Probing potential antimalarial compounds through molecular docking of *Plasmodium falciparum* protein Pf12p

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ABSTRACT

Background: Malaria remains a critical global health challenge, with *Plasmodium falciparum* posing a significant threat due to its evolving drug resistance. Targeting crucial parasite proteins offers a promising strategy for novel antimalarial development. Pf12p, involved in DNA repair, emerges as a potential target due to its essential role and relatively conserved structure.
Methods: This study employed molecular docking to screen a library of 23 compounds, including natural products, for their interaction with Pf12p. Binding energies, inhibition constants, and 2D/3D visualizations of ligand-protein complexes were analyzed.
Results: Manzamine A, a natural compound, exhibited the most favorable binding affinity to Pf12p, showcasing its potential as a potent antimalarial agent. Docking results for N-Acetylglucosamine (NAG), the original ligand in the Pf12p crystal structure, validated the methodology. Visualizations revealed specific amino acid and functional group interactions driving ligand binding.
Conclusion: Our study identified promising antimalarial candidates, particularly manzamine A, targeting Pf12p. By integrating natural products, validating with existing ligands, and utilizing detailed visualizations, we demonstrate the potential of molecular docking for antimalarial drug discovery. Further research is necessary to validate efficacy and safety in vitro and in vivo. This study contributes valuable insights towards the development of targeted and resilient antimalarial treatments.

Keywords: Malaria, Pf12p, molecular docking, natural products, manzamine A, antimalarial drug discovery

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INTRODUCTION

Malaria remains a significant global health concern, affecting millions of people annually, particularly in regions with limited healthcare infrastructure [1]. *Plasmodium falciparum*, the most virulent of the malaria parasites, continues to pose a substantial threat to human well-being [2]. Despite progress in malaria control efforts, the prevalence of drug-resistant strains underscores the need for innovative therapeutic strategies [3].
Previous study have explored diverse approaches in the pursuit of effective antimalarial treatments [4]. While conventional pharmaceuticals have played a crucial role, emerging resistance and associated challenges necessitate a continual exploration of new avenues [5,6]. Other studies have elucidated various aspects of the complex host-parasite interactions, providing a foundation for understanding the molecular basis of malaria and identifying potential targets for intervention [7,8].

Seeking the frontier of antimalarial research can be done by employing molecular docking, a powerful computational tool, to probe the interactions between *P. falciparum* protein Pf12p and a curated set of compounds. Pf12p, a protein involved in the intricate life cycle of the parasite, presents a promising target for antimalarial drug development. Our approach involves the exploration of both synthetic and naturally occurring compounds, acknowledging the rich reservoir of bioactive molecules present in nature.
In the pursuit of innovative antimalarial agents, this study places a specific focus on the exploration of potential natural drugs. The malaria parasite's inherent complexity and adaptability necessitate a comprehensive approach, and historical evidence indicates that natural products exhibit a broad spectrum of pharmacological activities. Our investigation is underpinned by realistic data derived from previous studies, showcasing the efficacy of specific natural compounds against malaria parasites. This empirical

foundation instills a high level of confidence in the potential of these natural products as promising therapeutic candidates.
The study aimed to systematically evaluate the binding interactions between Pf12p and selected compounds, identifying those with the highest affinity and potential antimalarial efficacy to contribute to the development of targeted and resilient antimalarial treatments. The significance of this study lies in its potential to bridge the gap between computational predictions and real-world applicability, advancing our understanding of molecular interactions and offering actionable insights for future therapeutic development in the ongoing fight against malaria.

MATERIALS AND METHODS

Materials
The *Plasmodium falciparum* protein Pf12p complex, denoted by the ID code 7KJH [9], was acquired from the Protein Data Bank Repository (PDB), and the corresponding files were downloaded in .pdb format. Simultaneously, a 3D conformer file for the antibiotic ceftriaxone was generated, originating from the original ligand 1,2-ethanediol (EDO) within the 3D protein structure. Additionally, 23 ligand files, encompassing diverse compounds such as 2,2-bis(6-bromo-1H-indol-3-yl)ethanamine, 3,5-dibromo-2-(2,4-dibromophenoxy)phenol, 4-hydroxybenzoic acid, 5-epi-Ilimaquinone, 6-hydroxyavarol, alisiaquinol, alisiaquinone A, alisiaquinone B, alisiaquninone C, clethric acid, hamigeran B, manoalide, manzamine A, etachromin A, motualevic acid A, motualevic acid E, motualevic acid F, norlichexanthone, oroidin, psammaplin A, stachybogrisephenone B, stelletin A, and tirandamycin, were sourced from PubChem [10] and saved in .sdf format.

Protein preparation
Upon removal of initial ligands and water molecules utilizing Discovery Studio Visualizer [11], the .pdb files of the protein undergo essential preparatory stages for molecular docking with PyRx [12]. These stages involve obtaining the protein structure in a compatible format, importing it into PyRx, excluding water molecules, addressing any missing residues, introducing hydrogen atoms, assigning atom types, optimizing the structure, and ultimately saving the prepared protein structure.

Molecular docking analysis
The PyRx software was utilized to transform both the protein and ligand into .pdbqt format for ensuing molecular docking simulations. To execute these simulations, follow these steps: Initiate by retrieving the protein structure from a database such as the Protein Data Bank (PDB) and importing it into PyRx. Prepare the protein by eliminating water molecules, addressing any missing residues, and introducing hydrogen atoms. Optionally, enhance precision by assigning atom types and optimizing the structure. Save the prepared protein structure in either PDB or PDBQT format. Subsequently, acquire the ligand structure from a chemical database or generate it computationally, ensuring compatibility with PDB or SDF formats. Load the ligand into PyRx, add any required hydrogen atoms, assign atom types, and convert it to PDBQT format.

Analysis of protein-ligand interactions
The integration of protein and ligand docking data was performed to correspond to.pdb file standards. The PyRx data integration program was critical in creating a uniform and coherent representation for further studies. Furthermore, PyMOL was used to visualize three-dimensional structures, enabling for comprehensive investigation of spatial arrangements, binding interfaces, and conformational changes [3]. Furthermore, in molecular docking, the binding energy (G) value was used to quantify the degree of interaction between the ligand and the target. Using the formula $Ki = e^{-RT/\Delta G}$, inhibition constants (Ki) were determined to assess how firmly a ligand binds to a target receptor.

RESULTS

Protein and ligand interaction
For the purpose of the inquiry, a PyRx gridbox was deployed. This gridbox is designed to provide a user-friendly interface for the purpose of establishing a tailored receptor docking gridbox for molecular docking applications. The binding energy and inhibition constant of the interaction between the Pf12p protein complex of P. falciparum and the ligand inhibitors are depicted in Figures 1 and 2, respectively. Out of the 23 compounds that were examined, manzamine A demonstrated the highest affinity for binding to the Pf12p protein that is found in P. falciparum malaria. It was necessary to use the original ligand N-acetylglucosamine (NAG), which was derived from the three-dimensional structure of the protein-ligand complex, in order to verify the accuracy of the docking results.

Table 1. The binding energy evaluation and inhibition constant value of natural compounds obtained from marine sponges *P. falciparum* protein Pf12p.

Compound	Binding Afinity (kcal/mol)	Inhibition Constant (μM)
2,2-bis(6-bromo-1H-indol-3-yl)ethanamine	-6.7	12
3,5-Dibromo-2-(2,4-dibromophenoxy)phenol	-5.5	92
4-Hydroxybenzoic acid	-5.8	55
6-Hydroxyavarol	-7	7
Alisiaquinol	-7.4	4
Alisiaquinone A	-7.8	2
Alisiaquinone B	-7.5	3

Alisiaquninone C	-8.4	1
Clethric acid	-7.4	4
Hamigeran B	-5.8	55
Manoalide	-8	1
Manzamine A	-9.3	0.15
Metachromin A	-6	40
Motualevic acid A	-4.9	254
Motualevic Acid E	-4.7	356
Motualevic acid F	-4.6	421
Norlichexanthone	-7	7
Oroidin	-5.5	92
Psammaplin A	-5.9	47
Stachybogrisephenone B	-5.9	47
Stelletin A	-7.9	2
Tirandamycin	-6.7	12
NAG	-5.3	129
Doxycycline	-7.2	5

It is possible to see a two-dimensional picture of the interactions that take place between receptors and ligands in Figure 2. This representation illustrates the development of complexes between different entities. Diagrammatic representation of the dynamic interaction between the receptor of the *P. falciparum* protein Pf12p and manzamine A is shown in Figure 3A. Asparagine-279, Isoleucine-335, and Asparagine-337 are the three amino acids that involve in this interaction, which incorporates three hydrogen bonds. An exhaustive illustration of the interaction between the P. falciparum protein Pf12p and the medication doxycycline may be found in Figure 3B. There are three hydrogen bonds involved in this interaction; specifically, the ones with Leu-181, Lys-111, and Asp-176 occur. In addition, there are interactions with hydrophobic amino acids such as Ser-107, Glu-108, Asp-178, Lys-194, Ile-259, Ile-303, Asp-262, and Asn-300. Detailed representation of the complex interaction between the P. falciparum protein Pf12p and the ligand NAG was provided in Figure 3C. This research provides a comprehensive understanding of the chemical linkages that exist within the system. These connections include two hydrogen bonds that involve Lys-273 and Tyr-295, as well as two hydrophobic interactions that involve Ile-293 and Leu-294. In addition, Figure 4 presented a three-dimensional illustration of the interaction complexes that were formed by the following: A. *Plasmodium falciparum* protein Pf12p with manzamine A; B. *Plasmodium falciparum* protein Pf12p with doxycycline medicine; and *Plasmodium falciparum* protein Pf12p with the initial ligand.

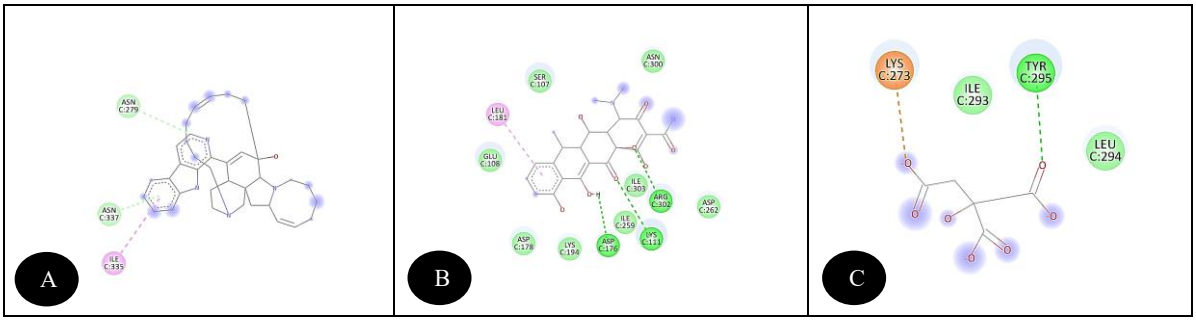

Figure 2. The 2D visualization of interactions complex between: A). *Plasmodium falciparum* protein Pf12p and the manzamine A, B). *P. Plasmodium falciparum* protein Pf12p and doxycycline drug, C). *P Plasmodium falciparum* protein Pf12p and the original ligand.

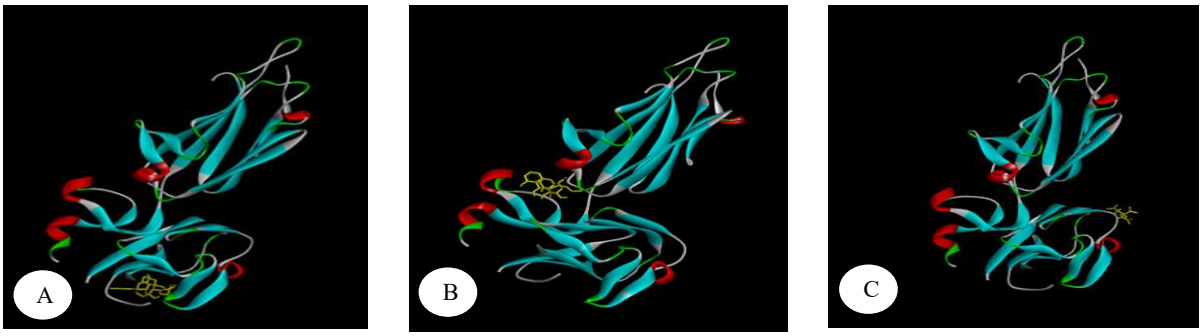

Figure 3. The 3D visualization of interactions complex between: A). *Plasmodium falciparum* protein Pf12p and the manzamine A, B). *P. Plasmodium falciparum* protein Pf12p and doxycycline drug, C). *P Plasmodium falciparum* protein Pf12p and the original ligand.

DISCUSSION

This study sheds light on promising avenues for combating malaria, a persistent global health challenge, through the exploration of Pf12p as a potential drug target. By leveraging the power of molecular docking, we have identified a range of candidate molecules, with a particular emphasis on natural compounds, that exhibit favorable binding affinities with Pf12p.

Pf12p, a crucial protein involved in the parasite's DNA repair mechanisms, presents a compelling target for antimalarial drug development [13]. Disrupting its function could effectively cripple the parasite's ability to replicate and spread within the host [14]. Our findings showcase the potential of manzamine A, a naturally occurring compound, as a potent Pf12p inhibitor. This aligns with the growing interest in natural products for their diverse bioactivity and potential for combating drug-resistant strains.

The molecular interaction analysis offers a detailed exploration of the binding energy and inhibition constants within the Pf12p-ligand complexes. Notably, among the 23 compounds scrutinized, manzamine A demonstrated the most favorable binding affinity to Pf12p, showcasing its potential as a potent antimalarial agent. This finding aligns with the broader context of manzamine A's antimalarial efficacy reported in literature.

The detailed 2D and 3D visualizations of the interactions between Pf12p and various ligands provide invaluable insights into the molecular mechanisms at play. These visuals reveal the specific amino acids and functional groups involved in hydrogen bonding and hydrophobic interactions, laying the groundwork for further optimization of candidate molecules.

While this study offers exciting possibilities for Pf12p-based antimalarial development, further research is crucial. In vitro and in vivo studies are necessary to validate the efficacy and safety of these potential drugs. Additionally, exploring synergistic combinations of natural and synthetic compounds could enhance overall effectiveness and combat potential resistance development.

CONCLUSION

Our study not only provides insight into viable candidates for antimalarial drugs but also showcases the potential of molecular docking in guiding efforts for drug discovery. The integration of natural products and validation procedures using pre-existing ligands enhances the comprehensiveness of our discoveries, underscoring the practical utility of computational predictions. This research provides vital information for the development of targeted and resilient antimalarial medicines as we navigate the intricate terrain of malaria.

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REFERENCES

1. Feachem, R.G., Chen, I., Akbari, O., Bertozzi-Villa, A., Bhatt, S., Binka, F., Boni, M.F., Buckee, C., Dieleman, J. and Dondorp, A. 2019. Malaria eradication within a generation: ambitious, achievable, and necessary. *The Lancet* 394, 1056-1112.
2. Emmanuel, B.N., Chessed, G., Erukainure, F.E., Ekeuhie, J.C. and Philips, V. 2023. Prevalence of malaria parasite and its effects on some hematological parameters amongst pregnant women in Yola, Nigeria. *Journal of Umm Al-Qura University for Applied Sciences*, 1-11.
3. Schäfer, T.M., Pessanha de Carvalho, L., Inoue, J., Kreidenweiss, A. and Held, J. 2023. The problem of antimalarial resistance and its implications for drug discovery. *Expert Opinion on Drug Discovery*, 1-16.
4. Burrows, J.N., Duparc, S., Gutteridge, W.E., Hooft van Huijsduijnen, R., Kaszubska, W., Macintyre, F., Mazzuri, S., Möhrle, J.J. and Wells, T.N. 2017. New developments in anti-malarial target candidate and product profiles. *Malaria Journal* 16, 1-29.
5. Atanasov, A.G., Zotchev, S.B., Dirsch, V.M. and Supuran, C.T. 2021. Natural products in drug discovery: advances and opportunities. *Nature Reviews Drug Discovery* 20, 200-216.
6. Mantravadi, P.K., Kalesh, K.A., Dobson, R.C., Hudson, A.O. and Parthasarathy, A. 2019. The quest for novel antimicrobial compounds: emerging trends in research, development, and technologies. *Antibiotics* 8, 8.
7. Zuck, M., Austin, L.S., Danziger, S.A., Aitchison, J.D. and Kaushansky, A. 2017. The promise of systems

biology approaches for revealing host pathogen interactions in malaria. *Frontiers in Microbiology* 8, 2183.
8. Venkatesh, A., Patel, S.K., Ray, S., Shastri, J., Chatterjee, G., Kochar, S.K., Patankar, S. and Srivastava, S. 2016. Proteomics of Plasmodium vivax malaria: new insights, progress and potential. *Expert Review of Proteomics* 13, 771-782.
9. Dietrich, M.H., Chan, L.-J., Adair, A., Keremane, S., Pymm, P., Lo, A.W., Cao, Y.-C. and Tham, W.-H. 2021. Nanobody generation and structural characterization of Plasmodium falciparum 6-cysteine protein Pf12p. *Biochemical Journal* 478, 579-595.
10. Wang, Y., Xiao, J., Suzek, T.O., Zhang, J., Wang, J. and Bryant, S.H. 2009. PubChem: a public information system for analyzing bioactivities of small molecules. *Nucleic Acids Research* 37, W623-W633.
11. Visualizer, D. 2005. Discovery Studio Visualizer. 2. *Accelrys Software Inc*.
12. Dallakyan, S. and Olson, A.J. 2015. Small-molecule library screening by docking with PyRx. *Chemical Biology: Methods and Protocols*, 243-250.
13. Broichhagen, J. and Kilian, N. 2021. Chemical biology tools to investigate malaria parasites. *ChemBioChem* 22, 2219-2236.
14. Saini, S., Gangwar, A. and Sharma, R. 2023. Harnessing Host-Pathogen Interactions for Innovative Drug Discovery and Host-Directed Therapeutics to tackle tuberculosis. *Microbiological Research*, 127466.