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Original Article

IDENTIFICATION OF NOVEL INHIBITORS AGAINST POTENTIAL TARGETS OF *CAMPYLOBACTER JEJUNI*

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ABSTRACT

Objective: The aim of the present study is the structure identification of UDP-N-acetyl muramate dehydrogenase and 4-hydroxy-3-methylbut-2-enyl diphosphate reductase for *Campylobacter jejuni* and designing their inhibitors using docking and simulation studies.

Methods: Uniprot, BLAST P, Discovery Studio, Verify 3D and Maestro Schrödinger suit have been used for structure identification, validation and docking studies.

Results: The structures of UDP-N-acetylmuramic dehydrogenase and 4-hydroxy-3-methylbut-2-enyl diphosphate reductase were predicted and validated generating 87.80% and 85.82% score respectively. For 4-hydroxy-3-methylbut-2-enyl diphosphate reductase, HTVS resulted in 5801 compounds while SP and XP resulted in 5781 ligands. For UDP-N-acetylmuramate dehydrogenase, HTVS resulted in 5474 compounds whereas SP and XP resulted in 5359 ligands.

Conclusion: The structures of UDP-N-acetylmuramate dehydrogenase and 4-hydroxy-3-methylbut-2-enyl diphosphate reductase were detected and verified. The list of top 10 inhibitors was acquired that can be considered as putative and potential drug targets.

Keywords: *Campylobacter jejuni*, Structure prediction, Active site, Docking, Inhibitor.

INTRODUCTION

Campylobacter jejuni is a gram negative pathogenic microorganism that is the principal instigator of diseases such as Campylobacteriosis which might lead to autoimmune disease such as Guillan–Barré syndrome [1]. It results in gastrointestinal ailments, disability, diarrhea, abdominal cramps, fever and can even lead to death. The infections caused by *Campylobacter* are increasing at a tremendous rate worldwide [2]. Its resistance to certain vital antibiotics has arisen as an acute public health matter [3]. Therefore, the need to identify novel and potential drug targets of *C. jejuni* has arisen. The availability of completed genome sequence of *C. jejuni* has bestowed the opportunity for utilizing it in order to identify prospective therapeutic targets [4]. Nowadays, gene essentiality has become a prominent benchmark for identi fication and prioritization of drug targets [5]. *In silico* analysis using the subtractive genomics approach for identification of essential genes facilitates in reducing any undesirable impact on host biology. The amalgamation of computational and experimental studies for gene essentiality prediction has led to progression in the identification of potent drug targets by reducing the time and resource expense. Several studies have shown certain genes and proteins to be essential in *C. jejuni* using the subtractive genomics approach that can be considered as putative drug targets [6, 7]. One such protein is 4-hydroxy-3-methylbut-2-enyl diphosphate reductase encoded by is pH gene [6]. It is involved in the metabolism of terpenoid and polyketide. Another protein UDP-N-acetylmuramate dehydrogenase encoded by murB gene is involved in carbohydrate metabolism and glycan biosynthesis and metabolism [6]. The identification of 3D crystal structure of UDP-N-acetylmuramate dehydrogenase and 4-hydroxy-3-methylbut-2-enyl diphosphate reductase for *Campylobacter jejuni* will aid stipulating novel prospects for inventing potent inhibitors [8].

In this study, structure identification and docking studies of UDP-N-acetylmuramate dehydrogenase and 4-hydroxy-3-methylbut-2-enyl diphosphate reductase for *Campylobacter jejuni* has been carried out for uncovering inhibitors preceding to the identification of novel drug targets.

MATERIALS AND METHODS

Retrieval of protein targets sequences

4-hydroxy-3-methylbut-2-enyl diphosphate reductase and UDP-N-acetylmuramate dehydrogenase are enzymes encoded by ispH gene and murB gene, found to be essential in *Campylobacter jejuni* [7]. In this study, the structural analysis and identification of inhibitors of these proteins has been done in order to decipher putative drug targets (fig. 1). The protein sequences of 4-hydroxy-3-methylbut-2-enyl diphosphate reductase (Q5HUR4) and UDP-N-acetylmuramate dehydrogenase (Q5HSB7) in *Campylobacter jejuni* were retrieved from Uniprot.

Protein structure identification

Further structure prediction studies were performed for the retrieved protein sequences. The protein structure identification was implemented using Discovery Studio [9]. The structural validation of the model was executed using Verify 3D (UCLA DOE) [10] in order to verify the accuracy of the overall fold/structure, certain stereochemical parameters and the errors present over the localized areas.

Preprocessing of target and active site prediction

The structures of 4-hydroxy-3-methylbut-2-enyl diphosphate reductase and UDP-N-acetylmuramate dehydrogenase were processed using the Protein Preparation Wizard (Prep Wizard) of the Maestro Schrödinger suite that is a flexible molecular modeling environment for state-of-the-art chemical simulation analysis. Processing of the protein is necessary in order to convert the protein from its raw state into a state that can be used for further calculations using Maestro Schrödinger suite by assigning bond orders, adding hydrogen atoms, creating disulfide bonds, calculating the protonation states of all ionizable groups and optimizing the orientation of hydroxyl groups, Asn, Gln and His residues [11, 12]. The active sites of the proteins were detected by performing an extensive literature survey that was used further for grid generation [13-15].

Grid generation and ligand preparation

A docking grid was generated using the Receptor Grid Generation of Schrödinger Suite incorporating the active sites obtained previously using the centroid of selected residues. The default values of the scaling factor and partial charge cut off were taken [16]. The ZINC Drug Database (Zdd) was downloaded in the sdf format. Ligand preparation was done using the Ligprep from the Schrödinger suite [17].

High throughput virtual screening (HTVS), Standard Precision (SP) and Xtra Precision (XP) docking

Docking studies were performed employing the *ZINC Drug Database* based on the active sites. The compounds were exposed to the Glide centered docking strategy in which the compounds were docked incorporating three stages which are High Throughput Virtual Screening (HTVS), Standard Precision (SP) and Xtra Precision (XP) [18]. Docking analysis lowered the low-energy conformers by the means of the docking filters and reduced the sampling and thoroughness of the ligand conformers. Glide module evaluated specific interactions, which are the ligand-protein interaction energies, hydrophobic interactions, hydrogen bonds, internal energy, π-π stacking interactions and root mean square deviation (RMSD) by reducing the number of false positives. Docking of the lead compounds was done in order to identify the probable ligands or protein inhibitors [19].

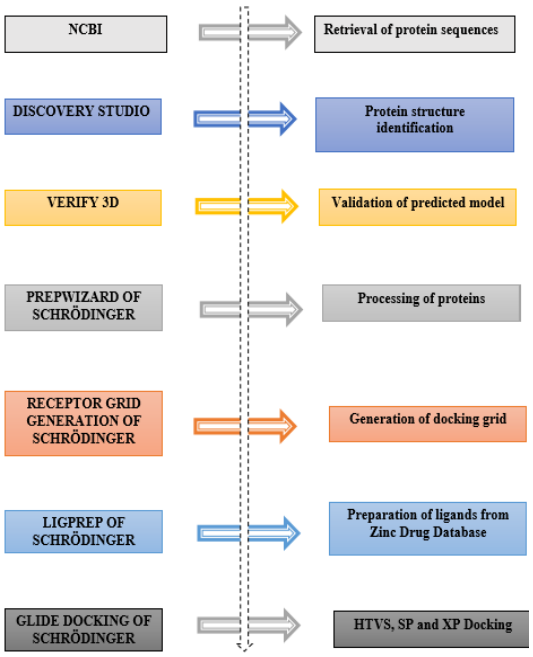


Fig. 1: Schematic workflow for the identification of inhibitors of 4-hydroxy-3-methylbut-2 enyl diphosphate reductase and UDP-N-acetylmuramate dehydrogenase of *Campylobacter jejuni*

Analysis and visualization of docking results

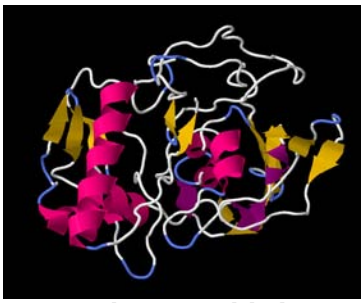
After performing the docking studies, the top 10 inhibitors were selected according to the docking score. The 3D chemical structures of these inhibitors were visualized using J mol interactive viewer [20].

RESULTS AND DISCUSSION

UDP-N-acetylmuramate dehydrogenase yielded 44% sequence identity with 1UXY_A (Chain A, Murb Mutant With Ser 229 Replaced By Ala, Complex With Enolpyruvyl-Udp-N-Acetylglucosamine) and 4-hydroxy-3-methylbut-2 enyl diphosphate reductase yielded 45% sequence identity with 3DNF_A (Chain A, Structure Of (e)-4-hydroxy-3-methyl-but-2-enyl Diphosphate Reductase, The Terminal Enzyme Of The Non-mevalonate Pathway. The structures were predicted using Discovery Studio [21]. These were visualized using J mols that are represented in fig. 2. The required score for protein models to be validated by Verify 3D is atleast 80% [22]. Verify 3D resulted in an 87.80% score for UDP-N-acetylmuramate dehydrogenase and 85.82% score for 4-hydroxy-3-methylbut-2 enyl diphosphate reductase (table 1). Proteins were prepared for further analysis using the Protein Preparation Wizard (Prep Wizard) of the Maestro Schrödinger suite [23, 24] by assigning the bond orders to the workspace and skipping residues with existing double and triple bonds. None of the bond orders were changed. Hydrogens were added to the Workspace structure, and metals were treated. Disulfide bonds were created. Waters were deleted farther than 5 angstroms from hets Optimization of H-bonds was performed. Certain parameters were set such as Water sampling: on, Minimize hydrogens of altered species: off and Use crystal symmetry: off. PROPKA with pH: 7.0 were used. The restrained minimization job was launched at RMSD: 0.3. The active sites of the proteins are represented in table 1. A receptor grid was generated using the active sites obtained from the literature available for further docking analysis (fig. 3) [25]. Zinc Drug Database (Zdd) is the assembly of all drugs approved for use and are commercially accessible as pure compounds. The size of this database is 3001. The ligands were prepared for molecular docking by assessing them through their pKa values, charges, tautomerization and their chiralities [26] against the Zdd. This database generated 6289 ligands in the mae format (table 2). For 4-hydroxy-3-methylbut-2-enyl diphosphate reductase, HTVS resulted in 5801 compounds. SP docking filtered out 5781 ligands and XP in 5781 ligands [27, 28]. For UDP-N-acetylmuramate dehydrogenase, HTVS resulted in 5474 compounds, SP in 5359 ligands and XP in 5359 ligands. The list of top 10 hits is provided in table 3 and 4 according to the docking score. These inhibitors can be used for identification of the ADME/Tox properties or pharmacophore modeling in future [29].

Table 1: Structure prediction, verification and active site prediction of 4-hydroxy-3-methylbut-2 enyl diphosphate reductase and UDP-N-acetylmuramate dehydrogenase of *Campylobacter jejuni*

Protein name	Gene name	Sequence identity (%)	Template sequence (PDB ID)	Verify 3D score	Active site residues
UDP-N-acetylmuramate dehydrogenase	murB	44%	1UXY_A	87.80%	142–142, 184–184, 254–254, 229-229, 325-325, 159-159
4-hydroxy-3-methylbut-2-enyl diphosphate reductase	ispH, lytB	45%	3DNF_A	85.82%	12–12, 41-41, 74-74, 96-96, 124-124, 167-167, 197-197, 269-269



UDP-N-acetylmuramate dehydrogenase

4-hydroxy-3-methylbut-2-enyl diphosphate reductase

Fig. 2: Predicted structures of protein sequences obtained from discovery studio

Table 2: Docking studies performed on 4-hydroxy-3-methylbut-2 enyl diphosphate reductase and UDP-N-acetylmuramate dehydrogenase of *Campylobacter jejuni*

Proteins	HTVS	SP	XP
4-hydroxy-3-methylbut-2-enyl diphosphate reductase	5801 ligands	5781 ligands	5781 ligands
UDP-N-acetylmuramate dehydrogenase	5474 ligands	5359 ligands	5359 ligands

Table 3: Top 10 hits (inhibitors) obtained after docking for 4-hydroxy-3-methylbut-2-enyl diphosphate reductase

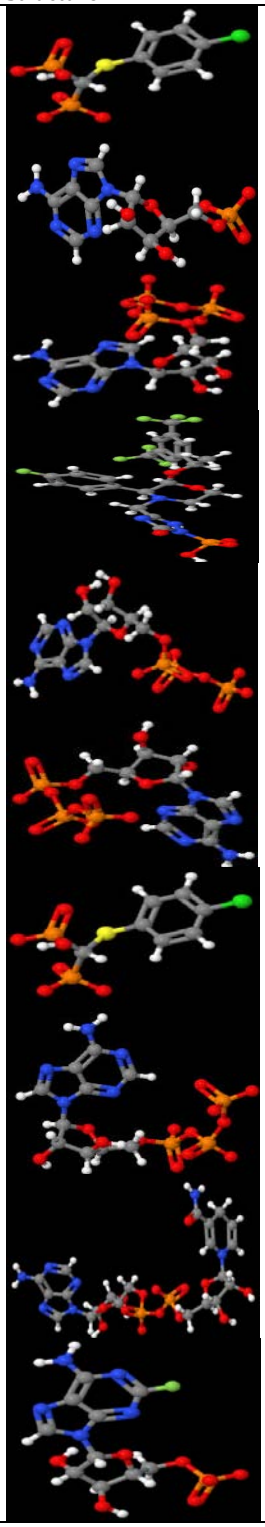
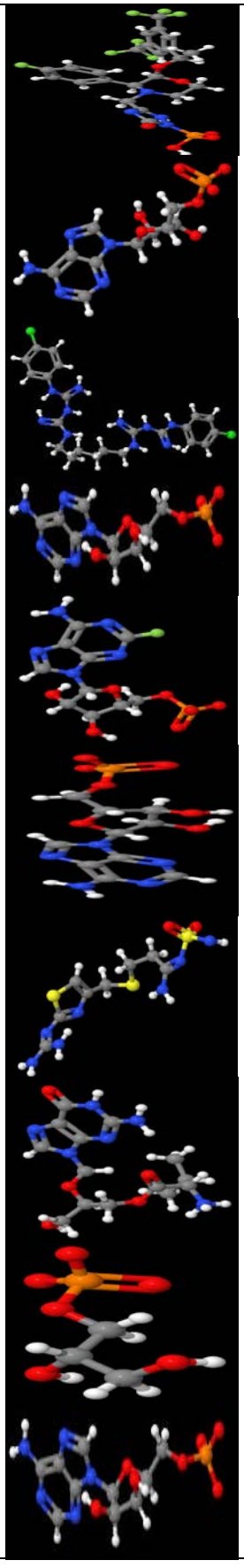
Ligand name	Docking score	Structure
ZINC22066984	-7.293	
ZINC01571045	-7.258	
ZINC03871613	-7.215	
ZINC28240499	-7.108	
ZINC18456332	-7.011	
ZINC03871615	-6.937	
ZINC22066984	-6.836	
ZINC03871614	-6.742	
ZINC53682927	-6.710	
ZINC03927870	-6.695	

Table 4: Top 10 hits (inhibitors) obtained after docking for UDP-N-acetylmuramate dehydrogenase

Ligand name	Docking score	Structure
ZINC28240499	-8.799	
ZINC02126310	-8.193	
ZINC18098320	-8.021	
ZINC03830180	-7.807	
ZINC03927870	-7.800	
ZINC03860156	-7.743	
ZINC12503222	-7.684	
ZINC01543916	-7.630	
ZINC03830895	-7.600	
ZINC03830180	-7.594	

UDP-N-acetylmuramate dehydrogenase **4-hydroxy-3-methylbut-2-enyl diphosphate reductase**

Fig. 3: Receptor grids generated using Schrödinger suite

CONCLUSION

Campylobacter jejuni leads to various infectious diseases such Campylobacteriosis. It might lead to autoimmune disorders such as the Guillan–Barré syndrome that can be fatal also. These infections are rising as a public health concern worldwide. Certain essential proteins such as 4-hydroxy-3-methylbut-2-enyl diphosphate reductase encoded by ispH gene and UDP-N-acetylmuramate de-hydrogenase encoded by murB gene are regarded as putative drug targets. Therefore, in order to decipher their inhibitors, structure prediction and docking of UDP-N-acetylmuramate dehydrogenase and 4-hydroxy-3-methylbut-2-enyl diphosphate against the ZINC Drug Database was carried out using the Discovery Studio and Maestro Schrödinger suite. For 4-hydroxy-3-methylbut-2-enyl diphosphate reductase, HTVS resulted in 5801 compounds. SP and XP resulted in 5781 ligands. For UDP-N-acetylmuramate dehydrogenase, HTVS resulted in 5474 compounds, whereas SP and XP resulted in 5359 ligands. The list of top 10 inhibitors was acquired that can be considered for further analysis such as the identification of the ADME/Tox properties, pharmacophore modeling or experimental studies towards the judicious construction of antibacterial drugs.

CONFLICT OF INTERESTS

Declare none

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