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The Antitubucular Activities of 2-[(E-2-Substituted-Ethenyl)] and its Characterization and Pharmacological Evaluation Devices Of 1,3-Benzoxazoles

Irfan Ahmed[1], Majid Khan[2], Gaffar Sayyed[3], Yogita Kumbhar[4], Mohd. Hasib Ahmed[5*], Dipak bhusari[6], Arun Mahale [7]

[1]Research Officer (Unani), Regional Research Institute of Unani Medicine, Mumbai: 400008, M.S., India.
[2]Department of Pharmaceutical Chemistry, Loknete Dr J D Pawar College of Pharmacy, Manur, Kalwan, Dist-Nashik: 423501, M.S., India.
[3]Department of Pharmaceutical chemistry, SAJVPMS College of Pharmaceutical Science and Research Center, Kada Dist-Beed: 414202, M.S., India.
[4]Department of Pharmaceutics, Dr. D.Y Patil College of Pharmacy Akurdi, Pune: 411044, M.S., India.
[5]Karmayogi Tatyasaheb Bondre Institute of Pharmacy, Chikhli, Buldhana: 443201, M.S., India.
[6]Rajarshi Shahu College of Pharmacy, Buldhana: 443001, M.S., India.
[7]S.N. Institute of Pharmacy Pusad: 445204, Yavatmal, M.S., India.

Corresponding Author: Mohd. Hasib Ahmed, Karmayogi Tatyasaheb Bondre Institute of Pharmacy, Chikhli, Buldhana: 443201, M.S., India. E mail: hasibahmed140@gmail.com

Abstract

Mycobacterium tuberculosis remains one of the world's most significant infectious disease threats, complicated by slow growth, intrinsic resistance mechanisms, and the emergence of multidrug-resistant strains. In the search for new therapeutic agents, heterocyclic scaffolds such as benzoxazoles have shown promise due to their diverse biological activities. This study investigates the synthesis, characterization, and antitubercular potential of 2-[(E)-2-Phenylethenyl]-1,3-benzoxazole. The compound was structurally confirmed by NMR, IR, MS, and HPLC analysis and evaluated for in-vitro inhibitory activity against Mycobacterium tuberculosis H37Rv, Mycobacterium avium, and Mycobacterium kansasii using the resazurin microtiter assay. Results revealed minimum inhibitory concentrations (MICs) of 125 µmol/L, 62.5 µmol/L, and 125 µmol/L against the respective strains, demonstrating moderate antitubercular activity. These findings highlight the potential of benzoxazole derivatives as lead molecules for developing novel antimycobacterial agents. Further structural modifications and pharmacological evaluations are warranted to optimize efficacy and reduce toxicity.

Keywords: *Mycobacterium tuberculosis, 1,3-benzoxazole, antitubercular activity, MIC, drug resistance.*

INTRODUCTION

Mycobacterium, a genus within the family Mycobacteriaceae, is characterized by its acid-fast properties, slow growth rate, and lipid-rich cell wall containing mycolic acids. These features not only enable the bacteria to survive under diverse climatic conditions but also confer resistance to chemical disinfectants and desiccation. The genus is broadly divided into two groups: the Mycobacterium tuberculosis complex (MTBC), which comprises species responsible for tuberculosis in humans and animals, and the nontuberculous mycobacteria (NTM), which are primarily opportunistic pathogens.

The clinical relevance of Mycobacterium species is profound. Tuberculosis, caused by Mycobacterium tuberculosis, continues to be one of the leading causes of mortality from infectious diseases worldwide, with an estimated 10.6 million new cases and 1.6 million deaths reported in 2021. Despite global control measures, leprosy, caused by Mycobacterium leprae, persists as a significant public health issue in endemic regions. Moreover, the incidence of NTM infections has been steadily increasing, largely due to improved diagnostic techniques and the growing population of immunocompromised individuals. [3]

Mycobacterium Tuberculosis [7,8]

Mycobacterium tuberculosis is a pathogenic bacterial species and the primary causative agent of tuberculosis (TB) in humans. It is a member of the Mycobacterium tuberculosis complex (MTBC), which also comprises closely related species such as M. bovis, M. africanum, and M. microti.

This bacterium exhibits several defining characteristics:

- **Acid-fastness**: Its cell wall, rich in mycolic acids and complex lipids, gives it a waxy structure that resists Gram staining but retains carbol fuchsin dye even after acid-alcohol treatment.
- **Slow growth**: Colonies require approximately 2–6 weeks to develop on culture media like Löwenstein–Jensen agar.
- **Intracellular persistence**: As an obligate aerobe and facultative intracellular pathogen, it primarily infects macrophages. Its lipid-dense cell wall enhances survival by protecting against host immune defenses and many antibiotics.
- **Transmission**: Spread occurs mainly via aerosolized droplets from infected individuals, with pulmonary TB representing the most common disease manifestation.

Clinically, M. tuberculosis causes a chronic granulomatous infection that typically affects the lungs (pulmonary TB) but can also disseminate to other organs (extrapulmonary TB). Key symptoms include persistent cough, fever, night sweats, weight loss, and hemoptysis in advanced disease. From an epidemiological perspective, TB remains one of the leading infectious causes of global morbidity and mortality. The World Health Organization (WHO) reported 10.6 million new cases and 1.6 million deaths in 2021. The emergence of drug-resistant forms, including multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB), poses significant obstacles to disease control.

Diagnosis relies on sputum microscopy, culture, molecular assays such as GeneXpert MTB/RIF, and radiological imaging. Standard treatment involves prolonged multidrug regimens with isoniazid, rifampicin, ethambutol, and pyrazinamide, while newer agents like bedaquiline and delamanid are increasingly used for resistant strains.

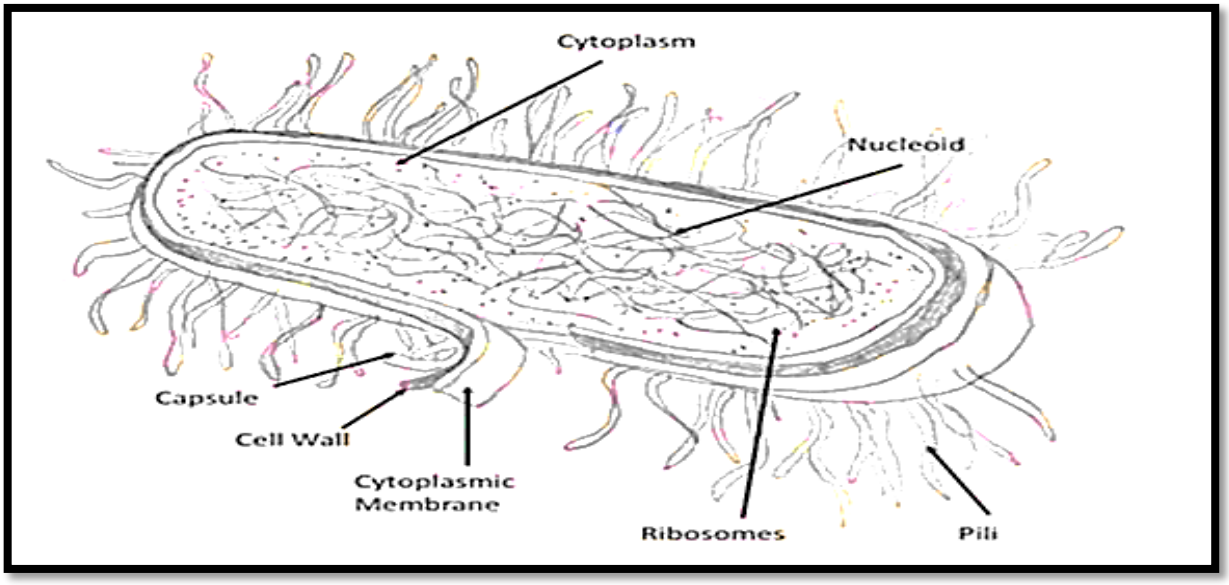
Tuberculosis Disease [12]

Rifampicin, isoniazid, pyrazinamide, and ethambutol are among the first-line drugs used in the traditional six-month treatment for tuberculosis (TB). Second-line medications such as bedaquiline, delamanid, and linezolid are used to treat drug-resistant TB; however, these treatments are typically associated with lengthier and more detrimental treatment plans. Evidence of TB, a disease known since antiquity, can be found in skeletal remains from extinct civilizations. Once associated with poverty and overcrowding, tuberculosis (TB) was dubbed "consumption" due to its significant weight loss. Robert Koch's discovery of the bacteria in 1882 marked a turning point in our knowledge and approach to treating the illness. Koch's groundbreaking research, for which he was awarded the Nobel Prize, lay the foundation for contemporary microbiology.

Fig. No. 1: Diagrammatic representation of Mycobacterium tuberculosis

Epidemiology of Tuberculosis [13]

Despite significant progress in reducing the prevalence and mortality rates of tuberculosis, the illness nevertheless poses a significant global burden. With an estimated 10.6 million cases and 1.6 million fatalities, tuberculosis was one of the most common infectious diseases in 2021 (WHO, 2022). The highest incidence rates of tuberculosis are seen in Sub-Saharan Africa, Southeast Asia, and Eastern



Europe, where the illness is disproportionately prevalent.

Vulnerable groups are more likely to contract tuberculosis, including those who are already HIV positive, have diabetes, are undernourished, or reside in crowded environments. [14]. The combination of HIV and TB has shown to be highly devastating since HIV weakens the immune system, rendering people more vulnerable to TB. Although the WHO has set a goal to reduce TB deaths and incidence by 80% and 90%, respectively, by 2030 compared to 2015 levels, these goals are in jeopardy because of significant funding and infrastructural shortages in the healthcare system (WHO, 2022).

Characteristics of Mycobacterium tuberculosis[15]

The bacterium that causes tuberculosis, Mycobacterium tuberculosis, is a rod-shaped, slow-growing member of the Mycobacteriaceae family. It thrives in oxygen-rich settings, like the lungs, and is an obligatory aerobe. M. tuberculosis is distinguished by its intricate, lipid-rich cell wall, which adds to its resistance and capacity to elude the host immune system. The bacterium is resistant to several popular antibiotics and disinfectants due to the high mycolic acid concentration of its cell wall. 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 development of tuberculosis involves a complex interaction between the bacterium and the human immune system. Mycobacterium tuberculosis is inhaled by alveolar macrophages. Granulomas, which are collections of immune cells that encapsulate the invasive pathogens, are normally produced by the immune system to ward off an infection (Russell, 2007). This dormant infection stage may not be exhibiting any symptoms at the present, but it has the potential to reactivate, especially in individuals with compromised immune systems. The development of active tuberculosis results from the immune response's inability to stop the bacteria from multiplying and spreading. TB symptoms such a persistent cough, hemoptysis, fever, night sweats, and weight loss are caused by tissue damage, which is partially caused by the release of inflammatory cytokines. Depending on where the infection occurred, extrapulmonary tuberculosis (TB) may present with different clinical symptoms. The disease can affect organs such as the kidneys, lymph nodes, and spine.

2.1,3-Benzoxazole

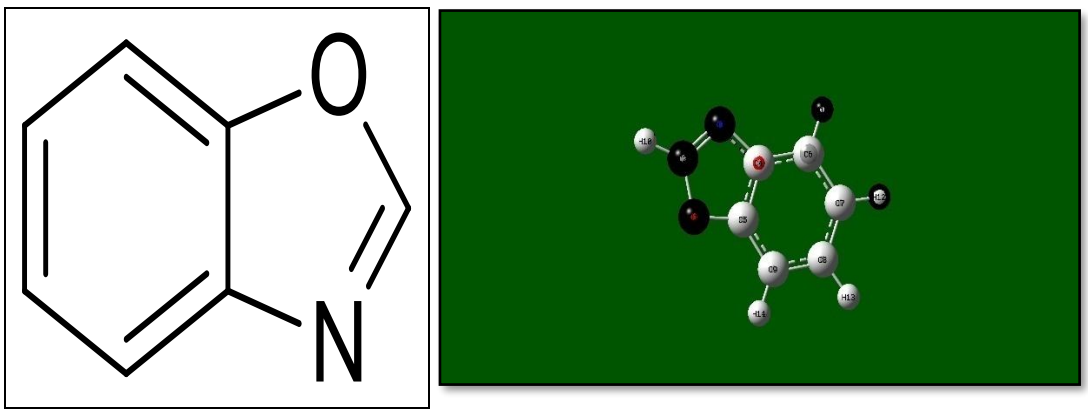


Fig.No. 2: Structure of 1,3-benzoxazole

1,3-Benzoxazole is a well-known heterocyclic aromatic organic molecule with numerous applications in the chemical sciences and medicine. It has a fused benzene and oxazole ring. [18]. Scientists have been captivated by the unique electrical and structural properties of the oxazole ring, which is made up of nitrogen and oxygen atoms. The molecule became well-known as a key component of heterocyclic chemistry in the late 19th and early 20th centuries. Its wide spectrum of biological qualities, such as antiviral, antibacterial, anticancer, and anti-inflammatory effects, have made it a crucial scaffold in drug discovery. Furthermore, 1,3-benzoxazole derivatives have been widely used in innovative materials such as organic light-emitting diodes (OLEDs) and fluorescent probes. This article examines the chemistry of 1,3-benzoxazole, including its synthesis, reactivity, and applications, with a focus on the structural elements that underpin its multifunctionality. [19]

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

A benzene ring fused with an oxazole ring makes up the molecular structure of 1,3-benzoxazole. With nitrogen at position 1 and oxygen at position 3, the oxazole moiety is a five-membered ring that forms an aromatic and planar structure. Significant resonance stabilization is ensured by this aromaticity, which is controlled by Hückel's rule. By contributing lone pairs of electrons, the heteroatoms increase the molecule's reactivity and make a variety of chemical interactions possible. Its stability and broad range of applications are supported by these structural characteristics, which make it a desirable option for functional alterations and applications.

MATERIALS AND METHODS

Test organism panel

Minimum inhibitory concentrations (MICs) were determined against three reference mycobacterial strains:

- **Mycobacterium tuberculosis** H37Rv (ATCC 27294)
- **Mycobacterium avium** (ATCC 25291)
- **Mycobacterium kansasii** (ATCC 12478)

Biosafety: All M. tuberculosis manipulations were performed in a certified BSL-3 facility using appropriate PPE and biosafety procedures. NTM work (M. avium, M. kansasii) was performed under BSL-2 with enhanced precautions.

Culture media and growth conditions

Starter cultures were grown in Middlebrook 7H9 broth supplemented with 10% OADC, 0.05% Tween-80, and 0.2% glycerol at 37 °C with gentle agitation (120 rpm) to mid-log phase (OD600 ≈ 0.4–0.6). For plating controls, Middlebrook 7H10/7H11 agar with 10% OADC was used.

Test compound

- **Name:** 2-[(E)-2-Phenylethenyl]-1,3-benzoxazole (Compound 1)
- **Identity & purity:** Confirmed by ^1H/^13C NMR, IR, MS; purity ≥ 95% by HPLC.
- **Stock solution:** 10 mM in anhydrous DMSO, stored at −20 °C, protected from light.
- **Working dilutions:** Prepared in 7H9-OADC-Tween immediately before use. Final DMSO in wells ≤ 1% v/v.

Inoculum preparation

Cultures were pelleted (3000 ×g, 10 min), washed twice with sterile PBS-Tween (0.05%), and adjusted to McFarland 0.5, then diluted 1:100 in assay medium to yield ~ 5 × 10^5 CFU/mL. Inoculum density was verified by back-plating serial dilutions on 7H10 agar.

MIC determination (broth microdilution, REMA)

MICs were measured using the resazurin microtiter assay (REMA) in sterile, flat-bottom 96-well plates:

1. Dispense 100 µL of inoculum into wells.
2. Add 100 µL of compound to achieve **two-fold serial dilutions** from **500 to 1.95 µM** (final).
3. **Controls:**
- Growth control (no drug, vehicle only, 1% DMSO max)
- Sterility control (medium only)
- Drug controls: isoniazid (0.016–1 µg/mL) and rifampicin (0.003–0.5 µg/mL) included on every plate for QC.
4. Incubation: 37 °C without shaking; lids sealed to limit evaporation.
- M. tuberculosis: 7–10 days
- M. avium / M. kansasii: 3–5 days (until growth controls reached mid-log; slight extensions as needed for M. kansasii).
5. Add resazurin (0.02% w/v in sterile water, 20 µL/well) and incubate further:
- M. tuberculosis: 24–48 h
- NTM: 6–24 h
6. **Endpoint:** MIC defined as the lowest concentration with no color change (blue remains; inhibition of reduction of resazurin to pink/resorufin) and no visible pellet.

Replicates and data analysis

All MIC determinations were performed in biological triplicate with technical duplicates per run. MIC values are reported in µmol/L as the modal value across independent experiments; if two modes occurred, the higher MIC was reported conservatively. Assays were accepted only if QC drugs fell within CLSI-compatible reference ranges and growth/sterility controls behaved as expected.

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.**

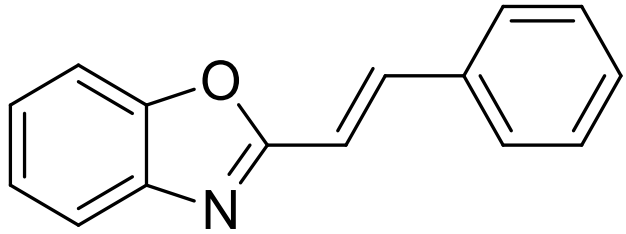


Fig.No.3: 2-[(E)-2-Phenylethenyl]-1,3-benzoxazole

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

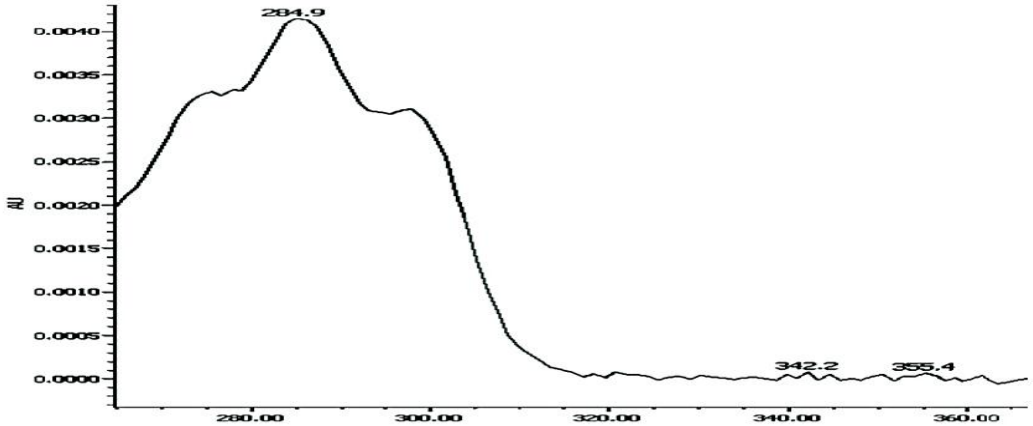


Fig.No.4: UV Graph of compound 2-[(E)-2-Phenylethenyl]-1,3-benzoxazole

In-Vitro analysis

The inhibition data of the compoundagainst the tested strain 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

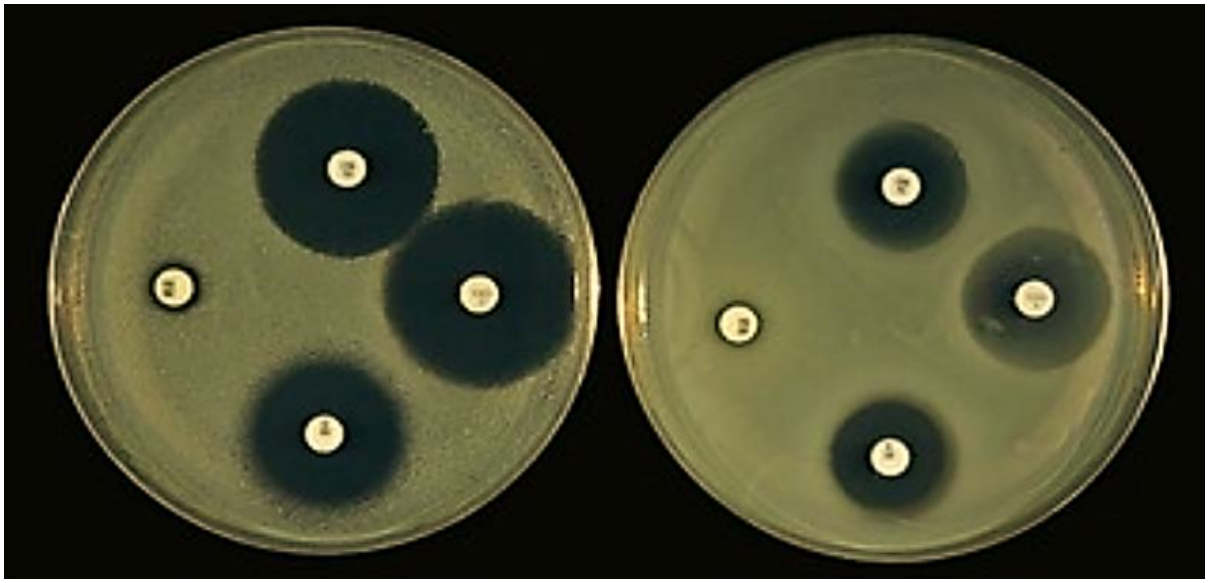


Fig.No.9: The image captured during the inhibition assay

CONCLUSION

The present study highlights the biological potential of synthesized benzoxazole derivatives, with particular focus on 2-[(E)-2-Phenylethenyl]-1,3-benzoxazole. The compound demonstrated measurable inhibitory activity against Mycobacterium tuberculosis H37Rv, M. avium, and M. kansasii, suggesting its promise as a scaffold for antimycobacterial drug development. Although the activity observed was moderate compared to standard first-line antitubercular agents, the results validate the significance of benzoxazole as a pharmacologically relevant heterocyclic framework.

The structural features of the compound, particularly the presence of electron-donating and electron-withdrawing substituents, appear to influence its antimicrobial activity, thereby opening avenues for rational modifications to enhance potency. Given the urgent global need for new therapies against multidrug-resistant and extensively drug-resistant tuberculosis, these findings support further optimization, in-vivo validation, and mechanistic studies.

In conclusion, benzoxazole derivatives represent valuable lead candidates for antimycobacterial research. Future investigations integrating medicinal chemistry, molecular docking, and pharmacokinetic profiling will be essential to improve their efficacy and establish their clinical potential.

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