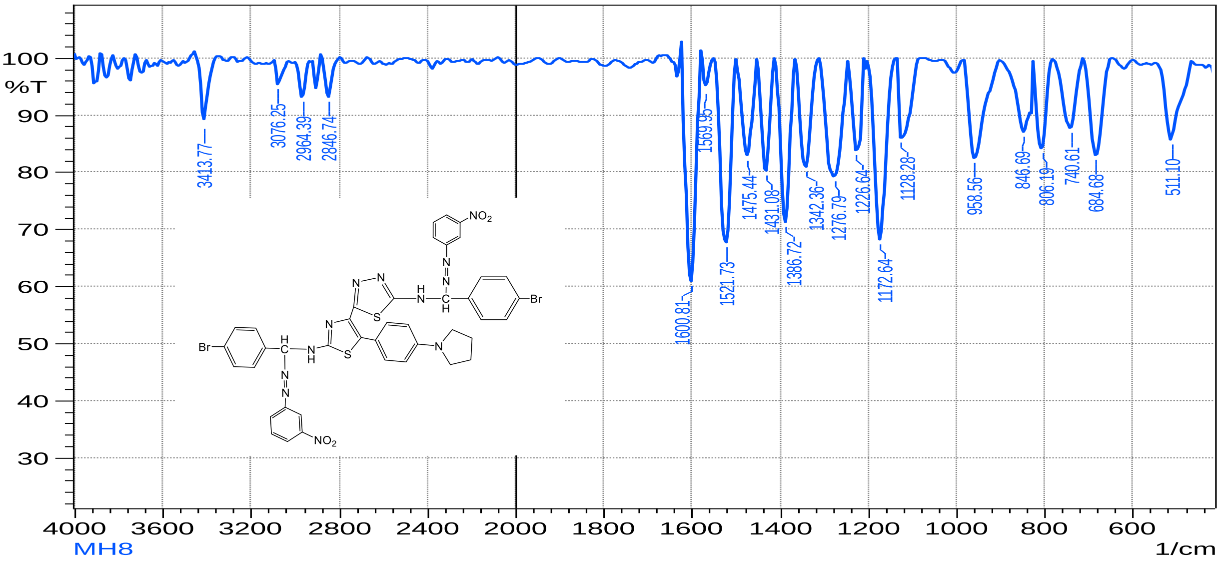


Figure 3. FT-IR of (HA6).

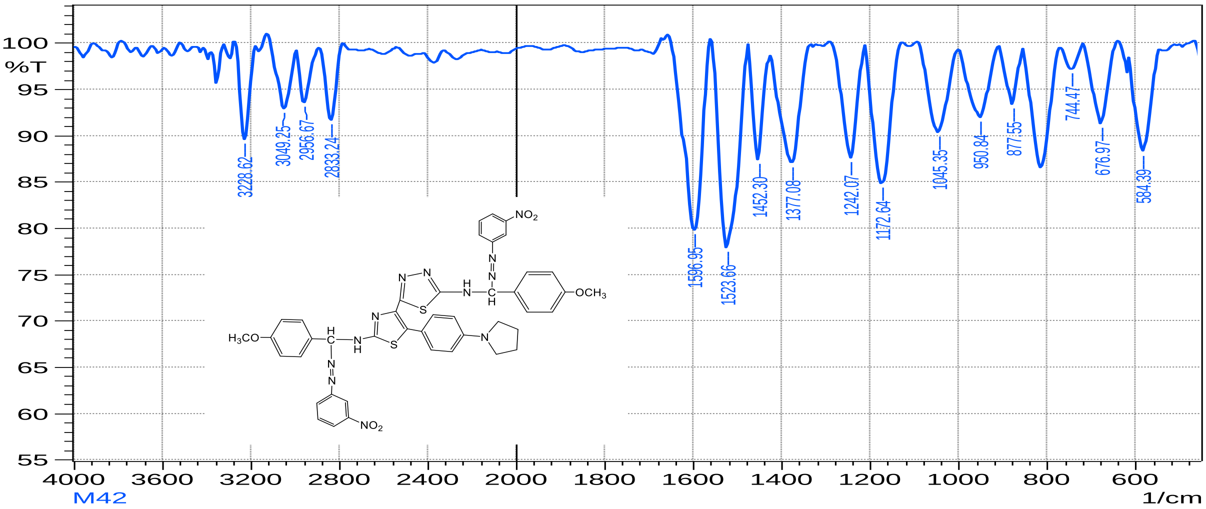


Figure 4. FT-IR of (HA11).

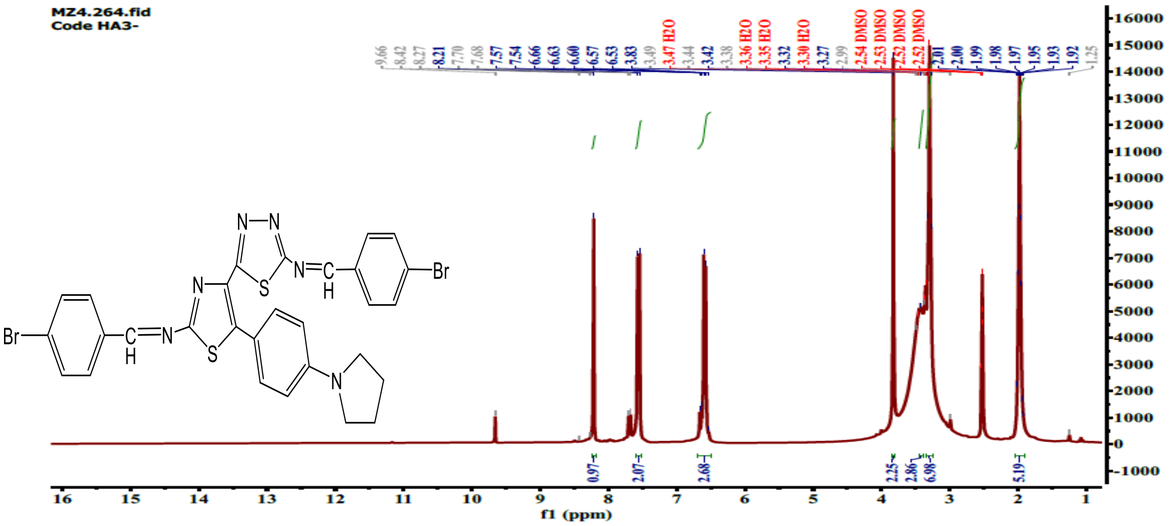


Figure 5. (^{1}H-NMR) of (HA3).

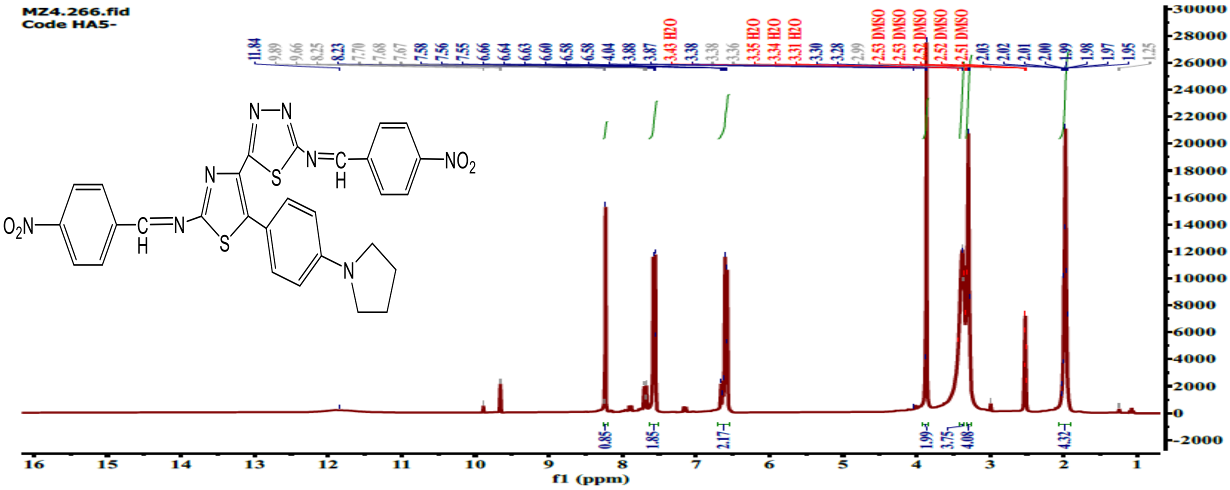


Figure 6. (^{1}H-NMR) of (HA5).

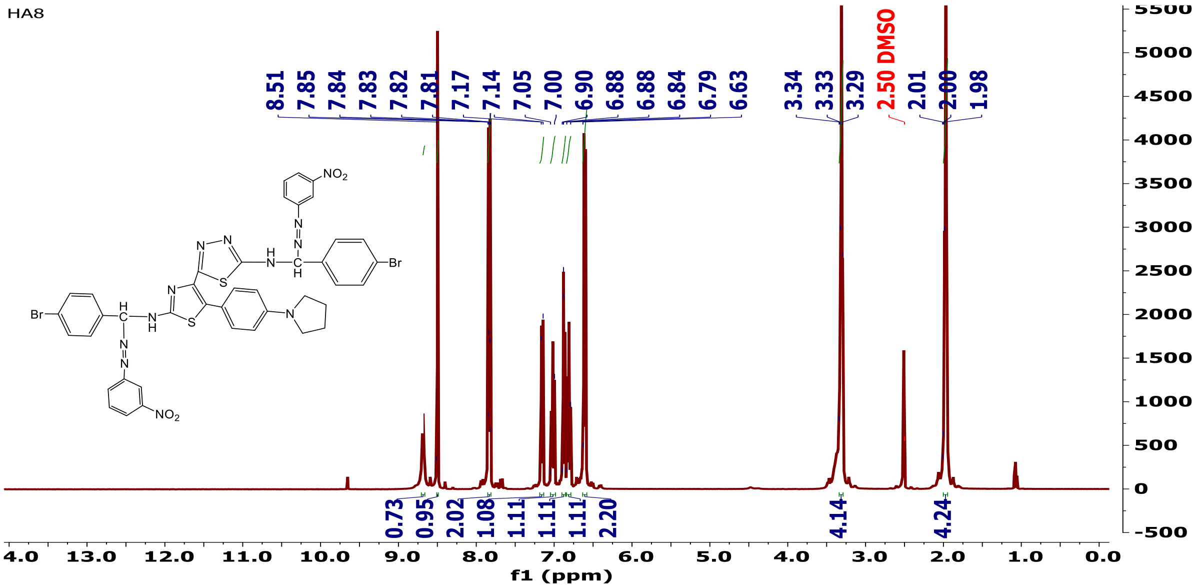


Figure 7. (^{1}H-NMR) of (HA$_8$).

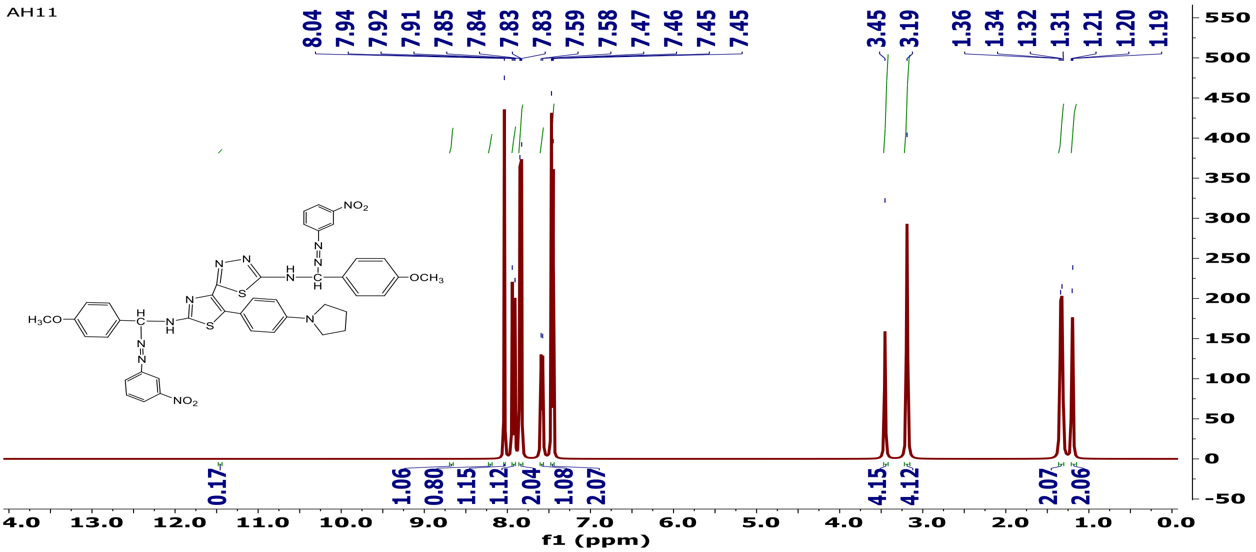


Figure 8. (^{1}H-NMR) of (HA$_{11}$).

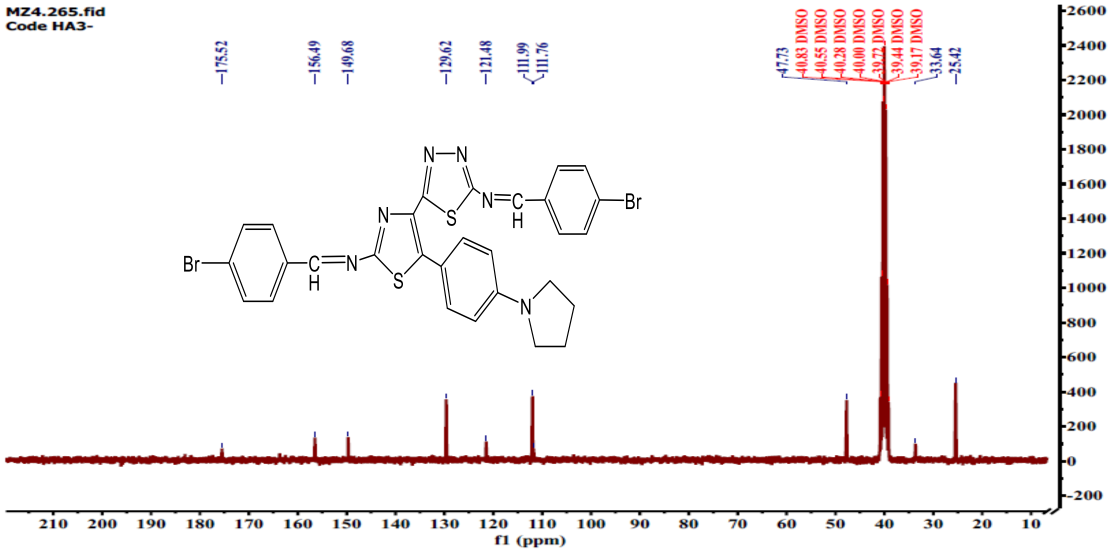


Figure 9. (^{13}C-NMR) of (HA$_3$)

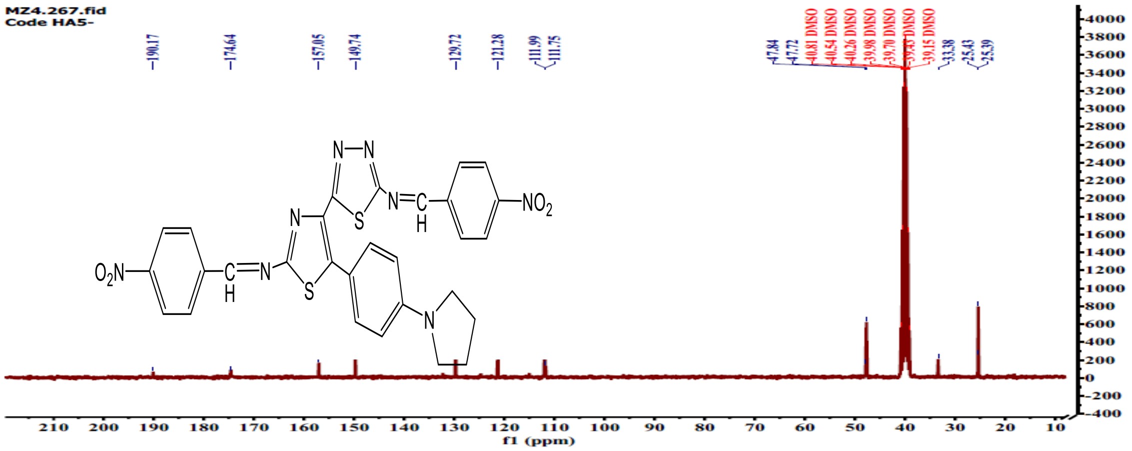


Figure 10. (^{13}C-NMR) of (HA_5)

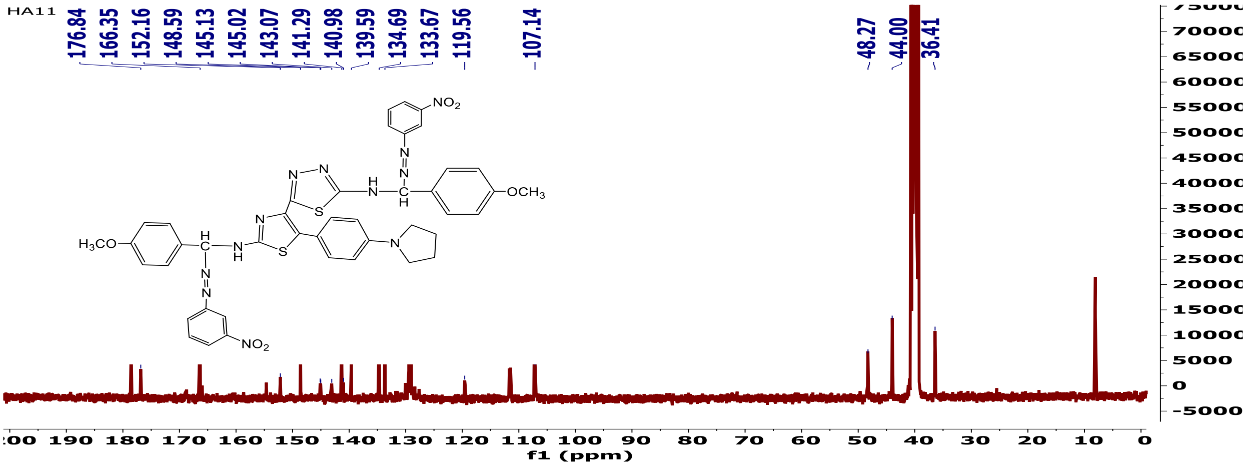
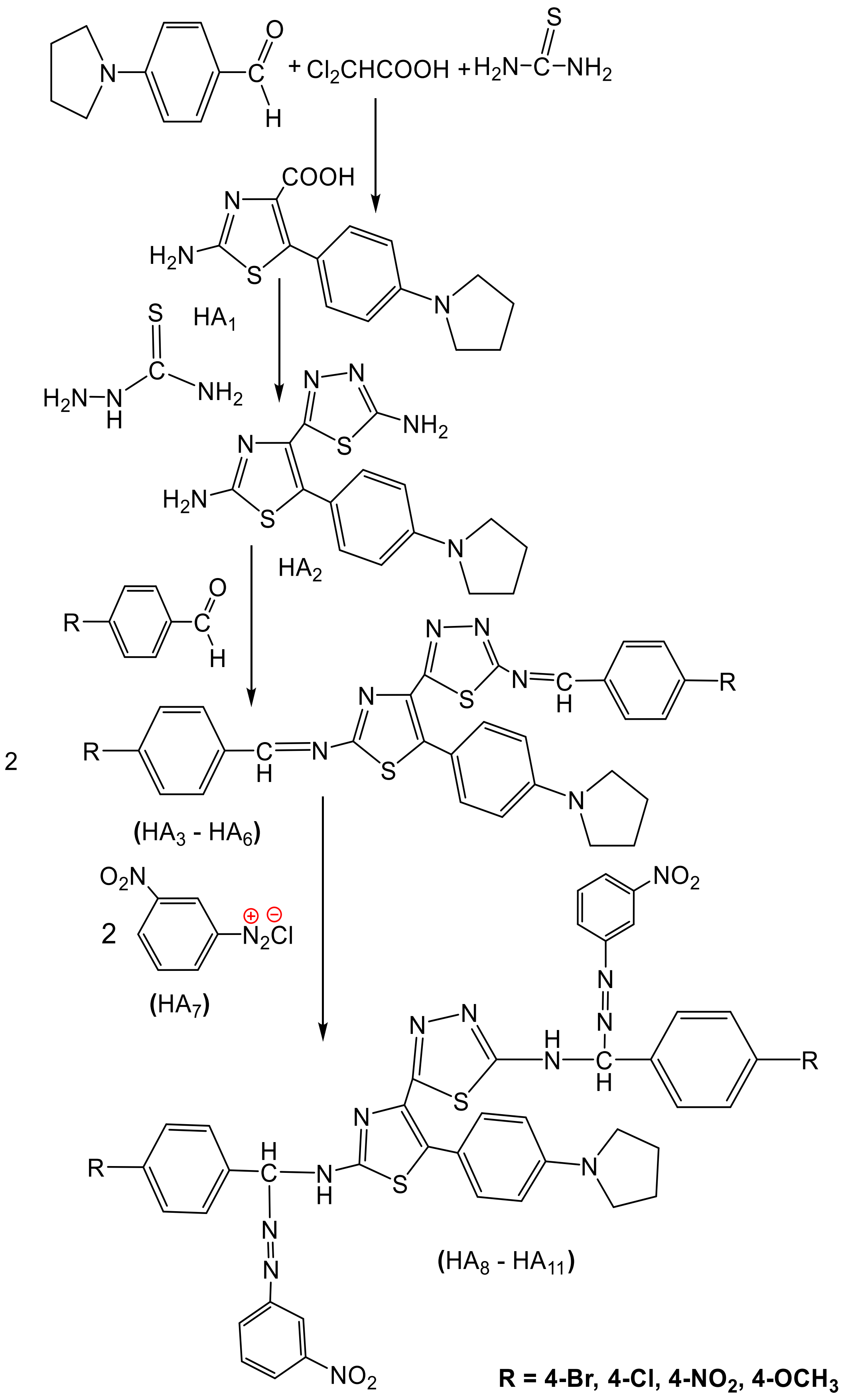


Figure 11. (^{13}C-NMR) of (HA_{11})



The laser activity was determined by utilizing a (helium-neon) laser device to irradiate some of the produced compounds (HA8, HA9, HA19, and HA11) for varying durations (20, 30, 40, and 50) seconds for each compound. The wavelength was 808 nm, the power was 1 milliwatt, and the distance between the beam source and the sample was 10 cm. The physical characteristics (color, melting point, and flow rate (Rf)) were then examined once again, and the alterations that took place in the created compounds were noted. Because the chemical compounds maintained their structural structure and physical properties and were not impacted by the laser beams, their physical properties did not alter over the study's time intervals of 20, 30, and 40 seconds.Over the course of the 50 seconds, all of the compounds under investigation had changes in their physical properties. and there was a noticeable drop in their melting points along with a shift in the thin-layer chromatography (TLC) flow rate (Rf) values. As indicated in Table, Results of measuring the laser activity of some prepared compounds, slight color changes are probably caused by the breakage of some bonds in the compounds, and new compounds may have been formed as a result of long and continuous exposure to high energy (laser beams) for 50 seconds.

Table 5. Effect of Solvent System Composition (20S–50S) on Rf Values and Physical Properties of Formazan Derivatives (HA8–HA11)

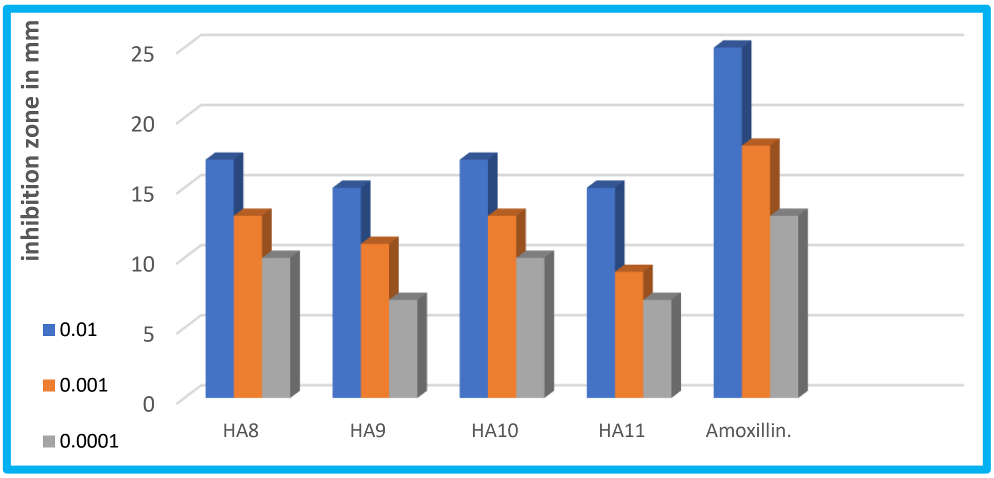
Comp No.	20 S			30 S			40 S			50 S		
	Color	M.P (^{0}C)	R*f*	Color	M.P (^{0}C)	R*f*	Color	M.P (^{0}C)	R*f*	Color	M.P (^{0}C)	R*f*
HA_8	Greenish	200-202	0.47	Greenish	200-202	0.47	Greenish	200-202	0.47	Light	122-124	0.72
HA_9	LightYellow	196-198	0.42	LightYellow	196-198	0.41	LightYellow	196-198	0.42	Light	128-130	0.32
HA_{10}	LightYellow	201-203	0.55	LightYellow	201-203	0.56	LightYellow	201-203	0.55	Light brown	118-120	0.34
HA_{11}	Yellow	186-188	0.59	Yellow	186-188	0.59	Yellow	186-188	0.59	Light Yellow	110-111	0.76

Evaluation of the biological activity of the prepared compounds

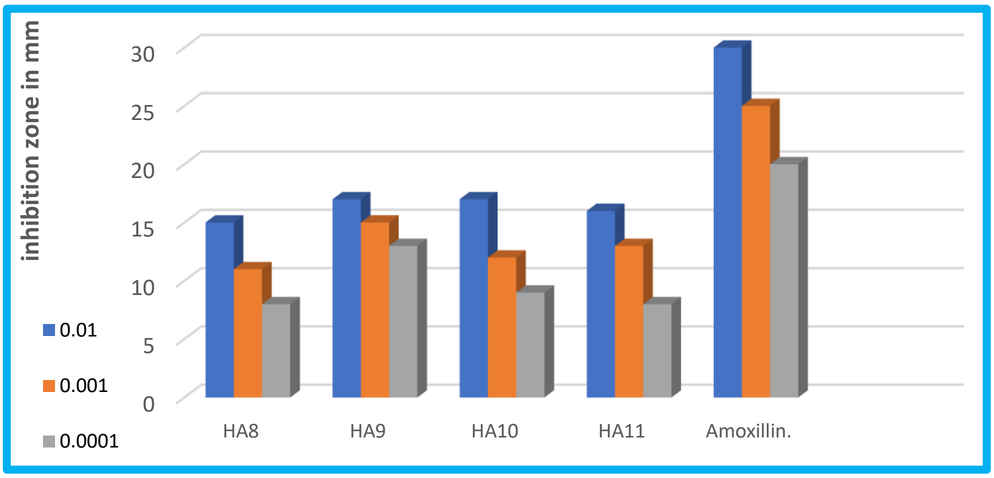
Each well of the hole should contain 40 µl of each of the three concentrations of the prepared compounds. The Petri dish should then be incubated at 37 degrees Celsius for 24 hours. To show the sensitivity of the derivatives used, the results should be read after 24 and 48 hours [8–10]. This depends on the apparent inhibitory diameter of the Petri dish surrounding the wells used, as an increase in the inhibitory diameter indicates an increase in the biological efficacy of the prepared compound [11,12]. Compounds HA8 and HA9 demonstrated strong inhibitory activity against bacteria with an inhibitory diameter of 20, 19 mm, and HA10 and HA11 demonstrated strong inhibitory activity against bacteria with an inhibitory diameter of 19, 18 mm. The greater the concentration, the greater the inhibitory effect, the concentration with the highest inhibitory ratio was 0.01 mg/ml[13,14,15].

Table 6. Inhibitory activity of compounds with diameter of mm

Comp No.	***Staphylococcus aureus* mg/ml**			***Escherichia coli* mg/ml**		
	0.01	**0.001**	**0.0001**	**0.01**	**0.001**	**0.0001**
HA_8	17	13	10	15	11	8
HA_9	15	11	7	17	15	13
HA_{10}	17	13	10	17	12	9
HA_{11}	15	9	7	16	13	8
Amoxillin.	25	18	13	30	25	20



Scheme 1. Inhibitory activity of compounds against Staphylococcus aureus



Scheme 2. Inhibitory activity of compounds against *Escherichia coli*

Conclusion

Spectroscopic measurements confirmed the accuracy of the synthesized products. Biological activity demonstrated varying abilities of these repositories to disinfect large areas of bacteria, with increasing inhibition capacity as concentration increased. Laser activity showed that the compounds exhibited high stability after 15-45 seconds of perspiration, but decreased stability after one minute of perspiration due to bond breakage.

REFERENCES

[1] M. N. Ibrahim and S. E. Sharif, “Journal of Chemistry,” vol. 4, no. 4, pp. 531–535, 2007.

[2] Z. K. Al-Dulaimy, A. A. Kadhim, A. Kh. Attia, and M. Z. Thani, “Synthesis and antibacterial study of new 2-amino-5-aryl-1,3-thiazole-4-carboxylic acid derivatives,” *Ibn Al-Haitham J. Pure Appl. Sci.*, vol. 26, no. 2, 2013.

[3] S. Jubie, R. Kalirajan, and P. Kumar Yadav, “Journal of Chemistry,” vol. 9, no. 2, pp. 980–987, 2012.

[4] H. M. Hassan, “Synthesis and identification of some heterocyclic compounds derived from 2,6-dipicolinic acid and study of biological activity,” M.S. thesis, Univ. Tikrit, 2019.

[5] D. A. Hassan, “Synthesis and spectral identification of azo dye derivative from 4-amino acetophenone,” *J. Basrah Res. (Sci.)*, vol. 21, no. 4, 2008.

[6] R. M. Desai and J. M. Desai, “Synthesis and antimicrobial activity of some new formazan derivatives,” *Indian J. Heterocycl. Chem.*, vol. 8, pp. 329–331, 1999.

[7] Y. K. Alasadi, F. H. Jumaa, and A. H. Dalaf, "Synthesis, identification, antibacterial activity and laser effect of new derivatives of bis-1,3-oxazepene-4,7-dione and 1,3-diazepine-4,7-dione," *AIP Conf. Proc.*, vol. 2394, no. 1, 2022.

[8] F. M. Muhammad, B. A. Khairallah, J. N. Saleh, and J. N. Saleh, "Preparation and characterization of new rings of oxazine derivatives and studying their biological and laser effectiveness and molecular docking," *Central Asian J. Theor. Appl. Sci.*, vol. 5, no. 4, pp. 190–201, 2024.

[9] M. M. Saleh, J. N. Saleh, F. F. Rokan, and M. J. Saleh, "Synthesis, characterization, and evaluation of bacterial efficacy and study of molecular substrates of cobalt (II) complex [Co(2-(benzo[d]thiazol-2-yloxy) acetohydrazide)(H_2O)(Cl_2)]," *Central Asian J. Med. Nat. Sci.*, vol. 5, no. 4, 2024.

[10] S. T. A. W. A., J. N. Saleh, M. J. Saleh, and H. M. Saleh Al-Jubori, "Preparation and characterization of new imidazole derivatives derived from hydrazones and study of their biological and laser efficacy," *Central Asian J. Theor. Appl. Sci.*, vol. 5, no. 4, pp. 202–211, 2024.

[11] A. W. A. S. Talluh, R. S. Najm, M. J. Saleh, and J. N. Saleh, "Synthesis, characterization, and evaluation of the biological activity of novel oxazepine compounds derived from indole-5-carboxylic acid," *Amer. J. Biosci. Clin. Integrity*, vol. 1, no. 8, pp. 10–19, 2024.

[12] M. J. Saleh *et al.*, "Preparation and characterization of some oxazolidine-5-one derivatives and evaluation of their biological activity," *South Asian Res. J. Nat. Prod.*, vol. 8, no. 1, pp. 74–84, 2025.

[13] M. J. Saleh *et al.*, "Use of solid basic catalysts in the preparation of cyclohexenone derivatives and evaluation of their bacterial activity," *Vital Annex: Int. J. Novel Res. Adv. Sci.*, vol. 3, no. 3, pp. 104–112, 2024.

[14] A. H. Dalaf, J. N. Saleh, M. J. Saleh, and A. W. A. S. Talluh, "Environmentally friendly synthesis, bioactivity evaluation and multi-faceted characterization of bis(5-((1H-imidazol-4-yl)methyl)-3-phenylimidazolidin-4-one) derivatives," *Amer. J. Biomed. Pharm.*, vol. 1, no. 7, pp. 104–114, 2024.

[15] J. N. Saleh and A. Khalid, "Synthesis, characterization and biological activity evaluation of some new pyrimidine derivatives by solid base catalyst Al_2O_3-OBa," *Central Asian J. Med. Nat. Sci.*, vol. 4, no. 4, pp. 231–239, 2023.