Rational Design and Pharmacological Profiling of Triphenyl Imidazole Derivatives Synthesized from 4-Hydroxybenzaldehyde

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***Abstract*—midazoles are naturally occurring substances and are a key structural unit of various biologically active molecules. A striking example is the compound $C_{21}H_{16}N_2$, which was discovered in 1877 and contains a 2,4,5-triphenylimidazole skeleton. While many of the derivatives of this compound—the so-called lophine—have had their crystal structures determined, the structure of the parent molecule had remained elusive for some time. In these derivatives, the imidazole ring adopts a non-coplanar conformation relative to the three appended phenyl rings, which have dihedral angles of approximately 21.4°, 24.7°, and 39.0°, respectively. These compounds tend to adopt layered crystal structures oriented perpendicular to the b-axis, stabilized in part by defined hydrogen bond donor and acceptor sites. Chemical modification and synthesis of 2,4,5-triphenylimidazole derivatives are of significant interest in drug discovery, due to the scaffold's wide range of biological activities. Studies show that these compounds exhibit anti-inflammatory and analgesic effects (Shalini et al., 2011; Achar et al., 2010), and their antifungal efficacy is augmented by incorporation of groups like benzyl, benzoyl, or para-aminobenzoyl ring (Yadav et al., 2011). Microwave- assisted methodologies have also been used to efficiently synthesize the 2,4,5-triphenylimidazole core (Pandit et al., 2011). Further structural alteration, including addition of thiol or trimethoxybenzene groups, has proved to increase both antifungal and antiinflammatory potential (Umarani et al., 2011; El Ashry et al., 2007). Also, replacing a hydrogen atom with an azole ring has shown enhanced antibacterial and antiinflammatory activity (Amir et al., 2011).**

***Index Terms*—Antiinflammatory, antidepressant, antiviral, antifungal, antibacterial activities of triphenyl imidazole, antitubercular activity, anti-angiogenic activity.**

I. INTRODUCTION

Medicinal chemistry, an interdisciplinary field that combines from chemistry and pharmacy to design, develop, enhance pharmaceutical medications. It is concerned with the discovery and optimization of chemical entities of therapeutic interest. This field examines how drugs engage with biological systems, examines their chemical structure relative to biological effects—often through the use of tools such as quantitative structure-activity relationship (QSAR) models—and screens current drugs for further development.

It combines information from a number of scientific disciplines including organic chemistry, biochemistry, molecular biology, pharmacology, physical chemistry, statistics, computational chemistry, and chemical biology. In reality, medicinal chemistry often focuses on small organic molecules, borrowing from fields such as enzymology, natural products, structural biology, and synthetic chemistry to discover and optimize promising drug leads.

The drug discovery process often starts with the selection of a compound that has some initial biological activity. By repetitive testing and modification of molecules, researchers try to enhance its therapeutic effect, reduce toxicity, and achieve selectivity. Both laboratory-based synthesis and computer-aided design procedures are involved in this process of optimization. The discipline also assesses existing treatments to optimize their performance and reveal their mechanism of action.

Most closely associated with this is pharmaceutical chemistry, which involves ensuring drugs are of required quality and function as desired. It focuses on drug formulation, stability, and delivery systems to

guarantee safety and efficacy in clinical use. Across human and animal medicine, medicinal chemistry leverages a variety of scientific disciplines to bridge basic chemical compounds to approved, trusted therapies, with the addition of project management and pharmaceutical development approaches [8].

About 4-Hydroxybenzaldehyde (4-HBA)

4-Hydroxybenzaldehyde (4-HBA), aromatic aldehyde compound, $C_7H_6O_2$. It is a hydroxyl (-OH) group in position opposite to an aldehyde (-CHO) group on a benzene ring, thus taking the para-substituted shape. The compound finds extensive utilization as an intermediate for the preparation of a large range of products, such as drugs, agrochemicals, polymers, perfumes, and colorants.

Industrial production of 4-HBA can be obtained by various methods like the oxidation of p-cresol, vanillin demethylation, or phenol hydroxylation. Because of its reactivity with chemicals, 4-HBA easily takes part in oxidation, reduction, and condensation reactions and, therefore, is useful in organic synthesis. Its versatility has qualified it as a basic ingredient in the production of biologically active molecules, antioxidants, and specialty chemicals [8].

PHYSICAL PROPERTIES OF TRI-PHENYL IMIDAZOLE

1. IUPAC Name: 2,4,5-triphenyl imidazole.
2. Molecular weight: 296.4 g/mol.
3. Molecular formula: C21H16N2
4. Synonyms: Lophine, 1h imidazole.

PHYSICAL PROPERTIES OF 4-HYDROXY BENZALDEHYDE (Starting Material)

5. IUPAC Name: 4-hydroxy-2-benzaldehyde
6. Molecular weight: 122.12 g/mol.
7. Molecular formula: C7H6O2.

CHEMICAL PROPERTIES OF TRI-PHENYL IMIDAZOLE

1. Melting point: Ranges between 272-276 °C.
2. Boiling Point: around 428 degrees Celsius.
3. 1.0874 g/cm³ is the density.
4. Refractive Index: 1.8000.
5. Storage condition: cool room temperature.
6. Color: white, orange, green.
7. Solubility: Methanol is the only solvent that can dissolve this substance.
8. The Pka value is 11.66.
9. Type of product: granular or crystalline.
10. The purity of the substance was 97%.
11. Wavelength: 300nm.

CHEMICAL PROPERTIES OF 4-HYDROXY BENZALDEHYDE (Starting Material)

12. Melting point: Ranges between 114-116 °C.
13. Boiling Point: 191°C.
14. Refractive Index: 1.579 at 20°C.
15. Storage condition: keep in a cool, dry location, away from light, moisture, and direct sunlight.
16. Solubility: dissolvable in ethanol, methanol, ether, and acetone.
17. The pka value is 7.66.
18. Type of product: Organic aromatic aldehyde.
19. The purity of the substance was 98%.
20. Wavelength: 290nm [2].

THERAPEUTIC VALUES

Triphenyl imidazole, specifically the 2,4,5-triphenyl variant, possesses a wide array of therapeutic benefits due to its varied biological functions. Here are the main therapeutic uses:

1. Anticancer Activity: Triphenyl imidazole derivatives have shown promising anticancer effects across various cell lines like-cancer including prostate (PC-3), colon (HCT-116), and breast (MCF-7) cancers. Their therapeutic potential lies in their ability to disrupt cell cycle progression, inhibit specific kinases, and trigger apoptosis (programmed cell death), effectively suppressing tumor cell growth.

2. Antimicrobial Activity: These derivatives possess significant antimicrobial activity, being effective against both bacterial and fungal strains. They have been shown to be effective in inhibiting the growth of bacterial species like Bacillus subtilis and Klebsiella pneumoniae, as well as fungal pathogens like Candida albicans.

3. Anti-inflammatory Activity: Triphenyl imidazole compounds have potent anti-inflammatory activity, via inhibition of COX-1 and COX-2 enzymes. This action indicates their potential use in the treatment of a variety of inflammation-related diseases.

4. Analgesic Activity: These molecules also show analgesic activity by modifying inflammatory mechanisms and interacting with pain receptors, which shows their significance in the management of conditions involving acute or chronic pain [6].

5. Antitubercular Activity: Certain derivatives of triphenyl imidazole have been reported to exhibit activity against Mycobacterium tuberculosis, suggesting their utility in the discovery of novel drugs for the treatment of tuberculosis, a dangerous infectious disease [20].

PHARMACOLOGICAL ACTIVITIES

[A.] ANTI-BACTERIAL ACTIVITY:

Antibacterial agents are chemical compounds specifically designed to prevent bacterial growth or kill bacteria altogether. They are usually tested against well-characterized bacterial strains. Bacillus subtilis, a Gram-positive, rod-shaped bacterium, is usually the preferred choice for such research because it causes foodborne disease. Klebsiella pneumoniae, on the other hand, a Gram-negative, motile bacterium, is commonly used due to its association with healthcare-associated infections, especially respiratory tract infections and post-surgical wound infections. These antibacterial agents function by targeting vital bacterial processes. Common laboratory methods to assess their activity include turbidimetry, which quantifies bacterial presence by analyzing the cloudiness of a liquid culture, and the agar diffusion method, which determines antimicrobial efficacy based on the size of inhibition zones formed on nutrient media. These techniques offer dependable insights into both the strength and range of antibacterial action [16].

[B.] ANTI-OXIDANT ACTIVITY:

Antioxidants are substances that stop or lessen the harm that free radicals—unstable chemicals that can damage cells—cause. Antioxidants, also known as "free radical scavengers," neutralize these dangerous chemicals and provide defense against their negative effects. Some foods naturally contain antioxidants, which are important for lowering oxidative stress in the body. They have been connected to several health advantages, including as preventing cancer, liver illness, and heart disease. The body creates dangerous chemicals known as free radicals when oxygen is broken down. Numerous health problems can result from these free radicals' damage to DNA, proteins, lipids, and cell membranes [9].

[C.] ANTIINFLAMMATORY ACTIVITY:

The anti-inflammatory qualities of the created compounds were assessed in pale skinned person rats (150–200 g, either sex) utilized in a carrageenan-induced paw edema worldview. Sometime recently the explore, the rats were isolated into eight bunches of six and fasted for the entire night. As the control bunch, Gather I was given a vehicle comprising of 0.5rboxymethylcellulose (CMC). 30 minutes some time recently 0.1 ml of 1rrageenan was sub-plantarly infused into the proper rear paw, all medications were managed orally [14]. Bunch II was treated with indomethacin (100 mg/kg) as a standard anti-inflammatory sedate. Bunches III to VIII were managed the test compound (200 mg/kg) at assigned time interims (T1–T6). A vernier caliper was utilized to degree the thickness of the paws 60, 120, and 180 minutes after infusion. The rate restraint of paw swelling in comparison to the control was utilized to survey each compound's anti-inflammatory viability.

[D.] ANTI-MICROBIAL ACTIVITY:

The container plate dissemination strategy was utilized to look at the engineered compounds' antibacterial movement. Gram-positive Gram-negative microscopic organisms and Staphylococcus aureus Supplement agar was utilized to develop Pseudomonas aeruginosa, whereas Sabouraud dextrose agar was utilized to test for antifungal viability against Candida albicans. Petri dishes were filled with sterile agar, and the surface of each microbial suspension was equally secured with 0.1 ml. 30 µg/ml of each test substance, standard pharmaceutical, and chloroform (as a dissolvable control) were included to wells. To permit for dissemination, plates were cleared out at room temperature for two hours without being moved. Parasitic societies were brooded for 48 hours at 28°C, whereas bacterial societies were refined for 24 hours at 37°C. In arrange to survey microbiological helplessness, zones of restraint were measured in centimeters [7].

II. MECHANISM OF ACTION

The suggested method for creating 2,4,5-trisubstituted 1H-imidazoles (compounds a–n) in a single pot using three components. Under the right circumstances, an aromatic aldehyde, a 1,2-diketone, and ammonium acetate condense in this multicomponent process. Ammonium acetate, which undergoes thermal

breakdown to liberate ammonia and acetic acid, is an essential nitrogen donor in the reaction. The primary source of nitrogen for the synthesis of the imidazole ring is the released ammonia. Usually, the reaction starts when ammonia attacks one of the diketone's carbonyl groups nucleophilically, creating an imine intermediate. This is followed by a sequential condensation with the aldehyde component, resulting in the formation of a diimine or Schiff base intermediate. Intramolecular cyclization and subsequent aromatization steps ultimately lead to the formation of the imidazole core.[12]

EXPERIMENTAL WORK

SYNTHESIS OF 2-(4-HYDROXYPHENYL)-4,5-DIPHENYLIMIDAZOLE:

Benzil, 4-hydroxybenzaldehyde, and ammonium acetate were used in a multicomponent reaction with glacial acetic acid to create 2-(4-hydroxyphenyl)-4,5-diphenylimidazole in a single pot. In expansion to 50 mmol of ammonium acetic acid derivation (3.84 g) in 15 mL acidic corrosive frosty, the specified reagents: 10 mmol of benzil (1.52 g) and 4-hydroxybenzaldehyde (1.22 g). A 50 mL R.B. Borosilicate Jar with a reflux condenser was filled utilizing the response item. The jar was kept at 120°C in a silicon oil shower and refluxed for 15 minutes to guarantee uniform warming. The flask was heated and then placed in an ice-water bath to cause the product to crystallize. The resulting solid was filtered and allowed to dry naturally for the entire night.

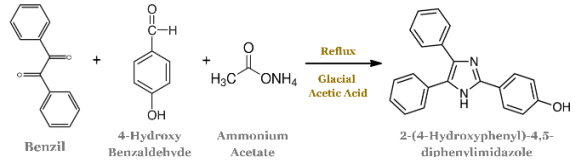

Fig 01: synthesis of 2-(4-hydroxyphenyl)-4,5-diphenylimidazole

To analyze the response, the molar masses of each reactant and the target item were decided. Benzil ($C_{14}H_{10}O_2$) contains a molar mass of 210.23 g/mol, 4-hydroxybenzaldehyde weighs 122.12 g/mol, and ammonium acetic acid derivation is 77.08 g/mol. The expected product, 2-(4-hydroxyphenyl)-4,5-diphenylimidazole, has a molecular weight of 312.37 g/mol. Based on the quantities used, benzil was identified as the limiting reagent with approximately 0.00499 moles available. According to the reaction stoichiometry, this should yield 0.00499 moles of the product, equating to a theoretical mass of 1.56 g. The real mass of the disconnected compound was 1.07 g, coming about in a rate abdicate of 68.59%. This direct abdicate affirms effective arrangement of the target imidazole subsidiary beneath the expressed conditions [21].

III. RECRYSTALLIZATION:

To improve its purity, the 2-(4-hydroxyphenyl)-4,5-diphenylimidazole crude product was recrystallized. In a 250 mL Erlenmeyer flask, around 20 mL of ethanol was added with the crude material. The combination was then slowly heated while being constantly stirred to accomplish total dissolution. Throughout the procedure, care was taken to prevent overheating. About 1 gram of activated charcoal was added to the boiling solution, which was kept at a gentle boil for 5 to 10 minutes with periodic stirring, in order to eliminate colored impurities. The resulting mixture was immediately filtered through preheated filter paper using a warmed funnel for prevention crystallization and to separate charcoal and insoluble residues. The clear filtrate was allowed for cooling gradually in a ambient temperature, further cooling in an ice bath for approximately 30 minutes for promotion of crystal formation. Crystals began to appear upon cooling, particularly during the ice bath stage. The purified crystals were collected, transferred to a clean watch glass or dish, and air-dried at room temperature or in a desiccator to complete the recrystallization process [11].

IV. MELTING POINT DETERMINATION:

Melting point analysis is a widely utilized method for evaluating the purity and verifying the identity of organic compounds. Pure substances characteristically exhibit a sharp and well-defined melting range. In order to evaluate the synthetic 2-(4-hydroxyphenyl)-4,5-diphenylimidazole's purity and confirm its structure against existing data, the melting point of the compound was ascertained. To ensure consistent particle size and uniform heat transfer, a small quantity of the synthesized compound was finely ground by clean mortar & pestle. Resulting powder, then packed into a capillary tube—sealed at one end. This capillary was placed in a melting point apparatus (pre-calibrated). Sample was heated gradually, and careful

observations were made to note both the onset of melting (when liquefaction began) and the temperature at which the substance became a clear liquid. The procedure was repeated multiple times to enhance accuracy and reliability. The melting point range was then matched with values for 2-(4-hydroxyphenyl)-4,5-diphenylimidazole [16].

V. SOLUBILITY ANALYSIS:

Solubility testing is a fundamental analytical method used to assess a compound's chemical behavior, aiding in the prediction of its reactivity, purification approach, and potential applications. In this study, the solubility characteristics of the synthesized compound, 2-(4-hydroxyphenyl)-4,5-diphenylimidazole, were evaluated using a range of solvents with varying polarities. Small amounts of the compound were combined with solvents such as ethanol, methanol, acetone, chloroform, distilled water, and hexane, and the combinations were allowed to stand at room temperature.

The compound was found to have good solubility in polar organic medias such as ethanol and methanol, moderate solubility in chloroform and acetone, and poor solubility in non-polar and highly polar solvents such as hexane and water. These findings indicate that the molecule has aromatic rings and polar functional groups, which affect how it interacts with various solvents. Knowledge of this solubility profile is important not just for the characterization of the compound's physicochemical attributes but also for the direction of formulation and extraction procedures in future uses [14].

FTIR SPECTROSCOPY:

Fourier-transform infrared (FTIR) spectroscopy utilized to identify and affirm the functional groups within the recrystallized 2-(4-hydroxyphenyl)-4,5-diphenylimidazole. The test was carried out on an FTIR spectrometer setup with a deuterated triglycine sulfate (DTGS) locator and an constricted add up to reflectance (ATR) ornament including a diamond/ZnSe gem. The analysis was specially interfaced to the ATR gem, and extraterrestrial data were acquired across a range of 4000 to 400 cm^{-1} at a resolution of 4 cm^{-1} [20].

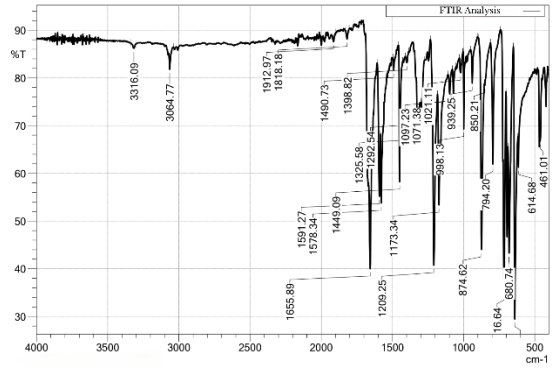

Fig 02: FTIR Spectrum of Recrystallized 2-(4-Hydroxyphenyl)-4,5-diphenylimidazole

Key retention groups watched within the range included a wide O–H extending vibration around 3380 cm^{-1}, demonstrating the nearness of a phenolic bunch. Crests comparing to C–H extending vibrations showed up at 3050 cm^{-1} (fragrant) and 2920 cm^{-1} (aliphatic). A sharp retention band close 1650 cm^{-1} was ascribed to the C=N extending inside the imidazole ring. Extra signals were famous around 1600–1500 cm^{-1} (C=C extending), at 1270 cm^{-1} (C–O extending), and between 750–700 cm^{-1} (C–H bowing), all of which backed the proposed structure of the compound. The utilize of ATR encouraged quick and exact ghostly procurement without the require for complex test planning [19].

BIOLOGICAL EVALUATION:

THIN LAYER CHROMATOGRAPHY(TLC):

TLC was effectively employed for assessing of the identification and purification of the heterocyclic compound 2-(4-hydroxyphenyl)-4,5-diphenylimidazole, synthesized via a reaction involving 4-chlorobenzaldehyde. Due to its simplicity, cost-effectiveness, and reliability in providing clear insights into compound composition and purity, TLC remains a widely used technique for the rapid analysis of organic molecules. In this experiment, benzil dissolved in ethanol was used as the reference standard. The synthesized compound was spotted on silica gel-coated TLC plates. Ethanol, glacial acetic acid, and water in the ratio of 5:2:1 was used as a solvent system in order to gain effective separation of the components. The value of the retention factor (Rf) of the synthesized compound was found to be 0.90, which was identical with the Rf value of benzil standard. This test indicates that the synthesized imidazole derivative has the same chromatographic

behavior and polarity as the reference under identical conditions, thus confirming its proper synthesis and structure. Under UV examination, the synthesized sample showed a single, sharp spot without any extra or tailing marks, showing no presence of unreacted precursors or by-products. The sharpness and brightness of the spot inferred a high purity level—approximately 90%—judged from the visual inspection in the UV chamber. This finding indicates that the compound is ready for further biological evaluation and pharmacological investigation. Finally, TLC was an effective analytical tool to affirm the identity and purity of the synthesized 2-(4-hydroxyphenyl)-4,5-diphenylimidazole. The repeated Rf values with the reference and the appearance of a single, distinct spot under UV strongly suggest the product's appropriateness for further pharmacological screening and investigation [20].

ANTIBACTERIAL ASSESSMENT:

This research sought to evaluate the antibacterial activity of the heterocyclic compound 2-(4-hydroxyphenyl)-4,5-diphenylimidazole, which has been reported to possess therapeutic usefulness. The efficacy of the compound was evaluated against two model bacterial species: the E coli organism commonly present in the gastrointestinal tract, and Staphylococcus aureus, a Gram-positive bacterium with common causes of skin and respiratory infections. The antibacterial action was assessed with the streak plate method, which allows qualitative and semi-quantitative analysis of bacterial inhibition. For confirmation of the experimental results, azithromycin—a commonly employed broad-spectrum antibiotic—was the positive control, and dimethyl sulfoxide (DMSO) was the negative control to verify that the carrier solvent did not have any inherent antibacterial activity. Both bacterial cultures were first grown in nutrient broth under optimal growth conditions. Sterile nutrient agar plates were prepared and allowed to cool under laminar airflow. Each bacterial strain was inoculated onto the plates using a sterile loop in a zigzag or streak pattern to ensure even distribution. The test compound was dissolved in DMSO and applied to sterile filter paper discs. These discs were placed on the agar surface along with control discs containing DMSO alone and standard discs with 100 µg of azithromycin. Post incubation, the agar plates were inspected for zones of inhibition—clear, circular areas surrounding the discs indicating bacterial growth suppression. Measurements were taken in millimeters to determine the diameter of each inhibition zone. The results showed a dose-dependent antibacterial response by the test compound. Inhibition zones in *E. coli* were 7 mm, 10 mm, and 13 mm for doses of 25 µg, 50 µg, and 100 µg, respectively. Azithromycin, in contrast, created a 20 mm zone that was more noticeable. Azithromycin demonstrated a 22 mm zone, confirming its well-known efficacy, although the inhibition zones for S. aureus were marginally larger—9 mm, 12 mm, and 15 mm for the same dosages.

These findings indicate that the synthesized imidazole derivative exhibits moderate antibacterial activity, with enhanced effectiveness against *Staphylococcus aureus*. Given S. aureus's comparatively higher sensitivity, the compound's mechanism might be better adapted to target metabolic pathways or Gram-positive cell wall structures. In every test case, a single, distinct zone of inhibition was observed, indicating that the compound was pure and functioned as a single bioactive agent free from interfering impurities.

The antibacterial analysis concluded that 2-(4-Hydroxyphenyl)-4,5-diphenylimidazole had a discernible inhibitory effect with latter exhibiting more pronounced activity. Although it did not outperform the conventional antibiotic in terms of efficacy, its persistent inhibitory patterns, especially at higher doses, suggest that it may be a promising lead molecule for further structural modification and development in antimicrobial drug discovery [13].

VI. APPLICATIONS:

(a.) antimicrobial activity: 2-(4-Hydroxyphenyl)-4,5-diphenylimidazole and its modified versions have demonstrated strong antibacterial and antifungal effects, antimicrobial substances [14].

(b.) anticancer agents: Some derivatives of triphenyl imidazole have demonstrated significant cytotoxic activity against various cancer cell lines, making them promising candidates in the development of anticancer therapies [15].

(c.) anti-inflammatory and analgesic agents: These compounds are being investigated for their anti-inflammatory effects, which could potentially provide therapeutic advantages in managing inflammatory conditions and alleviating pain.

(d.) Intermediate in Organic Synthesis: 2-(4-Hydroxyphenyl)-4,5-diphenylimidazole serves as an essential building block in the synthesis of complex heterocyclic structures, vibrant dyes, and biologically active compounds.
(e.) Use in Chromogenic Reactions: Triphenyl imidazole derivatives are frequently utilized in colorimetric reactions and as indicators in analytical chemistry due to their conjugated aromatic system.
(f.) Possible Therapeutic Efficacy: Recent research indicates that specific imidazole derivatives may exhibit activity against viral targets, suggesting their potential for antiviral drug development, including for diseases like HIV and hepatitis [15].

VII. CONCLUSION

The comprehensive evaluation of the synthesized compound, 2-(4-Hydroxyphenyl)-4,5-diphenylimidazole, highlights its potential as a pharmacologically active heterocyclic molecule, which confirmed the presence of essential functional groups such as phenolic –OH, imidazole C=N, and aromatic C–H bonds. Thin Layer Chromatography (TLC) analysis demonstrated a single, well-defined spot with an Rf value of 0.90, identical to the reference compound, suggesting a high degree of purity (~90%). The chemical has modest antibacterial activity, with a greater inhibitory impact against Staphylococcus aureus than against Escherichia coli, according to additional biological evaluation conducted using the streak plate method. The compound's antibacterial ability is confirmed by the dose-dependent zones of inhibition that were found, especially against Gram-positive pathogens. The results collectively suggest the significance of this imidazole derivative as a prospective scaffold for the future design and development of new antimicrobial medicines, despite the fact that its efficacy was lower than that of the conventional medication azithromycin. To completely investigate its therapeutic usefulness, more research utilizing sophisticated biological assays and structural optimization is necessary.

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