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In Silico Molecular Modeling and Docking Analysis of SARS-CoV-2 Helicase (YP_009725308) as Drug Target

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Abstract

Background: The ongoing outbreak of COVID-19 has become a global health emergency. The SARS-CoV-2 helicase (nsp13) play an important role in SARS-CoV-2 replication and could be serve as a target for antivirals to develop potential COVID-19 treatment. The objective of study is use Homology modeling and docking analysis of SARS-CoV-2 helicase (YP_009725308) as drug target.

Material and Methods: The structure and function of SARS-CoV-2 helicase (YP_009725308) predicted by *in silico* modeling studies. The SWISS-MODEL Structure Assessment tool was used for homology modeling and visual analysis of crystal structure of protein. The validation for structure models was performed by using PROCHECK. Model quality estimates based on the QMEAN and ProSA. The MCULE-1-Click docking, and InterEvDock-2.0 server were used for protein–ligand docking.

Results: The SARS-CoV-2 helicase (YP_009725308) model corresponding to probability conformation with 90.9% residue of core section that specifies accuracy of predicted model. The ProSA Z-score score -9.17; indicates the good quality of the model. Inhibitor N-[3-(carbamoylamino) phenyl] acetamide exhibited effective binding affinity against helicase (YP_009725308). The docking results revealed the Lys-146, Leu-147, Ile-151, Tyr-185, Lys-195, Tyr-224, Val-226, Leu-227, Ser-229 residues exhibit good binding interactions with inhibitor ligand N-[3-(carbamoyl amino) phenyl] acetamide.

Conclusion: Hence, the proposed inhibitor could potently inhibit SARS-CoV-2 helicase (YP_009725308), that recognized to play key roles during replication of viral RNAs. Overall findings demonstrate the SARS-CoV-2 helicase (nsp13) serve as a target for antivirals to cure COVID-19.

Keywords: SARS-CoV-2, COVID-19, Helicase, Homology Modeling, Docking

Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of the ongoing COVID-19 (coronavirus disease 2019) global public health emergency. Globally, 32,62,79,424 infections have been confirmed with 55,36,609 fatalities till 17th May 2022 [1, 2]. The novel coronavirus was first reported from Wuhan city, Hubei province of China in late 2019 [3,4,5]. The SARS-CoV-2 has been spread worldwide [6,7,8] within last one year. Angus Dalgleish and Birger Sorensen has claimed that the SARS-CoV-2 did not develop naturally, possibly developed artificially in Wuhan lab by reverse-engineering [9].

MERS-CoV and SARS-CoV-2 belong to the genus Beta coronavirus and family of *Coronaviridae*. Coronaviruses are enveloped, single-stranded RNA

viruses that RNA genomes size ranging approximately from 26 to 32 kb [10, 11, 12] consist at least 6 (14 in case of SARS-CoV-2) open reading frames (ORFs) [13, 14]. The first genome sequence of SARS-CoV-2 deposited in NCBI-Genbank was reported from Wuhan-Hu-1 (MN908947) as the ~30 kb isolate [15] which is used for sequence-related analyses in this article.

The ongoing outbreak of SARS-CoV-2 has caused tremendous losses in human lives and economy around the world, while there is no approved drug available for this virus. The current COVID-19 pandemic has prompted worldwide efforts for the rapid identification and development of vaccines and effective antiviral treatments [16, 17]. The following anti-coronaviral targets consider for research in past include, the main protease, spike protein (S), RNA-dependent RNA-polymerase (RdRp, nsp12), NTPase/helicase (nsp13) and papain-like protease (PLpro, part of nsp3) [18, 19, 20].

The SARS-CoV-2 nsp13 possesses the NTPase and RNA helicase activities and shown to be conserved [21]. The NTPase and helicase activities of SARS-CoV-2 nsp13, which may play an important role in SARS-CoV-2 replication and could be serve as a target for antivirals [20]. The study viewpoint is to present the potential application of inhibitors against SARS-CoV-2 helicase (YP_009725308).

Materials And Methods

Sequence retrieval and Homology Modeling

The amino acid sequence of SARS-CoV-2 helicase [YP_009725308] was retrieved from NCBI (601 aa) in FASTA format [22]. PSI-BLAST was used for alignment against Protein Databank (PDB) for suitable template search with query sequence [23]. The template with query sequence (PDB ID: 5WWP_A) was selected having 72.20% identity score and E value 0.00. The SWISS-MODEL (https://swissmodel.expasy.org) Structure Assessment tool was used for homology modelling and visual analysis of crystal structure of protein [24].

Model Evaluation

PROCHECK server used for validation of structure model [25, 26] and its results suggesting reliability of model [27] The overall G-factor, residue positions in φ-ψ plot regions analysis were used for the selection of suitable model. The quality of 3D model was verified by ERRAT, Verify3D and ANOLEA tools. ERRAT plot indicates the overall quality of model [28].

The protein stability was analysed by using SWISS-MODEL QMEAN (version 4.2.0) [29] and ProSA Z-score. ProSA (Protein Structure Analysis) [30] calculated an overall quality score of the predicted structure.

Molecular Docking

Molecular docking techniques dock small molecules into the protein binding site [31]. The MCULE-1-Click docking (https://mcule.com) and InterEvDock 2.0 (https://bioserv.rpbs.univ-paris-diderot.fr/) programs were used for docking calculations [32]. 1-Click docking is an online server for drug discovery platform performs docking [33].

Results

Protein Model Building: The alignment between target and template was performed by using PSI-BLAST [22]. The 3D ribbon model of SARS-CoV-2 helicase [YP_009725308] generated using SWISS-MODEL (https://swissmodel.expasy.org) Structure Assessment tool (Fig 1).

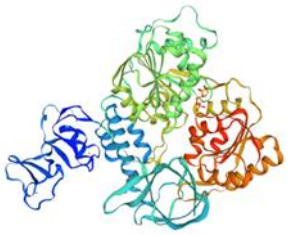

Fig 1. 3D ribbon structure model of helicase [YP_009725308]

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Model Reputation: SARS-CoV-2 helicase [YP_009725308] model corresponding to probability conformation with 90.9% residue of core section, 8.5% of allowed section and 0.4 % residue of outer section in φ-ψ plot [34] (Fig 2a, b). The above results indicate reliability of protein models (Table 1) [35, 36].

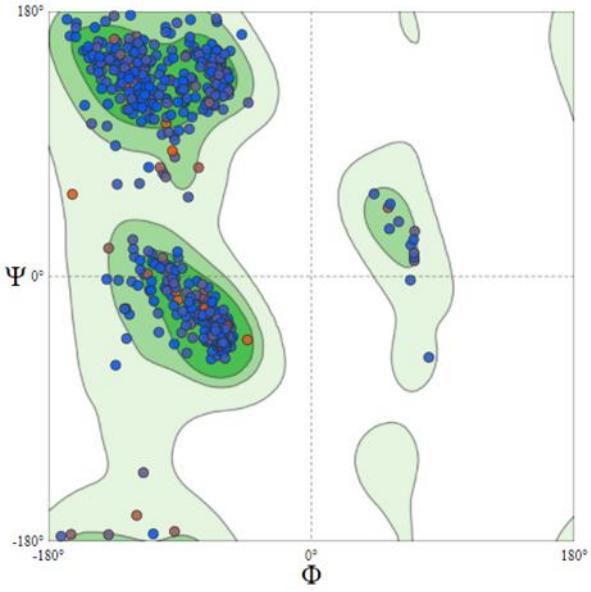

Fig 2. (a) The φ-ψ plot of SARS-CoV-2 helicase [YP_009725308]. Total number of residues were 90.9% in core, 8.5% in allowed and, 0.4 % in generously allowed regions; (b) φ-ψ plot showing separate Φ and Ψ distributions of residues in Glycine, Proline and Pre-Proline.

The verify-3D illustrates the compatibility of an atomic model (3D) with its own amino acid sequence (1D) by assigning a structural class based on its location and environment (alpha, beta, loop, polar, and nonpolar) [37]. ANOLEA recorded non-local energy of the helicase (E/kT units) was -6028 with Z-score -0.35. These scores indicate good quality of model (Table 1).

Table 1. Evaluation results of Model by PROCHECK, VERIFY3D and ANOLEA

Template	**PROCHECK**				**Verify-3D**	**ANOLEA**
YP_009725308	Core	Allowed	Generously outer	Disallowed	3D-ID Score	Z-Score
	90.9 %	8.5%	0.4 %	0.2 %	95.9	-0.35

ERRAT analyses the statistics of non-bonded interactions between different atom types and plots the value of the error function of versus position, that calculated by a comparison with statistics from highly refined structures [38]. ERRAT overall quality factor of model as 93.5829 with average probability value 5.05729 (Fig 3).

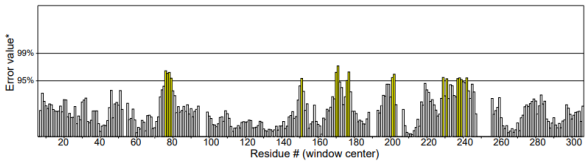

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Fig 3. ERRAT result showing an overall quality factor (93.5829) of model (error-axis showing the error values to reject regions that exceed error value).

Validation of Model: ProSA was used to figure out potential errors in 3D model of SARS-CoV-2 helicase [YP_009725308]. The archived ProSA Z-score score -9.17 indicates two aspects: overall model quality and energy deviation (Fig 4a, b).

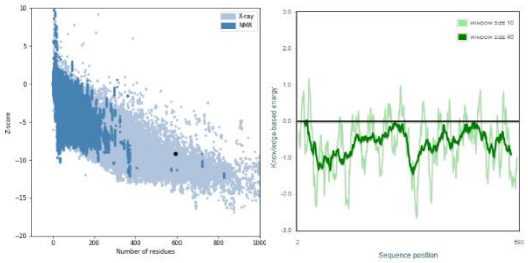

Fig 4. (a) ProSA examination of SARS-CoV-2 helicase [YP_009725308] overall model quality. Blue dot in plot showing the -9.17 z-score of predicted models; (b) Residue score plot showing energies of amino acids are less than zero that represent good local model quality.

The QMEAN4 value -0.99 observed for helicase [YP_009725308], that is very close to 0 and its illustrations acceptable values [42]. Assessed validity of model predictable among 0 and 1, that could be concluded from the density plot locus set for QMEAN score (Fig 5a, b). Figure 5 illustrations QMEAN scores for biological unit reference set that used as a tool for oligomeric protein assessment.

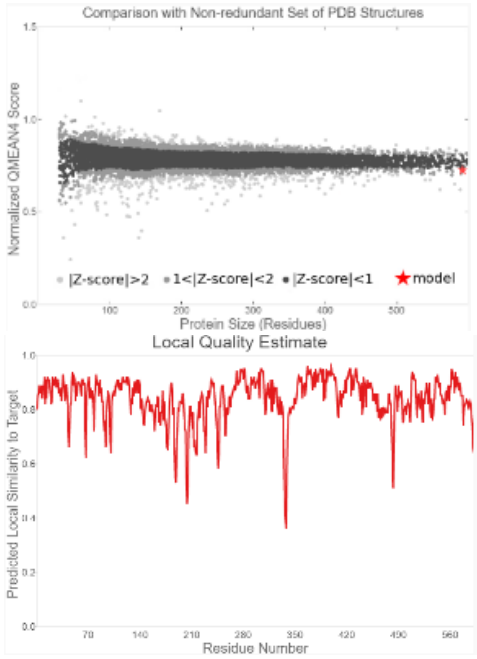

Fig 5. QMEAN scores for biological unit reference set of SARS-CoV-2 helicase [YP_009725308]. Plot showing Z-score (a). Local quality model for estimation of local summarily to target (b).

Molecular Docking

The binding pockets of SARS-CoV-2 helicase [YP_009725308] is not reported yet. So, the *in-silico* approaches utilized for the prediction of binding pockets using *in silico* docking study. MCULE-1-Click docking (https://mcule.com) and InterEvDock-2.0 server were employed to explore the binding of ligand to the respective protein. The top five docking models of binding pockets of SARS-CoV-2 helicase [YP_009725308] were identified which ranked based on the energy. More negative docking score indicate higher binding affinity (Table 2).

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The summary table contains two rows: the ranks and docking energy scores from the input structures. The Model 1 has a high accuracy with an interface docking score of -5.940 from the crystal structure (Table 2).

Table 2. Summary of the Top 05 Models. Row 1 (ranks of the models), Row 2 (docking energy scores)

Rank	1	2	3	4	5
Docking Score	-5.940	-5.920	-5.790	-5.440	-5.430

The binding pocket and interacting residues of selected inhibitor (N-[3-(carbamoyl amino) phenyl] acetamide) was analysed in 3D using both the server (Fig 6, 7). The binding residues of the cavities explored for fruitful binding of novel ligands. The energy range of predicted cavities also elaborates the efficacy of pockets. The mutational study of binding residues suggested that these residues could be used as clinical prospectus against effective treatment of COVID-19. The predicted binding residues lead to the drug designing of lead compounds against SARS-CoV-2 helicase [YP_009725308].

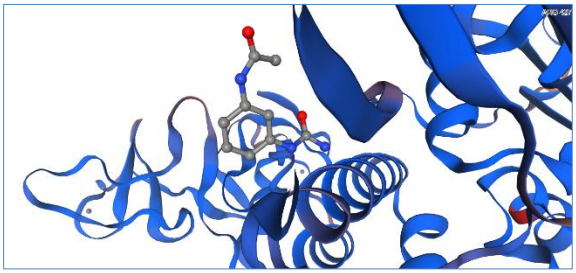

Fig 6. Binding pocket and interacting residues of analysed inhibitor (N-[3-(carbamoyl amino) phenyl] acetamide) using 1-Click docking server

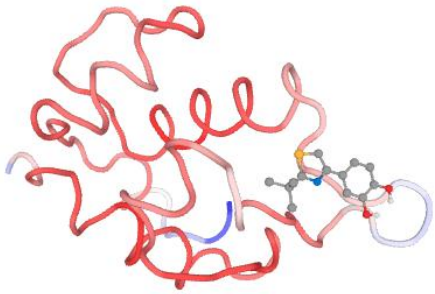

Fig 7. Binding pocket and interacting residues of analysed inhibitor (N-[3-(carbamoyl amino) phenyl] acetamide) using InterEvDock-2.0 server

The top five binding pockets of SARS-CoV-2 helicase [YP_009725308] enzyme was identified which ranked based on energy. The volume of the binding pockets was also analysed in 3D dimension (Table 3).

Table 3. Top five binding pockets with energy and dimension

Rank	**Energy (Kcal/mol)**	**Center**		
		X-axis	**Y-axis**	**Z-axis**
1	-5.940	46.48	13.16	25.11

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		20	00	90
2	-5.920	45.85 00	14.41 80	25.03 10
3	-5.790	45.47 30	15.13 20	26.19 20
4	-5.440	46.73 90	12.57 80	26.38 80
5	-5.430	46.34 40	13.28 50	27.54 60

The retrieved ligand N-[3-(carbamoylamino) phenyl] acetamide [PubChem CID: 828139] (Fig 7) for SARS-CoV-2 helicase [YP_009725308] is described in Table 4. The docking results revealed the Lys-146, Leu-147, Ile-151, Tyr-185, Lys-195, Tyr-224, Val-226, Leu-227, Ser-229 residues exhibit good binding interactions with inhibitor and mutational studies of these residues could be highly effective in further studies.

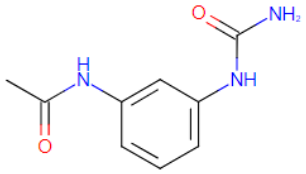

Fig 7. Structures of selected inhibitor N-[3-(carbamoyl amino) phenyl] acetamide

Table 4. Inhibitor N-[3-(carbamoyl amino) phenyl] acetamide (PubChem CID: 828139) properties and binding residues

Ligand Properties	$C_9 H_{11} N_3 O_2$
Molecular Weight (g/mol)	193.203
Component type	Non-polymer
Hydrogen Bond Donor Count	03
Hydrogen Bond Donor Count	02
Rotatable Bonds Count	02
Topological Polar Surface Area	84.2 Å²
Heavy Atom Count	14
Formal Charge	0
Interacting residues	Lys-146, Leu-147, Ile-151, Tyr-185,

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	Lys-195, Tyr-224, Val-226, Leu-227, Ser-229

It was observed that, the inhibitor binds at the binding residues between Lys-146 to Ser-229 (Table 4). Predicted structural and docking model described in this study may be further used for finding interactions with other SARS-CoV-2 enzymes to identify new anti-coronaviral targets.

Discussion

The SARS-CoV-2 helicase (YP_009725308) model corresponding to probability conformation with 90.9% residue of core section that specifies accuracy of prediction model. In verify-3D graph, 95.95% of the residues have averaged 3D-1D score >= 0.2 that illustrate the results of good structures. ANOLEA recorded non-local energy of the helicase (E/kT units) was -6028 with Z-score -0.35. These scores indicate good quality of model (Table 1). ERRAT overall quality factor of model as 93.5829 with average probability value 5.05729, which indicates much reliable and satisfactory model (Fig 3). The archived ProSA Z-score score -9.17 indicates two aspects: overall model quality and energy deviation (Fig 4a, b). The values of Z-score indicates less erroneous structures [39, 40]. Reliability of projected model based on scoring function of QMEAN that stated as 'Z-score' (Fig 5a, b) [41].

Assessed validity of model predictable among 0 and 1, that could be concluded from the density plot locus set for QMEAN score (Fig 5a, b). Fig 5 illustrations QMEAN scores for biological unit reference set that used as a tool for oligomeric protein assessment. The QMEAN value comparison with the non-redundant protein collection revealed different set of Z-values for different parameters. The diversion of total energy of helicase [YP_009725308] was measured by using Z-score [43].

Inhibitor N-[3-(carbamoylamino) phenyl] acetamide exhibited effective binding affinity against helicase (YP_009725308). The docking results revealed the Lys-146, Leu-147, Ile-151, Tyr-185, Lys-195, Tyr-224, Val-226, Leu-227, Ser-229 residues exhibit good binding interactions with inhibitor ligand N-[3-(carbamoyl amino) phenyl] acetamide.

Conclusion

The present study extends knowledge about one of the key replicative enzymes of SARS-CoV-2 and suggests that SARS-CoV-2 nsp13 can be a valuable target for antivirals, which may be helpful in the efforts to control this life-threatening virus.

The SARS-CoV-2 helicase (YP_009725308) model corresponding to probability conformation with 90.9% residue of core section that specifies accuracy of prediction model. The ProSA Z-score score -9.17; indicates the good quality of the model. Inhibitor N-[3-(carbamoylamino) phenyl] acetamide exhibited effective binding affinity against helicase (YP_009725308). The docking results revealed the Lys-146, Leu-147, Ile-151, Tyr-185, Lys-195, Tyr-224, Val-226, Leu-227, Ser-229 residues exhibit good binding interactions with inhibitor ligand N-[3-(carbamoyl amino) phenyl] acetamide. The predicted binding residues serve as base for drug designing of lead compounds against SARS-CoV-2 helicase, that recognized to play key roles during replication of viral RNAs.

Authors' Contributions

Data collection, interpretation and drafting of manuscript: Rajneesh Prajapat

Concept design of project and editing of article: Suman Jain and Sandeep Bhatnagar

Ethical Issues

The research project is the part of Ph.D research work. Research project approved by the ethics committee of Pacific Institute of Medical Sciences, Sai Tirupati University, Udaipur- 313003, Rajasthan, INDIA.

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