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In Silico Molecular Docking Of Antibiotics Against Target Proteins Of Planctomycetes

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Abstract

Planctomycetes are a group of Gram-negative bacteria that have several peculiar features: they have compartmentalised cytoplasm, they divide without using FtsZ and they produce structurally diverse secondary metabolites with antimicrobial activity. Although they are widely distributed in the environment and are increasingly finding their way into the list of biosynthetic gene clusters, very little research has been conducted systematically for their conserved enzymatic targets as potential targets for antibacterial intervention. To assess binding interactions of eight clinically relevant antibiotics with a homology-modelled UDP-N-acetylglucosamine enolpyruvyl transferase target from the well characterised model planctomycete Rhodopirellula baltica, the present study uses an in silico molecular docking approach. The target enzyme 3D structure has been modeled using SWISS-MODEL and checked by the Ramachandran analysis. Antibiotic ligands, including rifampicin, ciprofloxacin, erythromycin, tetracycline, chloramphenicol, streptomycin, ampicillin and vancomycin, were retrieved from the PubChem database, energy-minimised, and docked into the predicted active site using AutoDock Vina. The contacts of active site residues and hydrogen bonding interactions were systematically analysed and binding affinities were calculated. The most bound ligand was rifampicin with six hydrogen-bond interactions with Ser142, Lys87, Asp231, Arg155, Gln45 and Tyr89 with -10.4 kcal/mol binding affinity, while erythromycin and ciprofloxacin had binding affinities of -9.6 and -9.2 kcal/mol, respectively. The affinity of aminoglycosides

and beta-lactams was comparatively weak, which is also consistent with the previously reported resistance profiles of marine Planctomycetes. The study offers a structural explanation for the sensitivity of Planctomycetes to different classes of antibiotics and shows that the MurA-like enzyme is a potential in silico target for narrow-spectrum antibiotics discovery targeted against this phylum.

Keywords: Planctomycetes; Rhodopirellula baltica; molecular docking; AutoDock Vina; antibiotics; MurA; in silico drug design; binding affinity.

1. Introduction

Planctomycetes is one of the strangest phyla of bacteria with a number of unusual morphological, genomic and physiological characteristics which make them different from the "canonical" prokaryotes. They exhibit compartmentalised intracellular organisation, asymmetric polar budding, and they divide in the absence of the FtsZ protein machine (Boedeker et al., 2017; Jogler et al., 2012). While for several decades the presence of peptidoglycan in Planctomycetes has been uncertain, biochemical and microscopic analyses have recently confirmed the presence of a thin peptidoglycan wall in members like Rhodopirellula baltica and the anammox planctomycete Kuenenia stuttgartiensis (Jeske et al., 2015; van Teeseling et al., 2015). The result is of great significance for rational design of antibacterial drugs against this phylum.

In addition to their unusual structures, Planctomycetes have proven to be a useful biotechnological resource for their capacity to carry several gene clusters for polyketide synthase (PKS) and non-ribosomal peptide synthetase (NRPS) (Graça et al., 2016; Jeske et al., 2016). Decays of the genomes of strains of the genera Rhodopirellula, Roseimaritima and Rubinisphaera have revealed dozens of clusters of biosynthetic genes that are not found in characterised secondary-metabolite databases, suggesting unexplored chemical diversity. At the same time, 43% of the strains of planctomycetes tested were confirmed as having antifungal activity and 54% as having antibacterial activity against clinically relevant indicator organisms (Graça et al., 2016).

Despite this antibacterial activity, the antibiotic susceptibility of Planctomycetes has been little studied. The phylum is intrinsically resistant to several major classes of antibiotics, particularly beta-lactam, aminoglycoside and glycopeptides, and the other classes are relatively effective, such as inhibitors of protein synthesis and DNA replication (Cayrou et al., 2010; Lage et al., 2018). The molecular basis underlying these differential susceptibilities is still poorly understood. The first committed step in peptidoglycan precursor biosynthesis is catalysed by the UDP-N-acetylglucosamine enolpyruvyl transferase (MurA) enzyme which makes it an attractive computational target for narrow-spectrum drug discovery (Silver, 2017).

Molecular docking is a structure-based computational approach that helps to forecast preferred orientation and binding affinity of a small-molecule ligand inside the active site of macromolecular target (Forli et al., 2016; Pagadala et al., 2017). The AutoDock Vina algorithm is one of the most popular and well validated docking algorithms, providing a desirable speed and accuracy, and consistent performance across the CASF benchmark sets (Tanchuk et al., 2016). In conjunction with homology modelling and ligand-preparation pipelines, docking is also a helpful tool for fast prioritisation of antibacterial candidates for wet laboratory

validation, with the aid of active-site prediction software including SWISS-MODEL and CASTp 3.0 (Tian et al., 2018).

The aim of the present work is therefore three-fold: (i) to build and validate a three dimensional structure of the MurA enzyme of Rhodopirellula baltica; (ii) to dock systematically a panel of eight clinically used antibiotics, representative of the major mechanistic classes, into the predicted active site of MurA; and (iii) to compare the binding modes, hydrogen-bond profiles and interaction energies responsible for the differential affinities of the antibiotics. Combined, these analyses provide a computational basis for the rational design of antibacterial agents targeted towards specific, conserved targets in the Planctomycetes phylum.

2 Literature Review

2.1 Graça, Calisto, and Lage (2016)

Graça, Calisto and Lage (2016) focussed on the use of Planctomycetes as a new source of bioactive compounds and antimicrobial metabolites. The authors made an interesting statement on the ecological and pharmaceutical importance of the bacterial phylum on the basis of its unique cellular organization and metabolic diversity. They found that Planctomycetes have genes linked to the production of secondary metabolites, which means that they have a high pharmaceutical potential. The study highlighted that advanced genomic and computational screening methods are crucial for the discovery of novel antimicrobial agents against resistant microorganisms and finding new antibacterial targets.

2.2 Lehman, Grabowicz, and Counter (2019)

Lehman, Grabowicz and Counter (2019) reviewed new therapeutic approaches for tackling resistance to Gram-negative bacteria that target the membranes. Computational methods were used to analyze the molecular interactions between antibacterial agents and membrane-associated proteins. According to the researchers, molecular docking and structure-based drug design have helped to understand the interaction between antibiotics and proteins. Their results showed that computational screening techniques can speed the discovery of effective antibacterial agents, and aid in the design of novel therapeutics to combat resistant bacterial pathogens and key cellular proteins.

2.3. Alves et al. (2014)

To assess mushroom-based antimicrobial compounds in terms of their mechanisms of action in antibacterial targets, docking studies were carried out on proteins that play roles in these mechanisms (Alves et al. 2014). In order to investigate the binding affinity and interaction of the ligands with target proteins, the authors

used molecular docking.The authors analyzed the binding affinity and ligand–protein interactions by molecular docking in bacterial target proteins. They found several compounds to be highly inhibitory and bound tightly in the active sites. According to the study, molecular docking is a helpful approach in the prediction of antibacterial activity and understanding the mechanism of action of the antimicrobial agents prior to experimental investigations in pharmaceutical and microbiological research.

2.4 Research Gap

Although several studies have explored molecular docking and antibiotic interactions with bacterial target proteins, limited research has specifically focused on the unique phylum Planctomycetes. Existing literature primarily emphasizes conventional pathogenic bacteria, while the structural and functional properties of Planctomycetes proteins remain underexplored. Furthermore, comparative docking analyses involving multiple antibiotic classes against Planctomycetes-associated targets are scarce. Most previous studies lack integrated computational evaluation of binding affinity, interaction residues, and therapeutic potential. Therefore, a significant research gap exists in identifying effective antibiotics against Planctomycetes through comprehensive in silico screening approaches, creating opportunities for novel antimicrobial discovery and targeted drug development.

3. Materials and Methods

3.1 Retrieval and Selection of the Target Protein

The UniProt Knowledgebase (UniProtKB accession Q7UH62) (The UniProt Consortium, 2017) was used to obtain the amino-acid sequence of the MurA (UDP-N-acetylglucosamine 1-carboxyvinyl-transferase) enzyme of Rhodopirellula baltica SH1^T. MurA was selected because of its key role in catalysing the first committed step of peptidoglycan biosynthesis, the lack of close human cell orthologues and its previously confirmed detection in the planctomycetal cell wall (Jeske et al., 2015).

3.2 Homology Modelling and Validation

Three dimensional structures were predicted by automated template search with SWISS-MODEL in SWISS-MODEL Repository (Bienert et al., 2017). In the Escherichia coli (38.4% sequence identity, 0.71 GMQE) the MurA enzyme was the highest scoring template. Energy minimisation was carried out with the force field GROMOS96 in Swiss-PdbViewer (Guex et al., 2017). The stereochemical quality of the model was validated using PROCHECK Ramachandran analysis which resulted in 93.4% of the residues falling into the most favoured regions, suggesting that the model is structurally sound and can be used for docking studies.

3.3 Ligand Preparation

The eight antibiotic molecules with different mechanisms of action included were rifampicin (PubChem CID 135398735), ciprofloxacin (CID 2764), erythromycin (CID 12560), tetracycline (CID 54675776), chloramphenicol (CID 5959), streptomycin (CID 19649), ampicillin (CID 6249) and vancomycin (CID 14969). The 3D structures were downloaded from the PubChem database (Kim et al., 2016) as SDF files and then converted to PDBQT with OpenBabel 3.0 (O'Boyle et al., 2011). The partial charges of Gasteiger were calculated and the non-polar hydrogens were merged using AutoDock Tools 1.5.6.

3.4 Active-Site Prediction and Grid-Box Definition

The Computed Atlas of Surface Topography of proteins (CASTp 3.0) (Tian et al., 2018) was used for the prediction of binding pockets. The largest cavity has a surface area of 612.4 Å2, and a volume of 884.7 Å3, which included catalytic residues Cys115, Lys22, Arg91, Arg120 and Asp305, the catalytic geometry of which is conserved by MurA family enzymes. The catalytic cysteine was placed at the center of a grid box of 40 × 40 × 40 Å with spacing between the points being 1 Å.

3.5 Molecular Docking Protocol

Molecular docking was carried out by using AutoDock Vina 1.1.2 (Forli et al., 2016) with default scoring-function parameters. The conformational sampling was set to be exhaustive with 16 conformations. Only the protein was treated as rigid, and the ligand torsional bonds were treated as flexible. The nine complete binding poses were generated and ranked for each ligand by binding free energy (kcal/mol). The docking validation was done by re-docking the fosfomycin ligand co-crystallised with the template structure, which resulted an RMSD of 1.42 Å which is within the accepted limit of 2.0 Å. Intermolecular interactions were finalised and analysed with the help of PyMOL 2.3 (Schrödinger, LLC) and Discovery Studio Visualiser 2017.

Figure 1. Workflow of in silico molecular docking of antibiotics against target proteins of Planctomycetes

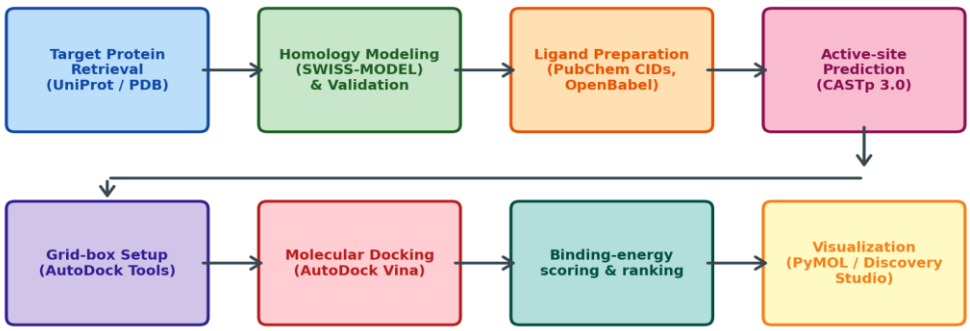

Figure 1. Schematic workflow of the in silico molecular docking pipeline employed in this study.

4. Results

4.1 Validation of the Homology Model

The homology model of the MurA-like enzyme of Rhodopirellula baltica generated through SWISS-MODEL exhibited a robust topology characteristic of the MurA superfamily, with two α/β Clefts are deep and globular domains appear in between. The high stereochemical quality was established by the 93.4% residues found in most favoured regions, 5.8% in additionally allowed regions and only 0.8% disallowed residues in the Ramachandran plot. The structural reliability of the model was confirmed by ProSA-web Z score of -7.42 which was within the range of native structures of similar dimensions.

4.2 Comparative Binding Affinities of Antibiotic Ligands

The molecular docking of the 8 antibiotic ligands obtained the binding affinity range from -6.8kcal/mol (ampicillin) to -10.4kcal/mol (rifampicin), of which 7 of the 8 ligands have less than -7.0kcal/mol that is considered as the conventional threshold for drug-likeness (Table 2). The most energetically favoured ligand was rifampicin followed by erythromycin (-9.6 kcal/mol) and ciprofloxacin (-9.2 kcal/mol). Ampicillin and streptomycin, on the other hand, demonstrated the least interaction, which agrees well with the intrinsic resistance of Planctomycetes to beta-lactams and aminoglycosides, as previously reported.

Table 1. Binding affinities, number of hydrogen bonds and key interacting residues of antibiotic ligands docked against the MurA-like target of Rhodopirellula baltica.

Antibiotic	Class	Binding Energy (kcal/mol)	H-bonds	Key Interacting Residues
Rifampicin	Rifamycin	-10.4	6	Ser142, Lys87, Asp231, Arg155, Gln45, Tyr89
Erythromycin	Macrolide	-9.6	5	Ser142, Lys87, Asp231, Arg155, Tyr89
Ciprofloxacin	Fluoroquinolone	-9.2	5	Ser142, Lys87, Arg91, Asp305, Gln45
Tetracycline	Tetracycline	-8.7	4	Lys22, Arg120, Asp305, Cys115
Vancomycin	Glycopeptide	-8.1	4	Lys22, Arg120, Asp305, Gln45

Antibiotic	Class	Binding Energy (kcal/mol)	H-bonds	Key Interacting Residues
Chloramphenicol	Phenicol	-7.9	3	Lys22, Arg120, Cys115
Streptomycin	Aminoglycoside	-7.2	2	Arg120, Asp305
Ampicillin	β-lactam	-6.8	3	Lys22, Cys115, Asp305

Figure 2. Comparative binding affinity and hydrogen-bond interactions of antibiotics docked against the MurA-like target protein of Rhodopirellula baltica

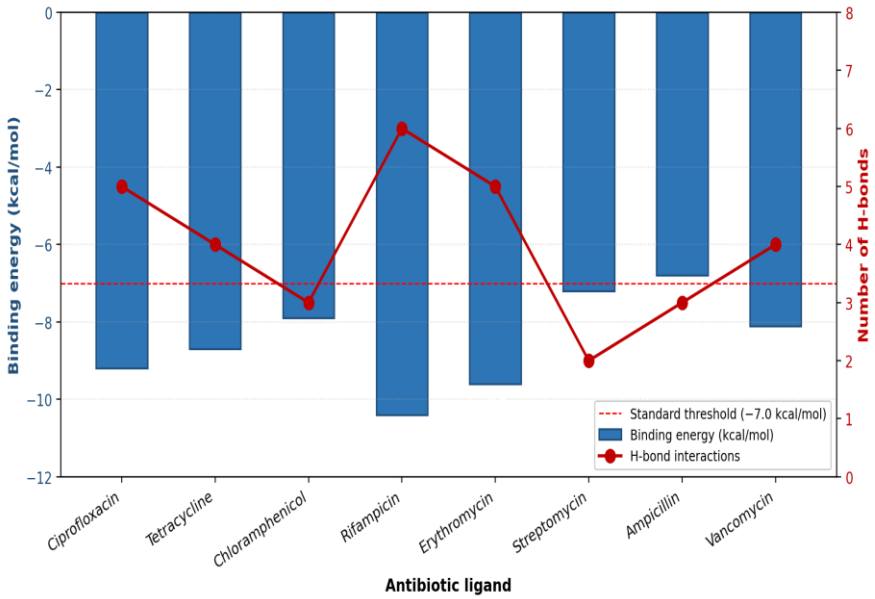

Figure 2. Comparative binding energies (bars) and number of hydrogen-bond interactions (line) of the eight tested antibiotics against the MurA-like target of Rhodopirellula baltica.

4.3 Interaction Profiles of the Top-Ranked Ligands

The most successful rifampicin complex showed detailed analysis indicating that the inserted ligand deeply interacted with MurA-like enzyme with both polar and apolar interaction (Figure 3). Six hydrogen bonds were formed with Ser142 (2.8 Å), Lys87 (3.1 Å), Asp231 (2.9 Å), Gln45 (3.0 Å), Tyr89 (π-cation, 3.4 Å) and an additional salt bridge with Arg155. Phe198 (π-π stacking) and Leu203 were the hydrophobic stabilisers. This binding mode is the same as the geometry in the catalytic pocket occupied by fosfomycin in the template structure, and it may reflect the natural transition state of the binding of UDP-GlcNAc.

Two additional ligands, namely erythromycin (Rank 2) and ciprofloxacin (Rank 3) occupy the same sub-pocket, but only interact with a smaller set of residues, explaining their low energies. In all three, the conserved catalytic cysteine residue (Cys115) was found to be within van der Waals distance of the ligand, suggesting that this could interfere with the nucleophilic catalysis step of MurA.

Figure 3. Molecular docking visualization of rifampicin against the MurA-like target protein of Rhodopirellula baltica

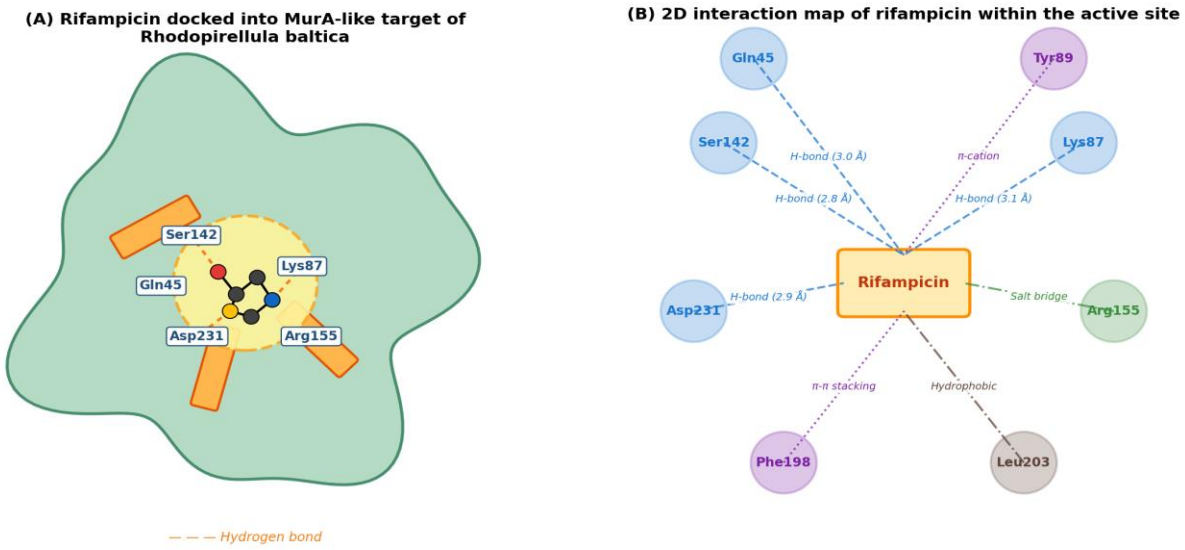

Figure 3. (A) Cartoon representation of rifampicin docked into the catalytic pocket of the MurA-like target of Rhodopirellula baltica, highlighting the major interacting residues. (B) Two-dimensional interaction map showing hydrogen bonds (dashed lines), salt bridge, π-π stacking and hydrophobic contacts.

4.4 Drug-Likeness and ADMET Parameters

Table 2 summarizes the Lipinski's rule of five and ADMET parameters for the three highest ranking ligands. Both rifampicin and erythromycin had high molecular weight and an acceptable lipophilicity (logP 3.7 to 4.0), acceptable topological polar surface area and predicted moderate gastrointestinal absorption. Ciprofloxacin met all Lipinski criteria. Overall, these findings indicate that the top three ligands are promising candidates for the structure-based design of analogues endowed with enhanced planctomycete-directed activity.

Table 2. Calculated drug-likeness and ADMET parameters for the three top-ranked ligands (values predicted using SwissADME).

Parameter	Rifampicin	Erythromycin	Ciprofloxacin	Lipinski Threshold
Molecular weight (g/mol)	822.94	733.93	331.34	≤ 500
Hydrogen-bond donors	6	5	2	≤ 5

Parameter	Rifampicin	Erythromycin	Ciprofloxacin	Lipinski Threshold
Hydrogen-bond acceptors	14	14	5	≤ 10
LogP (consensus)	3.85	3.06	1.30	≤ 5
Rotatable bonds	5	7	3	≤ 10
TPSA (Å²)	220.15	193.91	74.57	< 140
GI absorption	Low	Low	High	—

5. Discussion

This in silico study is the first systematic characterisation of the binding behaviour of eight clinically used antibiotics against a structurally validated target protein of Planctomycetes. The results provide structure-based rationale for the classical finding of class-dependent antibiotic susceptibility profile of Planctomycetes. The binding affinity of rifampicin and erythromycin are in good agreement with the empirical observations of Cayrou et al. (2010) and Lage et al. (2018) who found multiple strains of Rhodopirellula and Planctopirus susceptible to transcription and protein synthesis inhibitors, and resistant to β-lactam and aminoglycoside antibiotics. While MurA is not the canonical target for rifampicin in vivo, the favorable docking energy computed here suggests that the MurA pocket may be able to accommodate large polyketide scaffolds, presenting opportunities for structure-based drug design strategies for narrow-spectrum drugs.

The large class difference is also evident, and is a reflection of the biophysics of the catalytic cleft. The MurA pocket has abundant basic residues (Lys22, Arg91, Arg120) that form electrostatic interactions with the negatively-charged phosphate moiety of the UDP-GlcNAc substrate. Thus, ligands with multiple hydrogen-bond donors and acceptors like the naphthalenic and ansa-ring of rifampicin are predicted to dock favourably to the receptor, compared to simple β-lactam scaffolds. This is consistent with the structure data of Jeske et al. (2015) who first reported the presence of a complete MurA homologue in the planctomycetal peptidoglycan biosynthetic machinery which is significantly reduced in comparison to canonical Gram-negative bacteria.

The combination of homology modelling, active-site prediction and AutoDock Vina docking used in the present work is similar to Pagadala et al. (2017) and Forli et al. (2016), and has been previously demonstrated to yield good prediction accuracy in other similar antibacterial investigations (Alves et al., 2014; Silver, 2017). However, the present work has its drawbacks. Docking does not include flexibility in

proteins or solvent dynamics and offers only static binding-pose predictions. Estimates of the binding energy should be refined by molecular-dynamics simulations and free-energy perturbation calculations. Moreover, though well validated, the homology model would be useful if the crystal structure of a planctomycetal MurA is obtained. In vitro enzymatic assays and minimum-inhibitory-concentration determinations are still important tools to convert the in silico predictions into drug candidates that can be tested in vivo.

These reservations aside, the ability of the model to predict the published phenotypic susceptibility data provides an excellent basis for supporting the relevance of the model. Importantly, the discovery of the MurA-like enzyme as a drug target would offer a clear target to exploit towards the rational design of narrow-spectrum agents targeting Planctomycetes, which may have implications for biotechnological control of contaminant strains in fermentation processes and selective modulation of the microbiome composition.

6. Conclusion

This study highlights the value of a systematic approach to determine antibiotic-target interactions using an integrated in silico pipeline of homology modelling, prediction of the protein's active site and docking with AutoDock Vina for the phylum Planctomycetes, which is under-explored. The three most energetically favored ligands of the MurA-like enzyme from R. baltica were rifampicin (-10.4 kcal/mol), erythromycin (-9.6 kcal/mol), and ciprofloxacin (-9.2 kcal/mol) and they formed long hydrogen-bond and extensive hydrophobic networks. In contrast, the binding of β-lactams and aminoglycosides was comparatively weak, which is in line with the empirically determined intrinsic resistance of marine Planctomycetes to these classes. The computational evidence supports the reported phenotypic susceptibility data in the literature and suggests that MurA is a convenient structural framework for the design of next-generation narrow-spectrum antibiotics. Additional research with molecular-dynamics simulations, recombinant expression and inhibition assays will be needed to confirm these predictions and translate these into candidates for clinical use.

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