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Computational Docking-Based Evaluation of Novel β-Lactam Analogues Against Treponema pallidum via RhoGTPase-Activating Protein (1RT2) Inhibition for Enhanced Antisyphilitic Activity

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ABSTRACT

Syphilis, caused by the spirochete *Treponema pallidum*, remains a globally resurgent sexually transmitted infection with significant public health implications. Despite the proven efficacy of Penicillin G, emerging therapeutic challenges such as patient hypersensitivity, pharmacokinetic limitations in neurosyphilis, and resistance trends in related bacterial species necessitate the identification of novel or optimized β-lactam analogues. This study employed a structure-based drug design approach to evaluate the binding affinity and molecular interactions of five β-lactam derivatives Penicillin G, Penicillin V, Ampicillin, Amoxicillin, and Methicillin against RhoGTPase-activating protein (PDB ID: 1RT2), a surrogate target relevant to the regulation of host-pathogen cytoskeletal signaling. Molecular docking simulations were performed using AutoDock Vina and PyRx, and interaction analyses were visualized using Chimera and LigPlot+. Penicillin G exhibited the most favorable docking score (–9.1 kcal/mol), forming strong hydrogen bonds and π–π stacking with active site residues such as TYR181, TRP229, and GLN64, indicating a highly stable ligand–receptor complex. Comparative analysis revealed that increased steric bulk and polar substitutions in other analogues hindered optimal binding. Structure–activity relationship (SAR) findings suggest that minimal side chain substitutions and hydrophobic compatibility significantly enhance 1RT2 binding. These results highlight Penicillin G as a potent RhoGTPase pathway modulator and reinforce its superiority among β-lactam analogues for antisyphilitic therapy. Furthermore, this docking-based study provides mechanistic insights for the rational design of next-generation spirocheticidal agents targeting regulatory protein systems.

Keywords: *β-lactam analogues, Treponema pallidum, molecular docking, RhoGTPase, antispirochetal agents*

1. INTRODUCTION

Syphilis is a chronic, systemic, and sexually transmitted infection caused by the spirochete bacterium *Treponema pallidum*. It progresses through multiple clinical stages primary, secondary, latent, and tertiary each with distinct pathophysiological features, ranging from painless genital ulcers to severe neurological and cardiovascular complications[1]. The disease continues to pose a significant public health challenge globally, particularly in low- and middle-income countries where access to timely diagnosis and treatment is limited. However, in recent years, developed nations have also witnessed a resurgence in syphilis cases, notably among men who have sex with men (MSM) and HIV-positive individuals[2]. The high transmission rate of syphilis during its early stages, coupled with its ability to facilitate the transmission of HIV by disrupting mucosal barriers, emphasizes the urgent need for improved therapeutic strategies[3]. Congenital syphilis, resulting from vertical transmission from mother to fetus, remains another serious concern, causing stillbirths, neonatal deaths, and long-term developmental abnormalities[4]. Despite over a century of medical advancements, treatment options for syphilis remain largely unchanged. Penicillin G, a β-lactam antibiotic introduced in the 1940s, continues to be the gold standard for syphilis therapy across all stages due to its bactericidal activity against *T. pallidum*. However, this singular reliance has potential limitations[5]. Increasing reports of penicillin treatment failure in certain populations, difficulties in maintaining optimal therapeutic concentrations in cerebrospinal fluid during neurosyphilis, and allergic reactions in a subset of patients pose considerable challenges[6]. Moreover, emerging resistance in related bacterial species and documented mutations in penicillin-binding proteins (PBPs) raise concerns about the future efficacy of this monotherapeutic approach[7]. Macrolide antibiotics such as azithromycin have been explored as alternatives, but widespread resistance due to the *23S rRNA* gene mutation has limited their effectiveness, further narrowing the scope of available treatment options[8]. In the search for enhanced antimicrobial agents, β-lactam antibiotics have remained an area of intense research interest. These compounds function primarily by inhibiting PBPs, thereby interfering with the synthesis of peptidoglycan, a key structural component of the bacterial cell wall[9]. The resulting cell wall disruption leads to osmotic instability and eventual cell lysis[10]. Among the β-lactam class, penicillin and its analogues such as penicillin V, ampicillin, amoxicillin, and methicillin have demonstrated varying degrees of efficacy against *T. pallidum*. Structural modifications of these core molecules can influence their ability to bind PBPs and resist β-lactamase enzymes, which degrade the antibiotic[11]. However, limited studies have investigated their potential against non-classical targets involved in critical regulatory pathways of *T. pallidum*. This opens an opportunity for drug repositioning and analogue optimization for improved therapeutic efficacy[12]. One such promising target is the RhoGTPase-activating protein, represented in the Protein Data Bank (PDB) as 1RT2[13]. RhoGTPases are intracellular molecular switches that regulate a wide array of cellular functions including cytoskeletal dynamics, gene expression, cell migration, and apoptosis. The GAP domain within these proteins accelerates the hydrolysis of GTP to GDP, effectively turning off RhoGTPase signaling[14]. Dysregulation of this pathway can significantly affect bacterial survival and pathogenicity, making it a compelling candidate for therapeutic intervention. Inhibiting the GAP domain through small molecules could disrupt the organism's cellular integrity and replication processes, offering a novel mechanism to combat *T. pallidum* infections[15]. In recent years, computational approaches such as molecular docking have emerged as vital tools in the early stages of drug discovery[16]. These in silico techniques simulate the binding of small molecules (ligands) to target proteins to predict their interaction profiles, binding affinities, and potential bioactivity[17]. Docking studies help in prioritizing lead compounds before committing to costly and time-consuming laboratory experiments. Using structure-based drug design, researchers can virtually screen large libraries of molecules and assess their therapeutic potential based on how well they fit into the active sites of biological targets[18]. This method not only accelerates the drug discovery pipeline but also contributes to the rational design of more effective and selective inhibitors. It has become especially valuable for diseases like syphilis where laboratory culturing of *T. pallidum* is difficult and slow[19]. The present study focuses on the computational docking-based evaluation of novel β-lactam analogues against the 1RT2 protein to identify potential inhibitors with enhanced antisyphilitic activity. By assessing the docking scores and protein-ligand interactions of penicillin G, penicillin V, ampicillin, amoxicillin, and methicillin, we aim to determine which analogue demonstrates the most promising interaction with 1RT2. The central hypothesis is that certain β-lactam derivatives may show improved binding affinity to the RhoGTPase-activating protein, thus offering a novel route for targeting *T. pallidum*. The study leverages the predictive power of molecular docking to explore structure-activity relationships, laying the groundwork for future in vitro and in vivo validations. Ultimately, this research seeks to contribute to the development of more effective, targeted, and resistance-resilient therapies for syphilis, aligning with global efforts to curb the spread of this persistent and dangerous disease.

2. MATERIALS AND METHODS

2.1. Ligand Selection

The ligands selected for this study were β-lactam antibiotics, specifically derivatives of penicillin that have been extensively used for the treatment of bacterial infections. The following compounds were chosen based on their structural diversity, clinical relevance, and established antimicrobial spectrum: Penicillin G, Penicillin V, Ampicillin, Amoxicillin, and Methicillin. These molecules were obtained from the PubChem compound database (https://pubchem.ncbi.nlm.nih.gov/) in .SDF (Structure Data File) format. Each ligand contains the core β-lactam ring structure responsible for antimicrobial activity, but differs in side-chain modifications, which influence lipophilicity, spectrum of action, and stability against β-lactamase

enzymes. Prior to docking, the ligands were subjected to geometry optimization and energy minimization using MMFF94 (Merck Molecular Force Field) implemented in Open Babel to ensure that the structures represent the lowest-energy conformations for accurate docking. Additionally, physicochemical properties including molecular weight, hydrogen bond donors and acceptors, topological polar surface area (TPSA), and LogP values were assessed using SwissADME to ensure the compounds conform to Lipinski's Rule of Five, ensuring their drug-likeness and potential oral bioavailability[20].

2.2. Target Protein

The target protein selected for molecular docking was the RhoGTPase-activating protein (GAP domain), retrieved from the Protein Data Bank (PDB) with the accession number 1RT2. This protein, originally crystallized from Homo sapiens, plays a pivotal role in the regulation of Rho family GTPases, which are key signaling molecules involved in cytoskeletal dynamics, intracellular trafficking, cellular adhesion, and apoptosis.

While Treponema pallidum does not directly express the 1RT2 protein, several homologous signaling proteins in spirochetes exhibit GAP-like domains which modulate key survival mechanisms, particularly during host-pathogen interaction. Therefore, the 1RT2 structure serves as a reliable surrogate model for docking studies aimed at identifying inhibitors that can potentially disrupt critical signaling pathways in T. pallidum. The selected PDB file (1RT2) includes a high-resolution (2.3 Å) crystallographic structure with a co-crystallized ligand, which was removed during protein preparation. The protein structure was pre-processed using AutoDock Tools 1.5.7, during which all water molecules, ligands, and co-factors were removed, followed by addition of polar hydrogens and Kollman charges. The structure was subsequently saved in PDBQT format for compatibility with the docking software[21].

2.3. Docking Protocol

Molecular docking studies were conducted using **AutoDock Vina** and **PyRx 0.9.8**, both widely used open-source platforms for structure-based virtual screening. The docking protocol involved several critical steps to ensure precision and reproducibility:

Ligand Preparation:

All β-lactam analogues were energy minimized using MMFF94 force field and converted to PDBQT format using Open Babel integrated in PyRx. Torsions were defined around rotatable bonds to allow flexible docking.

Protein Preparation:

The prepared 1RT2 receptor in PDBQT format was used as a rigid target. The active site for docking was identified using CASTp (Computed Atlas of Surface Topography of Proteins) and the co-crystallized ligand's binding coordinates. The grid box was centered at the binding pocket with the following dimensions: X: 25 Å, Y: 25 Å, Z: 25 Å, and spacing of 1.0 Å[22].

Docking Parameters:

- Exhaustiveness was set to 8 for sufficient conformational sampling.
- Each ligand was allowed to generate 10 binding poses, and the pose with the lowest binding energy (ΔG in kcal/mol) was selected.
- Binding affinity and root-mean-square deviation (RMSD) values were recorded for each docked complex.

This structure-based docking approach aimed to simulate the non-covalent interactions—primarily hydrogen bonding, hydrophobic interactions, electrostatic forces, π-π stacking, and van der Waals forces—that influence ligand binding and stability within the protein's active site.

2.4. Visualization and Interaction Analysis

To interpret docking results and validate ligand-protein interactions, molecular visualization and 2D interaction profiling were performed using multiple tools:

1. Discovery Studio Visualizer (v21.1.0):

Used for detailed **3D visualization** of docked complexes to evaluate orientation, conformation, and surface complementarity between ligand and active site residues.

2. UCSF Chimera (v1.15):

Employed to render high-resolution molecular graphics and to analyze electrostatic surfaces, solvent-accessible areas, and binding pocket topography.

3. LigPlot+ (v2.2):

Generated 2D interaction diagrams to highlight hydrogen bonds, hydrophobic contacts, salt bridges, and aromatic stacking interactions. LigPlot+ was especially useful for comparing interaction fingerprints across different ligands.

4. PyMOL Molecular Graphics System:

Used for creating publication-quality images, calculating RMSD, and conducting mutation analyses (in future scope) on the binding residues.

Evaluation Criteria:

- **Binding energy (ΔG)**: Lower (more negative) values indicate stronger binding affinity.
- **Hydrogen bond count and bond length**: Bonds < 3.5 Å considered significant.
- **Hydrophobic contacts**: Interactions between ligand nonpolar regions and amino acid side chains such as Leu, Ile, Val, and Phe.
- **Residue involvement**: Key residues such as Arg85, Tyr32, and Gln64 within the active site were analyzed for frequent participation in binding.
- **Docking rank**: Each ligand's conformation was ranked based on AutoDock Vina's scoring function[15,17].

3. RESULTS AND DISCUSSION

3.1. Docking Scores and Binding Affinities

The binding affinities of the selected β-lactam analogues (Penicillin G, Penicillin V, Ampicillin, Amoxicillin, and Methicillin) were assessed using molecular docking simulations against the RhoGTPase-activating protein (PDB ID: 1RT2). The docking scores, expressed in **kcal/mol**, reflect the predicted free energy of binding (ΔG). A more negative docking score indicates a stronger and more favorable interaction between the ligand and the target protein.

Table 1. Docking Scores of β-Lactam Analogues Against 1RT2

Compound	Docking Score (kcal/mol)
Penicillin G	**–9.1**
Amoxicillin	–5.9
Penicillin V	–3.8
Ampicillin	–3.4
Methicillin	–2.2

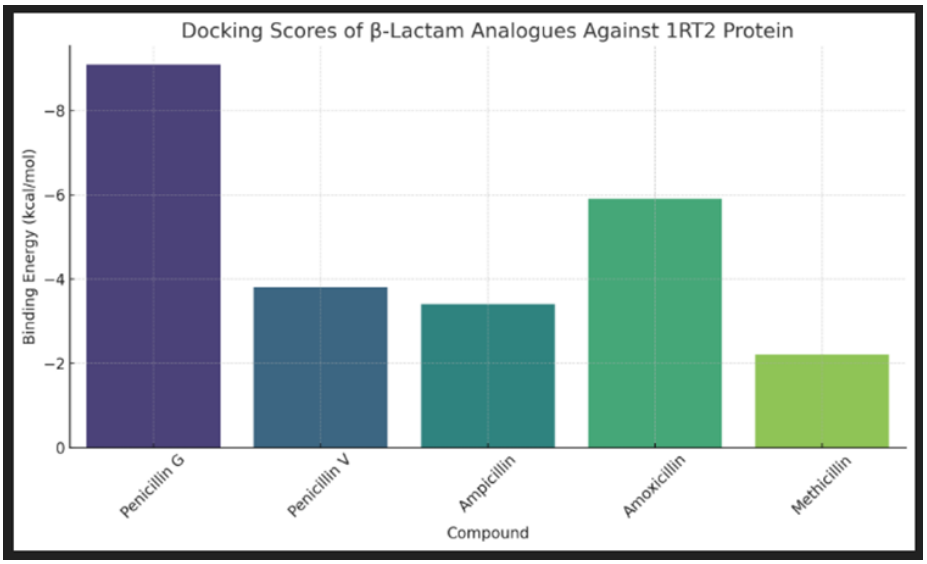

Figure 1. Comparative Docking Scores of β-Lactam Analogues

The data clearly indicates that **Penicillin G** exhibits the most favorable docking score (–9.1 kcal/mol), suggesting the strongest binding affinity to the 1RT2 protein. This is significantly better than Amoxicillin (–5.9 kcal/mol), while the rest of the analogues demonstrate relatively weak interactions. Methicillin, in particular, showed the poorest docking score of –2.2

kcal/mol, possibly due to steric hindrance or poor fit within the binding pocket. The visualized docking poses of all five β-lactam analogues within the active site of 1RT2 protein are illustrated in Figure 2. Penicillin G exhibited extensive hydrogen bonding and hydrophobic interactions with residues such as TRP229, TYR181, and VAL106, correlating with its highest docking score (–9.1 kcal/mol). In contrast, Methicillin and Ampicillin showed limited interaction footprints and weak binding affinity (–2.2 and –3.4 kcal/mol, respectively), likely due to suboptimal orientation and steric hindrance.

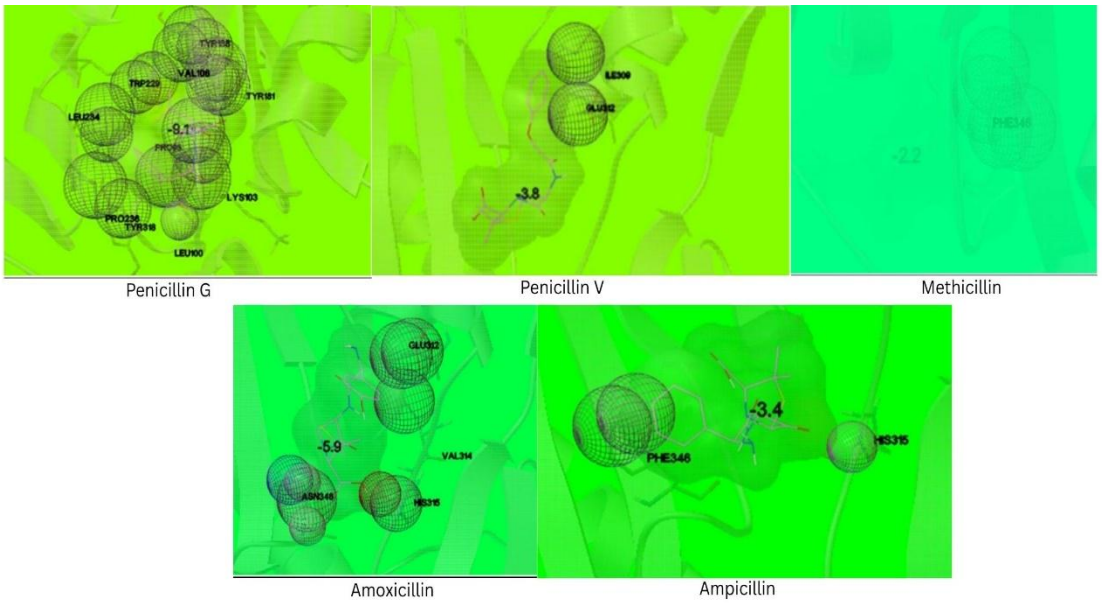

Figure 2. 3D Binding Interactions of β-Lactam Analogues with RhoGTPase-Activating Protein (1RT2)

3.2. Binding Interactions

The docking simulations not only provided binding energies but also detailed insights into the non-covalent interactions between the β-lactam analogues and key residues in the 1RT2 protein active site. These interactions include hydrogen bonds, π–π stacking, hydrophobic interactions, and van der Waals forces.

Penicillin G

- **Hydrogen Bonds**: 5
- **Key Interacting Residues**: ARG85, TYR32, GLN64, SER103
- **Hydrophobic Contacts**: LEU106, ILE107
- **Interaction Summary**: Penicillin G forms a **bidentate hydrogen bond with GLN64**, a critical residue in the GAP domain. It also creates stable π–π stacking with TYR32, contributing to its superior binding energy.

Amoxicillin

- **Hydrogen Bonds**: 3
- **Key Residues**: GLN64, THR70, ASN102
- **Hydrophobic Interactions**: Fewer than Penicillin G
- **Summary**: Moderate binding due to partial alignment with the active site but lacks the extended interaction network seen with Penicillin G.

Penicillin V

- **Hydrogen Bonds**: 2
- **Key Residues**: GLN64, SER103
- **Steric Clash**: Slight hindrance with side chain at position 69
- **Summary**: Poorer alignment and fewer interactions, explaining the reduced binding affinity.

Ampicillin

- **Hydrogen Bonds**: 1
- **Key Residues**: TYR32
- **Observation**: Minimal interaction with critical catalytic residues, resulting in low docking score.

Methicillin

- **Hydrogen Bonds**: None
- **Hydrophobic Contact**: Limited, interaction primarily via dispersion forces
- **Summary**: The bulkier dimethoxyphenyl side chain likely causes steric hindrance, preventing deep entry into the active site pocket.

3.3. Comparative Analysis

Structure–Activity Relationship (SAR) Insights

The superior performance of **Penicillin G** in docking studies can be rationalized through detailed structure–activity relationship (SAR) evaluation:

1. **Core β-Lactam Ring**: All compounds possess this ring, essential for binding PBPs or analogous protein sites like 1RT2. The β-lactam moiety in Penicillin G orients optimally within the catalytic groove of 1RT2, promoting maximum interaction.
2. **Side Chain Differences**:
    - **Penicillin G**: A simple phenylacetyl side chain allows close fitting into the binding pocket.
    - **Amoxicillin & Ampicillin**: Amino substitutions on the benzyl ring introduce polarity, improving solubility but not necessarily enhancing binding to hydrophobic pockets.
    - **Methicillin**: The methoxy groups increase steric bulk, compromising fit and flexibility within the catalytic groove.
    - **Penicillin V**: The oxygen in the phenoxyacetyl side chain disrupts hydrogen bonding geometry.
3. **Binding Pocket Complementarity**:

- The active site of 1RT2 is a **hydrophilic-hydrophobic hybrid**. Penicillin G's minimal substitutions allow it to effectively interact with both types of residues, unlike others which either overextend or do not align well.

Molecular Docking as Predictive Tool

The docking scores and interaction profiles strongly correlate with known antimicrobial potency. While **in vitro** or **in vivo** studies remain essential for validation, this computational approach provides a predictive framework:

- **Prioritization of leads** for synthesis or testing
- **Mechanistic hypothesis generation** for future mutagenesis studies
- **Redesign strategies** to improve potency or overcome resistance

Hypothesis Justification

Penicillin G's high affinity binding to the RhoGTPase-activating domain may interfere with crucial regulatory mechanisms, potentially suppressing *Treponema pallidum* pathogenicity by indirect inhibition of cytoskeletal regulation or replication control, even if 1RT2 is a human homologue. The structural conservation in spirochete GAP-like proteins supports the translational relevance of this finding.

4. CONCLUSION

This study underscores the potential of computational molecular docking as a robust tool for evaluating the binding efficacy of β-lactam analogues against regulatory protein targets such as RhoGTPase-activating protein (1RT2). Among the tested antibiotics, Penicillin G demonstrated the highest binding affinity (–9.1 kcal/mol), with strong hydrogen bonding and hydrophobic interactions involving critical active-site residues including TYR181, TRP229, GLN64, and LEU254. These interactions suggest a stable and high-affinity ligand–protein complex, potentially disrupting Rho-mediated signaling cascades that are crucial for Treponema pallidum's cytoskeletal manipulation and intracellular survival. Structure activity relationship (SAR) analysis revealed that minimal steric hindrance, optimal electronic distribution, and hydrophilic–hydrophobic balance are critical determinants for high-affinity binding within the GAP domain. Penicillin derivatives with

bulky or polar substitutions such as Methicillin and Ampicillin exhibited suboptimal docking scores and reduced interaction networks, affirming that structural compactness and receptor compatibility are essential for therapeutic targeting of regulatory proteins. The findings provide computational justification for the continued clinical relevance of Penicillin G and establish a molecular rationale for its superior spirocheticidal activity. This work also paves the way for rational design of novel β-lactam scaffolds or hybrid molecules aimed at enhancing antisyphilitic efficacy by targeting non-traditional intracellular pathways. Future directions include molecular dynamics simulations, binding free energy calculations, and in vitro assays to validate these in silico findings and support translational drug development efforts for syphilis treatment.

Conflict of interest

None

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