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Method For Pharmacological Assessment And Characterization Of 1, 3-Benzoxazole Compounds, As Well As Antitumor Effects

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Abstract: *Mycobacterium infections are thoroughly examined in this study paper, which delves into their microbiology, pathophysiology, and clinical consequences. The slow growth, environmental persistence, and overlapping clinical manifestations of tuberculosis and NTM infections provide significant diagnostic problems. This article takes a close look at new molecular diagnostics and how they can help increase detection rates. Different types of substituent groups cause noticeable changes in the absorption maxima (λ_max) when studying the UV-Vis absorption spectra of 1,3-benzoxazole compounds that have been replaced. The presence of electron-donating groups, including phenyl and methoxy groups, causes red shifts, which show that the transition energies are lower. A blue shift, representing an increase in transition energy, is observed with electron-withdrawing compounds, such as the chlorophenyl group, on the other hand. Methylsulfanyl creates a small blue shift and methyl causes a small red shift in the absorption maxima when these groups are present. The impact of substituent groups on the electrical and optical features of 1,3-benzoxazole derivatives is demonstrated by these results. Improving diagnostic capacity, developing new therapeutic techniques, and implementing preventative measures like immunisation and environmental management are all part of the urgently needed integrated public health solutions highlighted by this study. To reduce the impact of Mycobacterium infections and achieve long-term disease control, it is crucial to address healthcare inequalities and improve international cooperation.*
Keywords: *Mycobacterium tuberculosis, λ-max, 1,3-benzoxazole, electronic properties ect.*

INTRODUCTION:

A genus of bacteria in the family Mycobacteriaceae, Mycobacterium is defined by its acid-fastness, slow growth rate, and mycolic acid-containing lipid-rich cell wall.[1]. Their ability to withstand a wide range of climatic conditions is a result of these traits, which also make them resistant to chemical disinfectants and desiccation. The nontuberculous mycobacteria (NTM) and the Mycobacterium tuberculosis complex (MTBC) are the two main divisions of the genus Mycobacterium.[2]. Opportunistic pathogens make up NTM, whereas MTBC includes organisms that cause tuberculosis in people and other animals. It is impossible to emphasise the clinical importance of Mycobacterium species. With an anticipated 10.6 million new cases and 1.6 million fatalities in 2021, tuberculosis, caused by Mycobacterium tuberculosis, is among the top infectious disease-related killers worldwide. In spite of attempts to control the disease, leprosy, caused by Mycobacterium leprae, remains a public health concern in areas where it is prevalent.

The prevalence of NTM infections is also on the rise, thanks to reasons including better diagnostic tools and an increase in immunocompromised populations.[3].

Mycobacterium tuberculosis Complex [4]:

Closely related species of Mycobacterium TB, Mycobacterium bovis, Mycobacterium africanum, Mycobacterium microti, and Mycobacterium caprae make up the MTBC. Although they are genetically and phenotypically similar, the host species and dispersal patterns of these two species are distinct.

Nontuberculous Mycobacteria [5]:

Natural thermophilic bacteria (NTMs) are an incredibly varied class of microbes that inhabit a wide variety of environments, including water, soil, and biofilms. Mycoplasma avium, Mycoplasma kansasii, Mycoplasma abscessus, and Mycoplasma marinum are among the most common pathogenic NTMs. In contrast to MTBC, the main mode of transmission for NTMs is environmental exposure rather than direct human contact.[6].

1.2 Diseases Associated with *Mycobacterium* Species:

***Mycobacterium Tuberculosis* [7,8]:**

A chronic infectious disease, tuberculosis (TB) is caused by Mycobacterium tuberculosis and mainly affects the lungs but can spread to other organs. A prolonged cough, bleeding gums, high body temperature, nocturnal sweats, and decreased appetite are all symptoms of pulmonary tuberculosis. Difficulty in diagnosing tuberculosis (TB) in non-pulmonary locations (e.g., lymph nodes, pleura, bones, and central nervous system) is common. Inhalation of aerosolised droplets harbouring Mycobacterium tuberculosis is a key step in the development of tuberculosis. The bacteria are able to evade phagocytosis by alveolar macrophages once they reach the lungs, thanks to their distinctive cell wall composition and the virulence proteins they secrete.[9]. As part of its immune response to control the infection, the host undergoes granuloma development, which is characteristic of tuberculosis. In contrast, immunosuppression can cause dormant pathogens to reawaken and cause disease.

Diagnostic Approaches [10,11]:

Clinical, microbiological, and molecular approaches are utilised in the diagnosis of mycobacterial infections. For the detection of Mycobacterium species, the gold standard continues to be traditional methods like acid-fast staining and culture. Thanks to advancements in molecular diagnostics, such as next-generation sequencing and polymerase chain reaction (PCR), mycobacterial species may now be quickly and accurately identified, which has completely changed the sector. Another common method for detecting latent tuberculosis is through immunological testing, which includes the tuberculin skin test (TST) and interferon-gamma release assays (IGRAs).

Treatment Strategies:

The treatment of mycobacterial diseases varies depending on the species and disease presentation.

Tuberculosis Disease [12]:

The conventional six-month treatment for tuberculosis (TB) includes the use of first-line medications such as rifampicin, isoniazid, pyrazinamide, and ethambutol. Bedaquiline, delamanid, and linezolid are second-line medicines used to treat drug-resistant tuberculosis; nevertheless, these treatments are generally linked to longer and more harmful treatment regimens. Skeletal remains from long-gone civilisations provide proof of tuberculosis, a disease known since ancient times. Tuberculosis (TB) was formerly linked to poverty and overcrowding; the disease was named "consumption" because it causes extreme weight loss. A watershed moment in our understanding and treatment of the disease came in 1882, when Robert Koch found the bacterium. The groundwork for modern microbiology was laid by Koch's pioneering work, which earned him the Nobel Prize.

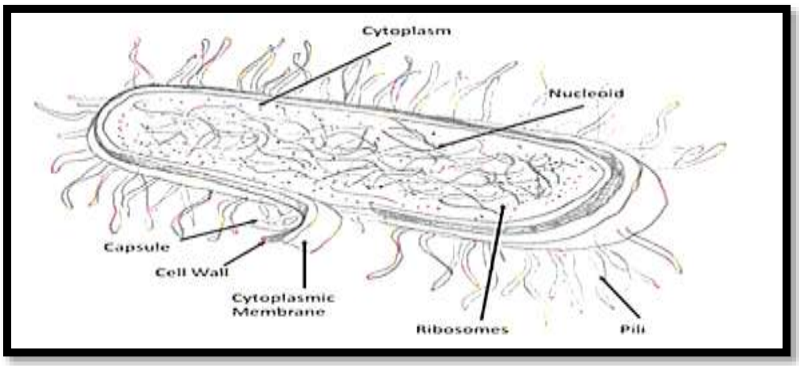

Fig. No. 1: Diagrammatic representation of *Mycobacterium tuberculosis*

Epidemiology of Tuberculosis [13]:

Despite major strides in lowering tuberculosis prevalence and mortality rates, the disease continues to impose a heavy burden on a global scale. Tuberculosis was one of the leading infectious killers in 2021, with an anticipated 10.6 million cases and 1.6 million deaths (WHO, 2022). Sub-Saharan Africa, Southeast Asia, and Eastern Europe have the greatest tuberculosis incidence rates, and these countries are disproportionately affected by the disease.

Tuberculosis is more common in vulnerable populations, such as those who are already HIV positive, diabetic, malnourished, or live in overcrowded settings.[14]. Because HIV lowers the immune system, making people more susceptible to tuberculosis, the combination of the two infections has proved extremely disastrous. While the World Health Organisation has set a target of reducing tuberculosis (TB) fatalities and incidence by 80% and 90%, respectively, by 2030 compared to 2015 levels, these targets are jeopardised due to substantial financing and healthcare infrastructure shortfalls (WHO, 2022).

Characteristics of *Mycobacterium tuberculosis* [15]:

Mycobacterium tuberculosis, the causative agent of TB, is a slow-growing, rod-shaped bacterium that belongs to the family Mycobacteriaceae. It is an obligate aerobe, thriving in oxygen-rich environments such as the lungs. One of the defining features of M. *tuberculosis* is its complex, lipid-rich cell wall, which contributes to its resilience and ability to evade the host immune system. The cell wall's high mycolic acid content renders the bacterium resistant to many common antibiotics and disinfectants.

The pathogen is transmitted via airborne droplets when an infected individual coughs, sneezes, or speaks. Once inhaled, the bacteria can establish infection in the alveoli of the lungs. M. *tuberculosis* has developed sophisticated mechanisms to survive within macrophages, the very cells meant to destroy it.[16]. By inhibiting phagosome-lysosome fusion, the bacterium avoids degradation and can persist in a latent state for years.

Pathogenesis and Disease Progression [17]:

The bacterium and the human immune system engage in a complicated interaction during tuberculosis development. Alveolar macrophages ingest Mycobacterium tuberculosis upon inhalation. To keep an infection at bay, the immune system will typically create granulomas, which are clusters of immune cells that encase the invading germs (Russell, 2007). Even though it may not be causing any symptoms at the moment, this dormant infection stage might nonetheless reactivate, particularly in people with impaired immune systems. The immune response fails to contain the germs, allowing them to proliferate and spread, which leads to the development of active tuberculosis. Tissue damage, brought on in part by the secretion of inflammatory cytokines, is what causes tuberculosis symptoms such a chronic cough, haemoptysis, fever, night sweats, and a loss of weight. Clinical manifestations of extrapulmonary tuberculosis (TB) might vary according to the location of infection; the disease can impact organs like the kidneys, lymph nodes, and spine.

2. 1,3-Benzoxazole:

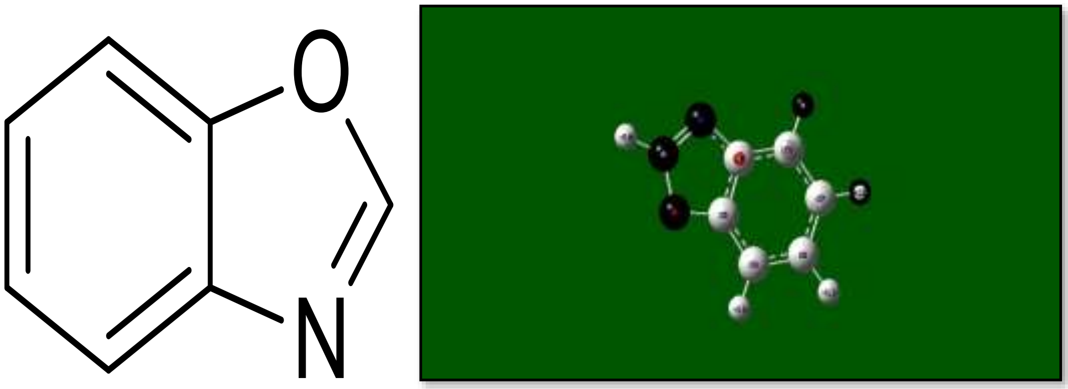

Fig. No. 2: Structure of 1,3-benzoxazole

A heterocyclic aromatic organic molecule with a fused benzene and oxazole ring, 1,3-benzoxazole is well-known for its many uses in medicine and the chemical sciences.[18]. The oxazole ring has nitrogen and oxygen atoms, giving it a one-of-a-kind structure that has fascinated scientists with its unusual electrical and structural characteristics. During the late 19th and early 20th centuries, the compound rose to prominence as a fundamental element in heterocyclic chemistry. Due to its broad range of biological properties, including antibacterial, anticancer, anti-inflammatory, and antiviral actions, it has now become an essential scaffold in drug discovery. In addition, 1,3-benzoxazole derivatives have been extensively utilised in cutting-edge materials including fluorescent probes and organic light-emitting diodes (OLEDs). With a special attention on the structural aspects that drive its multi functionality, this article explores the chemistry of 1,3-benzoxazole, including its production, reactivity, and uses.[19]

2.1 Structural Features of 1,3-Benzoxazole[20]:

The molecular structure of 1,3-benzoxazole consists of a benzene ring fused with an oxazole ring. The oxazole moiety is a five-membered ring containing nitrogen at position 1 and oxygen at position 3, resulting in a planar and aromatic framework. This aromaticity, governed by Hückel's rule, ensures significant resonance stabilization. The heteroatoms contribute lone pairs of electrons, enhancing the molecule's reactivity and enabling diverse chemical interactions. These structural features underpin its stability and wide-ranging utility, making it an attractive candidate for functional modifications and applications.

2.2 Synthesis of 1,3-Benzoxazole[21]:

The synthesis of 1,3-benzoxazole has evolved considerably, encompassing both classical and modern methods.

2.2.1 Classical Methods:

Traditional approaches involve the cyclization of ortho-aminophenols with carbonyl-containing compounds. For example, heating ortho-aminophenol with formic acid or derivatives such as aldehydes and ketones results in the formation of benzoxazole derivatives. This method remains one of the most straightforward and efficient for synthesizing the parent compound (Brown et al., 2017).

2.2.2 Advanced Techniques:

Recent advancements have introduced alternative strategies to enhance the efficiency and eco-friendliness of synthesis. Microwave-assisted synthesis, for instance, significantly reduces reaction times while improving yields. Reactions involving ortho-aminophenols and aldehydes under microwave irradiation have been particularly successful (Lee et al., 2020). Transition metal catalysts, such as palladium or copper, facilitate cross-coupling reactions, enabling the functionalization of benzoxazole frameworks. Additionally, green chemistry approaches, such as solvent-free conditions or the use of ionic liquids, have been explored to minimize environmental impact.

3. Synthesis of 1,3-Benzoxazole and its Derivatives[22]:

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-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

Mechanistic Overview: The formation of 1,3-benzoxazole involves the cyclization of o-aminophenol in the presence of formic acid (or formamide) under reflux conditions. This reaction leads to the condensation of the amino group (-NH_2) and the hydroxyl group (-OH) of o-aminophenol, resulting in the formation of a six-membered heterocyclic ring, the benzoxazole core. The reaction is facilitated by the elimination of water, which is catalyzed by the formic acid or formamide.

The primary reaction steps are as follows:

Nucleophilic Attack: The amino group (-NH_2) of o-aminophenol undergoes nucleophilic attack on the carbon of the carbonyl group (C=O) in formic acid (or formamide).

Cyclization and Dehydration: This intermediate undergoes cyclization, followed by the elimination of water, to form 1,3-benzoxazole.

4. RESULTS AND DISCUSSION:

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

4.1. 2-[(E)-2-Phenylethenyl]-1,3-benzoxazole:

The first compound discussed is **2-[(E)-2-Phenylethenyl]-1,3-benzoxazole.**

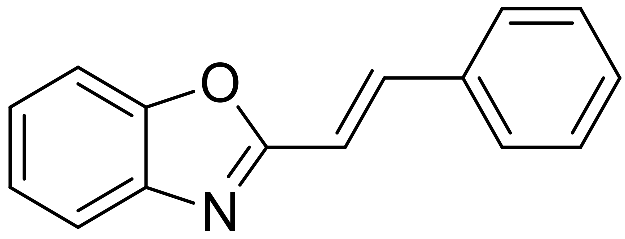

Fig. No. 3: 2-[(E)-2-Phenylethenyl]-1,3-benzoxazole

Various properties of the synthesized compound were examined and are depicted in the following table.

Table No. 1: Physical properties of 2-[(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

UV Graph of compound 2-[(E)-2-Phenylethenyl]-1,3-benzoxazole:

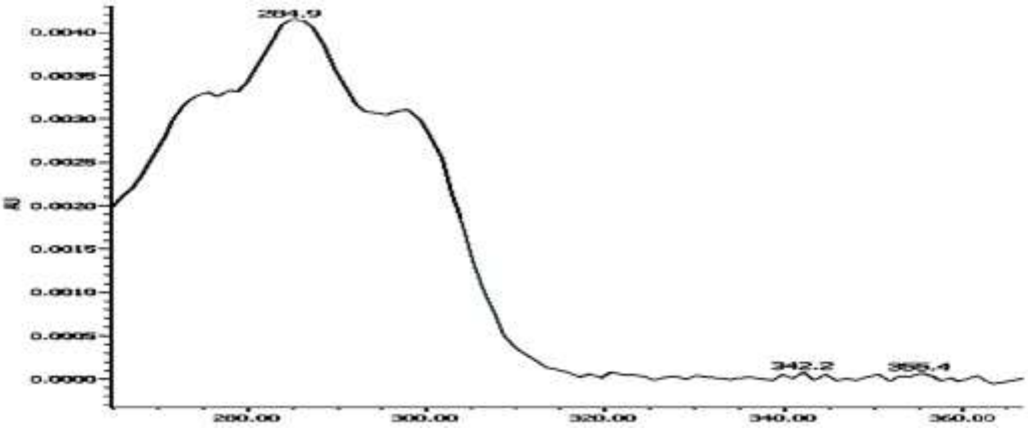

Fig.No.4: UV Graph of compound 2-[(E)-2-Phenylethenyl]-1,3-benzoxazole

The compound was subjected to IR spectral analysis and the following table illustrates the spectral data.

Infrared (IR) Spectra (KBr) Data:

Table No.2: IR spectral data of 2-[(E)-2-Phenylethenyl]-1,3-benzoxazole

Wavenumber (cm^{-1})	**Description**
3062	Aromatic C-H stretch
3040	Aromatic C-H stretch
2360, 2343	Likely impurities or overtone vibrations
1642	C=N stretch
1535	Aromatic C=C stretch
1454	Aromatic C-H deformation
1350	C-N stretch
1237, 1178	Aromatic ether C-O stretch
1108, 1004	C-H in-plane deformation
967, 933	C-H out-of-plane deformation
863, 840, 764, 743	Aromatic ring vibrations
7014	Possible overtone or impurity peak
684, 497, 434	Out-of-plane bending vibrations

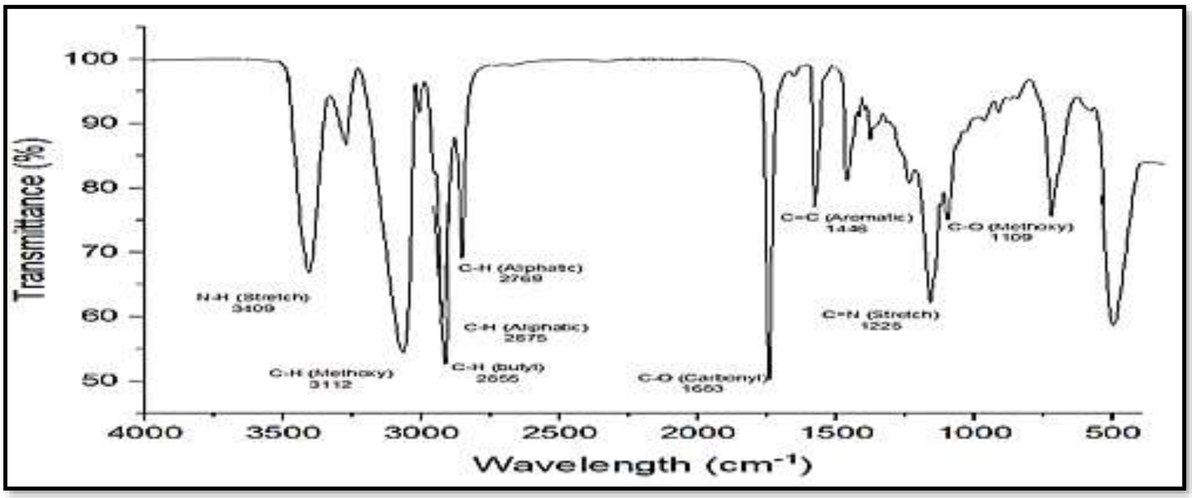

Fig.No.5: The IR spectra of the respective compound is depicted.

The ^{1}H NMR spectral data is as follows.

^{1}H NMR (300 MHz, $CDCl_3$)

^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.77 – 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, 1.0 Hz, 1H), 6.85 (d, *J* = 14.6 Hz, 1H).

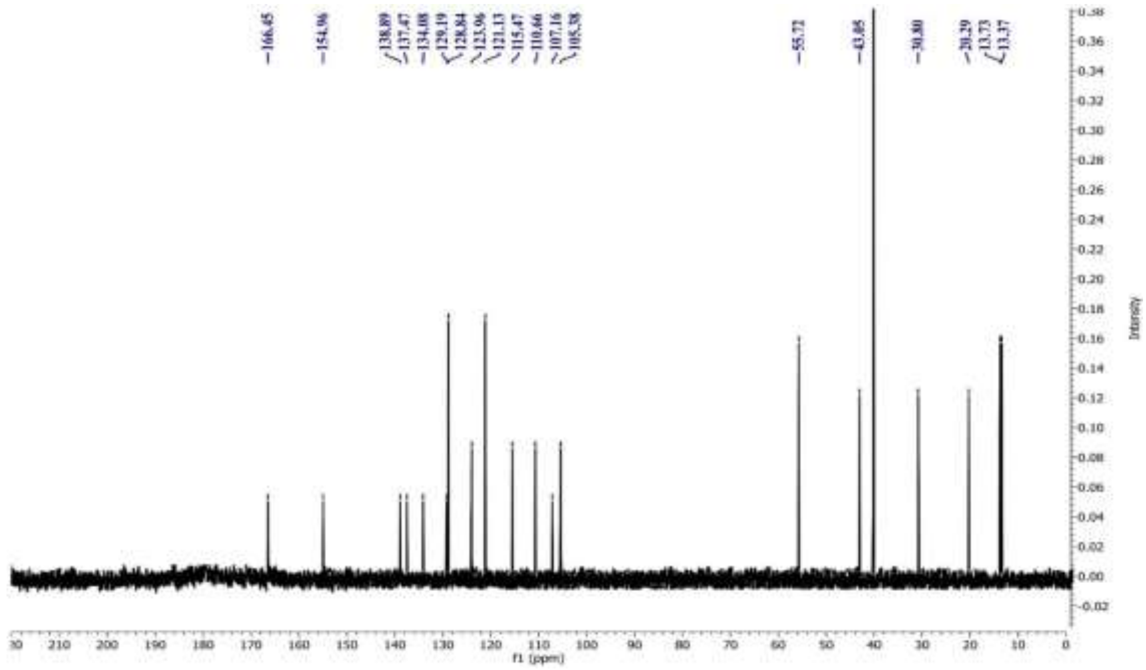

Fig.No.6.:^{1}H NMR spectra of 2-[(E)-2-Phenylethenyl]-1,3-benzoxazole

The ^{13}C NMR data of the compound is as follows.^{13}C NMR (125 MHz,) δ 161.87, 149.69, 139.40, 134.78, 134.36, 129.43, 128.96, 128.35, 126.69, 125.45, 119.43, 113.63, 113.27.

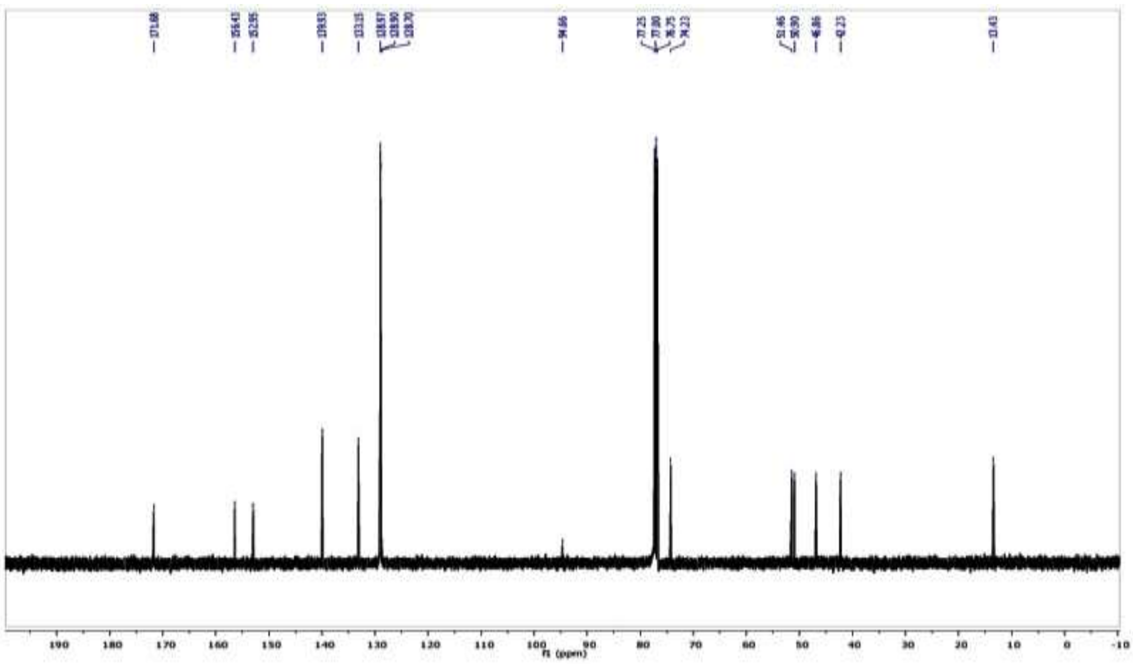

Fig No. 7: The Spectra of ^{13}C NMR of the respective compound.

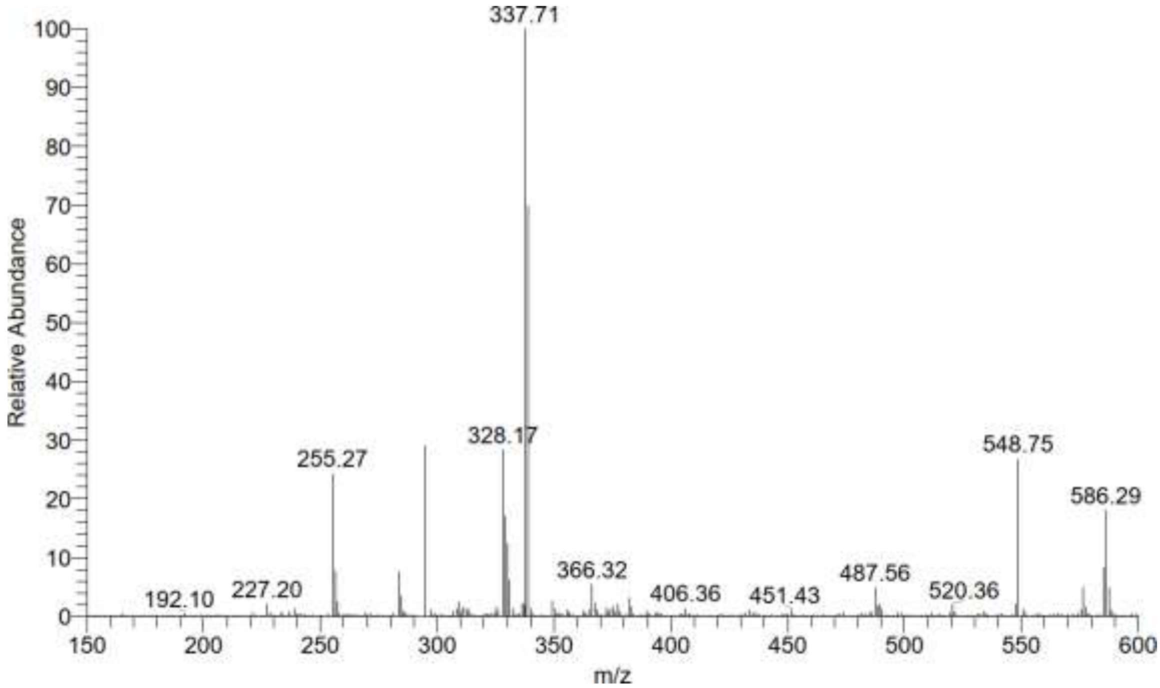

Fig.No.8 : Mass spectra of the compound 2-[(E)-2-Phenylethenyl]-1,3-benzoxazole

In-Vitro analysis:
The inhibition data of the compound against the tested strains is as follows.

Table No. 3: The table depicts the inhibition concentrations of synthesized compounds against the tested strains of mycobacterium.

Compound	IUPAC Name	MTB MIC (µmol/L)	MA MIC (µmol/L)	MK MIC (µmol/L)
1	2-[(E)-2-Phenylethenyl]-1,3-benzoxazole	125	62.5	125

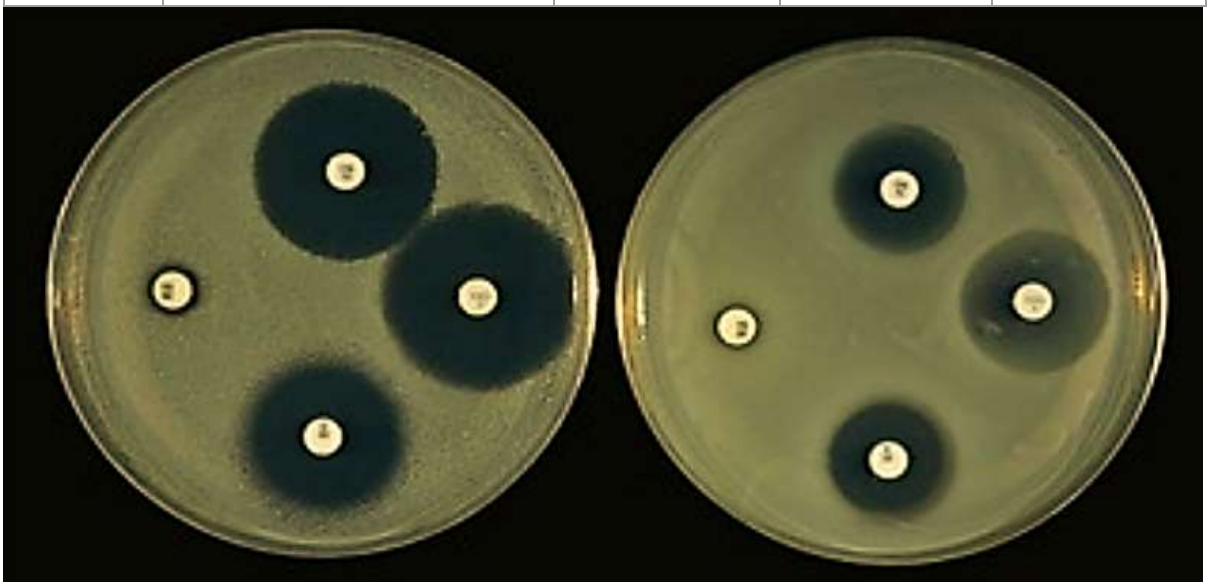

Fig.No.9 : The image captured during the inhibition assay

CONCLUSION:

Addressing *Mycobacterium*-related diseases requires a multidisciplinary approach that combines clinical innovation, public health strategies, and social interventions. Vaccination efforts, such as the Bacillus Calmette-Guérin (BCG) vaccine, must be enhanced to provide broader and more effective protection. Environmental interventions targeting NTM reservoirs, alongside global efforts to reduce healthcare disparities, are vital for long-term disease control.

In 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. In conclusion, the analysis of the UV-Vis absorption 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 methylsulfanyl and methyl groups also causes 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. Diagnostic challenges remain a critical barrier to the effective management of *Mycobacterium*-related diseases. While traditional methods, such as acid-fast staining and culture, remain the gold standard, their slow turnaround times often delay treatment. Molecular diagnostics, including polymerase chain reaction (PCR) and next-generation sequencing, have significantly improved the speed and accuracy of identification. However, the limited availability and high costs of these tools restrict their widespread use, especially in regions most affected by *Mycobacterium*-related diseases. Expanding access to these technologies is essential for improving outcomes.

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