*Indian J. Applied & Pure Bio.* Vol. 39(1), 239-256 (2024). **ISSN: 0970-2091**
**A Web of Science Journal**

# Exploring the Antibacterial potential of novel 2-Aminophenyl-2-(2,4,5-Triphenylimidazole) Acetate Derivatives: A comprehensive design and synthesis approach

**Piyusha Kolhe, *Rahul K. Godge and Amol S. Dighe**

Department of Pharmaceutical Chemistry, Pravara Rural College off Pharmacy
Pravaranagar, Loni-413713 (India)
*For Correspondence : Email: rahul.godge@pravara.in
Mobile: +91-9561282059

**Abstract**

Antibiotic resistance is a growing concern, and the development of new antibacterial agents is crucial. 2-aminophenyl-2-(2,4,5-triphenylimidazole) derivatives have shown potential as antibacterial agents in previous studies, and this study aims to further explore their potential. In this study, a series of 2-aminophenyl-2-(2,4,5-triphenylimidazole) derivatives were designed and synthesized. Their antibacterial activity was evaluated against Escherichia coli and Staphylococcus aureus using the disc diffusion method. The compounds were also evaluated for druglikeness and ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties. Molecular docking studies were conducted to investigate the binding modes of the compounds with biotin protein ligase. The synthesized compounds exhibited varying degrees of antibacterial 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 druglikeness, and exhibited favourable ADMET properties. The molecular docking studies revealed that the compounds had favourable binding modes with biotin protein ligase (PDB ID: 4DQ2). The 2-aminophenyl-2-(2,4,5-triphenylimidazole) derivatives designed and synthesized in this study exhibited promising antibacterial activity against E. coli and S. aureus. The compounds also demonstrated good Druglikeness 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 antibacterial agents and warrant further investigation.

**Key words :** Antibacterial, 2-aminophenyl-2-(2,4,5-triphenylimidazole) derivatives, druglikeness, ADMET, molecular docking.

The increasing prevalence of antibiotic-resistant bacterial infections has become a global public health concern, necessitating the development of novel antibacterial 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 antibacterial treatments. Among the promising scaffolds for such compounds, imidazole derivatives have garnered significant attention due to their diverse biological activities, including antimicrobial, antiviral, and anticancer properties.[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 antibacterial 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 antibacterial 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 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 antibacterial 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 antibacterial 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 antibacterial 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 and hydrophilic interactions, as well as to provide a versatile scaffold for the introduction of additional functional groups.

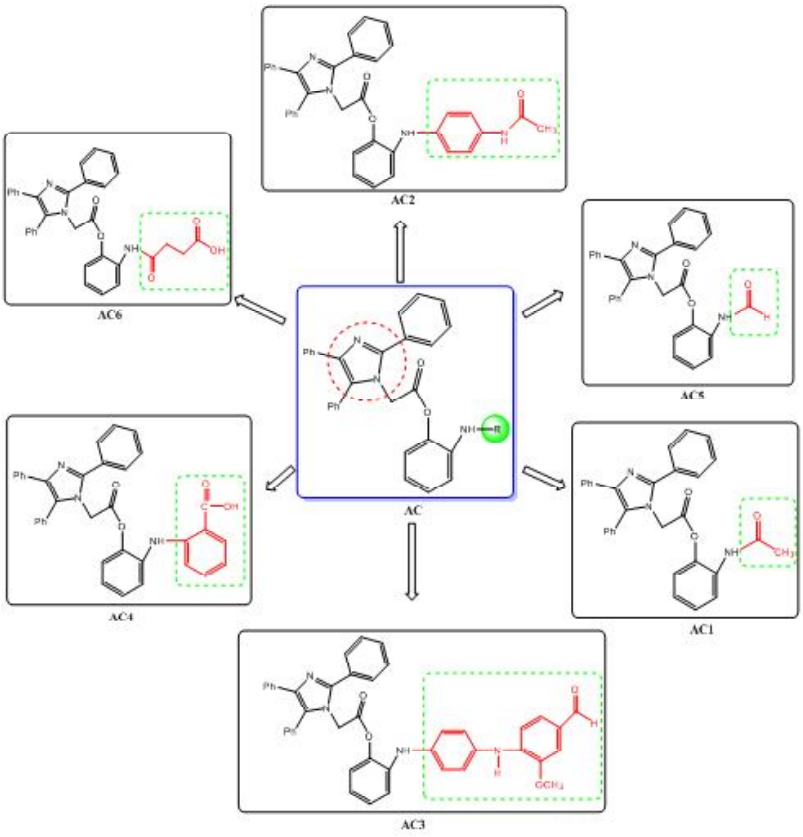



Figure 1: Designed for derivatives 2-aminophenyl-2-(2,4,5-triphenylimidazole) acetate

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 antibacterial 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 antibacterial agents.[8]

By unveiling the potential of these innovative compounds as a new class of antibacterial 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]

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 antibacterial activity assays, was obtained from RMI Laboratory (OPC Pvt. Ltd.).

*ADME parameter estimation :*

The absorption, distribution, metabolism, and excretion (ADME) properties of the synthesized compounds were estimated using the SwissADME web tool (http://www.swissadme.ch). 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 :*

Molecular docking studies were performed to investigate the binding interactions between the synthesized compounds and their bacterial target proteins using AutoDock Vina. The crystal structures of the target proteins were obtained from the Protein Data Bank (PDB). Prior to docking, the target proteins were prepared by removing water molecules and adding polar hydrogen atoms using the AutoDockTools software.[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 antibacterial 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 antibacterial 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 Antibacterial activity :*

The antibacterial activity of the synthesized compounds was evaluated using the agar disc diffusion method. The test organisms used were *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923). The bacterial strains were inoculated onto nutrient agar plates, and sterile filter paper discs were placed on the surface of the agar. A 100 µL solution of each test compound at a concentration of 100 µg/mL was applied to the filter paper discs. Ciprofloxacin was used as a positive control at the same concentration. The plates were incubated at 37°C for 24 hours. The zone of inhibition (ZOI) was measured in millimeters (mm) using a ruler. Each experiment was performed in triplicate, and the mean value and standard deviation were calculated. The results were interpreted based on the ZOI, where a larger zone of inhibition indicated higher antibacterial activity.[13,14]

*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 antibacterial 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 antibacterial properties, focusing on the substitutions to the amino group.[15]

The core scaffold of these derivatives comprises a 2-aminophenyl acetate moiety linked to a 2,4,5-triphenyl-1H-imidazole ring. The antibacterial activity of the synthesized compounds can be attributed to the presence

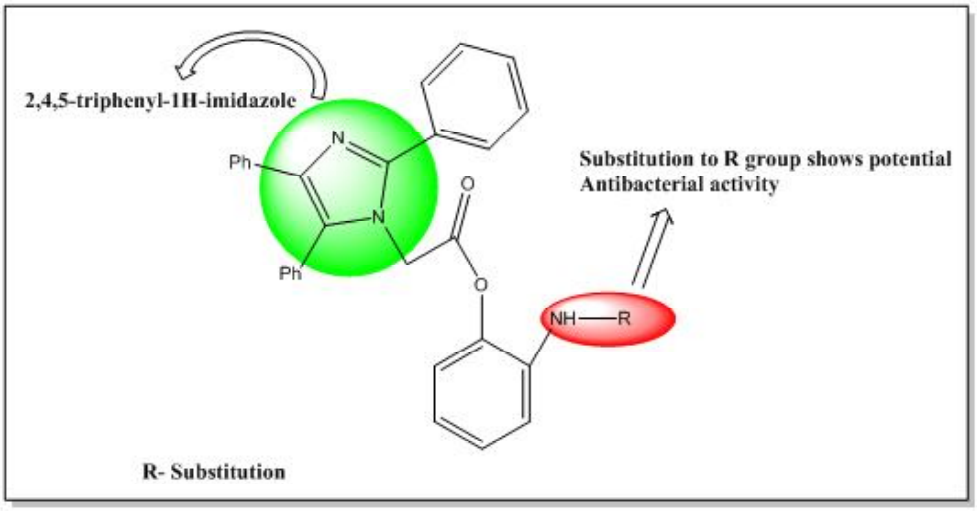



Figure 2. Design of 2-Aminophenyl-2-(2,4,5-triphenyl-1H-imidazole-1-yl) acetate derivatives.

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 antibacterial activity. It was observed that the presence of electron-donating groups, such as alkyl or alkoxy groups, increased the antibacterial 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 antibacterial potency of the derivatives.[17]

*Synthesis and spectral analysis of compounds (AC1-AC6) :*

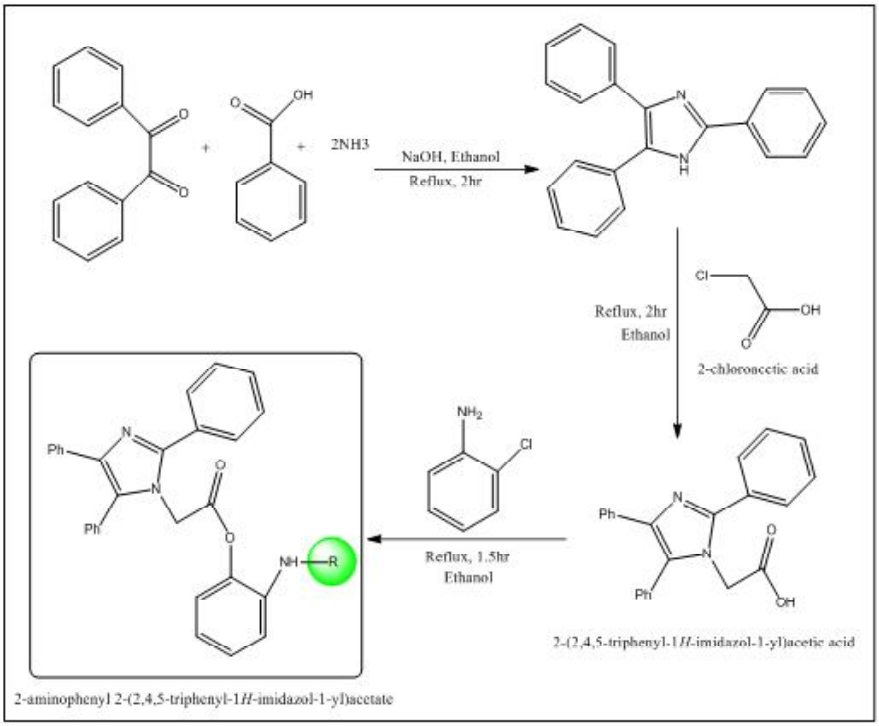



Figure 3. Scheme for synthesis of 2-Aminophenyl-2-(2,4,5-triphenyl-1H-imidazole-1-yl) derivatives (AC1 to AC6)

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

In a round-bottomed flask, we take 1gm of 2-(2,4,5-triphenyl-1H-imidazole-1-yl) acetic acid, 1ml 2-chloroaniline and 25ml glacial acetic acid (GAA) was mixed with each other. The mixture was heated in a water bath at 100° for 2 hours. 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 hours. The precipitated product was filtered and recrystallized with methanol to obtain 2-aminophenyl2-(2,4,5-triphenyl-1H-imidazole-1-yl)acetate.

*Synthesis of 2-acetamidophenyl 2-(2,4,5-triphenyl-1H-imidazol-1-yl)acetate (AC1):*

In a round-bottomed flask, we take 1gm of 2-aminophenyl 2-(2,4,5-triphenyl-1H-imidazole-1-yl) acetate, 1ml acetyl chloride and 25ml methanol was mixed with each other. The mixture was heated in a water bath at 100° for 2 hours. 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 hours. 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, 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 1gm of 2-aminophenyl2-(2,4,5-triphenyl-1H-imidazole-1-yl)acetate,1gm acetaminophen and 25ml methanol was mixed with each other. The mixture was heated in a water bath at 100° for 2 hours. 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 hours. The precipitated product was filtered and recrystallized with methanol to 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-methoxy-phenyl) amino)phenyl) amino)phenyl 2-(2,4,5-triphenyl-1H-imidazol-1-yl)acetate (AC3) :*

In a round-bottomed flask, we take 1gm of 2-aminophenyl2-(2,4,5-triphenyl-1H-imidazole-1-yl)acetate, 1gm vanillin and 25ml methanol was mixed with each other. The mixture was heated in a water bath at 100° for 2 hours. 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 hours. The precipitated product was 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-1H-imidazol-1-yl) acetoxy) phenyl) amino) benzoic acid (AC4) :*

In a round-bottomed flask, we take 1gm of 2-aminophenyl2-(2,4,5-triphenyl-1H-imidazole-1-yl) acetate,1gm salicylic acid and 25ml methanol was mixed with each other. The mixture was heated in a water bath at 100° for 2 hours. 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 hours. 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, $CH_2$), 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-triphenyl-1H-imidazol-1-yl)acetate (AC5):*

In a round-bottomed flask, we take 1gm of 2-aminophenyl2-(2,4,5-triphenyl-1H-imidazole-1-yl) acetate, 1ml formic acid and 25ml methanol was mixed with each other. The mixture was heated in a water bath at 100° for 2 hours. 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 hours. The precipitated product was 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 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 1gm of 2-aminophenyl2-(2,4,5-triphenyl-1H-imidazole-1-yl)acetate,1gm succinic acid and 25ml methanol was mixed with each other. The mixture was heated in a water bath at 100° for 2 hours. 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 hours. The precipitated product was

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 calculated Lipinski's rule of five and drug-likeness properties of the synthesized compounds (AC1-AC6) are presented in the table below. These properties are essential to evaluate the potential of these compounds as drug candidates.

According to Lipinski's rule of five, a compound is considered to have drug-like properties if it does not violate more than one of the following criteria: molecular weight less than 500 Da, log P less than 5, the number of hydrogen bond donors less than 5, and the number of hydrogen bond acceptors less than 10. Compounds AC1, AC2, AC3, AC5, and AC6 violate Lipinski's rule of five, indicating that they may have unfavourable pharmacokinetic properties. However, compound AC4 complies with the rule and can be considered as a potential drug candidate.

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 Å² 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

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 |

values are in the range of 70.14 Å² to 93.93 Å², well below the 140 Å² 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. 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).

Caco2 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

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

| Co-mp. | Absorption | | Distribution | | | Metabolism | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Caco2 permeability (log Papp in10-6 cm/s) | GI absorption | BBB perm. (log BB) | BBB Permeant | PPB (%) | CYP 3A4 substrate | CYP 1A2 inhibitor | CYP2C9 inhibitor, | CYP 3A4 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 |

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 :*

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 |

The molecular docking results presented in Table-3 indicate that the synthesized 2-aminophenyl-2-(2,4,5-triphenyl-1H-imidazole-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-1 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 antibacterial agents targeting biotin protein ligase.

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

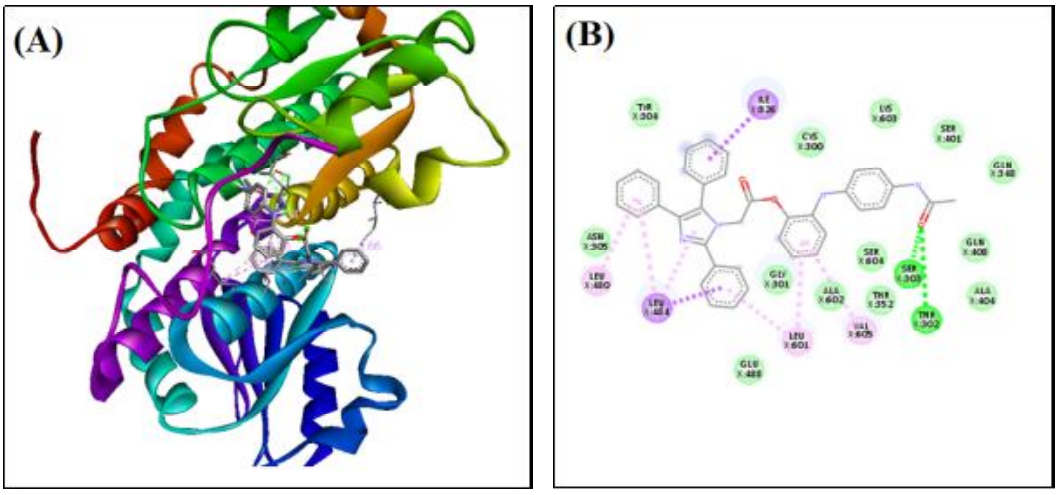



Figure 4. Binding of AC2 to active site of Biotin Protein Ligase
(A) along with the 2D binding diagram (B). The nucleophilic residue is labelled in green colour and the hydrogen bonds are depicted as green dotted lines.

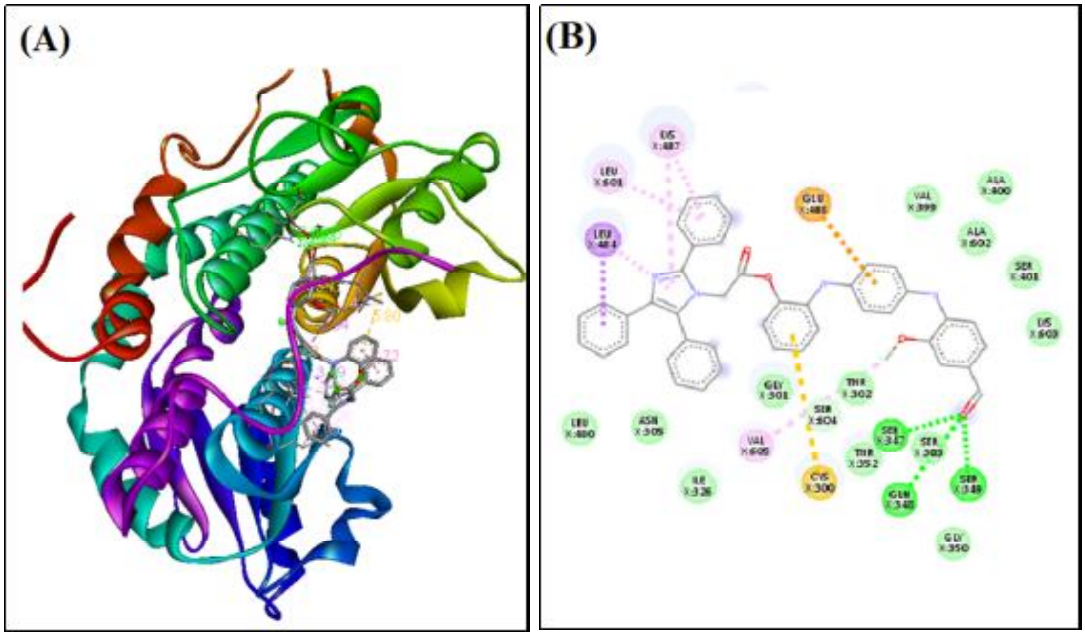
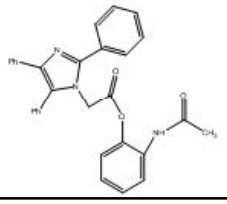
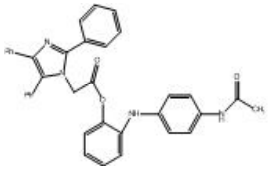
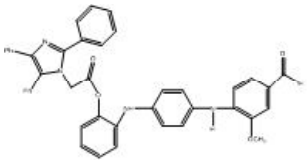
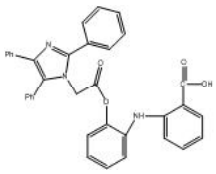
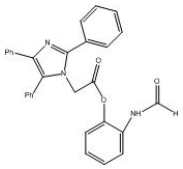
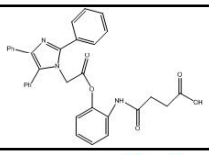
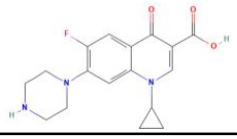



Figure 5: Binding of AC4 to active site of Biotin Protein Ligase
(A) along with the 2D binding diagram (B). The nucleophilic residue is labelled in green colour and the hydrogen bonds are depicted as green dotted lines.

*Results of Antibacterial Activity :*

Table-1. 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

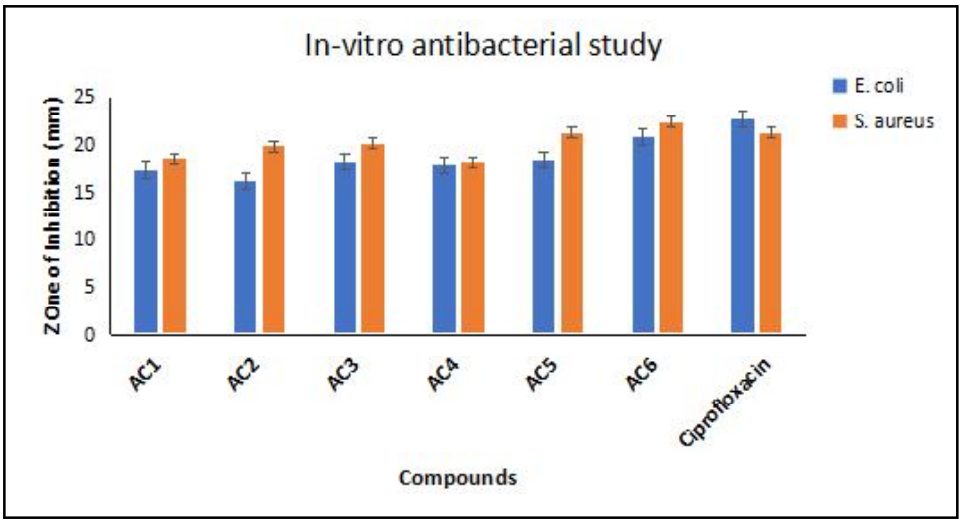



Figure 6. In-vitro antibacterial study of synthesized compounds (AC1 to AC6)

AC1 demonstrates moderate antibacterial 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 antibacterial 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 antibacterial 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 Gram-positive bacteria, but its overall activity against both strains is still considerable. AC5 shows a notable antibacterial 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 antibacterial 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 antibacterial 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 antibacterial activity to that of the reference compound, which highlights their potential as novel antibacterial agents.

In conclusion, the *in-vitro* antibacterial 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 antibacterial activity against both *E. coli* and *S. aureus*. Among the synthesized compounds, AC6 shows the most potent antibacterial activity, making it a potential candidate for further investigation and developmentas an antibacterial agent. The varying degrees of antibacterial 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 antibacterial 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' antibacterial activity. This information could guide the design of more potent and selective antibacterial agents that might help address the rising global threat of antibiotic resistance.

In addition to the results of the ADMET studies and the evaluation of the drug-like properties of the synthesized compounds, molecular docking studies were conducted to assess their potential binding affinity with the biotin protein ligase (PDB ID: 4DQ2). The molecular docking studies revealed the potential of the synthesized compounds to bind to the active amino residues of the target protein, indicating their potential efficacy as antibacterial agents. The compounds AC1-AC6 showed strong docking scores with the active amino residues of the target protein, suggesting their potential as effective inhibitors of biotin protein ligase. Furthermore, the in vitro antibacterial studies indicated that the synthesized compounds were effective against both *E. coli* and *S. aureus*. The compounds showed significant zone of inhibition against the tested bacterial strains, with AC6 showing the highest activity. In conclusion, the synthesized compounds exhibited promising drug-like properties with no violations of Lipinski's rule of five, good absorption, distribution, and low toxicity, and favorable binding affinities towards the target protein. Additionally, the compounds showed significant antibacterial activity against both Gram-positive and Gram-negative bacteria. Therefore, the synthesized compounds hold great potential for further development as antibacterial agents. Future studies should focus on in vivo testing to assess the pharmacokinetic properties and therapeutic efficacy of the compounds.

The study aimed to explore the antibacterial potential of novel 2-aminophenyl-2-(2,4,5-triphenylimidazole) acetate derivatives, which were designed and synthesized through

a comprehensive approach. The synthesized compounds were characterized using various spectroscopic techniques, and their drug-like properties were evaluated using in silico ADMET analyses. Molecular docking studies were performed to investigate the binding affinity of the compounds with biotin protein ligase. In vitro antibacterial studies were conducted to assess the compounds' antibacterial activity against Escherichia coli and Staphylococcus aureus. The results showed that all the synthesized compounds exhibited moderate to good drug-like properties and could potentially bind with biotin protein ligase. Furthermore, the antibacterial studies indicated that the synthesized compounds exhibited significant antibacterial activity against both *E. coli* and *S. aureus*. Compound AC6 was found to have the highest antibacterial activity among all the synthesized compounds.In conclusion, the study successfully designed and synthesized novel 2-aminophenyl-2-(2,4,5-triphenylimidazole) acetate derivatives, which exhibited promising drug-like properties and significant antibacterial activity against *E. coli* and *S. aureus*. The findings of this study could be useful for the development of new antibacterial agents to combat bacterial infections.

The authors would like to acknowledge the support and guidance provided by Principal Dr. Sanjay Bhawar, Pravara Rural College of Pharmacy, Loni, in conducting this research. His constant encouragement and valuable suggestions were instrumental in the successful completion of this study. The authors also express their gratitude to the institution for providing the necessary facilities and infrastructure to carry out this research work.

**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.

References :

1. Boyatzis S, and J. Nikokavouras (1986) *Molecular Crystals and Liquid Crystals. 137*(1): 403–12.
2. Frieri M, K Kumar, and A. Boutin (2017) *Journal of infection and public health. 10*(4): 369-78.
3. Gazieva GA, TB Karpova, T V. Nechaeva, and AN. Kravchenko (2016) *Russian Chemical Bulletin. 65*(9): 2172–82.
4. Jagadishbabu N, and K. Shivashankar (2017) *Journal of the Chinese Chemical Society. 64*(5): 474-80.

5. Jayachandran JP, and M L. Wang (2001) *Synthetic Communications. 31*(18): 2743-52.
6. Liu C, C Shi, F Mao, Y Xu, J Liu, B Wei, J Zhu, M Xiang, and J. Li (2014) *Molecules. 19*(10): 15653-72.
7. Marzouk AA, VM Abbasov, AH Talybov, and SK. Mohamed (2013) *World Journal of Organic Chemistry. 1*(1): 6-10.
8. McArthur AG, N Waglechner, F Nizam, A Yan, MA Azad, and AJ Baylay, *et al.* (2013) The comprehensive antibiotic resistance database. Antimicrob Agents Chemother. *57*(7): 3348–57.
9. Munita JM and CA. Arias (2016) *Virulence mechanisms of bacterial pathogens. 22:* 481-511.
10. Norinder U, and CA. Bergström (2006) *Chem Med Chem: Chemistry Enabling Drug Discovery. 1*(9): 920-37.
11. Petersen PJ, NV Jacobus, WJ Weiss, PE Sum, and RT. Testa (1999) *Antimicrobial agents and chemotherapy. 43*(4): 738-44.
12. Rajaraman D, G Sundararajan, R Rajkumar, S Bharanidharan and K. Krishnasamy (2016) *Journal of Molecular Structure. 1108:* 698-707.
13. Sakaino Y, H Kakisawa, T Kusumi, and K. Maeda (1979) *Journal of Organic Chemistry. 44*(8): 1241–4.
14. Tanino H, T Kondo, K Okada, and T. Goto (1972) *Bull Chem Soc Jpn. 45*(5): 1474–80.
15. Vijesh A A, A M Isloor, S Telkar, S K Peethambar, S Rai, and N. Isloor (2011) *European journal of medicinal chemistry. 46*(8): 3531-6.
16. Vijesh AM, AM Isloor, S Telkar, T Arulmoli, and HK. Fun (2013) *Arabian Journal of Chemistry. 6*(2): 197-204.
17. Yasodha A, A Sivakumar, G Arunachalam, and A. Puratchikody (2009) *Journal of Pharmaceutical Science and research. 1*(4): 127-30.