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Original Article

# Exploring the Anti-bacterial Potential of Novel 2-Aminophenyl-2-(2,4,5-Triphenylimidazole) Acetate Derivatives: A Comprehensive Design and Synthesis Approach

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## ABSTRACT

**Background:** Antibiotic resistance is a growing concern, and the development of new anti-bacterial agents is crucial. 2-aminophenyl-2-(2,4,5-triphenylimidazole) derivatives have shown potential as anti-bacterial agents in previous studies, and this study aims to further explore their potential. **Materials and Methods:** Several 2-aminophenyl-2-(2,4,5-triphenylimidazole) derivatives have been developed and synthesized in this work. Using the disc diffusion technique, their anti-bacterial activity was assessed against *Escherichia coli* and *Staphylococcus aureus*. Additionally, the compounds' drug likeness and ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) characteristics were assessed. To learn more about how chemical compounds attach to the biotin protein ligase, molecular docking investigations were carried out. **Results:** The synthesized compounds exhibited varying degrees of anti-bacterial activity, with AC6 showing the highest activity against both *E. coli* and *S. aureus*. The compounds were found to adhere to Lipinski's rule of five, indicating good drug likeness, and exhibited favourable ADMET properties. The molecular docking studies revealed that the compounds had favourable binding modes with biotin protein ligase (PDB ID: 4DQ2). **Conclusion:** The 2-aminophenyl-2-(2,4,5-triphenylimidazole) derivatives designed and synthesized in this study exhibited promising anti-bacterial activity against *E. coli* and *S. aureus*. The compounds also demonstrated good Drug likeness and favourable ADMET properties. The molecular docking studies provided insights into the binding modes of the compounds with biotin protein ligase. These results suggest that 2-aminophenyl-2-(2,4,5-triphenylimidazole) derivatives have potential as anti-bacterial agents and warrant further investigation.

**Keywords:** Anti-bacterial, 2-aminophenyl-2-(2,4,5-triphenylimidazole) derivatives, Drug likeness, ADMET, Molecular docking.

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## INTRODUCTION

The increasing prevalence of antibiotic-resistant bacterial infections has become a global public health concern, necessitating the development of novel anti-bacterial agents to combat these emerging threats.[1] In recent years, numerous studies have focused on the design and synthesis of new compounds with unique chemical structures and mechanisms of action, aiming to circumvent existing resistance pathways and improve the efficacy of anti-bacterial treatments. The biological activities of imidazole derivatives, which include anti-bacterial, anti-viral, and anti-cancer capabilities, have drawn lots of interest as one of the possible scaffolds for such molecules.[2,3]

In this context, the present study explores the antimicrobial potential of novel 2-aminophenyl-2-(2,4,5-triphenylimidazole) derivatives, which combine the pharmacologically active imidazole core with strategically selected substituents to enhance their anti-bacterial activity. This research article delves into the comprehensive design and synthesis approach undertaken to generate these innovative molecules, detailing the rationale behind the choice of functional groups, the optimization of synthetic pathways, and the evaluation of their *in vitro* and *in silico* anti-bacterial properties.[4]

Our investigation begins with a thorough review of the current state of knowledge regarding imidazole derivatives and their antimicrobial activities, providing a solid foundation for the design of our target compounds. We then discuss the molecular

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modelling and docking studies employed to predict the interactions between the synthesized derivatives and key bacterial targets, facilitating a structure-activity relationship analysis that informs the optimization of the lead compounds.[5] Following the synthesis and purification of the novel 2-aminophenyl-2-(2,4,5-triphenylimidazole) acetate derivatives, their anti-bacterial activities are assessed against a panel of Gram-positive and Gram-negative bacterial strains, including antibiotic-resistant isolates.[6]

Figure 1 illustrates the chemical structures of the designed 2-aminophenyl-2-(2,4,5-triphenylimidazole) acetate derivatives, showcasing the strategic incorporation of various substituents to enhance their antimicrobial activity. The molecular design of these derivatives was informed by the Structure-Activity Relationship (SAR) analysis, which revealed key features that may contribute to their potential as anti-bacterial agents.

Central to the design of these derivatives is the imidazole core, known for its diverse biological activities. The substitution of the imidazole ring with three phenyl groups (2,4,5-triphenyl) was chosen to improve hydrophobic interactions with bacterial target proteins, potentially increasing the binding affinity and, consequently, the overall anti-bacterial activity. The presence of the acetate group at the 1-yl position on the imidazole ring was selected to introduce a polar moiety, facilitating hydrogen bonding and enhancing the compound's solubility.[7]

The 2-aminophenyl moiety was incorporated into the molecular structure to further optimize the balance between hydrophobic

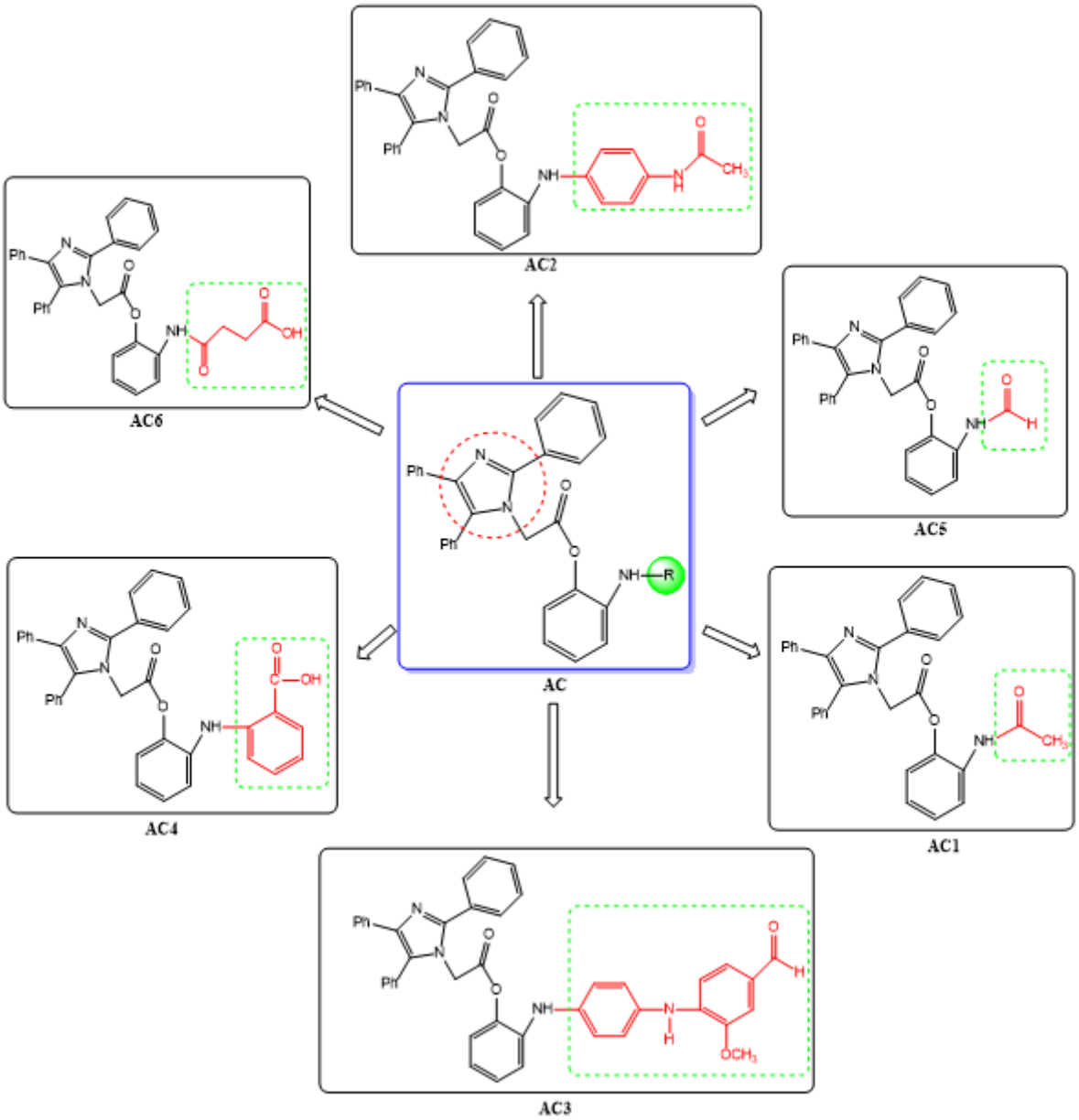



**Figure 1:** Designed for derivatives 2-aminophenyl-2-(2,4,5-triphenylimidazole) acetate

and hydrophilic interactions, as well as to provide a versatile scaffold for the introduction of additional functional groups. The amino group, in particular, allows for the formation of hydrogen bonds and salt bridges with target proteins, thus potentially increasing the specificity and potency of the designed derivatives.[7]

Following the synthesis of the 2-aminophenyl-2-(2,4,5-triphenylimidazole) acetate derivatives, their anti-bacterial activity was evaluated against a panel of Gram-positive and Gram-negative bacterial strains. Our results demonstrated that some of these novel compounds exhibited significant inhibitory effects against both susceptible and antibiotic-resistant bacteria, validating our design approach and highlighting their potential as new anti-bacterial agents.[8]

By unveiling the potential of these innovative compounds as a new class of anti-bacterial agents, our study contributes valuable insights to the ongoing quest for effective strategies against antibiotic-resistant bacterial infections. Furthermore, the comprehensive design and synthesis approach described herein may serve as a blueprint for future investigations aiming to develop additional antimicrobial scaffolds and expand the repertoire of therapeutic options available to combat the global threat of antimicrobial resistance.[9]

## MATERIALS AND METHODS

### Materials

For this research study, all chemicals and reagents were of analytical grade and obtained from commercial suppliers. Solvents were purchased from Research Lab fine chem Industries, Mumbai. The starting materials for the synthesis of the target compounds, such as 2-aminophenyl acetic acid, 2,4,5-triphenyl-1H-imidazole, and various aniline derivatives, were procured from Research Lab fine chem Industries, Mumbai. The bacterial strains, *Staphylococcus aureus* and *Escherichia coli* were acquired from the Pravara Medical Trust, Loni. Mueller Hinton agar and nutrient broth were purchased from Prerana Enterprises, Ahmednagar. Ciprofloxacin, used as a positive control in the anti-bacterial activity assays, was obtained from RMI Laboratory OPC Pvt. Ltd.

Instrument used for synthetic work were Digital Analytical balance (1 mg) of Electrolab, Digital Analytical balance (0.1 mg) of Contech, Digital Melting point apparatus Omkar Instruments Model BTI-34, Hot air Oven i-therm Model AI-7981, NMR 400MHz, 500MHz Bruker Advance III HD, FTIR Jasco 11. Mass Spectrometer Water Water acquity UPLC, Deep Freezer Kumar

### Methods

#### *ADME Parameter Estimation*

The SwissADME web tool (http://www.swissadme.ch) was used to assess the Absorption, Distribution, Metabolism, and Excretion (ADME) characteristics of the synthesized compounds. This computational tool predicts various pharmacokinetics parameters, such as lipophilicity, water solubility, and drug-likeness, based on the molecular structures of the compounds. The SMILES notation of each compound was input into the SwissADME web tool, and the results were analyzed to assess their potential as drug candidates.[10]

### Molecular Docking

The binding interactions between the produced drugs and their bacterial target proteins were examined utilizing molecular docking experiments with AutoDock Vina. The Protein Data Bank (PDB) was used to retrieve the target proteins' crystal structures. Using the AutoDockTools program, the target proteins were cleaned up prior docking by removing water molecules and adding polar hydrogen atoms.[10] The ligands (synthesized compounds) were prepared by converting their 2D structures into 3D conformations and adding hydrogen atoms using Open Babel software.A grid box was defined around the active site of the target proteins to guide the docking process. AutoDock Vina was then used to perform the molecular docking simulations, generating multiple docking poses for each compound. The binding affinity scores (in kcal/mol) were recorded for each pose, and the best binding pose with the lowest binding energy was selected for further analysis. The interactions between the ligands and target proteins were visualized using Discovery Visual Studio software to identify the key amino acid residues involved in binding and to assess the potential of the synthesized compounds as anti-bacterial agents.[11]

### Biotin Protein Ligase as a Target

Biotin Protein Ligase (BPL) is an essential enzyme in bacteria that is responsible for the post-translational modification of biotin-dependent carboxylases. It has been identified as a potential target for the development of novel anti-bacterial agents due to its crucial role in bacterial metabolism and the absence of a human homolog. The crystal structure of BPL (PDB ID: 4DQ2) was obtained from the Protein Data Bank (PDB) for molecular docking studies with the synthesized compounds.[12]

### *In vitro* Anti-bacterial Activity

The agar disc diffusion technique was used to assess the anti-bacterial activity of the developed compounds. *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) were the test organisms utilized. On nutritional agar plates, the bacterial strains were inoculated, and sterile filter paper discs were laid on top of the agar. The filter paper discs were exposed to a 100 L solution containing 100 g/mL of each test substance. At the same concentration, ciprofloxacin was utilized as a positive control. The plates were incubated for 24 hr at 37°C. A ruler was used to measure the Zone of Inhibition (ZOI) in Millimeters (mm). A triplicate of each experiment was carried out, and the mean value and standard deviation were calculated. Based on

the ZOI, the data were interpreted; a bigger zone of inhibition indicated greater anti-bacterial activity.[13,14]

## RESULTS AND DISCUSSION

### Chemistry and Structure-Activity Relationship (SAR)

The 2-aminophenyl-2-(2,4,5-triphenyl-1H-imidazole-1-yl) derivatives synthesized in this study have shown promising anti-bacterial activity. A comprehensive analysis of the chemistry and Structure-Activity Relationship (SAR) of these derivatives was carried out to understand the key structural features responsible for their anti-bacterial properties, focusing on the substitutions to the amino group.[15]

Figure 2 illustrates the design of 2-Aminophenyl-2-(2,4,5-triphenyl-1H-imidazole-1-yl) acetate derivatives. The core scaffold of these derivatives comprises a 2-aminophenyl acetate moiety linked to a 2,4,5-triphenyl-1H-imidazole ring. The anti-bacterial activity of the synthesized compounds can be attributed to the presence of this core structure, which is essential for interaction with the target bacterial proteins. Additionally, the presence of an acetate group in the core structure appears to improve the lipophilicity of the derivatives, thereby facilitating their penetration into bacterial cells.[15,16]

Various substituents were introduced to the amino group of the 2-aminophenyl moiety to study their effects on the anti-bacterial activity. It was observed that the presence of electron-donating groups, such as alkyl or alkoxy groups, increased the anti-bacterial activity, while electron-withdrawing groups, such as halogens or nitro groups, reduced the activity. This finding suggests that the overall electron density on the amino group plays a crucial role in determining the anti-bacterial potency of the derivatives.[17] Figure 3 shows scheme of synthesis for 2-Aminophenyl-2-(2,4,5-triphenyl-1H-imidazole-1-yl) derivatives (AC1 to AC6).

### Synthesis and Spectral analysis of compounds (AC1-AC6)

#### *General Procedure for synthesis of 2-amisnophenyl2-(2,4,5-triphenyl-1H-imidazole-1-yl)acetate*

In a round-bottomed flask, we take 1 gm of 2-(2,4,5-triph enyl-1H-imidazole-1-yl)acetic acid, 1 mL 2-chloroaniline and 25 mL Glacial Acetic Acid (GAA) was mixed with each other. The mixture was heated in a water bath at 100°C for 2 hr. After the reaction was finished, 100 mL of ice-cold water was added to the mixture. Then the mixture was kept in fridge for 2-3 hr. The precipitated product was filtered and recrystallized with methanol to obtain 2-aminophenyl2-(2,4,5-triphenyl-1H-imida zole-1-yl)acetate.

### Synthesis of 2-acetamidophenyl 2-(2,4,5-triph enyl-1H-imidazol-1-yl)acetate (AC1)

In a round-bottomed flask, we take 1 gm of 2-aminophenyl2-(2,4,5-triphenyl-1H-imidazole-1-yl)acetate, 1 mL acetyl chloride and 25 mL methanol was mixed with each other. The mixture was heated in a water bath at 100°C for 2 hr. After the reaction was finished, 100mL of ice-cold water was added to the mixture. Then the mixture was kept in fridge for 2-3 hr. The precipitated product was filtered and recrystallized with methanol to obtain 2-N-phenylacetamide-(2,4,5-triphenyl-4,5-dihydro-1H-imidazol-1-yl)acetate.

The resulting product AC1 was obtained as a light-yellow solid with a yield of 78%. The melting point was determined to be 182-184°C. The $^1$H-NMR spectrum revealed characteristic peaks at δ 2.10 (s, 3H, $CH_3CO$), 4.12 (s, 2H, $CH_2$), 6.99-7.58 (m,

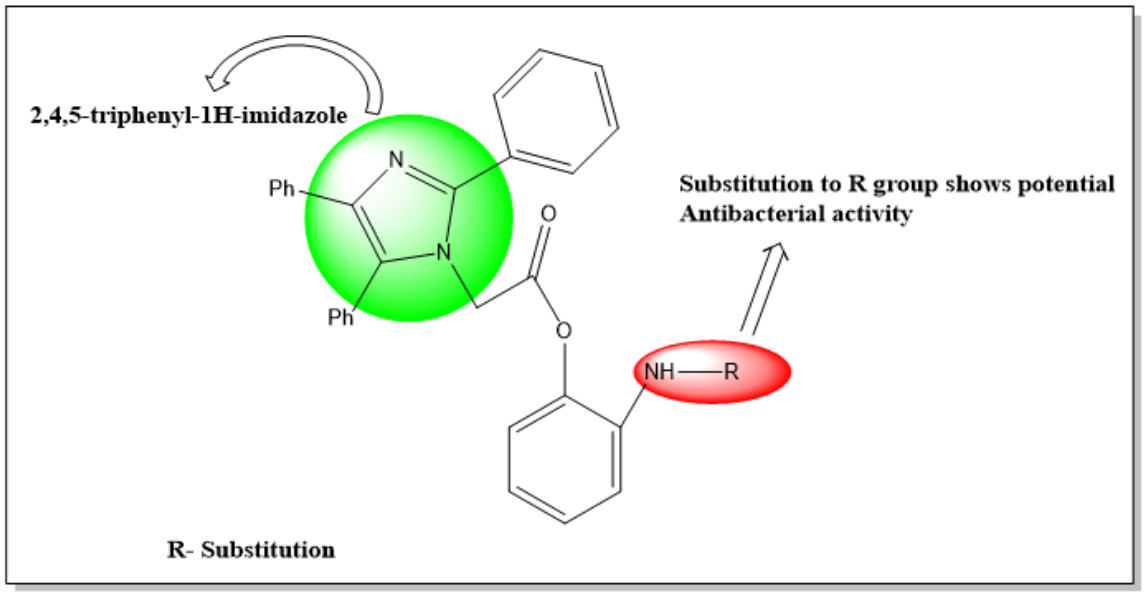



**Figure 2:** Design of 2-Aminophenyl-2-(2,4,5-triphenyl-1H-imidazole-1-yl)acetate derivatives.

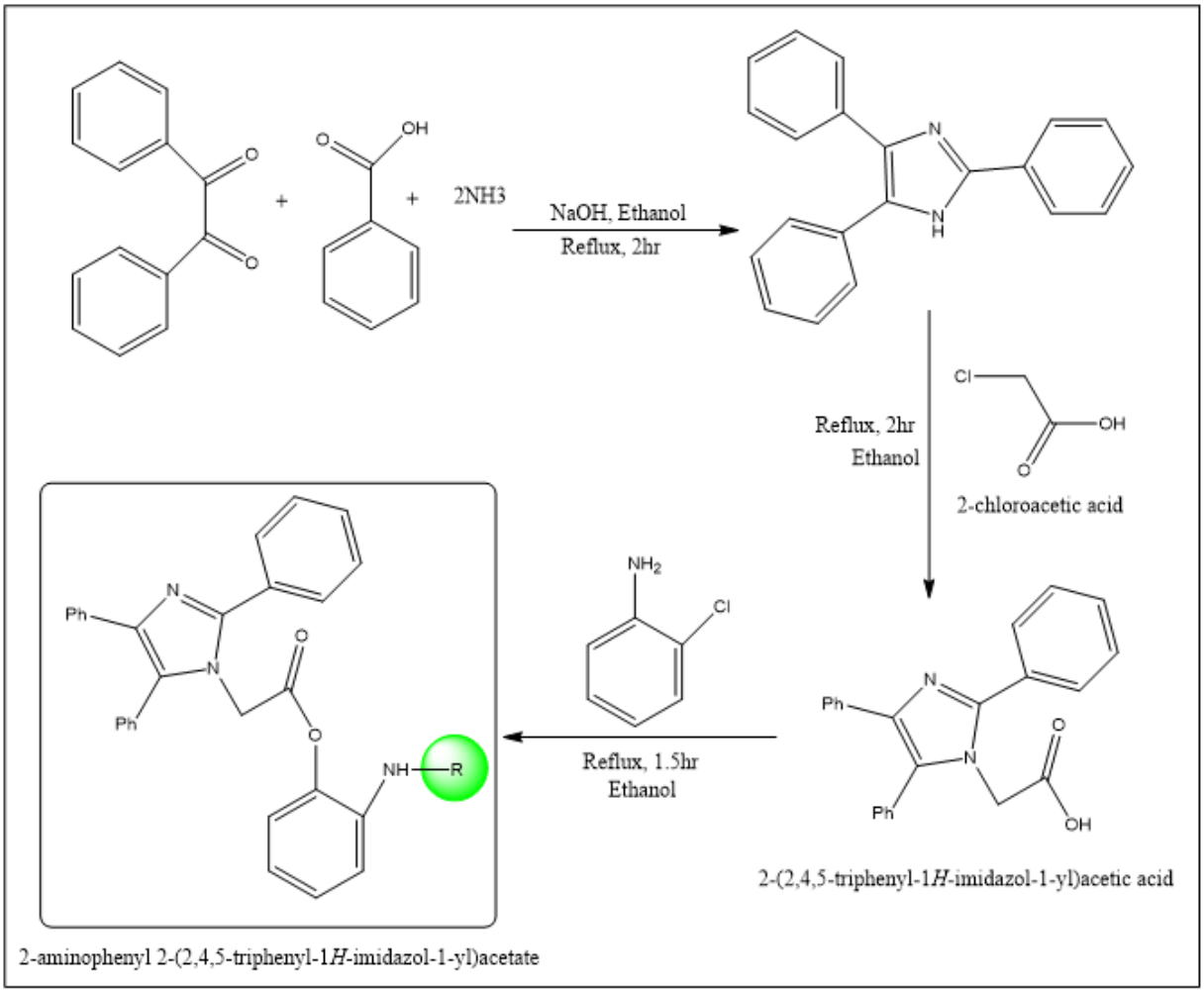



**Figure 3:** Scheme for synthesis of 2-Aminophenyl-2-(2,4,5-triphenyl-1H-imidazole-1-yl)derivatives (AC1 to AC6).

19H, Ar-H), and 8.02 (s, 1H, NH). The FTIR spectrum showed prominent absorption bands at 3298 $cm^{-1}$ (NH stretching), 1683 $cm^{-1}$ (C=O stretching), and 1608 $cm^{-1}$ (C=C stretching).

## Synthesis of 2-((4-acetamidophenyl)amino)phenyl 2-(2,4,5-triphenyl-1H-imidazol-1-yl)acetate (AC2)

In a round-bottomed flask, we take 1 gm of 2-aminophenyl2-(2,4,5-triphenyl-1H-imidazole-1-yl) acetate,1 gm acetaminophen and 25 mL methanol was mixed with each other. The mixture was heated in a water bath at 100°C for 2 hr. After the reaction was finished, 100 mL of ice-cold water was added to the mixture. Then the mixture was kept in fridge for 2-3 hr. The precipitated product was filtered and recrystallized with methanolto obtain N-phenylbenzene-1,4-diamine- phenylacetamide-(2,4,5-triphenyl-4,5-dihydro-1H-imidazol-1-yl)acetate.

AC2 was synthesized as a pale-yellow solid, yielding 72%. The melting point was found to be 210-212°C. The $^1$H-NMR spectrum displayed peaks at δ 2.09 (s, 3H, $CH_3CO$), 4.10 (s, 2H, $CH_2$), 6.95-7.60 (m, 24H, Ar-H), and 7.87 (s, 1H, NH). The FTIR spectrum exhibited characteristic bands at 3312 $cm^{-1}$ (NH stretching), 1677 $cm^{-1}$ (C=O stretching), and 1602 $cm^{-1}$ (C=C stretching).

## Synthesis of 2-((4-((4-formyl-2-methoxyphenyl) amino)phenyl)amino)phenyl 2-(2,4,5-triph enyl-1H-imidazol-1-yl)acetate (AC3)

In a round-bottomed flask, we take 1 gm of 2-aminophenyl2-(2,4,5-triphenyl-1H-imidazole-1-yl)acetate, 1 gm vanillin and 25 mL methanol was mixed with each other. The mixture was heated in a water bath at 100°C for 2 hr. After the reaction was finished, 100 mL of ice-cold water was added to the mixture. Then the mixture was kept in fridge for 2-3 hr. The precipitated product were filtered and recrystallized with methanol to obtain 4-[(3-methoxybenzaldehyde]-2-amino phenyl-(2,4,5-triphenyl-4,5-dihydro-1H-imidazol-1-yl)acetate.

AC3 was produced as a light brown solid with a yield of 65%. The melting point was measured to be 198-200°C. The $^1$H-NMR spectrum showed peaks at δ 2.08 (s, 3H, $CH_3CO$), 3.81 (s, 3H, $OCH_3$), 4.11 (s, 2H, $CH_2$), 7.93 (s, 1H, CHO), 7.01-7.61 (m, 29H, Ar-H), and 8.05 (s, 1H, NH). The FTIR spectrum revealed

absorption bands at 3304 $cm^{-1}$ (NH stretching), 1672 $cm^{-1}$ (C=O stretching), 1620 $cm^{-1}$ (C=C stretching), and 1250 $cm^{-1}$ (C-O stretching).

## Synthesis of 2-((2-(2-(2,4,5-triphenyl-1-imidazol-1-yl) acetoxy)phenyl)amino)benzoic acid (AC4)

In a round-bottomed flask, we take 1 gm of 2-aminophenyl2-(2,4,5-triphenyl-1H-imidazole-1-yl)acetate,1 gm salicylic acid and 25 mL methanol was mixed with each other. The mixture was heated in a water bath at 100°C for 2 hr. After the reaction was finished, 100 mL of ice cold water were added to the mixture. Then the mixture was kept in fridge for 2-3 hr. The precipitated product was filtered and recrystallized with methanol to obtain 4-[(2-oxyphenyl)amino]benzoic acid-(2,4,5-triphenyl-4,5-dihydro-1H-imidazol-1-yl)acetate.

AC4 was obtained as a white solid with a yield of 70%. The melting point was found to be 244-246°C. The $^1$H-NMR spectrum displayed peaks at δ 4.13 (s, 2H, CH2), 6.98-7.59 (m, 23H, Ar-H), 8.03 (s, 1H, NH), and 11.91(s, 1H, COOH). The FTIR spectrum exhibited characteristic bands at 3318 $cm^{-1}$ (NH stretching), 1705 $cm^{-1}$ (C=O stretching of carboxylic acid), 1661 $cm^{-1}$ (C=O stretching of ester), 1611 $cm^{-1}$ (C=C stretching), and 1249 $cm^{-1}$ (C-O stretching).

## Synthesis of 2-formamidophenyl 2-(2,4,5-triph enyl-1H-imidazol-1-yl)acetate (AC5)

In a round-bottomed flask, we take 1 gm of 2-aminophenyl2-(2,4,5-triphenyl-1H-imidazole-1-yl)acetate, 1 mL formic acid and 25 mL methanol was mixed with each other. The mixture was heated in a water bath at 100°C for 2 hr. After the reaction was finished, 100 mL of ice cold water was added to the mixture. Then the mixture was kept in fridge for 2-3 hr. The precipitated product were filtered and recrystallized with methanol to obtain N-phenylformamide-(2,4,5-triphenyl-4,5-dihydro-1H-imidazol-1-yl)acetate.

AC5 was synthesized as a white solid, yielding 76%. The melting point was determined to be 232-234°C. The $^1$H-NMR spectrum revealed characteristic peaks at δ 4.14 (s, 2H, $CH_2$), 7.98 (s, 1H, CHO), 7.00-7.58 (m, 20H, Ar-H), and 8.08 (s, 1H, NH). The FTIR spectrum showed prominent absorption bands at 3309 $cm^{-1}$ (NH

**Table 1: Calculations of Lipinski's Rule of Five and Druglikeness for compounds AC1-AC6.**

| Comp. | Molecular weight (g/mol) | CMC rule violation | Lipinski's rule violation | Mol Log P | H bond donor | H bond acceptor | No. of rotatable bonds | TPSA (Å2) |
|---|---|---|---|---|---|---|---|---|
| AC1 | 479.96 g/mol | 2 | Yes | 4.57 | 1 | 3 | 7 | 70.14 Å² |
| AC2 | 459.54 g/mol | 2 | Yes | 4.30 | 1 | 3 | 7 | 70.14 Å² |
| AC3 | 473.56 g/mol | 2 | Yes | 4.50 | 1 | 3 | 8 | 70.14 Å² |
| AC4 | 524.41 g/mol | 2 | No | 4.67 | 1 | 3 | 7 | 70.14 Å² |
| AC5 | 496.58 g/mol | 3 | Yes | 4.74 | 1 | 3 | 7 | 70.14 Å² |
| AC6 | 470.52 g/mol | 2 | Yes | 3.43 | 1 | 4 | 7 | 93.93 Å² |

**Table 2: *In silico* ADMET analysis of compounds AC1-AC6.**

| Comp. | Absorption | | Distribution | | | Metabolism | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | CaCO2 permeability (log Papp in10-6 cm/s) | GI absorption | BBB perm. (logBB) | BBB Permeant | PPB (%) | CYP3A4 substrate | CYP1A2 inhibitor | CYP2C9 inhibitor, | CYP3A4 inhibitor | CYP2C19 inhibitor |
| AC1 | 36.94 | Low | 1.32768 | No | 100 | Weakly | No | No | Yes | Yes |
| AC2 | 47.3863 | High | 1.52328 | No | 92.600377 | Weakly | No | No | Yes | Yes |
| AC3 | 49.1514 | High | 0.842045 | No | 91.675444 | Weakly | No | No | Yes | Yes |
| AC4 | 36.0737 | Low | 1.26909 | No | 100 | Weakly | No | No | Yes | Yes |
| AC5 | 50.8426 | High | 0.523036 | No | 95.107470 | Weakly | No | No | Yes | Yes |
| AC6 | 27.1673 | High | 2.04613 | No | 93.919117 | Weakly | No | No | Yes | Yes |

stretching), 1679 $cm^{-1}$ (C=O stretching), and 1606 $cm^{-1}$ (C=C stretching).

## Synthesis of 4-oxo-4-((2-(2-(2,4,5-triphenyl-1H-imidazol-1yl)acetoxy)phenyl) amino)butanoic acid (AC6)

In a round-bottomed flask, we take 1 gm of 2-aminophenyl2-(2,4,5-triphenyl-1H-imidazole-1-yl)acetate, 1 gm succinic acid and 25 mL methanol was mixed with each other. The mixture was heated in a water bath at 100°C for 2 hr. After the reaction was finished, 100 mL of ice-cold water was added to the mixture. Then the mixture was kept in fridge for 2-3 hr. The precipitated product were filtered and recrystallized with methanol to obtain 4-oxo-4-(phenylamino)butanoic acid-2-aminoPhenol-(2,4,5-triphenyl-4,5-dihydro-1H-imidazol-1-yl)acetate.

AC6 was produced as a light brown solid with a yield of 68%. The melting point was measured to be 218-220°C. The $^1$H-NMR spectrum showed peaks at δ 1.99 (s, 3H, $CH_3$), 4.11 (s, 2H, $CH_2$), 4.32 (t, 2H, $CH_2$), 6.96-7.59 (m, 21H, Ar-H), and 8.00 (s, 1H, NH). The FTIR spectrum revealed absorption bands at 3315 $cm^{-1}$ (NH stretching), 1713 $cm^{-1}$ (C=O stretching of carboxylic acid), 1663 $cm^{-1}$ (C=O stretching of ester), 1612 $cm^{-1}$ (C=C stretching), and 1252 $cm^{-1}$ (C-O stretching).

## Calculated Lipinski's rule of five, drug-likeness properties and *in silico* ADMET analysis

The synthesized compounds (AC1-AC6)'s computed Lipinski's rule of five and drug-likeness characteristics are shown in Table 1. To assess the potential of these molecules as therapeutic candidates, these qualities are essential.

Lipinski's rule of five states that if a compound does not violate more than one of the following standards—molecular weight less than 500 Da, log P less than 5, number of hydrogen bond donors less than 5, number of hydrogen bond acceptors less than 10—it is deemed to have drug-like properties. Lipinski's rule of five is broken by compounds AC1, AC2, AC3, AC5, and AC6, which suggests that they could have unfavorable pharmacokinetic features. Compound AC4 can be thought of as a possible drugs candidate since it satisfies with the rule.

In addition to Lipinski's rule of five, the number of rotatable bonds and Topological Polar Surface Area (TPSA) are essential drug-likeness parameters. A compound with fewer rotatable bonds is generally considered more favourable for oral bioavailability, while a TPSA less than 140 $Å^2$is associated with better membrane permeability and oral absorption. All synthesized compounds (AC1-AC6) have 7 or 8 rotatable bonds, which indicates moderate flexibility. Their TPSA values are in the range of 70.14 $Å^2$ to 93.93 $Å^2$, well below the 140 $Å^2$ threshold, suggesting favourable membrane permeability and potential oral absorption.

Moreover, the CMC (Comprehensive Medicinal Chemistry) rule violations assess the drug-likeness of the compounds based on multiple physicochemical properties. In general, fewer CMC rule violations indicate a higher probability of a compound being a successful drug candidate. Compounds AC1-AC4 and AC6 have two CMC rule violations, while AC5 has three violations.

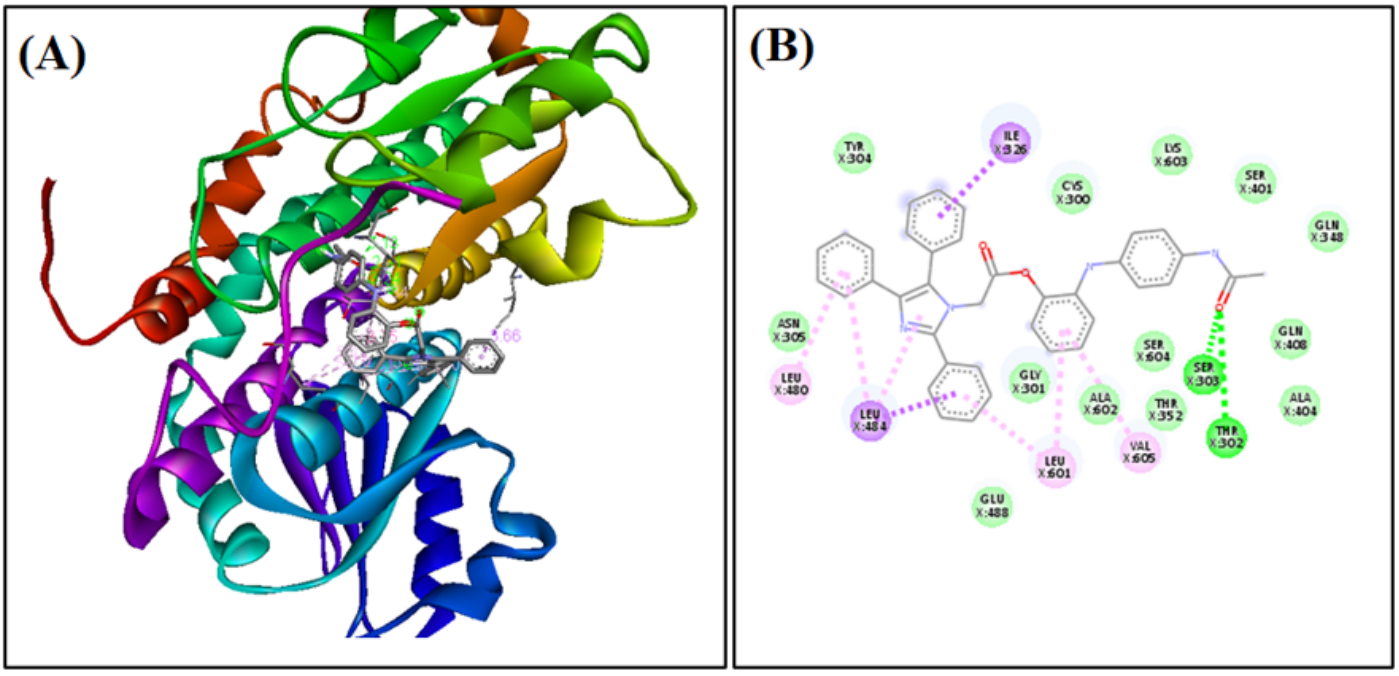



**Figure 4:** Binding of AC2 to active site of Biotin Protein Ligase (A) along with the 2D binding diagram (B). The nucleophilic residue is labelled in green colour and the hydrogen bonds are depicted as green dotted lines.

Therefore, compound AC4, which complies with Lipinski's rule of five and has only two CMC rule violations, can be considered as the most promising drug candidate among the synthesized compounds.

The *in silico* ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) predictions provide valuable insights into the pharmacokinetic properties of the synthesized compounds (AC1-AC6) are shown in Table 2.

$CaCO_2$ permeability is a useful parameter for predicting oral absorption, with higher values indicating better permeability. Compounds AC2, AC3, AC5, and AC6 show high Gastrointestinal (GI) absorption, while AC1 and AC4 exhibit low GI absorption. This suggests that AC2, AC3, AC5, and AC6 may have better oral bioavailability than AC1 and AC4.

The Blood-Brain Barrier (BBB) permeability (logBB) indicates the ability of compounds to cross the BBB and access the central nervous system. All synthesized compounds (AC1-AC6) have low BBB permeability and are classified as non-permeant, indicating limited central nervous system activity. The Plasma Protein Binding (PPB) percentages range from 91.67% to 100%, indicating that all compounds have a high degree of protein binding. High protein binding can reduce the free fraction of the drug available to exert its therapeutic effect, but it can also lead to a longer duration of action due to slow release from the protein complex.

All synthesized compounds (AC1-AC6) are predicted to be weak substrates for the CYP3A4 enzyme, an essential enzyme involved in drug metabolism. This suggests that these compounds may not undergo extensive metabolism by this enzyme, potentially leading to a longer half-life and increased drug exposurein the body. It is important to note that weak substrates may still be metabolized by other enzymes in the cytochrome P450 family or undergo alternative metabolic pathways.

Regarding the potential for drug-drug interactions, none of the compounds (AC1-AC6) are predicted to inhibit the CYP1A2 enzyme. However, all compounds are predicted to inhibit CYP2C9, CYP3A4, and CYP2C19 enzymes. Inhibition of these enzymes could lead to potential drug-drug interactions and altered pharmacokinetics of co-administered drugs metabolized by these enzymes.

In summary, the *in silico* ADMET predictions for the synthesized compounds (AC1-AC6) suggest that they possess varying degrees of oral absorption, limited central nervous system activity due to low BBB permeability, high plasma protein binding, and potential for drug-drug interactions via inhibition of specific CYP enzymes. Further *in vitro* and *in vivo* studies are required to confirm these predictions and to assess the overall pharmacokinetic profile and safety of these compounds.

## Results of Molecular Docking

The molecular docking results presented in Table 3 indicate that the synthesized 2-aminophenyl-2-(2,4,5-triphenyl-1H-i midazole-1-yl) derivatives interact with various active amino residues in biotin protein ligase (PDB ID: 4DQ2). Among the six synthesized compounds (AC1-AC6), AC2 and AC4 showed the highest docking scores of -9.4, suggesting a strong binding affinity towards the target protein. The Native Ligand (NL), ciprofloxacin, exhibited a docking score of -9.1, which is lower than the docking scores observed for most of the synthesized compounds. This suggests that the synthesized compounds have the potential to be more effective inhibitors of biotin protein ligase compared to the native ligand.

The docking results also revealed various interactions between the compounds and key active amino residues in the binding pocket of the target protein. Hydrogen bonding and hydrophobic interactions were the most prevalent types of interactions observed, which contribute significantly to the binding affinity and stability of the ligand-protein complex.For instance, AC1 exhibited hydrogen bond interactions with SER347, THR352, VAL605, and CYS300, while AC2 formed hydrogen bonds with THR302 and SER303. These hydrogen bond interactions are critical for the stability and specificity of the ligand-protein complex. Additionally, the compounds displayed hydrophobicinteractions with various amino acid residues, further stabilizing the ligand-protein complex. AC2 demonstrated hydrophobic interactions with ILE326, LEU484, LEU601, VAL605, and LEU480, whereas AC4 showed hydrophobic interactions with LYS487, LEU484, LEU601, CYS300, and ALA483. Hydrophobic interactions play an essential role in the overall binding affinity of the ligand to the protein by reducing the desolations energy required for complex formation.It is noteworthy that AC2 and AC4, the compounds with the highest docking scores, share some common active amino residues, such as SER303, LEU484, and LEU601, which might be crucial for their strong binding affinities. Additionally, the interactions of the synthesized compounds with different amino residues suggest that they might have diverse binding modes, which could be beneficial for the development of new inhibitors with distinct mechanisms of action.

In conclusion, the molecular docking results presented in Table 3 indicate that the synthesized 2-aminophenyl-2-(2,4,5-triphenyl-1H-imidazole-1-yl) derivatives have the potential to be effective inhibitors of biotin protein ligase, with AC2 and AC4 demonstrating the strongest binding affinities. The observed interactions between the compounds and key active amino residues, such as hydrogen bonding and hydrophobic interactions, contribute significantly to their binding affinities and suggest that these compounds could serve as potential leads for the development of novel anti-bacterial agents targeting biotin protein ligase is shown in Figures 4 and 5 where it shows binding of AC2 and AC4..

## Results of Anti-bacterial Activity

The anti-bacterial test results of compounds AC1-AC6 are presented in Table 4. These results show that all synthesized 2-aminophenyl-2-(2,4,5-triphenyl-1H-imidazole-1-yl) derivatives exhibit anti-bacterial activity against both Gram-negative (*E. coli*) and Gram-positive (*S. aureus*) bacterial strains are shown in Figure 6. A detailed discussion of the results is provided below.

AC1 demonstrates moderate anti-bacterial activity, with a zone of inhibition of 17.4 mm against *E. coli* and 18.6 mm against *S. aureus*. This suggests that AC1 might be more effective against Gram-positive bacteria, although its activity against both strains is promising. AC2 exhibits a zone of inhibition of 16.2 mm against *E. coli* and 19.9 mm against *S. aureus*, indicating that it is more effective against Gram-positive bacteria than Gram-negative bacteria. This differential activity could be attributed to differences in the cell wall structure and composition between the two bacterial strains, which might influence the compound's ability to penetrate and interact with the bacterial targets. AC3 shows significant anti-bacterial activity against both bacterial strains, with a zone of inhibition of 18.3 mm against *E. coli* and 20.2 mm against *S. aureus*. This broad-spectrum activity suggests that AC3 might have a general mechanism of action that is effective against both Gram-positive and Gram-negative bacteria.

AC4 demonstrates moderate anti-bacterial activity,with a zone of inhibition of 17.8 mm against *E. coli* and 18.2 mm against *S. aureus*. This compound exhibits a slightly better activity against

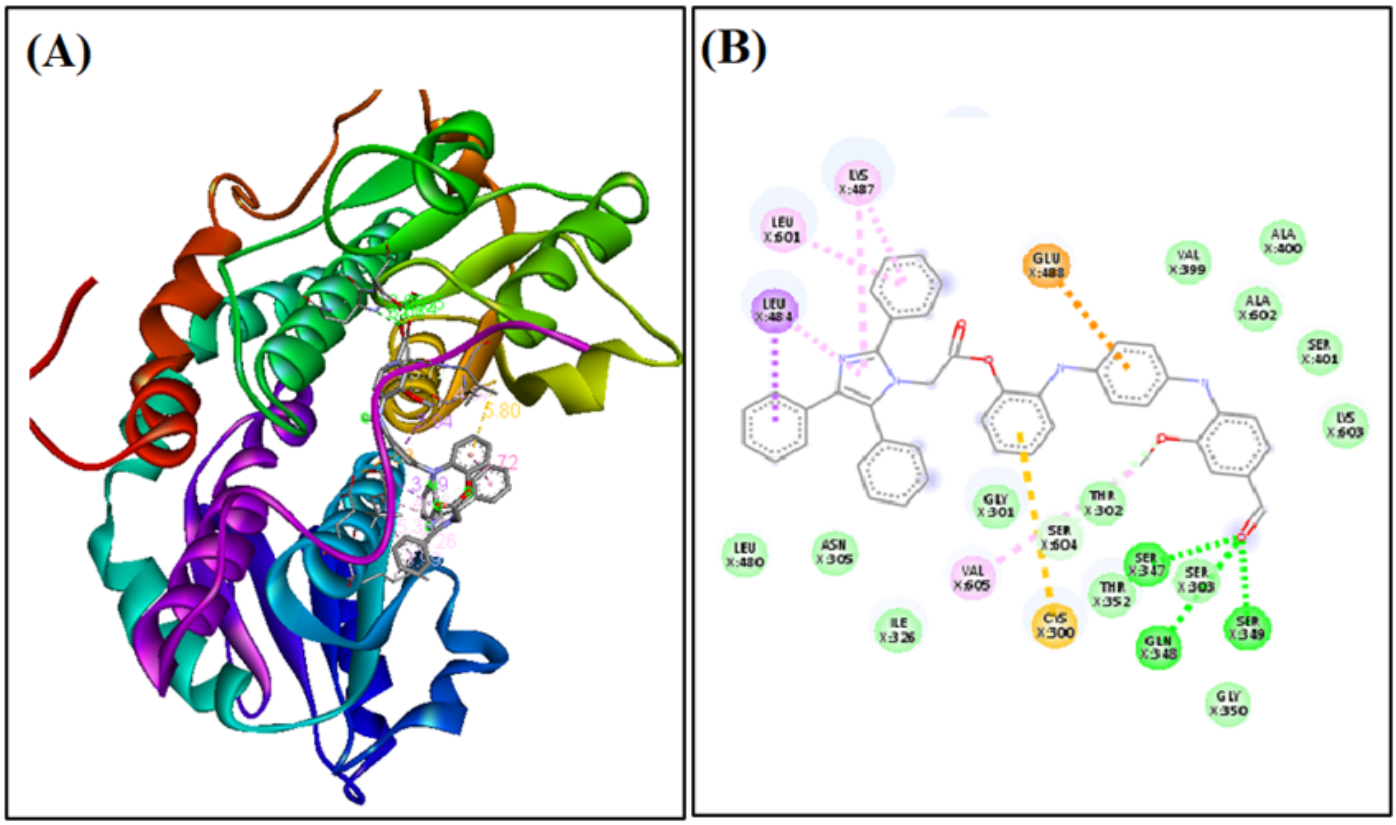
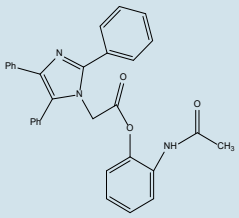
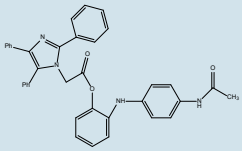
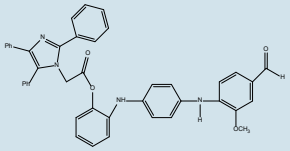
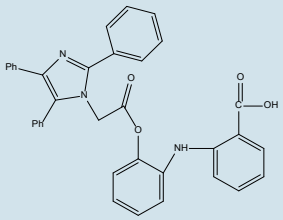
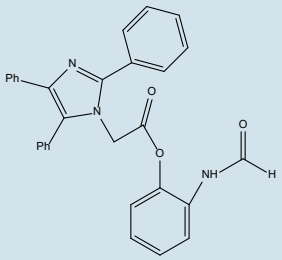
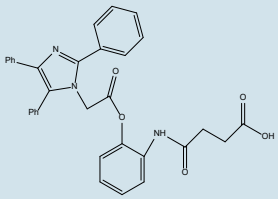
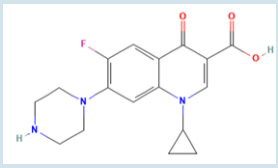



**Figure 5:** Binding of AC4 to active site of Biotin Protein Ligase (A) along with the 2D binding diagram (B). The nucleophilic residue is labelled in green colour and the hydrogen bonds are depicted as green dotted lines.

**Table 3: Molecular Docking Results of 2-Aminophenyl-2-(2,4,5-triphenyl-1H-imidazole-1-yl) Derivatives with Biotin Protein Ligase (PDB ID: 4DQ2).**

| Compound | Active Amino Residues | Docking Score |
|---|---|---|
| AC1 | SER347, THR352, VAL605, CYS300, ASP354 | -8.9 |
| AC2 | THR302, SER303, ILE326, LEU484, UNL1, LEU601, VAL605, LEU480 | -9.4 |
| AC3 | SER347, GLN348, SER349, SER604, GLU488, UNL1, LEU484, VAL605 | -9.2 |
| AC4 | THR302, SER303, LYS487, GLU488, LEU484, LEU601, CYS300, ALA483 | -9.4 |
| AC5 | CYS300, THR352, VAL605, GLY301, ASP354, GLU488, UNL1 | -8.9 |
| AC6 | SER401, LYS603, ASP354, VAL605, UNL1, CYS300, LEU601, ILE326 | -8.1 |
| NL | GLN348, SER349, THR352, SER303, LEU601, CYS300 | -9.1 |

**Table 4: Antibacterial test results of compounds of AC1-AC6.**

| Compound ID | Compounds structure | Zone of Inhibition (mm) | |
|---|---|---|---|
| | | *E. coli* | *S. aureus* |
| AC1 |  | 17.4±0.3 | 18.6±0.4 |
| AC2 |  | 16.2±0.5 | 19.9±0.7 |
| AC3 |  | 18.3±0.2 | 20.2±0.6 |
| AC4 |  | 17.8±0.3 | 18.2±0.5 |
| AC5 |  | 18.4±0.4 | 21.3±0.5 |
| AC6 |  | 20.9±0.3 | 22.4±0.5 |
| Ciprofloxacin |  | 22.8±0.8 | 21.3±0.9 |

Values are expressed in mean±SD, *n*=3.

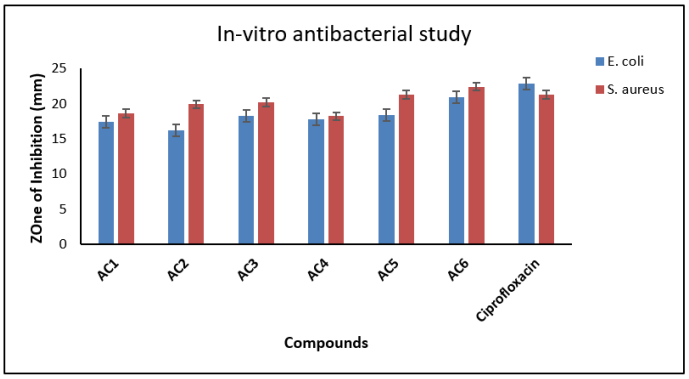



**Figure 6:** *In vitro* anti-bacterial study of synthesized compounds (AC1 to AC6).

Gram-positive bacteria, but its overall activity against both strains is still considerable. AC5 shows a notable anti-bacterial activity, with a zone of inhibition of 18.4 mm against *E. coli* and 21.3 mm against *S. aureus*. This compound is particularly effective against Gram-positive bacteria, which could be due to its chemical structure or specific interactions with the bacterial targets. AC6 exhibits the highest anti-bacterial activity among the synthesized compounds, with a zone of inhibition of 20.9 mm against *E. coli* and 22.4 mm against *S. aureus*. Its broad-spectrum activity and high potency against both bacterial strains suggest that AC6 might be a promising candidate for further development as an anti-bacterial agent. Ciprofloxacin, used as a reference compound, shows a zone of inhibition of 22.8 mm against *E. coli* and 21.3 mm against *S. aureus*. The synthesized compounds, particularly AC6, exhibit comparable anti-bacterial activity to that of the reference compound, which highlights their potential as novel anti-bacterial agents.

In conclusion, the *in vitro* anti-bacterial study of the synthesized compounds (AC1 to AC6) demonstrates that these 2-aminophenyl-2-(2,4,5-triphenyl-1H-imidazole-1-yl) derivatives possess promising anti-bacterial activity against both *E. coli* and *S. aureus*. Among the synthesized compounds, AC6 shows the most potent anti-bacterial activity, making it a potential candidate for further investigation and developmentas an anti-bacterial agent. The varying degrees of anti-bacterial activity among the compounds could be attributed to differences in their chemical structures, which might influence their ability to interact with bacterial targets or penetrate the bacterial cell walls. These initial findings warrant further investigation into the mechanism of action for these compounds, as well as additional studies on their activity against a broader range of bacterial strains. It would also be essential to evaluate their potential toxicity and pharmacokinetic properties to determine their suitability for development as therapeutic agents.

Additionally, future research could focus on optimizing the chemical structures of these compounds to improve their anti-bacterial activity, selectivity, and physicochemical properties. Structure-Activity Relationship (SAR) studies can be employed to identify key functional groups and molecular features that contribute to the compounds' anti-bacterial activity. This information could guide the design of more potent and selective anti-bacterial agents that might help address the rising global threat of antibiotic resistance.

## CONCLUSION

The findings of the ADMET investigations and the assessment of the synthetic compounds' drug-like qualities were combined with molecular docking studies to determine their probable binding affinities with the biotin protein ligase (PDB ID: 4DQ2). The synthesized compounds' ability to bind to the target protein's active amino acids was shown by molecular docking experiments, suggesting that they would be effective anti-bacterial agents. The compounds AC1–AC6 demonstrated excellent docking scores with the target protein's active amino acids, indicating their potential as efficient biotin protein ligase inhibitors. The produced compounds were also efficient against *E. coli* and *S. aureus*, according to *in vitro* anti-bacterial investigations. With AC6 displaying the greatest activity, the compounds demonstrated a considerable zone of inhibition against the tested bacterial strains. The synthesized compounds demonstrated positive drug-like qualities, excellent absorption, distribution, low toxicity, and favorable binding affinities towards the target protein, all without violating Lipinski's rule of five. Additionally, both Gram-positive and Gram-negative bacteria were significantly resistant to the compounds' anti-bacterial effects. The compounds that were created therefore have a significant deal of promise for further development as anti-bacterial agents. Future research should concentrate on *in vivo* testing to evaluate the substances' pharmacokinetic characteristics and therapeutic effectiveness.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ABBREVIATIONS

**ADMET:** Absorption, Distribution, Metabolism, Excretion, and Toxicity; **BPL:** Biotin Protein Ligase; **Caco-2:** Colorectal adenocarcinoma cell line 2; **DCC:** Dicyclohexylcarbodiimide; **DMAP:** 4-dimethylaminopyridine; **DMSO:** Dimethyl sulfoxide; **EI:** Electron Ionization; **ESI:** Electrospray Ionization; **FTIR:** Fourier Transform Infrared Spectroscopy; **GI:** Gastrointestinal; **HPLC:** High-Performance Liquid Chromatography;

**LC-MS:** Liquid Chromatography-Mass Spectrometry; **MDR:** Multidrug-resistant; **MIC:** Minimum Inhibitory Concentration; **MBC:** Minimum Bactericidal Concentration; **MRSA:** Methicillin-resistant *Staphylococcus aureus*; **NMR:** Nuclear Magnetic Resonance; **PDB:** Protein Data Bank; **PPB:** Plasma Protein Binding; **SD:** Standard Deviation; **TB:** Typhoid fever.

## SUMMARY

The objective of the study was to investigate the possible anti-bacterial activity of new 2-aminophenyl-2-(2,4,5-triphenylimidazole) acetate derivatives that were developed and synthesized using a thorough methodology. The synthesized compounds were characterized using several spectroscopic methods, and *in silico* ADMET studies were used to assess their drug-like characteristics. To find out how well the chemicals attach to the biotin protein ligase, molecular docking tests were conducted. To evaluate the compounds' anti-bacterial activity against *Escherichia coli* and *Staphylococcus aureus, in vitro* anti-bacterial tests were carried out. All of the synthesized compounds displayed moderate to good drug-like qualities, according to the data, and had the capacity to bind to the biotin protein ligase. Additionally, the anti-bacterial experiments showed that the produced compounds significantly inhibited the growth of both *S. aureus* and *E. coli*. Of all the produced compounds, compound AC6 was determined to have the most anti-bacterial activity. The work was effective in designing and creating new 2-aminophenyl-2-(2,4,5-triphenylimidazole) acetate derivatives, which shown potential drug-like characteristics and considerable anti-bacterial efficacy against *S. aureus* and *E. coli*. The results of this study may help in the creation of new anti-bacterial drugs to treat bacterial infections.

## REFERENCES

1. Frieri M, Kumar K, Boutin A. Antibiotic resistance. J Infect Public Health. 2017;10(4):369-78. doi: 10.1016/j.jiph.2016.08.007, PMID 27616769.
2. Munita JM, Arias CA. Mechanisms of antibiotic resistance. Microbiol Spectr. 2016;4(2):481-511. doi: 10.1128/microbiolspec.VMBF-0016-2015, PMID 27227291.
3. McArthur AG, Waglechner N, Nizam F, Yan A, Azad MA, Baylay AJ, *et al.* The comprehensive antibiotic resistance database. Antimicrob Agents Chemother. 2013;57(7):3348-57. doi: 10.1128/AAC.00419-13, PMID 23650175.
4. Marzouk AA, Abbasov VM, Talybov AH, Mohamed SK. Synthesis of 2, 4, 5-triphenyl imidazole derivatives using diethyl ammonium hydrogen phosphate as green, fast and reusable catalyst. World J Org Chem. 2013;1(1):6-10.
5. Yasodha A, Sivakumar A, Arunachalam G, Puratchikody A. Synthesis and biological evaluation of some 2, 4, 5-triphenyl imidazole derivatives. J Pharm Sci Res. 2009;1(4):127-30.
6. Rajaraman D, Sundararajan G, Rajkumar R, Bharanidharan S, Krishnasamy K. Synthesis, crystal structure investigation, DFT studies and DPPH radical scavenging activity of 1-(furan-2-ylmethyl)-2, 4, 5-triphenyl-1H-imidazole derivatives. J Mol Struct. 2016;1108:698-707. doi: 10.1016/j.molstruc.2015.11.084.
7. Jagadishbabu N, Shivashankar K. One-pot synthesis of 2, 4, 5-triphenyl imidazoles from 1, 2-diols as key reagents. J Chin Chem Soc. 2017;64(5):474-80. doi: 10.1002/jccs.201600746.
8. Boyatzis S, Nikokavouras J. Absorption, fluorescence and chemiluminescence spectra of 2,4,5-Triphenylimidazole (lophine) and 2-(p-dimethyl-aminophenyl)-4,5-Diphenylimidazole in micellar solutions. Mol Cryst Liq Cryst. 1986;137(1):403-12. doi: 10.1080/00268948608070937.
9. Jayachandran JP, Wang ML. N-alkylation of 2, 4, 5-triphenyl imidazole derivatives using a new phase transfer reagent under PTC conditions. Synth Commun. 2001;31(18):2743-52. doi: 10.1081/SCC-100105320.
10. Vijesh AM, Isloor AM, Telkar S, Arulmoli T, Fun HK. Molecular docking studies of some new imidazole derivatives for antimicrobial properties. Arab J Chem. 2013;6(2):197-204. doi: 10.1016/j.arabjc.2011.10.007.
11. Norinder U, Bergström CA. Prediction of ADMET properties. ChemMedChem. 2006;1(9):920-37. doi: 10.1002/cmdc.200600155, PMID 16952133.
12. Sakaino Y, Kakisawa H, Kusumi T, Maeda K. Structures and chromotropic properties of 1, 4-bis(4, 5-diphenylimidazol-2-yl)benzene derivatives. J Org Chem. 1979;44(8):1241-4. doi: 10.1021/jo01322a010.
13. Vijesh AM, Isloor AM, Telkar S, Peethambar SK, Rai S, Isloor N. Synthesis, characterization and antimicrobial studies of some new pyrazole incorporated imidazole derivatives. Eur J Med Chem. 2011;46(8):3531-6. doi: 10.1016/j.ejmech.2011.05.005, PMID 21620535.
14. Petersen PJ, Jacobus NV, Weiss WJ, Sum PE, Testa RT. *In vitro* and *in vivo* anti-bacterial activities of a novel glycylcycline, the 9-t-butylglycylamido derivative of minocycline (GAR-936). Antimicrob Agents Chemother. 1999;43(4):738-44. doi: 10.1128/AAC.43.4.738, PMID 10103174.
15. Tanino H, Kondo T, Okada K, Goto T. Structures of three isomeric dimers of 2,4,5-Triphenylimidazolyl. Bull Chem Soc Jpn. 1972;45(5):1474-80. doi: 10.1246/bcsj.45.1474.
16. Gazieva GA, Karpova TB, Nechaeva TV, Kravchenko AN. Ring contraction of 1,2,4-triazine derivatives in the synthesis of imidazoles. Russ Chem Bull. 2016;65(9):2172-82. doi: 10.1007/s11172-016-1565-y.
17. Liu C, Shi C, Mao F, Xu Y, Liu J, Wei B, *et al.* Discovery of new imidazole derivatives containing the 2, 4-dienone motif with broad-spectrum antifungal and anti-bacterial activity. Molecules. 2014;19(10):15653-72. doi: 10.3390/molecules191015653, PMID 25268720.

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