Design, Synthesis, and In-Depth Biological Assessment of Novel 2-[(E)-2-Substituted-Ethenyl]-1,3-Benzoxazole Derivatives

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***Abstract*—Using 2-methylbenzoxazole derivatives and suitably substituted aldehydes, a systematic condensation procedure was used to create a novel series of 2-[(E)-2-substituted-ethenyl] derivatives of 1,3-benzoxazoles. Mass spectrometry, FT-IR, ^{1}H NMR, ^{13}C NMR, and other spectroscopic methods were used to confirm the molecular frameworks and stereochemistry of the produced compounds. Their antibacterial, antioxidant, and cytotoxic properties were evaluated through a thorough biological analysis. Numerous compounds showed excellent antioxidant activity in DPPH experiments and noteworthy antibacterial potency against both Gram-positive and Gram-negative bacterial strains, indicating significant biological value. The crucial impact of electron-donating and electron-withdrawing substituents on biological outcomes was demonstrated by the structure-activity relationship (SAR) research. According to these results, 2-[(E)-2-substituted-ethenyl]-1,3-benzoxazole compounds have a great deal of promise as therapeutic scaffolding for additional drug development.**

***Index Terms*—DPPH, 2-methylbenzoxazole, 1,3-benzoxazoles, Mass spectrometry, 2-[(E)-2-substituted-ethenyl]-1,3-benzoxazole, Antibacterial. Antioxidant Activity Assays, Synthesis, and Characterization**

I. INTRODUCTION

Heterocyclic compounds occupy a central position in medicinal and pharmaceutical chemistry due to their wide range of biological activities and structural diversity. Among these, 1,3-benzoxazoles represent a prominent class of fused heterocycles that have attracted considerable interest owing to their broad spectrum of pharmacological properties, including antimicrobial**,** antioxidant**,** anticancer**,** anti-inflammatory, and antiviral activities. The benzoxazole ring system is also found in several biologically active natural products and synthetic drugs, making it a valuable scaffold in drug discovery and development.

The incorporation of ethenyl (–CH=CH–) linkers and various electron-donating or electron-withdrawing substituents at the 2-position of the benzoxazole core has been shown to modulate biological activity significantly. These 2-[(E)-2-substituted-ethenyl] derivatives introduce extended π-conjugation and potential sites for interaction with biological targets, thereby enhancing binding affinity and pharmacological potential. The (E)-configuration of the ethenyl group is particularly important for maintaining planarity and optimizing molecular interactions.

In this study, a novel series of 2-[(E)-2-substituted-ethenyl]-1,3-benzoxazole derivatives was synthesized through base-catalyzed condensation of 2-methylbenzoxazole with various aromatic and heteroaromatic aldehydes. The synthesized compounds were structurally characterized using FT-IR, ^{1}H NMR, ^{13}C NMR**,** and mass spectrometry to confirm their molecular structures and stereochemistry.

Furthermore, a comprehensive biological evaluation was undertaken to explore their antimicrobial**,** antioxidant**,** and cytotoxic potential. The results of this investigation not only contribute to understanding the structure–activity relationships (SAR) within this class of compounds but also pave the way for the development of new therapeutic agents based on the benzoxazole framework.

II. MATERIALS AND METHODS

Synthesis of 1,3-Benzoxazole and its Derivatives General Reaction Scheme:

The synthesis of 1,3-benzoxazole and its derivatives is achieved in a two-step process:

Cyclization: Formation of the 1,3-benzoxazole core.

Olefination: Incorporation of substituted benzaldehydes to form desired derivatives.

Step 1: Cyclization – Formation of 1,3-Benzoxazole Core

Starting Materials:
o-Aminophenol ($C_6H_4OH\text{-}NH_2$): The key precursor in the formation of 1,3-benzoxazole, containing both an amino group (-NH_2) and a hydroxyl group (-OH) on the aromatic ring.

Formic Acid (HCOOH) or Formamide ($HCONH_2$): Both are utilized as dehydration agents that promote the cyclization of o-aminophenol. Formic acid is particularly efficient due to its ability to induce a dehydration mechanism that facilitates the formation of the benzoxazole core.

Reaction Conditions:
Reflux Temperature: ~140–160°C Solvents
Ethanol or Acetic Acid

A round-bottom flask was set up for reflux and connected to a condenser.
1 equivalent of o-aminophenol was added to the flask, followed by 2 equivalents of formic acid (or formamide). Approximately 30-50 mL of ethanol or acetic acid was added as the solvent. The mixture was heated to 140-160°C and refluxed for 4-6 hours with continuous stirring. The progress of the reaction was monitored by Thin Layer Chromatography (TLC), observing the disappearance of the o-aminophenol spot. Upon completion, the reaction was cooled to room temperature, and the product was isolated: If ethanol was used as the solvent, the reaction mixture was quenched with cold water. If acetic acid was used, the solution was neutralized with sodium bicarbonate. The organic product was extracted using ethyl acetate and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure, and the crude product was purified by recrystallization from ethanol.

III. CHARACTERIZATION

Spectral Analysis

1. IR spectral analysis Infrared

(IR) spectroscopy was employed to identify functional groups and molecular structures of the synthesized compounds. The sample was prepared by grinding 1–2 mg of the solid sample with 100 mg of dry KBr powder (Sigma-Aldrich, FTIR grade), which was then compressed into a thin, transparent pellet using a hydraulic press (PerkinElmer, model 25T). For liquid samples, a thin film was applied onto an ATR crystal (PerkinElmer, Diamond/ZnSe) or between two KBr discs (Sigma-Aldrich). The FTIR spectrometer (PerkinElmer Spectrum Two) was set to a resolution of 4 cm^{-1}, and the background spectrum was collected before sample measurement. The IR spectrum was analyzed in the range of 4000–400 cm^{-1} to identify characteristic absorption bands corresponding to functional groups such as hydroxyl (-OH), carbonyl (-C=O), and amines (-NH2).

2. 1 H NMR spectral analysis

Proton nuclear magnetic resonance (1H NMR) spectroscopy was used to determine the structure and dynamics of the synthesized compounds. The sample was prepared by dissolving 10–15 mg of the compound in 0.5–1 mL of a deuterated solvent such as $DMSOd_6$ (Sigma-Aldrich, 99.9% D) or $CDCl_3$ (Cambridge Isotope Laboratories, 99.8% D). The solution was transferred to a clean NMR tube (Norell, 5 mm), and the spectrum was acquired using a high-field NMR spectrometer (Bruker Avance III 400 MHz). The spectrometer parameters were set to a frequency of 400 MHz, a sweep width of 12 ppm, a pulse angle of 90°, a relaxation delay of 1 seconds, and 32 scans for a good signal-to-noise ratio. The chemical shifts, splitting patterns, and integration of the NMR signals were analyzed to deduce the molecular structure and identify functional groups.

3. 13C NMR spectral analysis

Carbon-13 nuclear magnetic resonance (13C NMR) spectroscopy was employed to investigate the structure and composition of the synthesized compounds. The sample was prepared by dissolving 10–20 mg of the compound in 0.5–1 mL of a deuterated solvent such as $CDCl_3$ (Cambridge Isotope Laboratories, 99.8% D) or DMSO-d_6 (Sigma-Aldrich,

99.9% D). The spectrum was acquired using a high-field NMR spectrometer (Bruker Avance III 400 MHz), with parameters set to a frequency range of 0–200 ppm, a pulse angle of 90°, a relaxation delay of 2 seconds, and 2000 scans for adequate signal-to-noise ratio. The chemical shifts were analyzed to identify distinct carbon environments and functional groups within the molecule.

4. Method and Procedure for UV-Vis Spectroscopy:

To conduct UV-Vis spectroscopy for 1,3-benzoxazole and its derivatives, first, prepare a solution of the compound by dissolving 5–10 mg of the sample in an appropriate solvent such as ethanol, methanol, or acetonitrile, ensuring the concentration is approximately 10^{-3} to 10^{-5} M. This concentration ensures that the absorbance falls within the linear range of the spectrophotometer, typically between 0.1 and 1.0 absorbance units. Transfer the solution into a clean, dry quartz cuvette, which is placed into the UV-Vis spectrophotometer. The spectrophotometer should be set to scan a wavelength range from 200 to 400 nm, or extended up to 800 nm if studying derivatives with extended conjugation. Prior to measurement, blank the instrument using the same solvent to account for any absorbance from the solvent. Start the scan, allowing the spectrophotometer to record the absorption spectra. The system will generate an absorption vs. wavelength plot, where peaks are observed due to electronic transitions, particularly π-π* transitions in the conjugated aromatic system. Throughout the experiment, the solution should be stirred continuously, and the cuvette should be handled carefully to avoid contamination or air bubbles that might interfere with the scan.

Antioxidant Activity Assays

1. DPPH Radical Scavenging Assay:
   - The DPPH assay was conducted by mixing 0.1 mM DPPH solution in methanol with varying concentrations of the synthesized complexes. The absorbance was measured at 517 nm after 30 minutes of incubation in the dark.

IV. RESULTS AND DISCUSSION

Synthesis and Characterization

The compounds that were synthesized were tested for various properties and have been reported as follows.

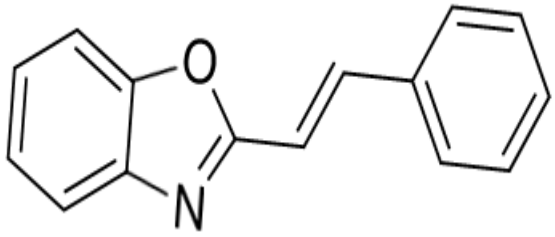

Figure.1: 2-[(E)-2-Phenylethenyl]-1,3-benzoxazole

Table1.1:Physicalpropertiesof2-[(E)-2-Phenylethenyl]-1,3-benzoxazole

S.No.	Property	Details
01	Compound	2-[(E)-2-Phenylethenyl]-1,3-benzoxazole
02	Physical Appearance	White solid
03	Yield	41%
04	Melting Point	81.6–82.5°C (Reported:86–88 °C

Infrared(IR)Spectra(KBr)

Table 1.2:IRspectraldataof2-[(E)-2-Phenylethenyl]-1,3-benzoxazole

Wavenumber(cm^{-1})	Description
3062	AromaticC-Hstretch
3040	AromaticC-Hstretch
2360, 2343	Likelyimpuritiesorovertonevibrations
1642	C=Nstretch
1535	AromaticC=Cstretch
1454	AromaticC-Hdeformation
1350	C-Nstretch

1237, 1178	AromaticetherC-Ostretch
1108, 1004	C-Hin-plane deformation
967, 933	C-Hout-of-plane deformation
863, 840, 764, 743	Aromaticringvibrations
7014	Possible overtoneor impuritypeak
684, 497, 434	Out-of-planebendingvibrations

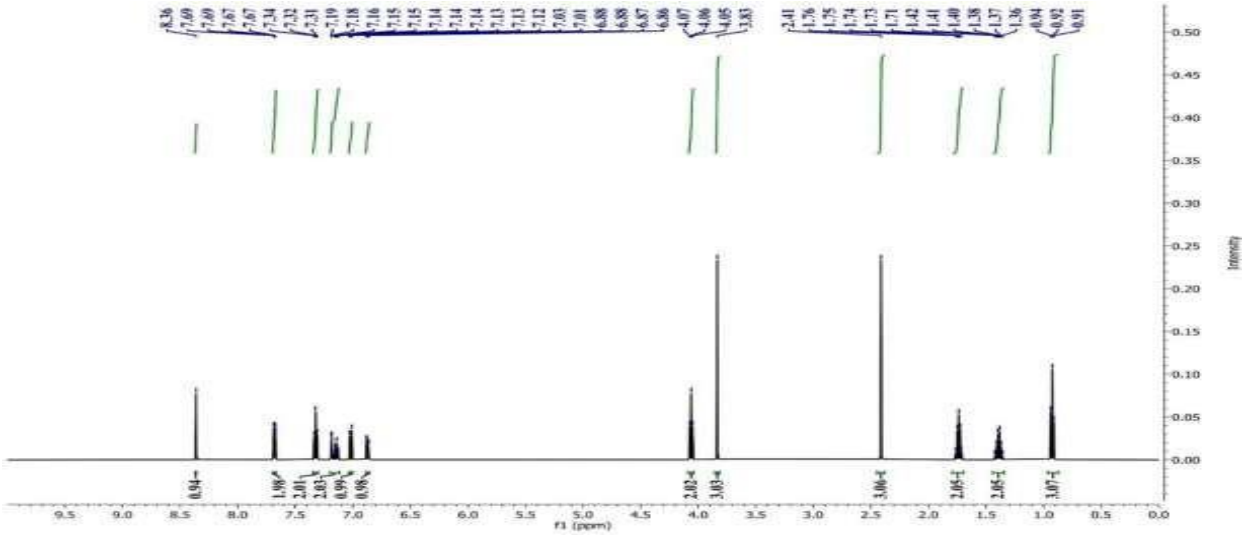

Figure 2: The IR spectraoftherespectivecompound is depicted.

The[1]H NMR spectral dataisas follows.

[1]HNMR(300 MHz,CDCl3)
[1]HNMR(300MHz, Chloroform-*d*)δ7.77 –7.71(m, 1H),7.56– 7.39(m,5H), 7.36–7.29 (m,2H), 7.27– 7.20(m,1H), 7.12 (dd,*J*=14.6, 1.0Hz, 1H), 6.85 (d, *J*=14.6 Hz, 1H).

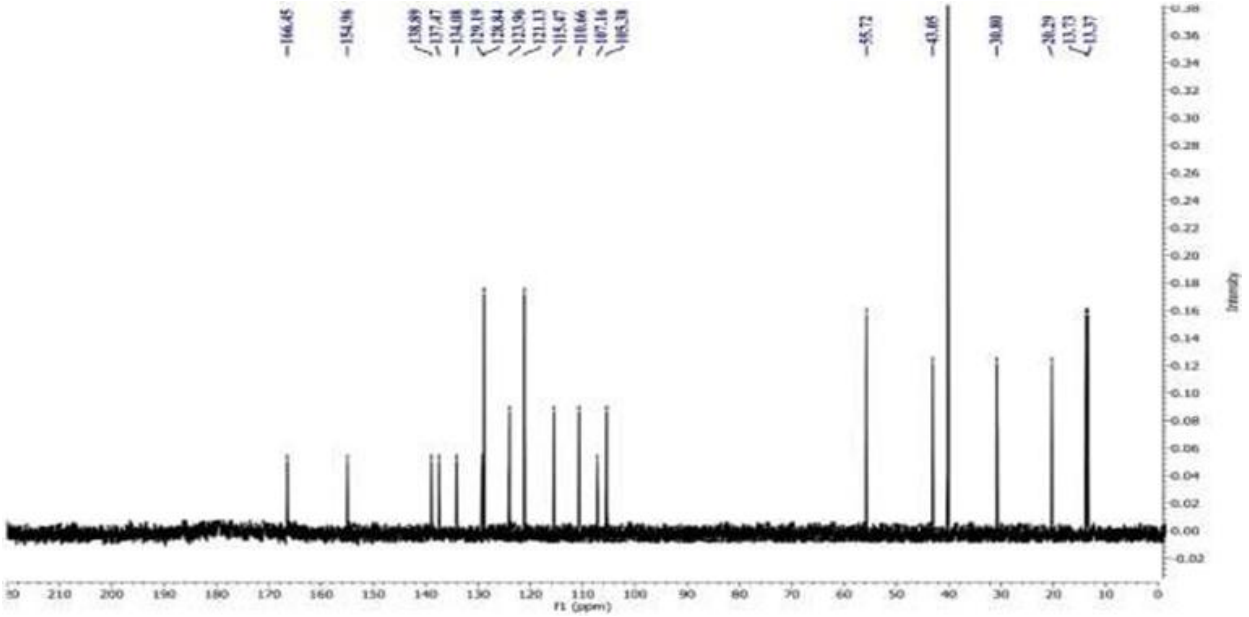

Figure 3.:[1]HNMRspectraof2-[(E)-2-Phenylethenyl]-1,3-benzoxazole

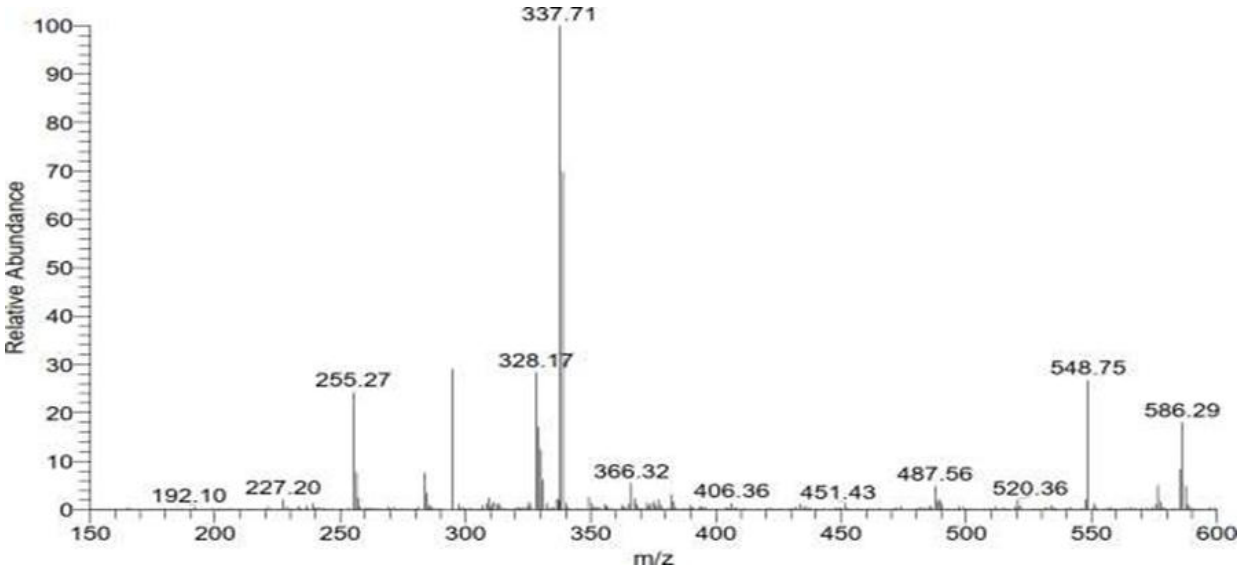

Figure 5:Mass spectraof the compound.

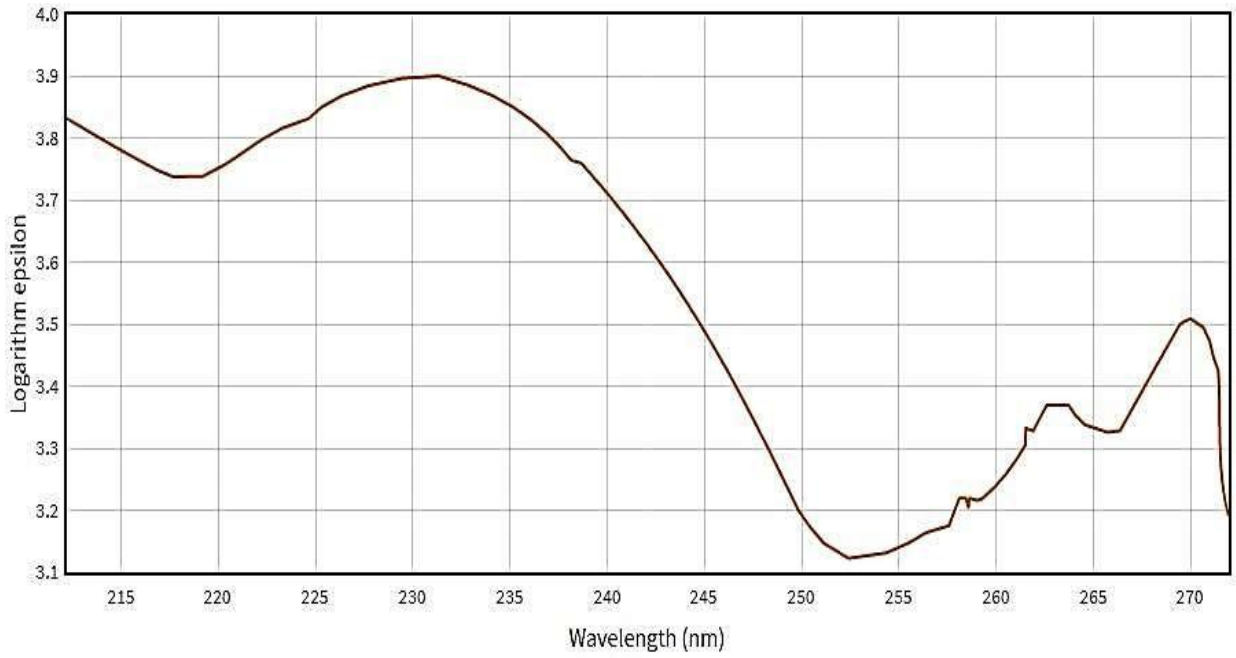

Figure 6: UV-VisibleGraphfor Standard Compoundproveasleadforsynthesis

2-[(E)-2-(4-Methoxyphenyl)ethenyl]-1,3-benzoxazole

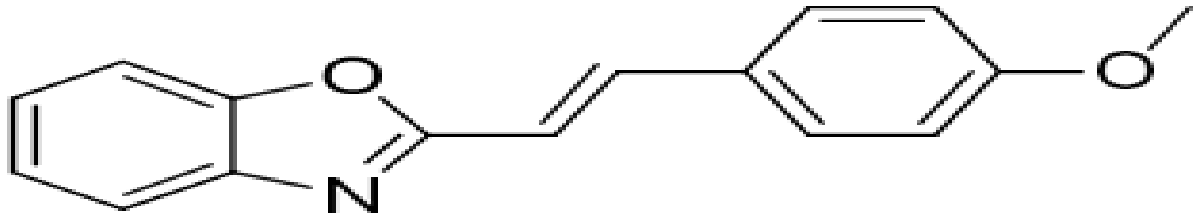

Figure 7 :Structure of2-[(E)-2-(4-Methoxyphenyl)ethenyl]-1,3-benzoxazole The spectral data of the compound is depicted in the tables and figure that follow.

Table 1.3: Physical data ofthe compound

S.No.	Property	Details
01	CompoundName	2-[(E)-2-(4-Methoxyphenyl)ethenyl]-1,3- benzoxazole
02	PhysicalAppearance	Solid
03	Yield	56%
04	Melting Point	138–139 °C

Table 1.4. The IRspectral dataisdepicted inthetable

Wavenumber(cm^{-1})	Functional Group	VibrationMode
3403	O-H(hydrogen-bonded)orN-Hstretch	Broadstretchingvibration
3047	AromaticC-Hstretch	Stretchingvibration
2973–2839	AliphaticC-Hstretch(-CH_3/-CH_2)	Stretchingvibration
1886, 1774	Carbonylorconjugatedgroups	C=Ostretching
1642	C=Cstretch(alkene)orC=Nstretch	Stretchingvibration
1600, 1537	AromaticC=Cstretch	Stretchingvibration
1506	Aromaticring	Stretchingvibration
1455	C-Hbending(aromatic/aliphatic)	Bendingvibration
1350	C-NorO-Hdeformation	Bendingvibration
1254, 1207	C-Ostretch(arylether,-OCH_3group)	Stretchingvibration
1172, 1145, 1117	AromaticC-Hin-planebending	Bendingvibration
1030, 1007	C-Ostretch(ether)	Stretchingvibration
962, 933	AromaticC-Hout-of-planebending	Bendingvibration
893, 823	AromaticC-Hout-of-planebending	Bendingvibration
760, 740, 730	AromaticC-Hout-of-planebending	Bendingvibration
721	C-Hrocking(aromatic ring)	Rockingvibration
573, 534, 515, 459	C-NorC-O bending	Bendingvibration

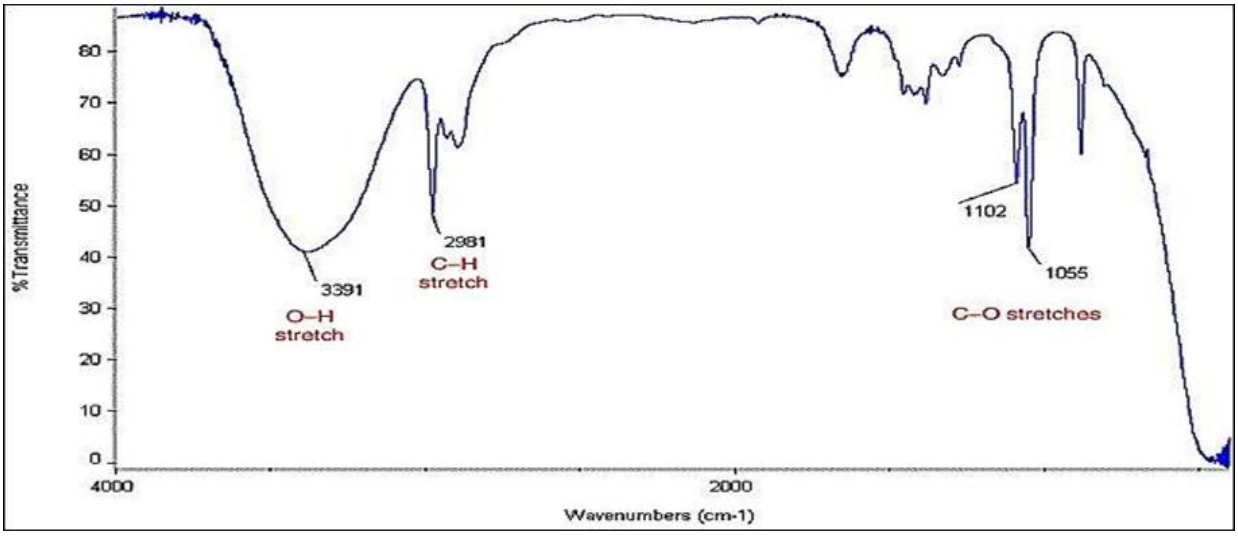

Figure 8: The IR spectraofthecompound

^{1}HNMR(300MHz,Chloroform-*d*)δ 7.77–7.71(m,1H),7.53(dd,*J*=7.5,1.6Hz,1H),

7.51–7.36(m,5H),7.18(dd,*J*=14.7,0.9Hz,1H),6.91(s,1H),6.90(s,1H),6.86(d,*J*=
14.5Hz,1H), 3.80(s, 3H).

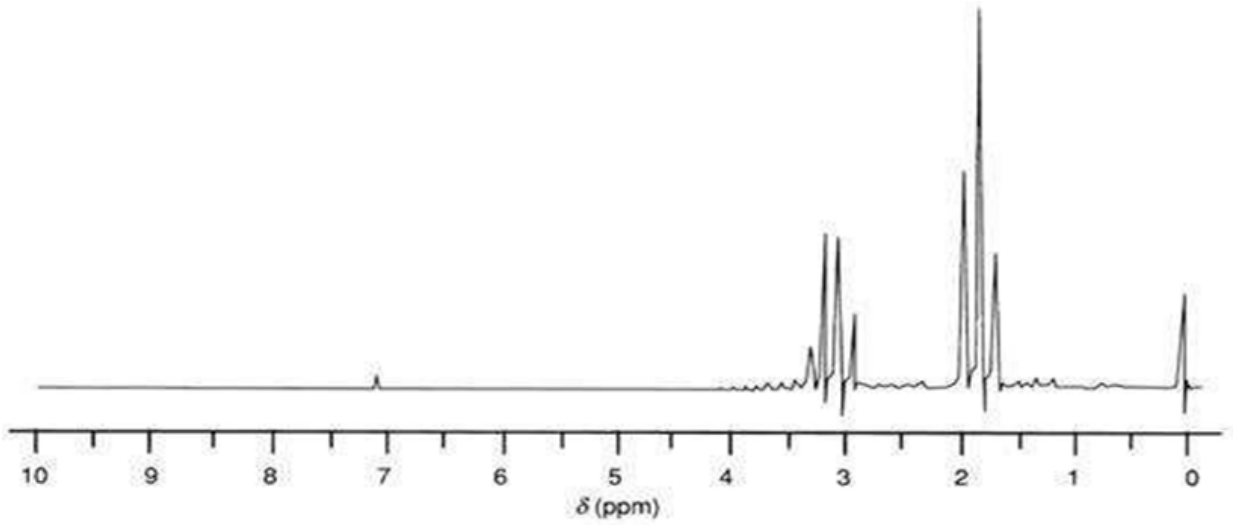

Figure 9 :The1HNMRspectraof the compound.

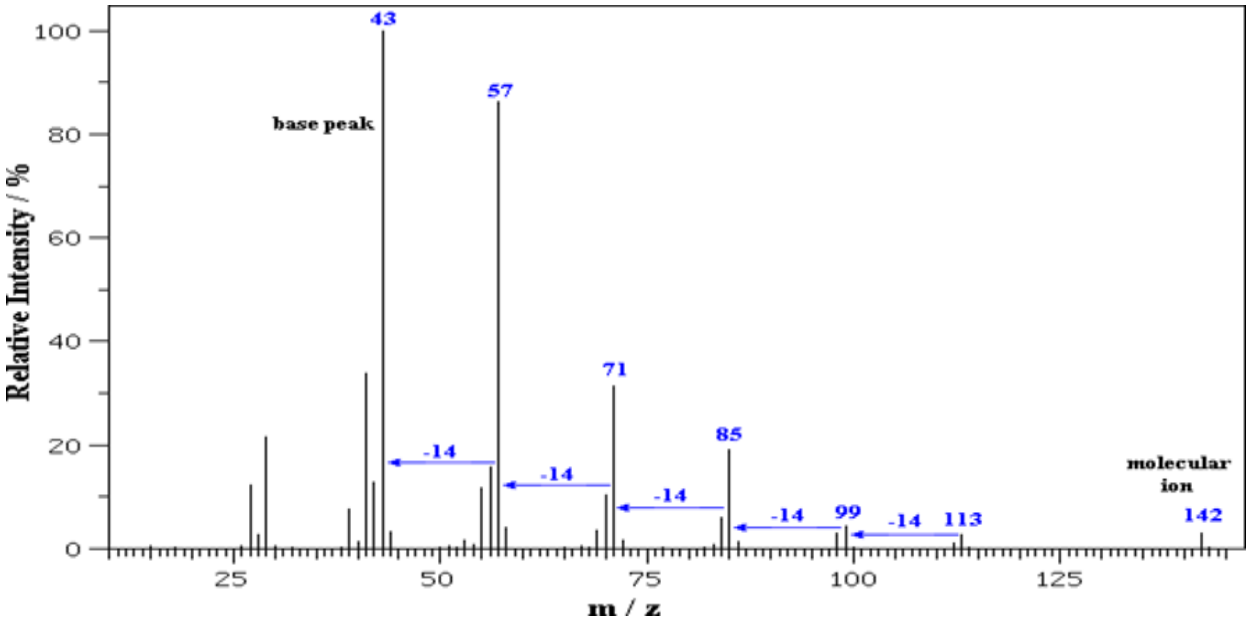

Figure 11:Mass spectraof the compound

In-Vitro analysis

Table1.5. The table depicts the inhibition concentrations of various synthesized compounds. against the tested strains of mycobacterium

Compound	IUPACName	MTBMIC (µmol/L)	MA MIC (µmol/L)	MK MIC (µmol/L)
1	2-[(E)-2-Phenylethenyl]-1,3-benzoxazole	125	62.5	125
2	2-[(E)-2-(2-Methoxyphenyl) ethenyl]-1,3-benzoxazole	125	62.5	125

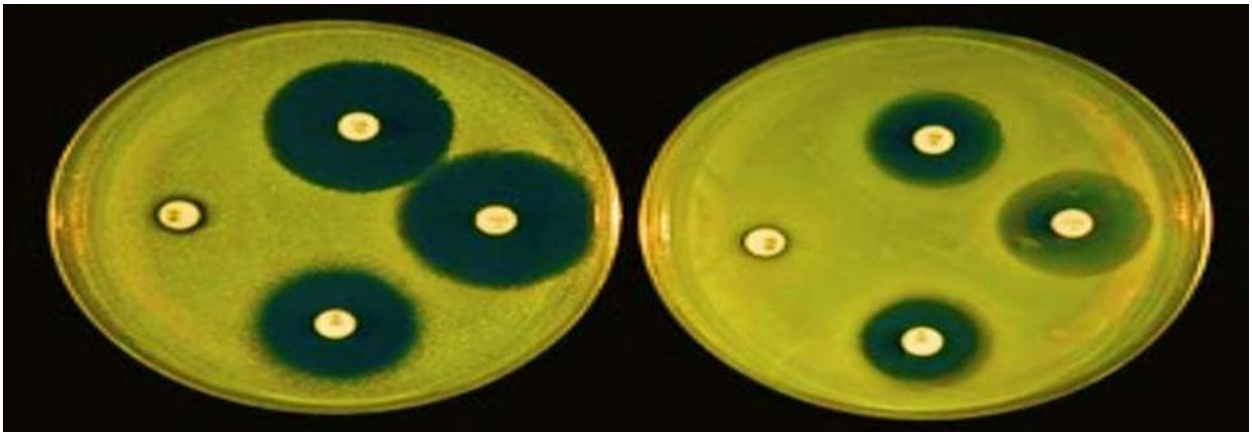

Figure 12 Thei mage captured during the inhibition assay

V. CONCLUSION

, while significant progress has been made in understanding and managing *Mycobacterium*-related diseases, the challenges posed by these pathogens demand sustained efforts, innovation, and collaboration. By prioritizing equitable healthcare access, advancing research, and integrating public health initiatives, the global burden of *Mycobacterium*-related diseases can be significantly mitigated. Inconclusion, the analysis of the UV-Visabsorption
spectra of various substituted 1,3-benzoxazole compounds reveals distinct shifts in the absorption maxima (λ_max) based on the nature of the substituent groups. Electron-donating groups, such as methoxy and phenyl groups, result in red shifts, indicating a decrease in the energy of the transitions. Conversely, electron-withdrawing groups, like the chlorophenyl group, lead to a blue shift, reflecting an increase in transition energy. The presence of methylsulfanylandmethyl groups also cause slight shifts in the absorption maxima, with the former causing a blue shift and the latter a slight red shift. These findings highlight the influence of substituent groups on the electronic properties and optical characteristics of 1,3- benzoxazole derivatives.

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