

Research Article

Homology Modelling and Molecular Docking Studies to Discover Potent Inhibitors of CD169 in PRRSV Infection

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Abstract

CD169 is one of the putative receptors of porcine reproductive and respiratory syndrome virus, also plays a major role in PRRSV infection. Computational methods including, homology modelling, molecular docking analysis and molecular dynamics simulations carried out to investigate 3D structure and potent inhibitors of CD16. Homology modelling and molecular docking were done by Maestro 10.6. A 3D structure of CD169 was obtained through homology modelling. It was later subjected protein-ligand interaction by molecular docking study. The docking results showed top ten hits compounds with the docking score energies, among those compounds MOL002433 (3R,8S,9R,10R,13R,14S,17R)-3-hydroxy-4,4,9,13,14-pentamethyl-17-[(E,2R)-6-methyl-7-[(2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-[(2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxymethyl]oxan-2-yl]oxyhept-5-en-2-yl]-1,2,3,7,8,10,12,15,16,17-decahydr) had the best docking score energy -8.095 kcal/mol and showed significant binding affinity and interactions with CD169 receptor active site, respectively form H bond with residues ASP-40, SER-104, LYS-107 and ASN-92. Furthermore, MD (molecular dynamics) simulations were performed by Amber 16 to investigate the stability of a ligand-protein complex. The analysis of root mean square deviation (RMSD) of CD169/(3R,8S,9R,10R,13R,14S,17R)-3-hydroxy-4,4,9,13,14-pentamethyl-17-[(E,2R)-6-methyl-7-[(2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-[(2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxymethyl]oxan-2-yl]oxyhept-5-en-2-yl]-1,2,3,7,8,10,12,15,16,17-decahydr) complex revealed that CD169 protein has more stability when it interacts with the inhibitor. These findings have given us a better understanding of the functional properties and the reaction mechanism of CD169 receptor. Our results will help to identify new leads for drug discovery in PRRSV infection.

Keywords

PRRSV, Homology Modelling, Molecular Docking, MD Simulation, CD169

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1. Introduction

PRRS (Porcine reproductive and respiratory syndrome) is one of the most economically critical diseases in the swine industry. The infection was first reported in the late 1980s in the USA, and it has since spread worldwide to become endemic in countries with a high level of swine rearing [1]. It has been an economically significant swine disease worldwide for over two decades, and has been estimated to cost the US swine industry at least \$600 million annually [2]. PRRSV (Porcine reproductive and respiratory syndrome virus), the causative agent of the disease, is an enveloped virus containing a 15 kb positive-strand RNA genome. PRRSV belongs to the family Arteriviridae of order Nidovirales. Its genome RNA is a positive-strand, 3-polyadenylated molecule of 15kb in length containing 11 known (ORFs) open reading frames. PRRSV also has 7ORFs with a set of 8 structural proteins, including a small non-glycosylated protein and a set of glycosylated ones: GP2ab, GP3, GP4, GP5, and GP5a, M and N proteins [3, 4].

PRRSV is known to have specific cell tropism (infecting only Porcine Alveolar Macrophages and MARC-145 *in vitro*) due to the presence of specific receptors in such cells [5]. Molecules or factors including HS (heparan sulfate), vimentin, a CD151 (cluster of differentiation 151), CD163 (cluster of differentiation 163), CD169, Sn (sialoadhesin), DC-SIGN (CD209) and MHC II-A or MYH9 (non-muscle myosin heavy chain 9) are among the several proteins described as putative receptors for PRRSV [6, 3]. CD163 plays a significant role in the viral uncoating, and releasing of the genome in the cytoplasm; this is an essential phase in PRRSV infection [7, 1]. While CD169 which is also known as Sn or sieglic-1 is one of the PRRSV putative receptors that mediates the attachment of the virus to susceptible cells [8, 1]. From the foregoing, researchers have proven that there is an urgent need for a better and effective alternative method to control the PRRS by

stopping the spread of the virus among herds through the inhibition of the replication of PRRSV and more effectively by blocking the virus from attaching to/and penetrating target cells.

CD169 was first identified as a sialic acid-dependent sheep erythrocyte receptor on bone marrow macrophages, and it was then considered to be the receptor of PRRSV infection, it was also involved in different physiological functions [1]. CD169's extracellular region is made up of 17 consecutive Ig-like domains, which are categorized into an amino-terminal V-set Ig domain and 16 C2-set Ig domains [9]. It is also the primary sialic acid binding receptor. The location of CD169 at the lower plasma membrane in the CD169 PRRSV cell surface, suggests that CD169 also plays a significant role in the internalization of viral particles during infection. The major glycoprotein GP5 of PRRSV was found to interact with CD169. The interaction between the sialic acid of PRRSV GP5 and the N-terminal V-set-Ig like domain of CD169 is the principal mechanism responsible for binding the virus to the protein [10, 11].

In this study, the 3D structure of CD169 was built through the homology modelling process [Figure 1](#): the model was significantly suitable and reliable. The 3D structure of CD169 was then used for molecular docking studies and the discovery of more CD169 potent inhibitors. After finding the 3D structure of CD169 protein, some of the best active compounds were prepared for molecular docking studies and examined their potent therapy against PRRSV through the inhibition of CD169. This study further explains the interaction between CD169 and its main inhibitors through protein-ligand interaction based on the docking results. The results were further supported by molecular Dynamics Simulations.

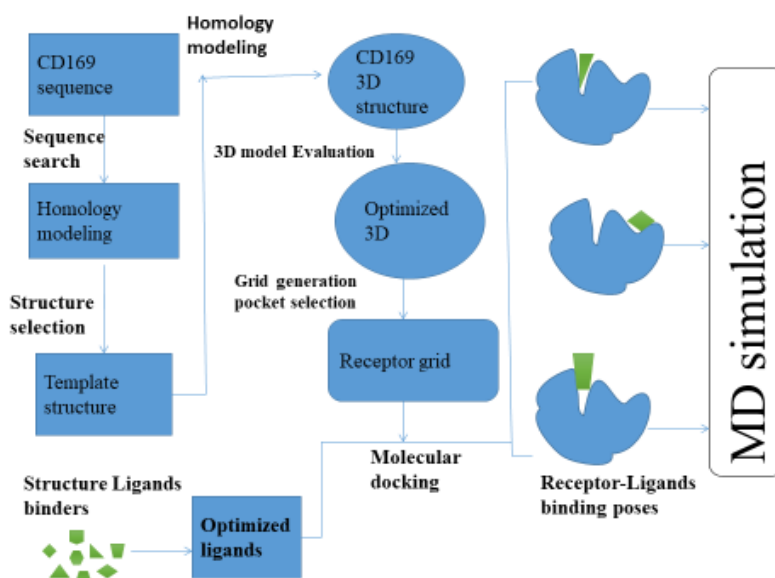


Figure 1. The work flow applied to discover the novel CD169 inhibitor.

2. Methodology

2.1. Homology Modelling, Sequence Alignment and Structure Prediction

Searching through the RCSB Protein Data Bank (<http://www.rcsb.org/>), it was confirmed that the tertiary structure of CD169 was not available [12]. Since, the PDB database (<https://www.rcsb.org/>) does not contain the crystal structure of CD169 protein, the primary focus of further research is on building a 3D model of CD169 [13].

The sequence of CD169 was downloaded from the UniProt (UniProt entry: DQ176853) (<http://www.uniprot.org/>). The template for sequence alignment was identified by searching the CD169 sequence on PDB using the BLASTp program provided by Uniprot with default parameters. The 3D structure of CD169 (UniProt ID: A7LCJ3, residues Val23-Val135) was downloaded from PDB (PDB ID: 1QFP) as the template structure and the template structure had 64% level similarity with a resolution of 2.8 Å making it an excellent template [14] and then the model was generated using Easy Modeller 4 [15]. The steps involved in this process were as previously described by [16]: (i) the amino acid sequence of the selected template protein was identified by the software. (ii) The sequence of pSn V-set Ig-like domain aligned to the template sequence(s). (iii) The backbone and loop modeling of the model was generated. (iv) The complete protein model of pSn V-set Ig-like domain was formed and initially optimized [13].

2.2. Model Evaluation

After optimizing, the 3D model of CD169 was evaluated by the PROCHECK [17] ERRAT [18] and VERIFY 3D [19] programs available from the Structural Analysis and Verification Server (SAVES) (<http://nihserver.mbi.ucla.edu/SAVES>). PROCHECK was used for the assessment of the stereochemical quality of the protein structure and the Verify3D program was used to observe the compatibility between an atomic model (3D) and its amino acid sequence (1D) in order to evaluate the 3D protein structure.

2.3. Protein Preparation

To analyse molecular docking studies, the optimized 3D model of CD169 was prepared by protein preparation wizard workflow Maestro 10.6 in Schrödinger Suite to prevent any interaction that is not physical and to ease the process of assigning bond orders. Bond orders were assigned and hydrogen atoms were added to the protein. The structure was then minimized to reach the converged RMSD of 0.30 Å with the OPLS_2005 force field [14].

2.4. Molecular Docking

The highest scoring molecules obtained from the initial screening were submitted to the LigPrep utility with the default parameters. The OPLS 2005 Force field was used to minimize energy on 32 isomers or tautomers. The minimized energy structures were then used for molecular docking analysis in XP mode of the Glide module. The vdW scaling factor of 0.8 and the potential charge cut-off of 0.15 were applied. After ligand docking, post dock minimization was done and 5 poses per ligand interactions were recorded within 12.0 Å centroid of the specified grid. The highest-scoring pose was generated and then evaluated using Maestro's Pose Viewer tool and XP Visualize [21].

2.5. Ligands Preparations

All ligands used for docking were downloaded from TCMSP (Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform) serves as a data repository and analytical platform that enables users to conduct comprehensive research on TCM (Traditional Chinese Medicines) includes the identification of active components, screening of drug targets, and the generation of compounds-targets-diseases networks. Additionally, it provides detailed drug pharmacokinetic information such as DL (drug-likeness), OB (oral bioavailability), BBB (blood-brain barrier), intestinal epithelial permeability (Caco-2), ALogP, FASA (fractional negative surface area), and Hdon/Hacc (the number of H-bond donor/acceptor). So far, TCMSP has attracted broad attention and several groups have published a lot of papers using TCMSP database [20]. To further increase ligands likeness with ADME, we set the criterion of OB to be greater than 40% and DL greater than 0.18. And then the 3D structure of ligands was prepared by LigPrep10.6 in Schrödinger Suite with an OPLS_2005 force field. The ionization states of the compounds were generated at a pH of 7.0 ± 2.0 using Epik2.2 in the Schrödinger Suite. A maximum of 32 potential stereoisomers per ligand were maintained.

2.6. Molecular Dynamics Simulations

The interactions between the CD169/(3,4,5-trihydroxy-6-[(2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxymethyl]-3R,8S,9R,10R,13R,14S,17R)-3-hydroxy-4,4,9,13,14-pentamethyl-17-[(E,2R)-6-methyl-7-[(2R,3R,4S,5S,6R)-oxan-2-yl]oxyhept-5-en-2-yl]-1,2,3,7,8,10,12,15,16,17-decahydr) complex were analysed using MD simulation in Amber 16 [22, 23]. The general AMBER force field (gaff) and ff14SB force field were used for the ligand and protein, respectively [24, 25].

The energy minimization and equilibration protocol were performed by the Sander program [26]. The preferential

binding mode of CD169/(3R,8S,9R,10R,13R,14S,17R)-3-hydroxy-4,4,9,13,14-pentamethyl-17-[(E,2R)-6-methyl-7-[(2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-[[[(2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxymethyl]oxan-2-yl]oxyhept-5-en-2-yl]-1,2,3,7,8,10,12,15,16,17-decahydr) was performed with 20-ns MD simulation based on the docking results. In order to check whether the protein-ligand interactions made by docked ligands are stable. The equilibration of quantities, such as the RMSD (root-mean-square deviation) was used to assess the equilibration of the trajectory with respect to the initial structure. The specific details of the complex system have been previously described [13].

3. Results and Discussion

3.1. Homology Modelling and Model Evaluation

The structural model of CD169 was built based on the template structure of mouse (PDB: 1QFP with a sequence similarity of 64.5%) with a resolution of 2.8 Å. when a template structure has a 30% sequence similarity to a known structure, then it can be considered to be the threshold limit for accurate homology modelling [27]. A look at our template reveals that it has a high sequence similarity, and it is very reasonable to be used as a model and in building a tidy structural model. The sequences alignment between the model and the template

showed the similarity, as shown in Figure 2a. For further structure characterization, we generated the protein superposition of the target (CD169) and the template. It also showed a high level of similarity as shown in Figure 2b. the RMSD (Root Mean Square Deviation) in the backbone position is about 0.359 Å indicating that both template and target have a high sequence similarity and also have similar folds.

Subsequently, the quality of models was validated using PROCHECK (Program to check the stereochemical quality of protein structures), a program that relies on Ramachandran plots for structure verification. Based on this software, a model of high quality would usually have more than 90% of its residues located in the most favored region. The Ramachandran plot for the predicted model revealed that 93% of residues were in the most favourable region, while 5.3% were in the allowed region, 1.1% of residues were generously allowed confirming that the predicted model is of good quality Figure 2c. ERRAT is also called “overall quality factor”. It shows the range for non-bonded atomic interactions, with higher scores and higher quality. The generally accepted range is >50 for a high-quality model. For the current 3D model, the overall quality factor predicted by the ERRAT server was 73.33%. The Verify 3D server predicted that 100% of the residues in pSn had an average 3D-1D score > 0.2, thereby verifying the model. The results from validation indicate that the 3D model of CD169 was entirely suitable and it could be used in further studies.

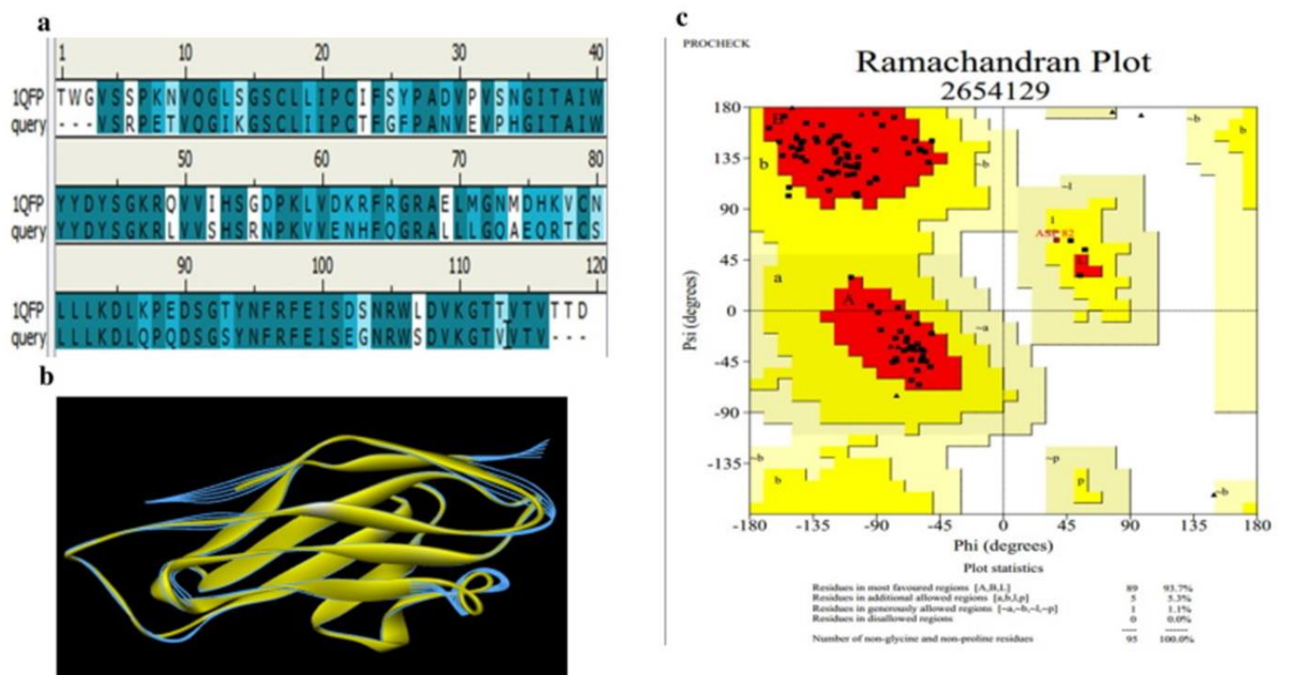


Figure 2. (a) Comparison of sequence alignment between model (CD19) and template (1QFP). (b) Superposition of the target (CD169) and template (1QFP) (RMSD: 0.359 Å). (c) Ramachandran plot 'calculation of CD169 model provided by PROCHECK: 93% residues in favorable regions; 5.3% residues in additional allowed regions; 1.1% residues in generously allowed regions; 0% residues in disallowed regions. Regions; 1.1% residues in generously allowed regions; 0% residues in disallowed regions.

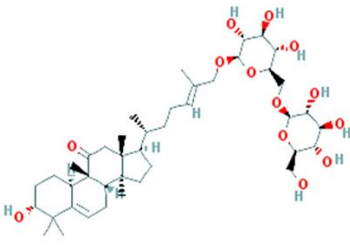
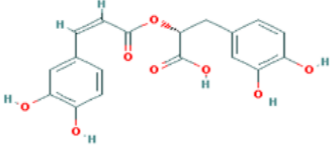
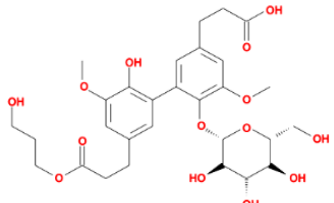
3.2. Docking Result

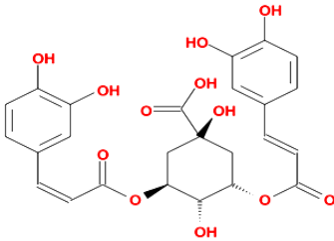
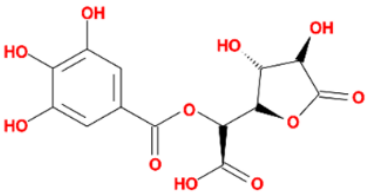
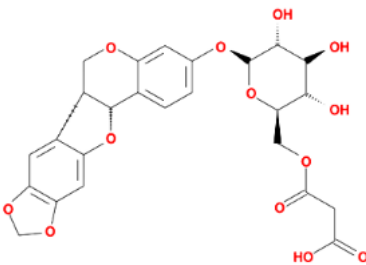
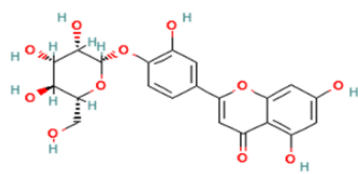
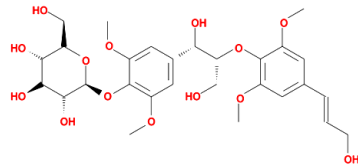
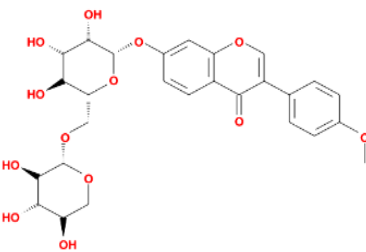
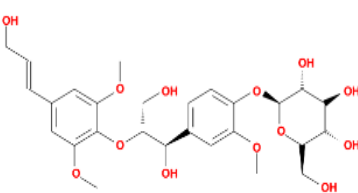
Molecular docking defines as the predominant approach for evaluating protein-ligand interactions. Docking is the most efficient technique used to predict the potential ligand-binding sites on the whole protein [28]. Molecular docking was done by Maestro 10.6 in Schrödinger Suite in extra precision to study the keys amino acid residues at the binding site of CD169 protein that interacts with the compound. The interaction between the amino acid residues and the compound leads to the stability of the ligand-protein complex which mostly leads to the inhibition of CD169 catalytic activity. Docking facilitates the acceptance of a chemical moiety that is compatible with the catalytic site of the receptor and highlights the ones that conform to the receptor pocket [21]. According to the docking results, ten compounds proved that they have the best docking score Table 1. The compound

MOL002433 (3R,8S,9R,10R,13R,14S,17R)-3-hydroxy-4,4,9,13,14-pentamethyl-17-[(E,2R)-6-methyl-7-[(2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-[(2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxymethyl]oxan-2-yl]oxyhept-5-en-2-yl]-1,2,3,7,8,10,12,15,16,17-decahydr) inhibitor was found to have the best docking score -8.095 kcal/mol among all the top hits. The

negative and the low value of the compound inhibitor demonstrated that there is a strong bond interaction between CD169 and MOL002433 inhibitor while ligand tries to fit into the binding pocket of the protein molecule. Furthermore, the complex displayed 3 H-bonds backbone and 3 H-bond sidechains *via* residues ASP-40, SER-104, LYS-107 and ASN-92 Figure 3a, b. According to the known previous literature studies, amino acid residues Ser-103, Arg-97, Tyr-44, Trp-106, Leu-107 and Arg-105 suggest having potential links with the catalytic substrates 3' Sialyllactose and DSLc4 or oxamido-Neu5Ac of CD169 sialic acid [29, 30]. Figure 2A showed that the key amino acid residues Arg-94, Tyr-41, Trp-103 and Arg-102 of CD169 are appropriately aligned by sequence similarity. However those amino acids have no direct interaction but they may be involved in the catalysis process. The amino acids residues Ser-103 and Leu-107 changed to align with Gly-100 and Ser-104, the direct interaction amino acid is Ser-104. These amino acids are nearly close to the entire active cavity. It is suggesting that the compound has a strong binding affinity with protein active site and it governs the strong interaction stability between ligand-protein complexes. Hence, the present study successfully demonstrates that the docked compounds will be the promising next generation chemotherapeutic drugs, which could be used in the treatment of PRRS.

Table 1. Chemical structures, names, docking scores and interactions of the top 10 ligands binders from docking study.

SN	Molecule Names	Structure	Docking scores/ kcal/mol	Protein-ligand interactions
1	(3R,8S,9R,10R,13R,14S,17R)-3-hydroxy-4,4,9,13,14-pentamethyl-17-[(E,2R)-6-methyl-7-[(2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-[(2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxymethyl]oxan-2-yl]oxyhept-5-en-2-yl]-1,2,3,7,8,10,12,15,16,17-decahydr		-8.095	Lys107, Asn92, Asp40, Ser104
2	(2R)-3-(3,4-dihydroxyphenyl)-2-[(Z)-3-(3,4-dihydroxyphenyl)acryloyl]oxy-propionic acid		-7.722	Ser94, Ser20, Arg94, Ser104
3	Dichotomaside		-7.502	Arg94, Ser42, Ser104

SN	Molecule Names	Structure	Docking scores/ kcal/mol	Protein-ligand interactions
4	(E,E)-3,5-Di-O-caffeoylquinic acid		-7.407	Val106, Arg45, Arg94, Arg104, Tyr41
5	Mucic acid 1,4-lactone 5-O-gallate		-7.240	Arg45, Arg94, Hie60, Glu96, Tyr38
6	(-)-Maackiain-3-O-glucosyl-6'-O-malonate		-7.164	Arg102, Gly100
7	Luteolin-4'-glucoside		-7.003	Asn92, ARG102, Ser104, Arg94
8	Picraquassioside C		-6.845	Ser104, Tyr41, Gly96, Arg92
9	Kushenol O		-6.826	Lys44, Gyl43, Tyr38, Arg52, Glu58
10	Citrusin B		-6.725	Tyr41, Arg94, Val106, Asn92, Arg102, Glu99

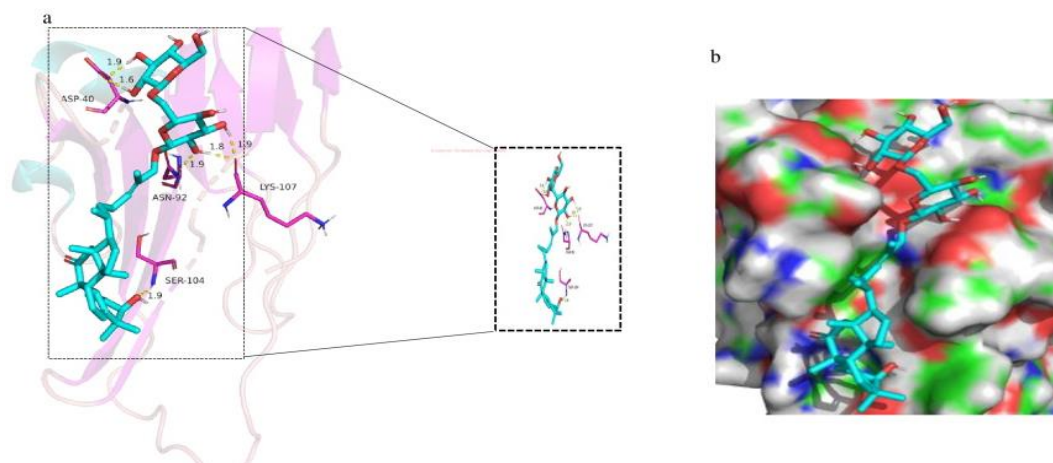


Figure 3. (a) Protein-ligand interactions (binding mode) of MOL002433 against CD169. MOL002433 was shown in a aqua color stick model. The binding site residues are shown as a stick model lavender color. The yellow dots indicate H bonds. (b) The protein-ligand interactions are shown in surface. MOL002433 was shown in aqua color stick mode, and CD169 was shown in an electrostatic surface.

3.3. Molecular Dynamics Simulations Result

MD (Molecular dynamics simulation) is a helpful accurate method to investigate molecular interactions possible in a protein-ligand complex that is responsible for its stability. MD simulations were performed for 20ns to examine the stability of the complex [31]. Initially, the RMSD values of backbone C α atoms are evaluated to examine whether each system reaches equilibrium, and Figure 4, shows that the complex was found to reach equilibrium at 15 ns. It clearly shows that the RMSD values of the CD169/(3R,8S,9R,10R,13R,14S,17R)-3-hydroxy-4,4,9,13,14-pentamethyl-17-[(E,2R)-6-methyl-7-[(2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-[(2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydr

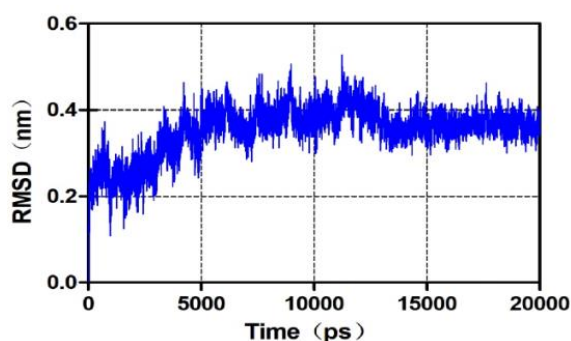


Figure 4. The molecular dynamics (MD) simulation of the CD169/MOL002433 complex. Shows the root-mean-square deviation (RMSD) calculated for the backbone atoms of the protein during MD simulation.

4. Conclusion

The current research revealed that CD169 protein could be a major drug target in PRRSV infection, our work aimed to build the 3D structure of CD169 protein in order to identify novel therapy inhibitors for PRRSV. The model was then optimized and evaluated in order to assess the stereochemical and amino acid quality environments. The 3D structure of CD169 was then used for molecular docking studies and the discovery of more CD169 potent inhibitors. After finding the 3D structure of CD169 protein, some of the best active compounds were prepared for molecular docking studies and examined their potent therapy against PRRSV through the inhibition of CD169. And the results showed that MOL002433 was one the best and bound with ASP-40, SER-104, LYS-107 and ASN-92 which make strong binding affinity and also occupying large binding cavity space. The overall results of MD simulations have shown protein stability and RMSD values remain the same with little deviations. The molecular docking result and MD simulations result give us a significant fact that MOL002433 might be a potential lead in the discovery of potent inhibitor CD169 in PRRSV treatment compared with other molecules we used in our study.

Abbreviations

MD	Molecular Dynamics
RMSD	Root Mean Square Deviation
TCM	Traditional Chinese Medicines
TCMSP	Traditional Chinese Medicine Systems
	Pharmacology Database and Analysis Platform
PRRS	Porcine Reproductive and Respiratory
	Syndrome
ORFs	Open Reading Frames

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Author Contributions

Nsabimana Eliphaz: Conceptualization, Formal Analysis, Investigation, Methodology, Software, Visualization, Writing – original draft, Writing – review & editing

Wen-Qiang Cui: Methodology, Software, Writing – review & editing

Han Xiao: Conceptualization, Investigation, Writing – review & editing

God'Spover Bello-Onaghise: Formal Analysis, Investigation, Writing – review & editing

Tang Yang: Investigation, Software, Writing – review & editing

Yu Fei: Data curation, Investigation, Methodology, Writing – review & editing

Zhang Yue Feng: Data curation, Formal Analysis, Methodology, Writing – review & editing

Jun-Jie Qin: Conceptualization, Investigation, Writing – review & editing

Wen-Xin Guo: Investigation, Methodology, Writing – review & editing

Yan-Hua Li: Project administration, Resources, Supervision, Visualization

Conflicts of Interest

The authors report no conflict of interest.

References

- Jie, H., Rui, L., Hongfang, M., Songlin, Q. J. F. Structural prediction of porcine sialoadhesin V-set Ig-like domain sheds some light on its role in porcine reproductive and respiratory syndrome virus (PRRSV) infection. *Front. Agr. Sci. Eng.* 2016, 3(1): 65–71. <https://doi.org/10.15302/J-FASE-2016086>
- Holtkamp, D. J., Kliebenstein, J. B., Neumann, E., Zimmerman, J. J., Rotto, H., Yoder, T. K., et al. Assessment of the economic impact of porcine reproductive and respiratory syndrome virus on United States pork producers. *Journal of Swine Health and Production* 2013, 21(2), 72. <https://doi.org/10.2460/javma.2005.227.385>
- Lunney, J. K., Fang, Y., Ladinig, A., Chen, N., Li, Y., Rowland, B., et al. Porcine reproductive and respiratory syndrome virus (PRRSV): pathogenesis and interaction with the immune system. *Annual Review of Animal Biosciences* 2016, 4, 129–154. <https://doi.org/10.1146/annurev-animal-022114-111025>
- Huang, C., Zhang, Q., and Feng, W.-h. J. V. r. Regulation and evasion of antiviral immune responses by porcine reproductive and respiratory syndrome virus. *Virus Research* 2015, 202, 101–111. <https://doi.org/10.1016/j.virusres.2014.12.014>
- Du, T., Shi, Y., Xiao, S., Li, N., Zhao, Q., Zhang, A., et al. Curcumin is a promising inhibitor of genotype 2 porcine reproductive and respiratory syndrome virus infection. *BMC Veterinary Research* 2017, 13(1), 298. <https://doi.org/10.1186/s12917-017-1218-x>
- Gao, J., Xiao, S., Xiao, Y., Wang, X., Zhang, C., Zhao, Q., et al. MYH9 is an essential factor for porcine reproductive and respiratory syndrome virus infection. *Scientific Reports* 2016, 6, 25120. <https://doi.org/10.1038/srep25120>
- Fraser, I., and Gordon, S. J. E. j. o. c. b. Murine erythroleukemia (MEL) cells bear ligands for the sialoadhesin and erythroblast receptor macrophage hemagglutinins. *European journal of cell biology* 1994, 64(2), 217–221.
- Guo, C., Zhu, Z., Wang, X., Chen, Y., and Liu, X. J. V. M. Pyrithione inhibits porcine reproductive and respiratory syndrome virus replication through interfering with NF-κB and heparanase. *Veterinary Microbiology* 2017, 231–239. <https://doi.org/10.1016/j.vetmic.2017.01.033>
- Crocker, P. R., Hartnell, A., Munday, J., and Nath, D. J. G. j. The potential role of sialoadhesin as a macrophage recognition molecule in health and disease. *Glycoconjugate journal* 1997, 601–609.
- Delpitte, P. L., and Nauwynck, H. "Porcine Arterivirus Entry in Macrophages: Heparan Sulfate-Mediated Attachment, Sialoadhesin-Mediated Internalization, and a Cell-Specific Factor Mediating Virus Disassembly and Genome Release," in *The Nidoviruses*. Springer) 2006, 247–252.
- Van Breedam, W., Van Gorp, H., Zhang, J. Q., Crocker, P. R., Delpitte, P. L., and Nauwynck, H. J. J. P. p. The M/GP5 glycoprotein complex of porcine reproductive and respiratory syndrome virus binds the sialoadhesin receptor in a sialic acid-dependent manner. *PLoS Pathogens* 2010, 6(1), e1000730. <https://doi.org/10.1371/journal.ppat.1000730>
- Messaoudi, A., Belguith, H., Hamida, J. B. J. T. B., and Modelling, M. Homology modeling and virtual screening approaches to identify potent inhibitors of VEB-1 β-lactamase. *Theoretical Biology and Medical Modelling* 2013, 10(1), 22.
- Cui, W.-Q., Qu, Q.-W., Wang, J.-P., Bai, J.-W., Bello-Onaghise, G. s., Li, Y.-A., et al. Discovery of Potential Anti-infective Therapy Targeting Glutamine Synthetase in *Staphylococcus xylosus*. *Frontiers in Chemistry* 2019, 7. <https://doi.org/10.3389/fchem.2019.00381>
- Shen, J., Zhang, W., Fang, H., Perkins, R., Tong, W., and Hong, H. (Year). "Homology modeling, molecular docking, and molecular dynamics simulations elucidated α-fetoprotein binding modes", in: *BMC bioinformatics*: BioMed Central), S6.
- Kuntal, B. K., Aparoy, P., and Reddanna, P. J. B. r. n. EasyModeller: A graphical interface to MODELLER. *BMC Research notes* 2010, 3(1), 226.

- [16] Krieger, E., Nabuurs, S. B., and Vriend, G. Homology modeling. *Methods Biochem. Anal* 2003, 44, 509–523. <https://doi.org/10.1002/0471721204.ch25>
- [17] Laskowski, R. A., MacArthur, M. W., Moss, D. S., and Thornton, J. M. J. J. o. a. c. PROCHECK: a program to check the stereochemical quality of protein structures. *Journal of Applied Crystallography* 1993, 26(2), 283–291. <https://doi.org/10.1107/S0021889892009944>
- [18] Colovos, C., and Yeates, T. O. J. P. s. Verification of protein structures: patterns of nonbonded atomic interactions. *Protein Science* (1993) 2(9), 1511–1519. <https://doi.org/10.1002/pro.5560020916>
- [19] Lüthy, R., Bowie, J. U., and Eisenberg, D. J. N. Assessment of protein models with three-dimensional profiles. *Nature* 1992, 356(6364), 83.
- [20] Lu, P., Chen, J., Zhao, H., Gao, Y., Luo, L., Zuo, X., et al. In silico syndrome prediction for coronary artery disease in traditional chinese medicine. *Evidence-based Complementary and Alternative Medicine* 2012. <https://doi.org/10.1155/2012/142584>
- [21] Usman, M. M., Bharbhuiya, T. K., Mondal, S., Rani, S., Kyal, C., and Kumari, R. J. Combined protein and ligand based physicochemical aspects of molecular recognition for the discovery of CDK9 inhibitor. *Gene Reports* 2018, 13, 212–219.
- [22] Case, D. A., Cheatham III, T. E., Darden, T., Gohlke, H., Luo, R., Merz Jr, K. M.,... Woods. The Amber biomolecular simulation programs. *J Comput Chem*. 2005 December; 26(16): 1668–1688. <https://doi.org/10.1002/jcc.20290>
- [23] Pearlman, D. A., Case, D. A., Caldwell, J. W., Ross, W. S., Cheatham III, T. E., DeBolt, S. Kollman. AMBER, a package of computer programs for applying molecular mechanics, normal mode analysis, molecular dynamics and free energy calculations to simulate the structural and energetic properties of molecules. *Computer Physics Communications Volume 91, Issues 1–3, 2 September 1995, Pages 1–41*. [https://doi.org/10.1016/0010-4655\(95\)00041-D](https://doi.org/10.1016/0010-4655(95)00041-D)
- [24] Maier, J. A., Martinez, C., Kasavajhala, K., Wickstrom, L., Hauser, K. E., Simmerling. ff14SB: improving the accuracy of protein side chain and backbone parameters from ff99SB. *J. Chem. Theory Comput* 2015, 11(8), 3696–3713. <https://doi.org/10.1021/acs.jctc.5b00255>
- [25] Wang, J., Wolf, R. M., Caldwell, J. W., Kollman, P. A., & Case, D. Development and testing of a general amber force field. *J Comput Chem*. 2004 25(9), 1157–1174. <https://doi.org/10.1002/jcc.20035>
- [26] Fothergill, A. W. Antifungal susceptibility testing: clinical laboratory and standards institute (CLSI) methods. In *Interactions of yeasts, moulds, and antifungal agents* 2012 (pp. 65–74): Springer.
- [27] Xiang, Z. Advances in homology protein structure modeling. *Current Protein and Peptide Science* 2006, 7(3), 217–227. <https://doi.org/10.2174/13892030677452312>
- [28] Suri, S., Waseem, R., Bandi, S., & Shaik, S. Homology Modeling of Mus Musculus CDK5 and Molecular Docking Studies with Flavonoids. *International Journal of Pharmaceutical and Clinical Research* 2017; 9(6): 480–484.
- [29] May, A., Robinson, R., Vinson, M., Crocker, P., & Jones, E. Crystal structure of the N-terminal domain of sialoadhesin in complex with 3' sialyllactose at 1.85 Å resolution. *Molecular Cell* 1998, 1(5), 719–728. [https://doi.org/10.1016/S1097-2765\(00\)80071-4](https://doi.org/10.1016/S1097-2765(00)80071-4)
- [30] Attrill, H., Takazawa, H., Witt, S., Kelm, S., Isecke, R., Brossmer, R., Crocker. The structure of siglec-7 in complex with sialosides: leads for rational structure-based inhibitor design. *Biochem. J*. 2006, 397(2), 271–278. <https://doi.org/10.1042/BJ20060103>
- [31] Itteboina, R., Ballu, S., Sivan, S. K., Manga, V. J. Molecular docking, 3D-QSAR, molecular dynamics, synthesis and anti-cancer activity of tyrosine kinase 2 (TYK 2) inhibitors. *Journal of Receptors and Signal Transduction* 2018, 38(5–6), 462–474. <https://doi.org/10.1080/10799893.2019.1585453>