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Vanillin-derived heterocycles via esterification with malic anhydride: synthesis, characterization, and antibacterial properties

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ABSTRACT:

This study focuses on the synthesis of three heterocyclic compounds (M1a, M1b, M1c) through the esterification of vanillin with malic anhydride. The ester (M) was synthesized by reacting vanillin (4-hydroxy-3-methoxybenzaldehyde) with malic anhydride. A Schiff base was then formed by reacting the vanillin ester (M) with aniline, followed by an addition reaction to the C=N double bond, yielding the heterocyclic compounds. The compounds were characterized by thin-layer chromatography (TLC) and melting point determination, as well as by Fourier-transform infrared spectroscopy (FTIR), proton nuclear magnetic resonance (^{1}H-NMR), and carbon-13 nuclear magnetic resonance (^{13}C-NMR). The synthesized compounds demonstrated superior antibacterial activity against both Gram-positive (Staphylococcus aureus) and Gram-negative (Escherichia coli) bacteria compared to ciprofloxacin. The inhibition zones for the compounds ranged from 18 to 25 mm for *E. coli* and *S. aureus*, compared to 12 to 15 mm for the drug. The heterocyclic compounds exhibited stronger antibacterial activity, with larger inhibition zone diameters compared to ciprofloxacin.

Keywords: Vanillin Schiff's bases, Heterocyclic compounds, Staphylococcus aureus, Escherichia coli.

INTRODUCTION

Vanillin (4-hydroxy-3-methoxybenzaldehyde) is an aromatic compound with functional groups including an aldehyde, phenol, and ether (Fig. 1), which can be derived from plant extracts [1-4]. Schiff bases are formed through a condensation reaction between primary amines and carbonyl compounds, first discovered by Schiff in 1864 [5-6]. A defining feature of Schiff bases is the imine group, with the general formula RHC = N-R1, where R and R1 can be alkyl, aryl, or heterocyclic groups, often substituted. These compounds are also known as imines or azomethines [7-8].

Heterocyclic compounds play a key role in medicinal chemistry, being integral to many biomolecules, including vitamins, enzymes, and bioactive substances. These include antimicrobial, antibacterial, anti-allergic, antioxidant, enzyme inhibitory, anticancer, and herbicidal agents [9-11]. The lone electron pair in the sp^2 hybrid orbital of the nitrogen atom in the azomethene group is crucial for its chemical and biological activity. Schiff bases are

particularly valued for their simple synthesis and the unique properties of the C=N bond [12-13]. Their significance has grown in various pharmacological fields, including antibacterial, antifungal, antitumor, and antipyretic applications. Aryl-substituted Schiff bases are especially stable and easy to produce.

The increasing prevalence of multidrug-resistant infections and the emergence of antibiotic-resistant strains highlight the urgent need for novel antibacterial agents [14-16]. This study aims to synthesize heterocyclic compounds containing nitrogen, oxygen, and sulfur from vanillin esterification for potential medical and biological applications.

EXPERIMENTAL

Instruments

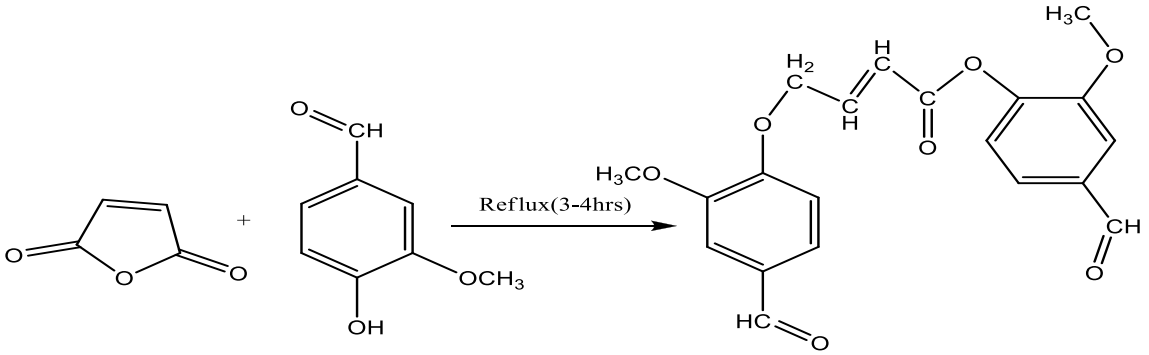
The techniques used in the investigation of the prepared compound were a melting point apparatus type MFB-600-Gallenkamp, an NMR instrument of type Varian-Ultra Shield- 300 MHz, Switzerland, with DMSO-d6, and an infrared spectrophotometer (FT-IR) IR Affinity- 1S (Shimadzu, Japan).

Chemical reagents and standard solutions

All chemicals and reagents used in this investigation were of high purity and were employed without further purification.

Synthesis of vanillin esterification (compound M)

The preparation procedure involved adding 0.06 mol of vanillin to a 250 mL round-bottom flask containing 100 mL of dioxane. Then, 0.03 mol of malic anhydride was introduced, and the mixture was refluxed and stirred for 3–4 hours at 90–100 °C. After completion of the reaction, the solvent was removed under reduced pressure using a rotary evaporator. The resulting compound was washed with diethyl ether, yielding a brown solid. The synthesis diagram is shown in Scheme 1.

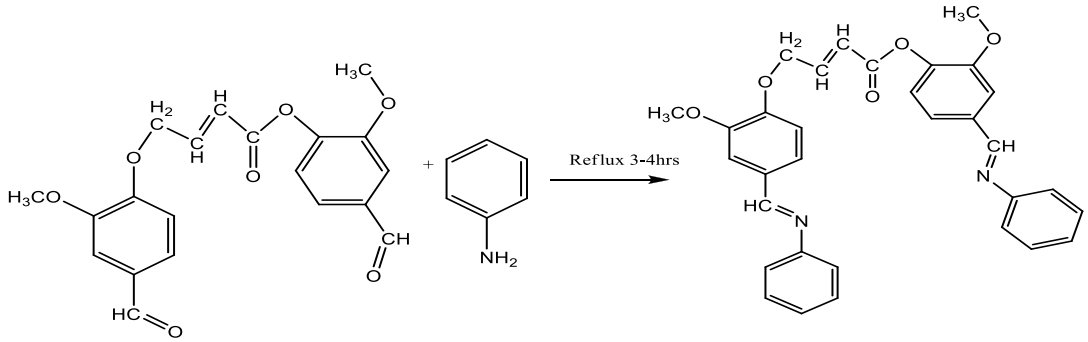


Scheme. 1. Synthesis of Vanillin and maleic anhydride compound ((E)-4-formyl-2-methoxyphenyl 4-(4-formyl-2-methoxyphenoxy)but-2-enoate ester(M)

Synthesis of Schiff Base Ester

A Schiff base, (E)-2-methoxy-4-((E)-(phenylimino)methyl)phenyl 4-(2-methoxy-4-((E)-(phenylimino)methyl)phenoxy)but-2-enoate (M1), was synthesized by reacting 0.008 mol of aniline with 0.004 mol of the ester (compound M) in the presence of a few drops of glacial acetic acid. The reaction mixture was dissolved in 100 mL of dichloromethane and placed in a 250 mL

round-bottom flask, then stirred for 3–4 hours at 90–100 °C. After the reaction was complete, the solvent was removed under reduced pressure using a rotary evaporator. The resulting solid was filtered, washed with ethanol, and dried. The melting point of the product, compound M1, was determined to be 170 °C, as shown in scheme 2.



Scheme. 2. Chemical structure of M_1 compound.

Synthesis of Heterocyclic compounds [17-18]

Synthesis of (E)-2-methoxy-4-(1-phenyl-2,5-dihydro-1H-tetrazol-5-yl)phenyl 4-(2-methoxy-4-(1-phenyl-2,5-dihydro-1H-tetrazol-5-yl)phenoxy)but-2-enoate ester (M_1a)

A solution containing 0.002 moles of compound M1 and 0.004 moles of sodium azide was prepared by dissolving the reagents in a 250 mL round-bottom flask. This solution was then combined with 100 mL of dimethylamine (DMA) solution and stirred for 20–24 hours at 90–110 °C. After the reaction, the solvent was removed under reduced pressure using a rotary evaporator. The progress of the reaction was monitored by thin-layer chromatography (TLC). The product was isolated by filtration and purified through recrystallization from benzene. Compound M1a was obtained with a melting point of 195 °C, as shown in Scheme 3.

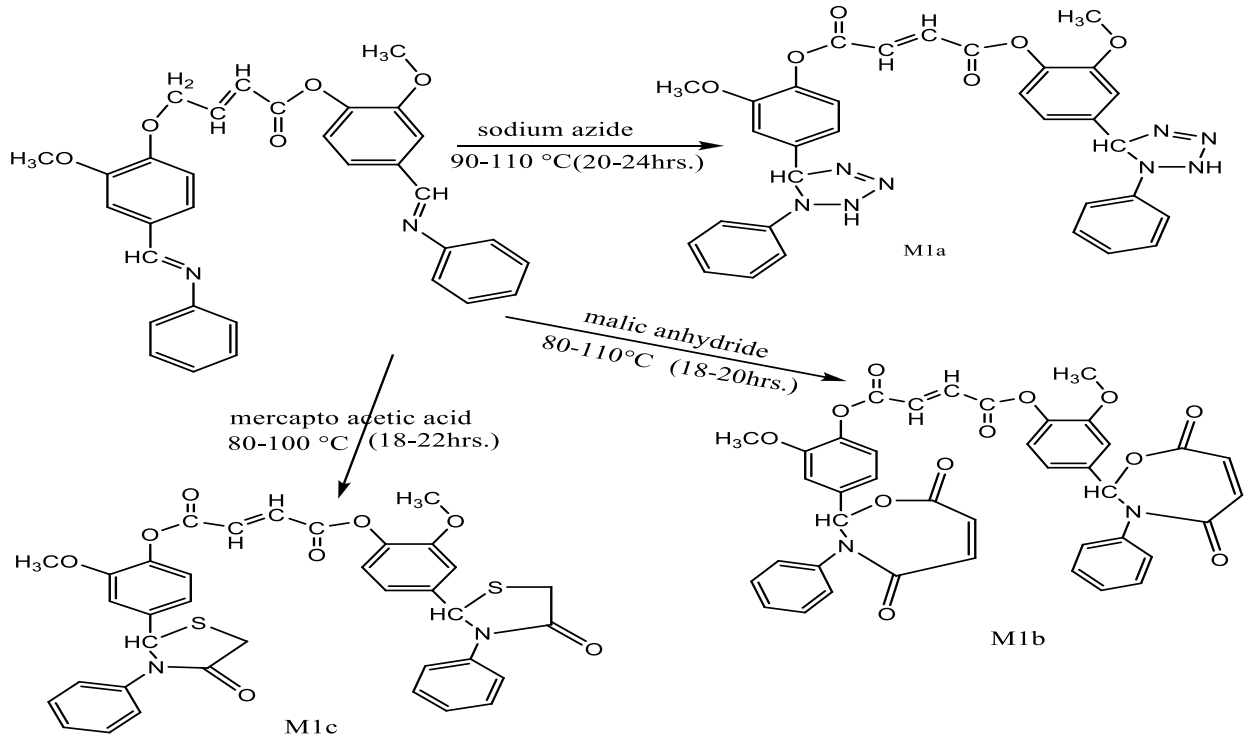
Synthesis of bis(4-(4,7-dioxo-3-phenyl-2,3,4,7-tetrahydro-1,3-oxazepin-2-yl)-2-methoxyphenyl) fumarate (M_1b)

To prepare compound M1b, 0.003 moles of compound M1 and 0.006 moles of malic anhydride were dissolved in 150 mL of Dimethylformamide (DMF) in a 250 mL round-bottom flask. The mixture was stirred for 18–20 hours at 80–110 °C. After the reaction, the solvent was removed under reduced pressure using a rotary evaporator. The reaction progress was monitored by thin-layer chromatography (TLC). The product was isolated by filtration and purified by recrystallization with diethyl ether. Compound M1b was obtained with a melting point of 156 °C, as shown in Scheme 3.

Synthesis of bis(2-methoxy-4-(4-oxo-3-phenylthiazolidin-2-yl)phenyl) fumarate (M_1c)

To prepare compound M1c, 0.003 moles of compound M1 and 0.006 moles of mercaptoacetic acid were added to a 250 mL round-bottomed flask. The mixture was dissolved in 100 mL of dioxane and stirred for 18–22 hours at 80–100 °C. After the reaction, the solvent was removed under reduced pressure using a rotary evaporator. The progress of the reaction was monitored by thin-layer chromatography (TLC). The product was isolated by filtration and

purified by recrystallization with diethyl ether. Compound M1c was obtained with a melting point of 170 °C, as shown in Scheme 3.



Scheme. 3. Synthesis of M1a, M1b and M1c Heterocyclic Compounds

Antibacterial activity [19]

The disk diffusion method was employed to assess the antibacterial activity of the synthesized heterocyclic compounds against the Gram-positive bacterium Staphylococcus aureus and the Gram-negative bacterium *Escherichia coli*. Agar and Petri dishes were sterilized by autoclaving at 121 °C for 15 minutes. The sterilized agar was poured into Petri dishes and allowed to solidify. Four 6-mm holes were then punched in the agar, and 0.1 mL of the prepared compound solution (0.01 g in 2 mL of Dimethylformamide) was added to each hole. The plates were incubated at 37 °C for 24 hours to assess antibacterial activity.

Results and discussions

The FTIR Spectrum of vanillin and malic anhydride ester ((E)-4-formyl-2-methoxyphenyl 4-(4-formyl-2-methoxyphenoxy)but-2-enoate) ester (M) [20] is shown in Figure 1.

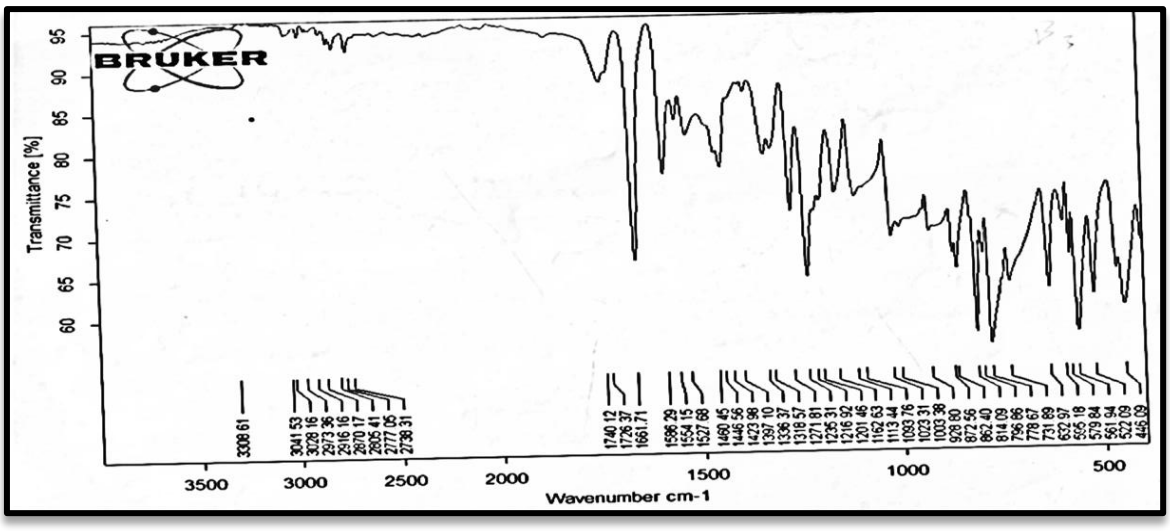


Figure 1. FT-IR spectrum of compound M

The M compound was characterized by FTIR spectroscopy. Two prominent peaks were observed: The C=O ester stretch of the ester group at 1726.37 cm^{-1} and the C=C aromatic stretch at 1661.71 cm^{-1}. Additionally, the hydroxyl (OH) group was absent, as evidenced by the disappearance of the broad peak around 3100 cm^{-1}. In the ^{1}H-NMR spectrum, recorded using dimethyl Sulfoxide (DMSO) as the solvent, the aldehyde proton appeared as a signal at 9.5 ppm. A CH_2 signal was observed at 6.5 ppm, while the aromatic ring protons produced signals around 6.8 ppm. The methyl group protons were observed as a signal at 3.8 ppm.

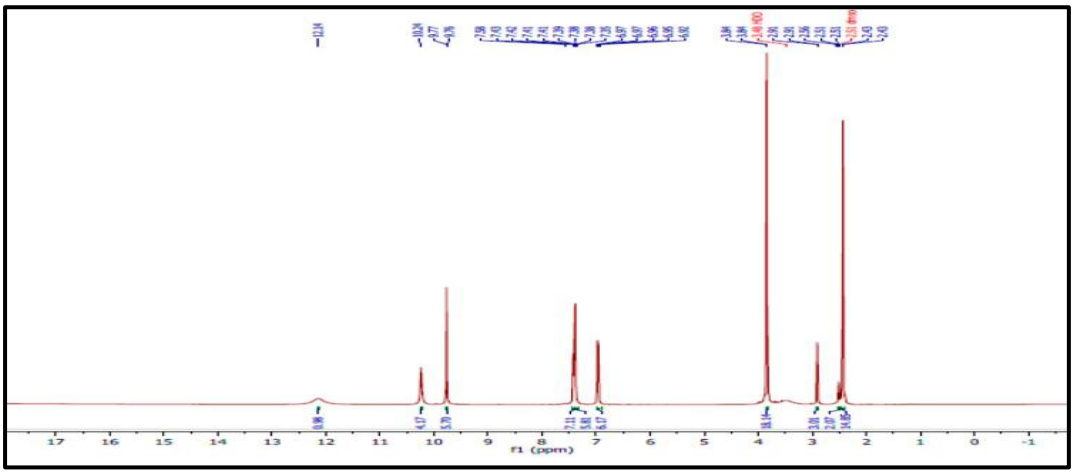


Figure. 2. ^{1}H- NMR spectrum of compound M

The reaction between ester vanillin (M) with primary amines, such as aniline, formed a novel compound (M1). The M1 compound was further characterized using several analytical techniques. In the IR spectrum (KBr, cm^{-1}), notable peaks included 1583.61 cm^{-1} (C=N, imine), 3061.13 cm^{-1} (C-H, aliphatic), 3271.38 cm^{-1} (C-H, aromatic), and 1510 cm^{-1} (C=C), along with the disappearance of the primary amine group (NH_2) in the 3500–3600 cm^{-1} range. In the ^{1}H-NMR spectrum(Figure 4), recorded in DMSO, a proton signal for the azomethine group (HC=N) was observed at 8.3 ppm, while the aromatic ring protons appeared at 6.8 ppm, and the methyl

group protons were detected at 3.8 ppm. The melting point (M.P.) of compound M1 was determined to be 170 °C..

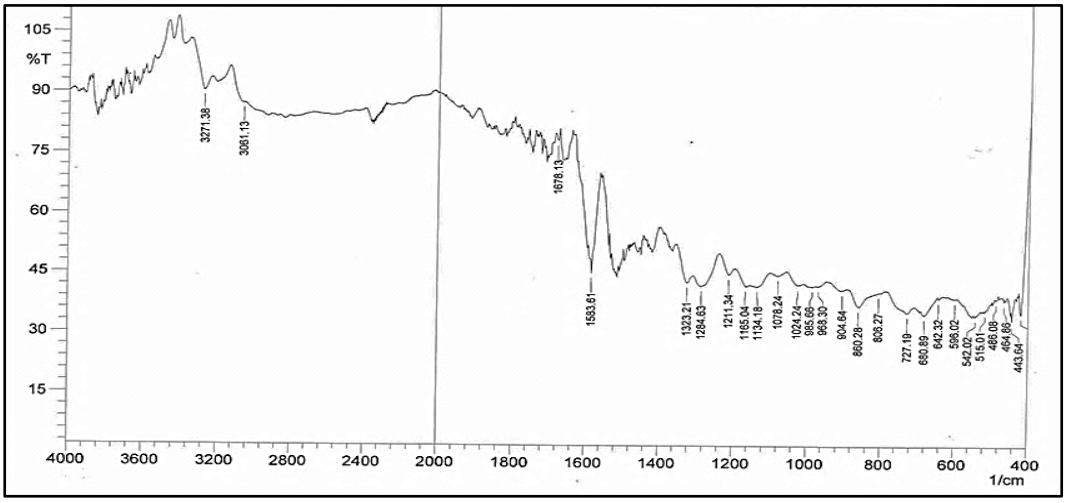


Figure 3. FT-IR spectrum of compound M1

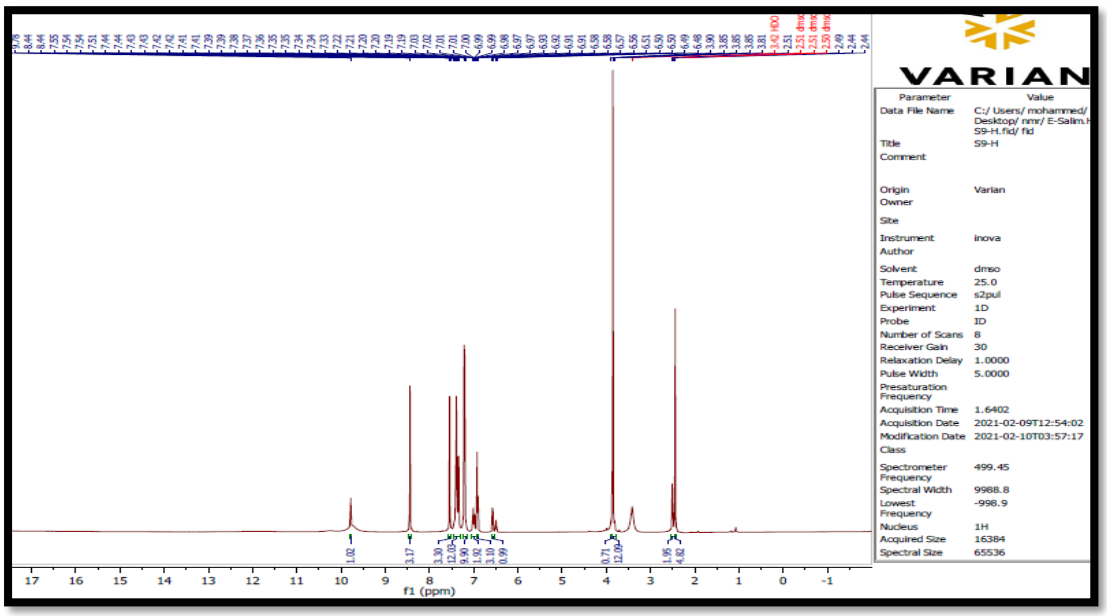


Figure. 4.[1]H-NMR spectrum of compound M1

The heterocyclic compounds (M1a, M1b, M1c) were identified and confirmed using FTIR, H-NMR, and 13C NMR spectra.

Through the addition reaction to the C=N double bond in compound M1, three new heterocyclic compounds were synthesized by reacting the Schiff base (M1) with sodium azide, malic anhydride, and mercaptoacetic acid. This reaction led to the formation of the corresponding heterocyclic compounds [21-22]

Figure 5 shows the FTIR spectrum of compound M1a, which exhibits the following characteristic peaks: 3171.08–3217.37 cm^{-1} (-NH) and 1496.81–1481.38 cm^{-1} (N=N) corresponding to the tetrazole ring. The peak at 1583.61 cm^{-1}, associated with the azomethine group (HC=N), disappears, indicating the cleavage of the azomethine bond and the loss of its absorption band.

In the ^{1}H-NMR spectrum of compound M1a, recorded in DMSO, a singlet appears at 3.85 ppm (CH=N) and another singlet at 8.3 ppm (N-H) corresponding to the tetrazole ring. The ^{13}C-NMR spectrum(Figure 7)shows a singlet at 109.94 ppm (CH=N), a singlet at 167.21 ppm (C=O, ester group), and a singlet at 136.99 ppm (C-O, phenol), as shown in Figure 6. The melting point of compound M1a was determined to be 195 °C.

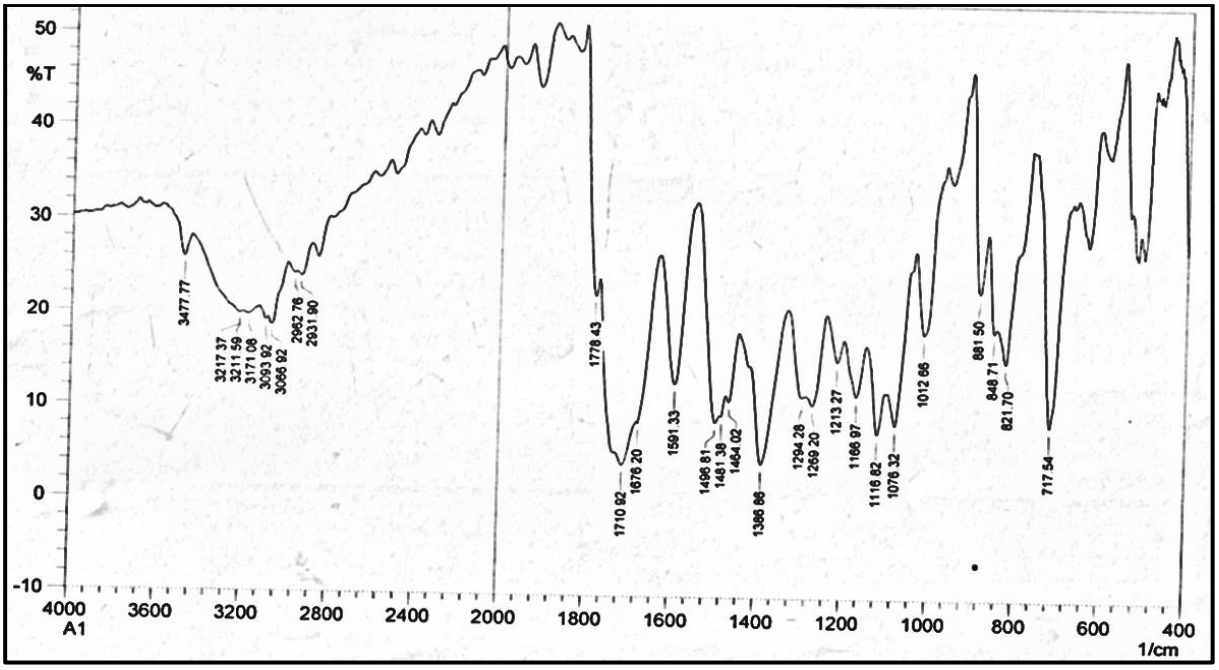


Figure 5. FT-IR spectrum of compound M1a

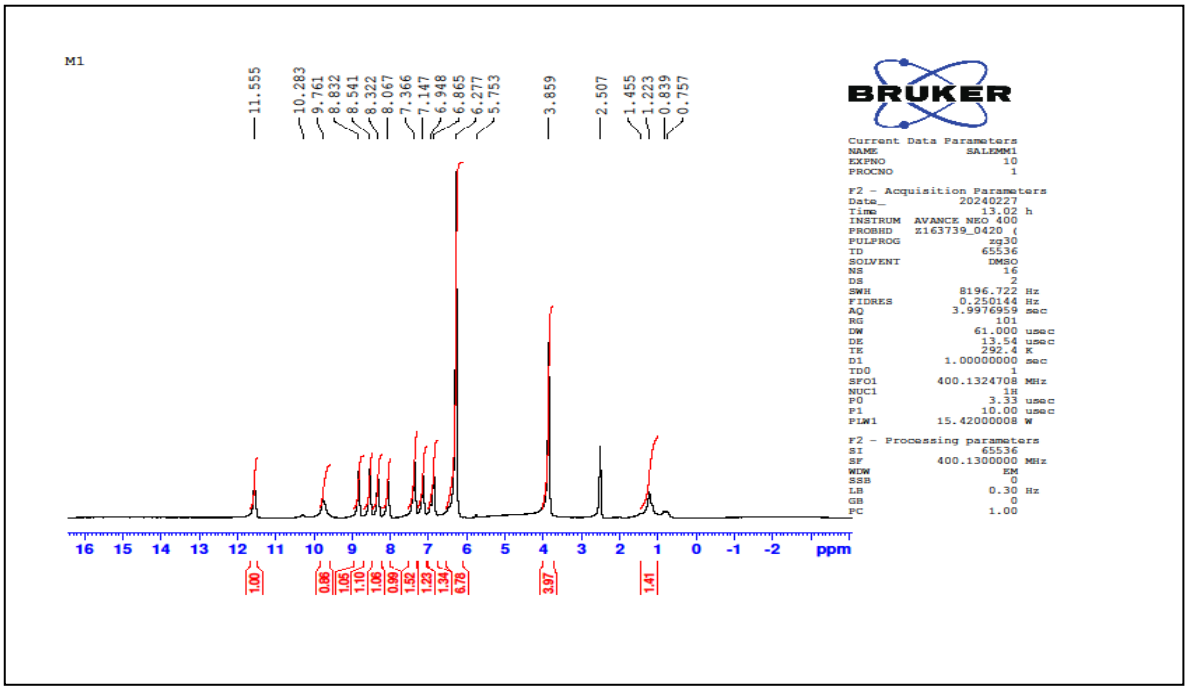


Figure. 6.1H-NMR spectrum of compound M1a

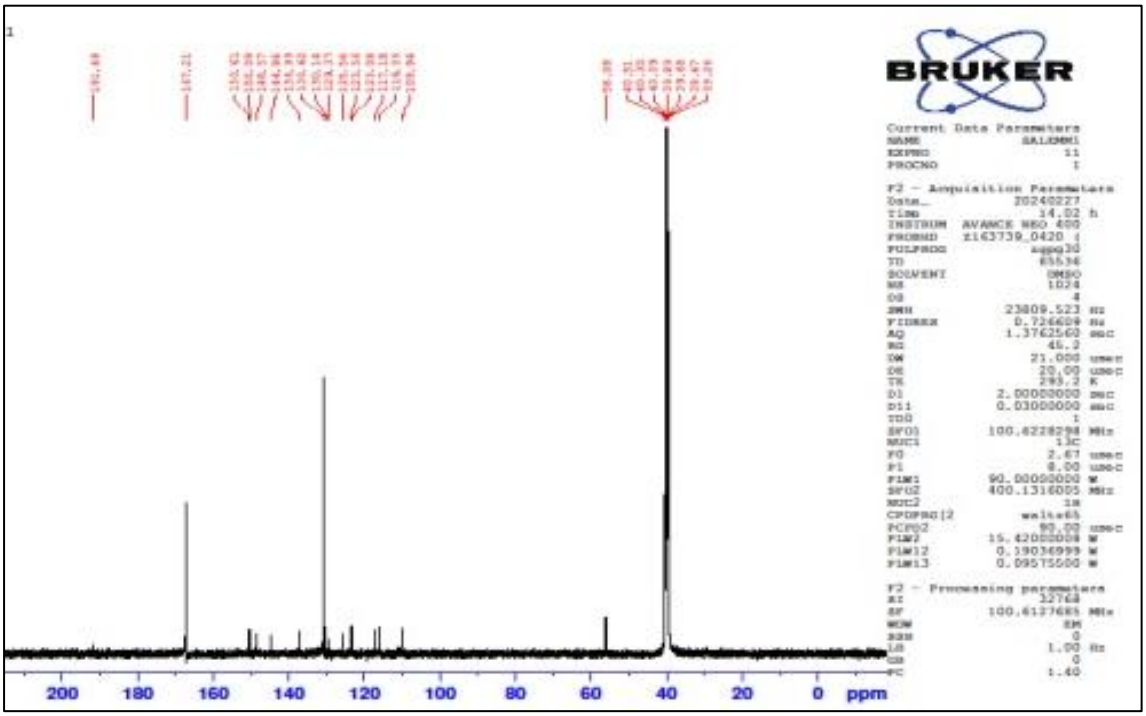


Figure 7.^{13}C-NMR spectrum of compound M1a

The FT-IR spectrum of compound M1b(Figure 8)displays characteristic peaks at 1722.49 cm^{-1} (C=O, lactone) and 1618.33 cm^{-1} (C=O, lactam), as well as the disappearance of the azomethine group (HC=N) at 1583.61 cm^{-1}, indicating its cleavage.

In the ^{1}H-NMR spectrum of M1 (Figure 9), recorded in DMSO, a singlet appears at 8.4 ppm (CH=N), a singlet at 6.98 ppm (C-H) corresponding to the Oxazipine ring, and the signal for the azomethine proton disappears at 9.0 ppm. The ^{13}C-NMR spectrum shows a singlet at 167.12 ppm (C=O, ester group), a singlet at 136.99–117.18 ppm (C=C), and a singlet at 56.08 ppm (N-C-O) in the heptagonal ring, as shown in Figure 10. The melting point of compound M1 was determined to be 156 °C.

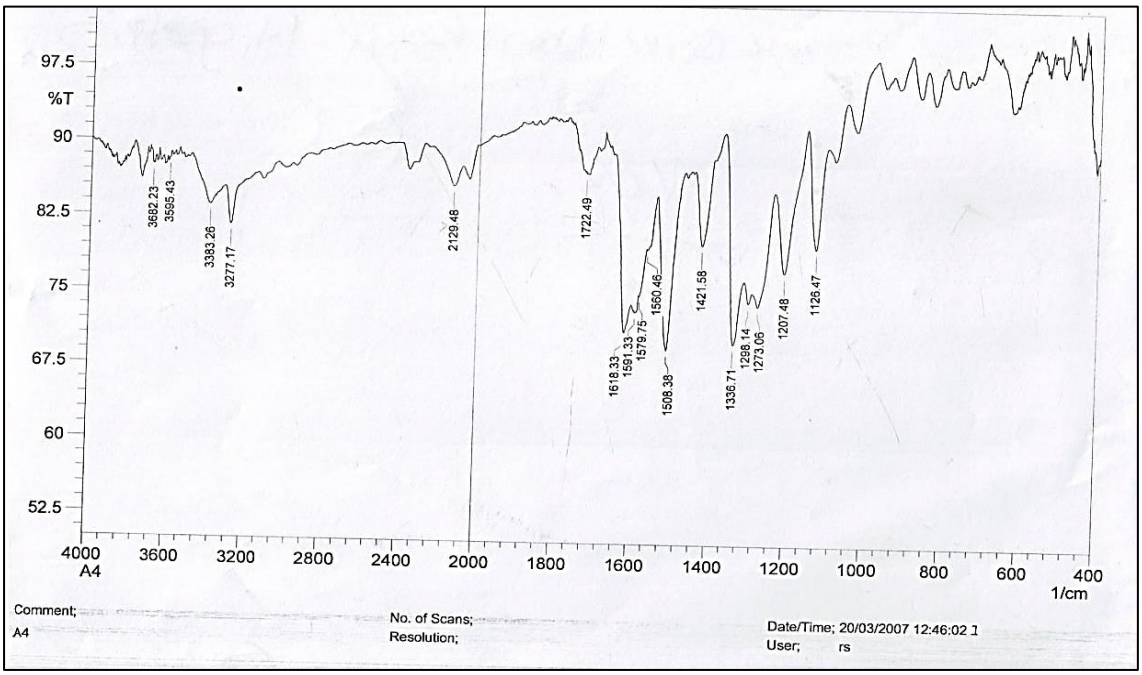


Figure 8. FT-IR spectrum of compound M1b

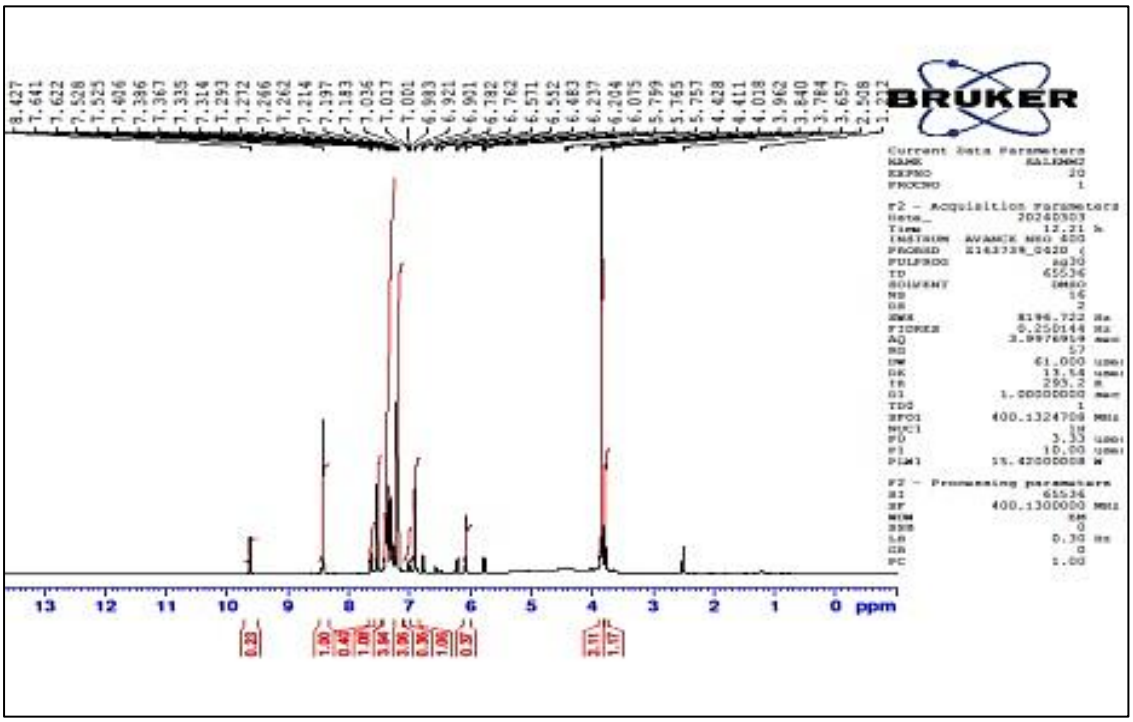


Figure 9. ^{1}H-NMR spectrum of compound M1b

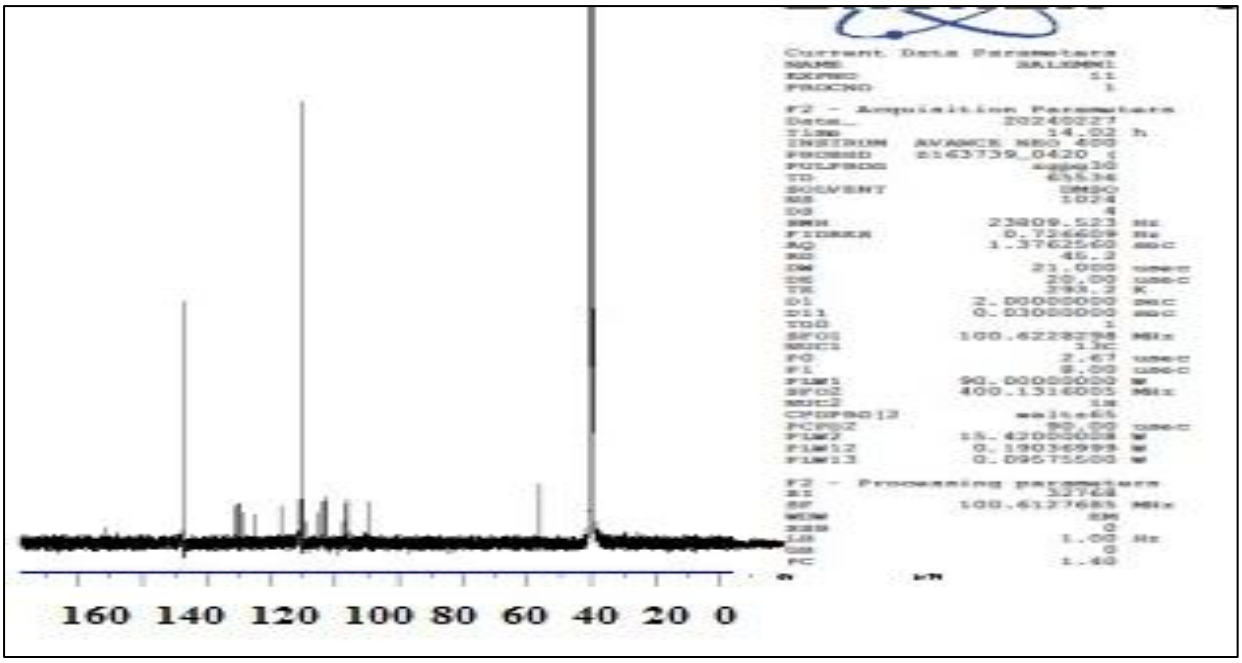


Figure 10. ^{13}C-NMR spectrum of compound M1b

The FT-IR spectrum of compound M1c (Figure 11) shows the following characteristic peaks: 1502.6 cm^{-1} (C-N), 1651.12 cm^{-1} (C=C, aromatic), 1708.99–1705.13 cm^{-1} (C=O), and 1587.47 cm^{-1} (C-S) corresponding to the pentagonal ring.

In the ^{1}H-NMR spectrum of M1c(Figure 12), recorded in DMSO, a signal appears at 7.22 ppm (CH=N) and another at 3.67 ppm (S-CH_2-CO) in the thiazolidine ring. The ^{13}C-NMR spectrum shows a singlet at 167.13 ppm (C=O) and a singlet at 59.48–39.37 ppm (C-S-C) in the pentagonal ring, as shown in Figure 13. The melting point (M.P.) of compound M1c was determined to be 170 °C.

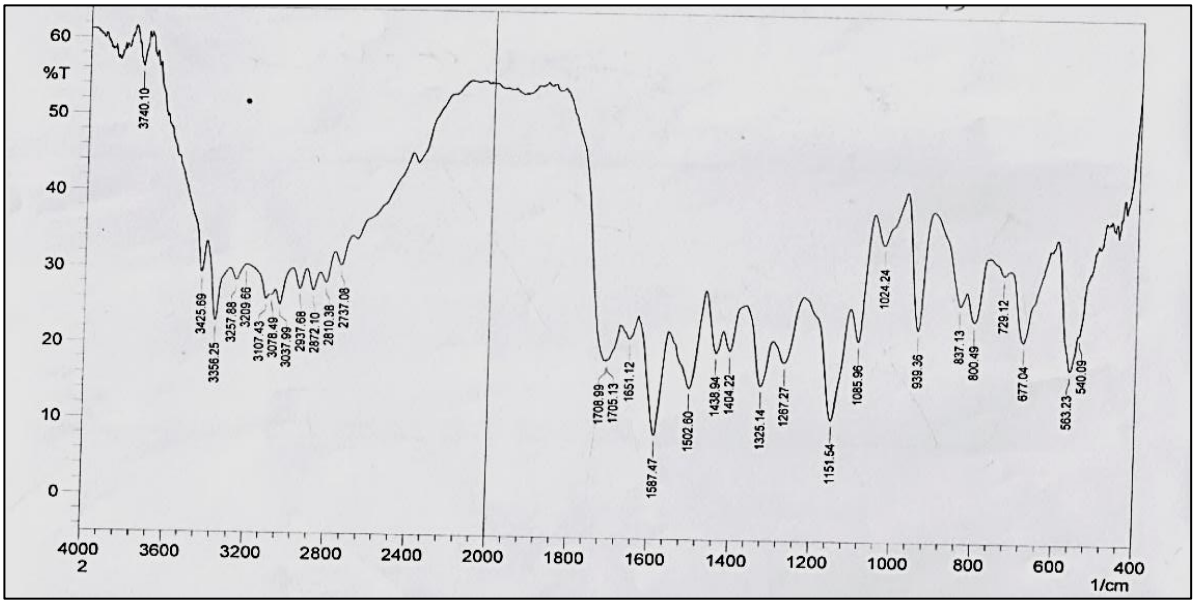


Figure 11. FT-IR spectrum of compound M1c

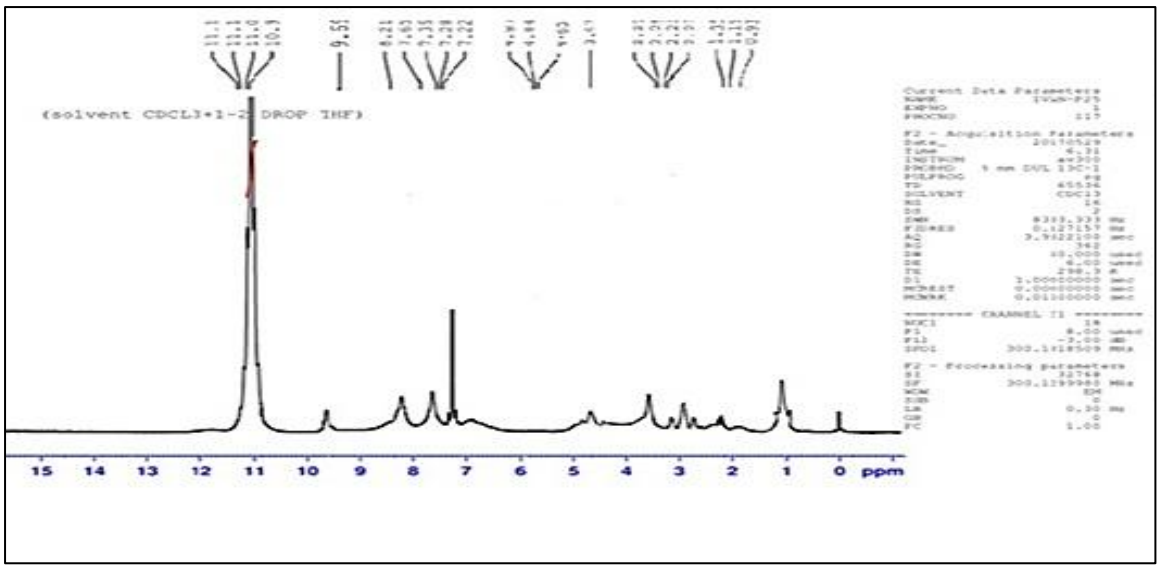


Figure 12. [1]H-NMR spectrum of compound M1c

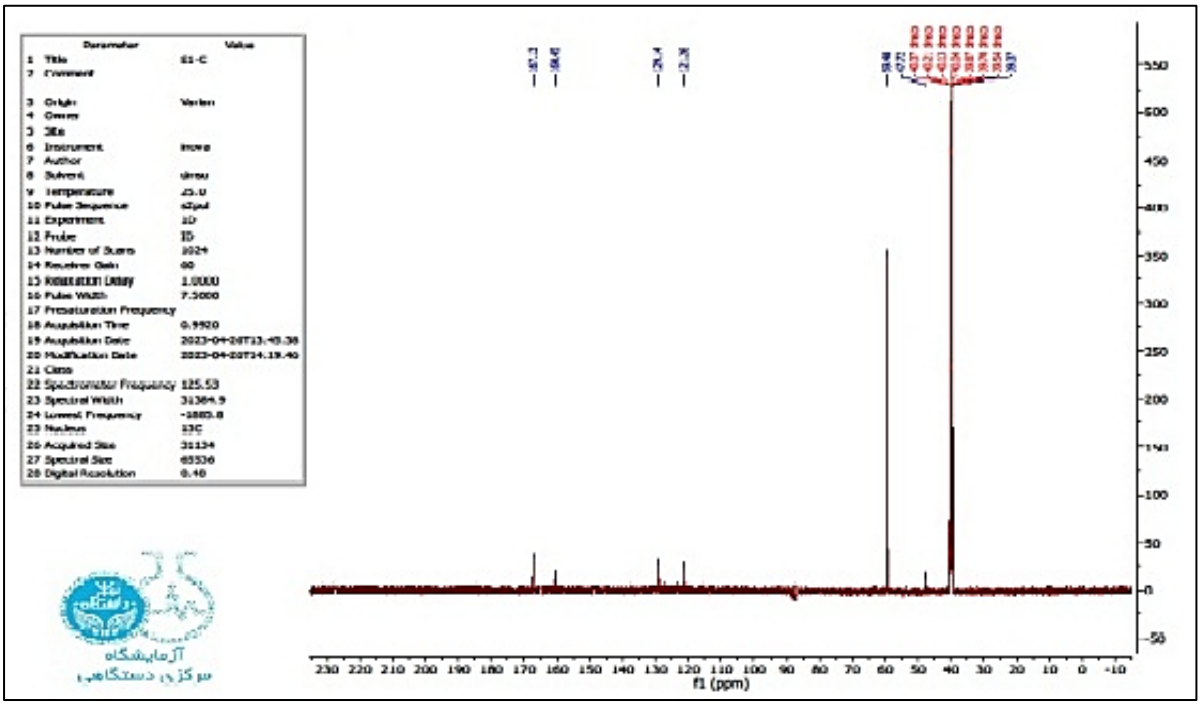


Figure 13.[13]C-NMR spectrum of compound M1c

Mass spectrum of the prepared compounds (M1a, M1b, and M1c):

According to the obtained results from mass spectra (MS), the compounds with the symbols (M1a, M1b, and M1c their recorded mass was found to match the calculated value for their molar mass, as shown in figures 14,15, and 16.

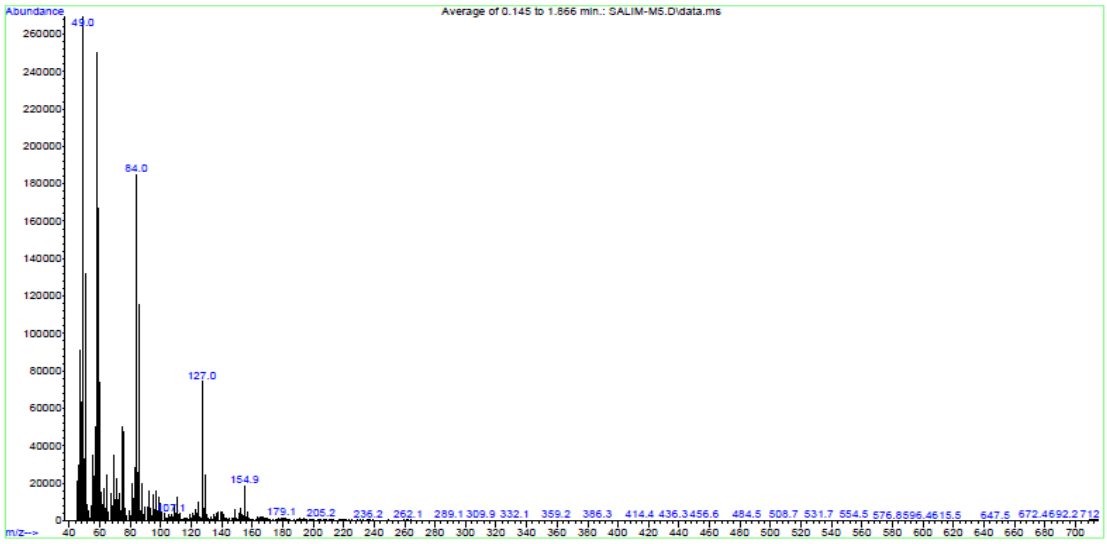


Figure 14: Mass Spectra of M1a Complex

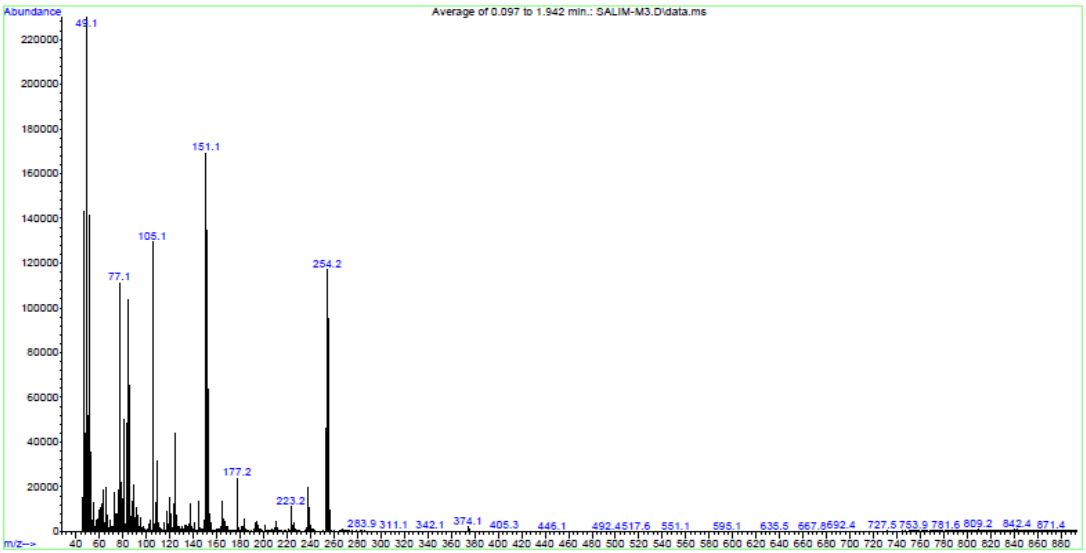


Figure 15: Mass Spectra of M1b Complex

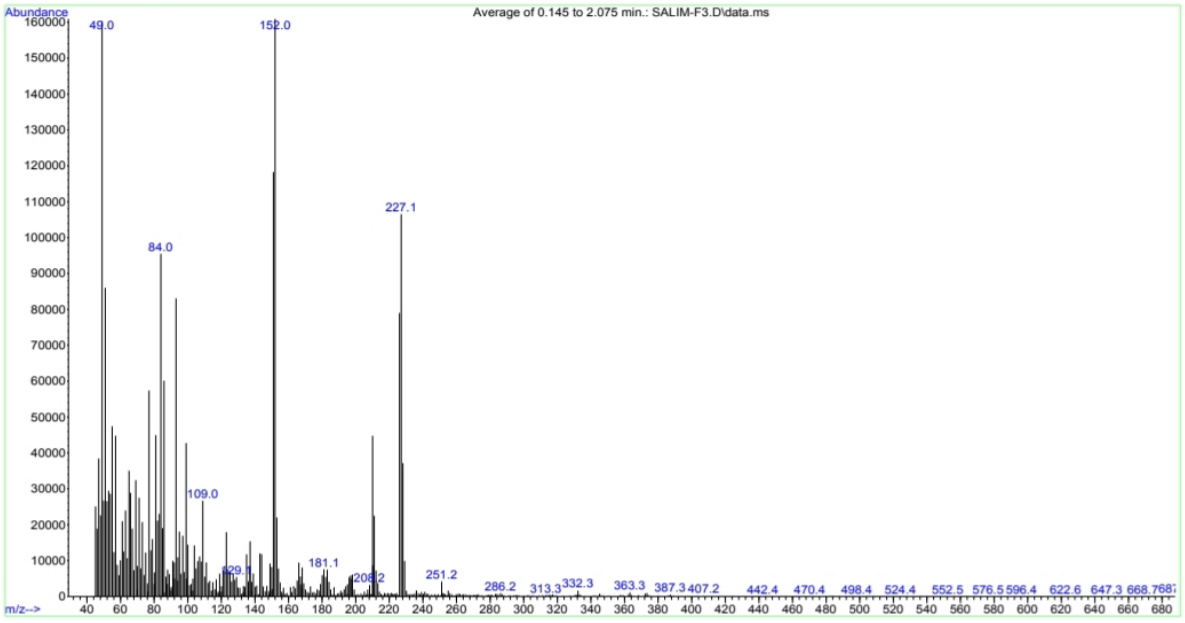
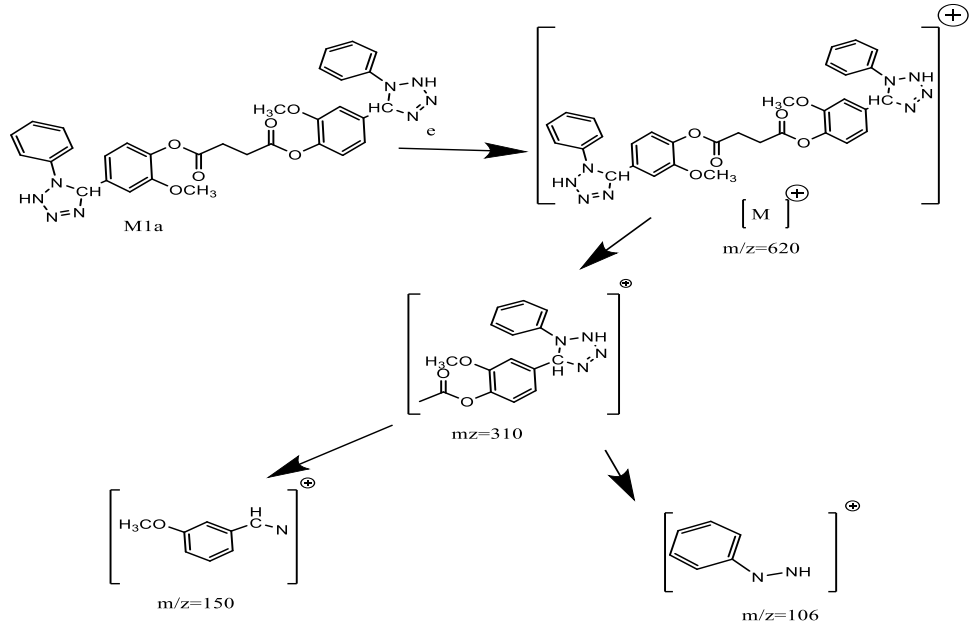


Figure 16: Mass Spectra of M1c Complex

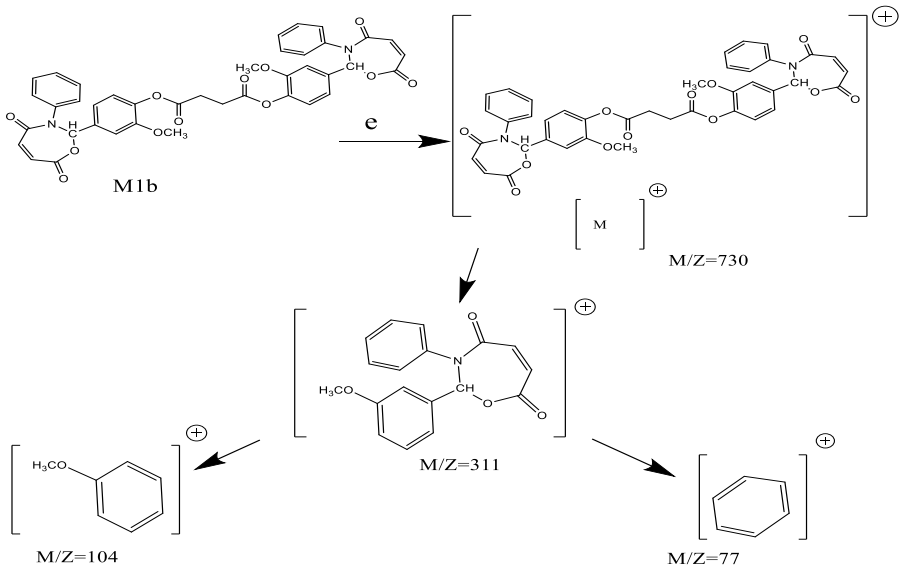
The molecular weight of the compound M1a($C_{32}H_{28}N_8O_6$) is similar to that of an ionic molecular compound. It was found that there was a similarity between the molecular weight of the formed fragments and that of the interested compound.

The proposed mechanism for the fragmentation processes is shown in Scheme 4.

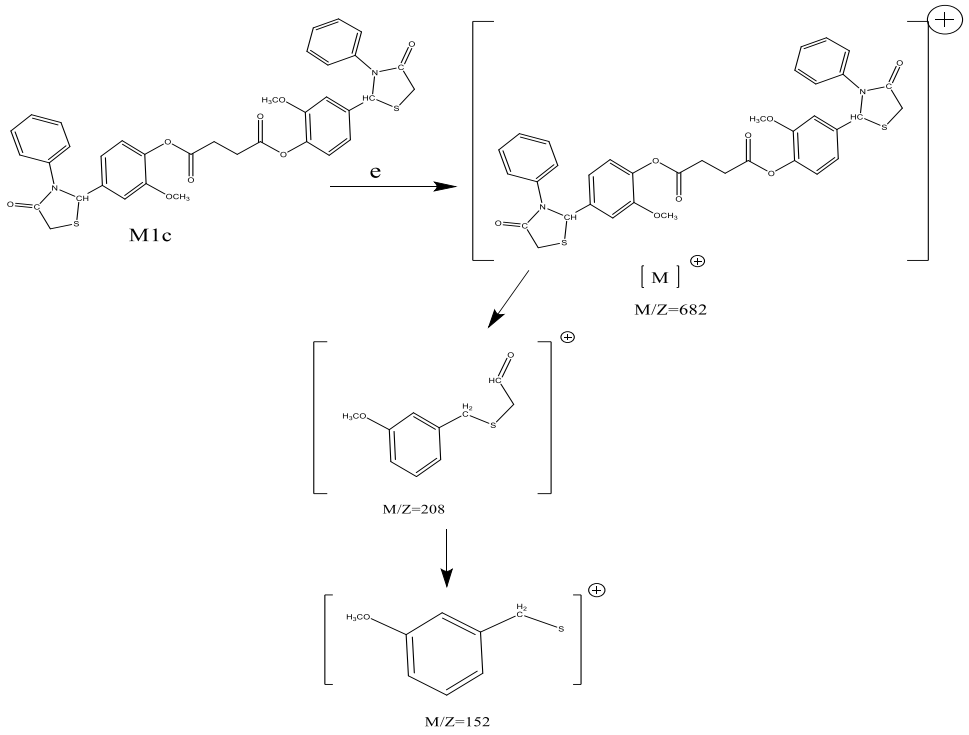


Scheme (4): Suggested Fragmentation of the M1a Compound

Formation of products that were derived from the two synthesized compounds, No.2 (M1b) and No.3 (M1c), was also confirmed, as shown in schemes 5 and 6.



Scheme (5): Suggested Fragmentation of the M1b Compound



Scheme (6): Suggested Fragmentation of the M1c Compound

It was found that the molecular weight of the compound with chemical formula M1b($C_{40}H_{30}N_2O_{12}$) was found to be (m/z)=730, and its calculated molecular weight was 730. On the other hand, the molecular weight of the compound with chemical formula M1c ($C_{36}H_{30}N_2O_8S_2$) was found to be (m/z) =682, and its calculated molecular weight was 682.

Antibacterial activity [23-25]

The antibacterial activity was evaluated using the disk diffusion method against *Escherichia coli* and *Staphylococcus aureus* (Figure 6). The zone of inhibition increased significantly from 18 to 25 mm for the heterocyclic compounds, compared to 12–15 mm for the ciprofloxacin drug, as shown in Table 1. The results demonstrated strong antibacterial activity for compounds M1a, M1b, and M1c, surpassing the activity of ciprofloxacin.

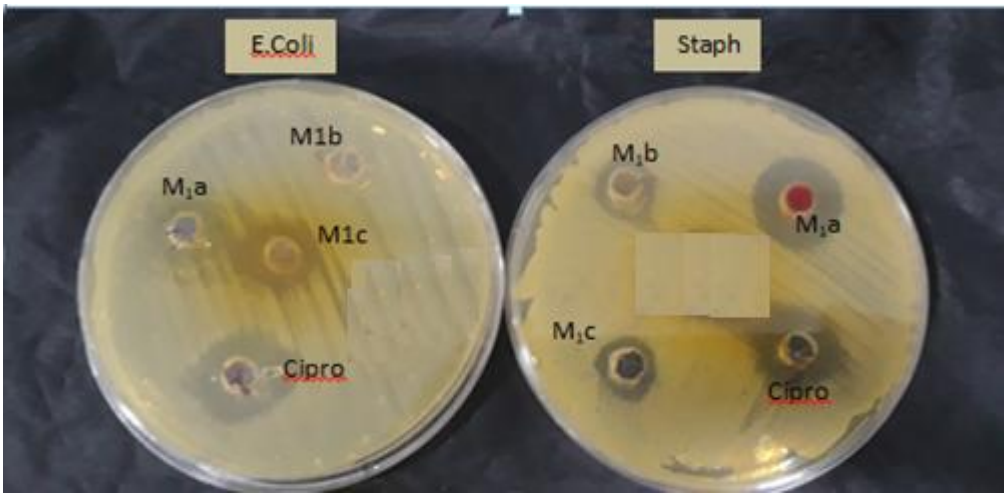


Figure 17. Effect of compounds on bacterial growth.

Table 1. Antibacterial activity of heterocyclic compounds.

Sample ID	Inhibition Zone (mm)	
	E.coli	Staph
M1a	23	25
M1b	25	22
M1c	18	20
Cipro	12	15

CONCLUSIONS

The Schiff base components were synthesized as starting materials for the preparation of three heterocyclic compounds containing nitrogen, oxygen, and sulfur through an addition reaction. Upon evaluating the biological activity of the prepared compounds and comparing them with ciprofloxacin, the results revealed that the heterocyclic compounds exhibited superior antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* compared to the drug.

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