

***In silico* antimalarial investigation of some bioactive molecules targeting *Plasmodium falciparum*'s Sortilin protein**

^{1,2}Ja'afar, J. N., ³Ibrahim, A. and ³Zailani, H. A.

¹Department of Biotechnology, Faculty of Life Sciences, Modibbo Adama University, Yola, Adamawa State, Nigeria.

²Chevron Biotechnology Center, Modibbo Adama University, Yola, Adamawa State, Nigeria.

³Department of Biochemistry, Faculty of Life Sciences, Modibbo Adama University, Yola, Adamawa State, Nigeria.

Paper History

Received: 30th May, 2023

Accepted: 26th June, 2023

Published: 06th July, 2023

Abstract:

Plasmodium falciparum (*P. falciparum*) is the most prevalent malaria parasite in Africa, accounting for 99.7% of estimated malaria cases in 2017. *P. falciparum* Sortilin protein has a potential role as a protein escorter to the Rhoptry organelle. Rhoptry organelle is an essential protein for erythrocyte invasion by *P. falciparum*. Hence, this study was aimed at identifying natural bioactive molecules from the natural product library (NPLib) of RPBs webserver through an *in-silico* approach that can inhibit *P. falciparum* Sortilin protein from binding cargo proteins. Of the 652 ligands screened and docked from the NPLib, ten (10) ligands with the least binding energy scores were selected and their physiochemical and Absorption, distribution, metabolism, elimination and toxicity (ADMET) properties were checked using Swiss institute of bioinformatics webserver. Further molecular docking studies carried out revealed that lutein and β -carotene were the most potent inhibitors of *P. falciparum* Sortilin protein with binding affinities of -20.9 kcal/mol and -18.9 kcal/mol, respectively. The findings of this study are believed to serve as a baseline information for further wet lab investigations by pharmaceutical organizations toward the development of new drugs for combating the endemicity of malaria in this region of the globe.

Corresponding author

Ja'afar, N. J.

injaafar@mau.edu.ng

Keywords: β -carotene, *In silico*, Lutein, *Plasmodium falciparum*, Rhoptry, Sortilin

1. Introduction

Malaria, a life-threatening disease caused by *Plasmodium* parasite, is transmitted to humans through the bite of infected female *Anopheles* mosquito. Malaria still represents an enormous burden in several tropical and subtropical regions of the globe. For instance, of the 219 million cases recorded in 2017, majority of the 435,000 deaths were due to *Plasmodium falciparum* (*P. falciparum*) [1]. More so, Nigeria accounts for 25% of all malaria cases globally [1].

Like other apicomplexans, *Plasmodium* spp. are obligate intracellular parasites, with a polarized secretory system consisting of micronemes, rhoptries and dense granules, which are required for host cell invasion [2]. Invasion of erythrocytes by the malaria merozoite is a multistep process driven by the highly coordinated sequential release of organelles that form the apical complex: the rhoptries, micronemes, and dense granules [3]. These organelles are developed *de novo* during schizogony stage of the *Plasmodium*'s life cycle [3]. However, the mechanism of their biogenesis to apical complex in *P. falciparum* is poorly understood [4]. Nonetheless, Richard, *et al.* [5] had earlier hypothesized that rhoptry-associated membrane antigen (RAMA), a glycosyl phosphatidylinositol (GPI)-anchored *P. falciparum* rhoptry protein, is serving as an escorter for a number of other

rhoptry proteins found in the low molecular weight rhoptry complex, rhoptry-associated protein (RAP) complex [5].

As the rhoptry organelle is critical for *P. falciparum* to invade erythrocyte, Hallee, *et al.* [4] proved that *P. falciparum* sortilin protein is acting as an escorter for the trafficking of RAMA to the rhoptries. Furthermore, the authors demonstrated the significance of *P. falciparum* sortilin protein in erythrocyte invasion by decreasing its expression. This resulted in the lack of formation of the inner membrane complex thereby abrogating the generation of new merozoites that will invade the erythrocytes [6].

Because of the significance and potential role of *P. falciparum* sortilin in invading erythrocyte through escorting RAMA to the rhoptries and subsequent formation of the apical complex, it makes sortilin a suitable pharmaceutical target towards preventing the invasion of human erythrocyte, thus indirectly reducing the burden of malaria. Hence, this study aimed at *in silico* identification and docking of biomolecules that would bind *P. falciparum* sortilin protein thereby blocking its functionality in binding and escorting RAMA to the rhoptries.

2. Materials and Methods

2.1 Protein sequence retrieval

Amino acid sequence of *P. falciparum*'s sortilin protein (PF3D7_1451800) was retrieved from NCBI database.

2.2 Target protein preparation

Preparation of the target protein and binding site prediction. The three-dimension (3D) structure of *P. falciparum*'s sortilin protein was retrieved using *Genome3D* web server *Genome3D* [7]. In preparation for docking, the protein was processed by adding polar hydrogens, removing hetero-atoms, and then converting the data to ".pdbqt" file format. Furthermore, the binding site of the protein was determined based on blind docking procedure [8].

2.3 Preparation of ligands for docking

Six hundred and fifty-two (652) compounds available at the Natural Product Library (NPLib) were virtually screened using Resource *Paradienne en Bioinformatique Structurale* (RPBS) web server [9]. Subsequently, the 3D structures and common names of the top ten (10) ligands were downloaded from PubChem database [10] in ".sdf" format and converted to ".pdbqt" format. Physiochemical properties of these ligands, such as Lipinski rule of five, log *P* (lipophilicity) and binding affinity (kcal/mol) were checked using Swiss Institute of Bioinformatics webserver [11].

2.4 ADMET Analysis

ADMET properties were checked using Swiss institute of bioinformatics webserver [11]. The ten (10) top ligands were screened for drug likeness by their adherence to Lipinski's "rule of five" with no more than one violation [12]. Other features such as blood-brain barrier penetration, gastrointestinal absorption (bioavailability) and molecular weight were identified from the AdmetSAR server [13].

2.5 Molecular docking using AutoDock

The ten (10) top ligands were each docked with *P. falciparum*'s sortilin protein using AutoDock 4.2 suite. The energy values for each docked molecule were noted and calculated in kcal/mol from the output files. Also, AutoDock inhibition constant (*K_i*) was predicted from the docking process. In the present study, the binding site of each ligand was selected based on the active site component and the substrate-binding site of the *P. falciparum* sortilin protein. The substrate binding site residue was covered as grid box. The grid box size was set as Size X = 69.52 Å, Size Y = 70.51 Å and Size Z = 72.68 Å. The grid box centre was set as Centre X = - 27.11 Å, Centre Y = 20.35 Å and Centre Z = 55.36 Å. AutoGrid program supplied with AutoDock was used to produce grid maps. The spacing between grid points was 0.361 Å. Docked conformations were clustered using a tolerance of 2.0 Å RMSD. The Pymol Molecular Graphics System and DS Visualizer [14] (Accelrys, Inc., USA) were employed to visualize, analyse and modify the receptor and ligand structures.

3. Results and Discussion

Putative binding mode of each ligand with *Plasmodium falciparum* Sortilin protein were shown in Figure 1. Table 1 described some selected ligands from natural product library (NPLib) showing their docking score, inhibitory constant (*K_i*) values, ADMET properties, and interacting residues between the ligands and *Plasmodium falciparum* sortilin protein. A more negative binding affinity indicates stronger binding.

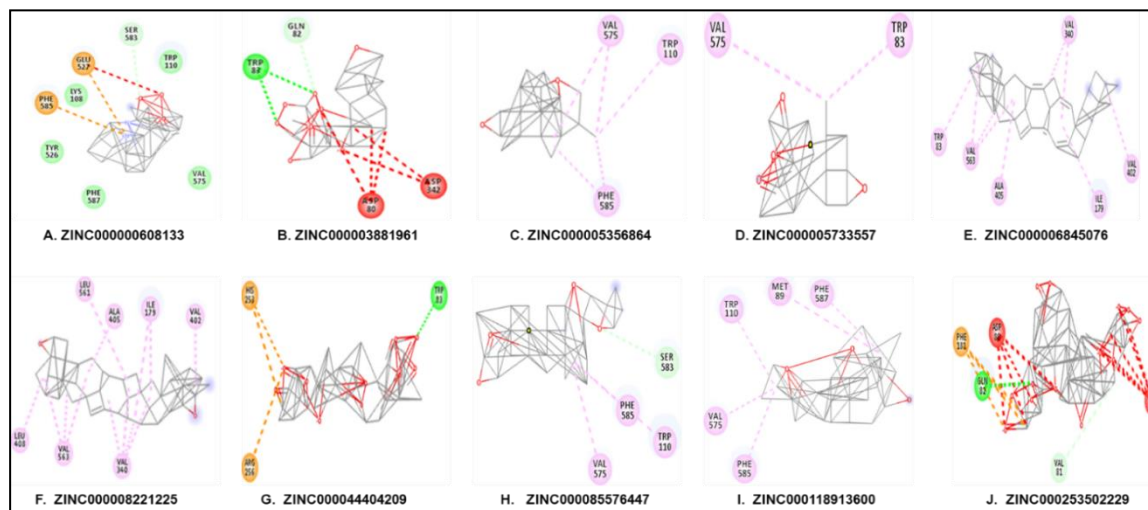


Figure 1: Putative binding mode of each ligand with *Plasmodium falciparum* Sortilin protein

Molecular docking of *P. falciparum*'s sortilin protein with different biomolecules hypothetically blocks its function to bind cargo proteins. To identify a biomolecule that could inhibit *P. falciparum*'s sortilin protein from serving its function in binding cargo proteins, this study screened 652 ligands from the NPLib and docked the best ten (10) ligands (Table 1) with the protein. Of the ten ligands, ZINC000008221225 (Lutein), ZINC000253502229 and ZINC000006845076 (β -Carotene), respectively displayed the least binding energy (docking score) against *P. falciparum*'s sortilin protein (Table 1). The lower the binding energy score, the stronger the

interaction between the receptor and ligand [15]. Carotenoids are divided into two classes according to their chemical structures: the carotenes (e.g., β -carotene) and the xanthophylls (e.g., lutein) [16]. Lutein confers a yellow or orange colour to various common foods such as cantaloupe, orange/yellow peppers, pasta, corn, carrots, salmon, and fish [17]. Similarly, the results showed that the three (3) compounds respectively formed seven, five and six hydrogen bonds (Fig 1) within the active site of *P. falciparum*'s sortilin protein with different amino acid residues.

Table 1: Selected ligands from natural product library (NPLib)

Ligand	Docking Score (kcal/mol)	AutoDock K _i (pM)	Lipinski rule of five	Log P	GI absorption	Blood-Brain Barrier penetration	Interacting residues with <i>Pf</i> Sortilin	Molecular weight (g/mol)	Common name
ZINC000008221225	-20.9	0.0018	2 violations	9.21	Low	No	Ile179, Val340, Val402, Ala405, Leu408, Leu561, Val563	568.87	Lutein
ZINC000253502229	-19.4	0.0210	3 violations	0.94	Low	No	Asp80, Val81, Gln82, Phe181, Asp342	712.9	-
ZINC000006845076	-18.9	0.0473	2 violations	11.11	Low	No	Trp83, Ile179, Val340, Val402, Ala405, Val563	536.87	β-Carotene
ZINC000118913600	-16.3	3.2200	1 violation	5.21	High	Yes	Met89, Trp110, Val575, Phe585, Phe587	416.64	Epitigogenin
ZINC000044404209	-16.2	3.7900	1 violation	3.37	Low	No	Trp83, His253, Arg256	542.49	-
ZINC000085576447	-14.5	59.800	1 violation	4.32	High	Yes	Trp110, Val575, Ser583, Phe585	432.64	-
ZINC000005356864	-12.9	803.00	Yes	3.33	High	Yes	Trp110, Val575, Phe585	270.37	Uden
ZINC000003881961	-12.1	2940.0	1 violation	0.62	High	No	Asp80, Gln82, Trp83, Asp342	390.38	-
ZINC000000608133	-11.7	5630.0	Yes	2.46	High	Yes	Lys108, Trp110, Tyr526, Glu527, Val575, Ser583, Phe585, Phe587	354.44	β-Yohimbine
ZINC000005733557	-9.8	123000	Yes	2.81	High	Yes	Trp83, Val575	268.26	Pratol

However, ZINC000253502229 had to be excluded from further studies because it has been discontinued. Nonetheless, binding of the compounds to *P. falciparum*'s sortilin protein suggests their inhibitory potential for *P. falciparum* to invade erythrocytes. In contrast, Nortey-Mensah, *et al.* [18] and Tonhosolo, *et al.* [19] had demonstrated the active capability of intra erythrocytic stages of *P. falciparum* in carotenoid biosynthesis as it plays an important role in the parasite's metabolism.

The study does not negate the binding of both lutein and β -carotene to *P. falciparum*'s sortilin protein, rather a possible alternative pathway in the utilization of carotenoids. More so, the current study proposes a preventive measure of inhibiting *P. falciparum* from invading erythrocyte. Interestingly, previous studies have shown the potential of these ligands as antimalarials based on different pharmacokinetic pathways [20]. However, most of these studies have not explored the relationship between the binding affinities of the compounds with respect to *P. falciparum*'s sortilin protein.

Grace, *et al.* [21] had described the antiplasmodial activity of lutein from the leaf extract of *Cassia fistula* plant via *in vitro* studies. Further cytotoxicity studies on Chinese hamster ovary (CHO) showed lutein to be non-toxic even at high doses [21]. Nonetheless, Kopec, *et al.* [22] demonstrated the inhibitory effect of lutein on β -carotene absorption but not on its cleavage, suggesting caution when administering foods with these compounds. Similarly, a low level of β -carotene compared to lutein was observed between severely and non-severely *P. falciparum* anaemic individuals before and after seven days post treatment [23]. From a different perspective, other researchers have argued that the merozoite surface protein 1 (MSP1) functions in erythrocyte invasion. Supporting this are reports that MSP1 binds to erythrocyte glycophorin A [24], Band 3 [25, 26], and heparin-like molecules [27]. Although MSP-1 is the most abundant and functionally conserved protein on the merozoite and is associated with the parasite's membrane via a GPI anchor [28], eight other surface-bound GPI-anchored proteins have been identified, some of which have EGF [29] or 6-Cys domains [30, 31]. Nonetheless, the mechanistic pathway of MSP 1 invasion of erythrocyte it is still unclear, as some of the proteins are believed to be involved in regular protein-protein interactions in all *Plasmodium* spp. [32, 33] and cyto-adherence [34].

4. Conclusion

Although several compounds have been identified as potential inhibitors of *P. falciparum*'s sortilin protein, the specific mode of inhibition of these compounds is yet to be understood. The ability of this study to elucidate the relationship between binding affinities of the various biomolecules with respect to inhibition of *P. falciparum*'s sortilin protein could perhaps, serve as a baseline information in developing new antimalarial agents.

References

- [1]. World Health Organization. (2018). World malaria report. World Health Organization, Geneva, Switzerland. 2018, <https://www.who.int/malaria/publications/world-malaria-report-2018/report/en>
- [2]. Mayer, D. C. G. (2021). Protein sorting in *Plasmodium falciparum*. *Life*, 11(9), 937.
- [3]. Cowman A. F., Drew, B. and Jake, B. (2012). The cellular and molecular basis for malaria parasite invasion of the human red blood cell. *Journal of Cell Biology*, 198(6), 961–971
- [4]. Hallée, S., Boddey, J. A., Cowman, A. F. and Richard, D. (2018a). Evidence that the *Plasmodium falciparum* protein sortilin potentially acts as an escorter for the trafficking of the rhoptry-associated membrane antigen to the rhoptries. *Mosphere*, 28, 3(1), e00551-17.
- [5]. Richard, D., Kats, L. M., Langer, C., Black, C. G., Mitri, K., Boddey, J. A., Cowman, A. F. and Coppel, R. L. (2009). Identification of rhoptry trafficking determinants and evidence for a novel sorting mechanism in the malaria parasite *Plasmodium falciparum*. *PLoS pathogens*, 5(3), p.e1000328.
- [6]. Hallée, S., Counihan, N. A., Matthews, K., de Koning-Ward, T. F. and Richard, D. (2018b). The malaria parasite *Plasmodium falciparum* Sortilin is essential for merozoite formation and apical complex biogenesis. *Cellular Microbiology*, 20(8), e12844.
- [7]. Genome3D Web Server, (2023). Three-dimension (3D) structure of *P. falciparum*'s sortilin protein. Retrieved in 2023 from Genome3D server <http://www.genome3d.eu/uniprot/id/Q8IKV8/annotations>
- [8]. Hassan, N. M., Alhossary, A. A., Mu, Y. and Kwoh, C. K. (2017). Protein-ligand blind docking using QuickVina-W with inter-process spatio-temporal integration. *Scientific Reports*, 7(1), 15451.
- [9]. RPBS Web Server, (2023). *Resource Paradienne en Bioinformatique Structurale*. Virtually screened using RPBS Web Server in 2023: <https://mobylye.rpbs.univ-paris-diderot.fr/cgi-bin/portal.py#jobs::overview>
- [10]. PubChem database (2023). The 3D structures and common names of the top ten (10) ligands. Downloaded in 2023 from PubChem database: <http://pubchem.ncbi.nlm.nih.gov>
- [11]. Swiss Institute of Bioinformatics webserver, (2023). Physiochemical properties of these ligands, such as Lipinski rule of five, log *P* (lipophilicity) and binding affinity (kcal/mol) were checked in 2023 using Swiss Institute of Bioinformatics webserver: <https://www.sib.swiss>
- [12]. Lipinski, C. A., Lombardo, F., Dominy, B. W. and Feeney, P. J. (2012). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced Drug Delivery Reviews*, 64, 4-17.
- [13]. AdmetSAR server (2023). Blood-brain barrier penetration, gastrointestinal absorption (bioavailability) and molecular weight were identified from the AdmetSAR server: <http://lmmd.ecust.edu.cn/admetSar2/>

- [14]. Accelrys, Inc., (2023). Visualization, analyses and modification of the receptor and ligand structures. Conducted using Pymol Molecular Graphics System and DS Visualizer, (Accelrys, Inc., USA).
- [15]. Deborah, A. F. and Ijidakinro, O. D. (2021). In Silico Antiparasitic investigation of compounds derived from *Andrographis paniculata* on some parasites validated drug targets. *African Journal of Biological Sciences*, 3(3), 93-110.
- [16]. Perrone, S., Tei, M., Longini, M. and Buonocore, G. (2016). The multiple facets of lutein: a call for further investigation in the perinatal period. *Oxidative Medicine and Cellular Longevity*, 2016, 5381540.
- [17]. Mitra, S., Rauf, A., Tareq, A. M., Jahan, S., Emran, T. B., Shahriar, T. G., Dhama, K., Alhumaydhi, F. A., Aljohani, A. S., Rebezov, M. and Uddin, M. S. (2021). Potential health benefits of carotenoid lutein: an updated review. *Food and Chemical Toxicology*, 154, 112328.
- [18]. Nortey-Mensah, R., Teye, M. A. and Ofori, M. F. (2021). The carotenoid biosynthesis pathway in the asexual intraerythrocytic stages of *Plasmodium falciparum*. *Asian Journal of Tropical Biotechnology*, 18(1), 13-27.
- [19]. Tonhosolo, R., Fabio, L. D., de Rosso, V. V., Gazarini, M. L., Matsumura, M. Y., Peres, V. J., Merino, E. F., Carlton, J. M., Wunderlich, G., Mercadante, A. Z. and Kimura, E. A. (2009). Carotenoid biosynthesis in intraerythrocytic stages of *Plasmodium falciparum*. *Journal of Biological Chemistry*, 284(15), 9974-9985.
- [20]. Klein, P. W., Easterbrook, J. D., Lalime, E. N. and Klein, S. L. (2008). Estrogen and progesterone affect responses to malaria infection in female C57BL/6 mice. *Gender Medicine*, 5(4), 423-433.
- [21]. Grace, M. H., Lategan, C., Graziose, R., Smith, P. J., Raskin, I. and Lila, M. A. (2012). Antiplasmodial activity of the ethnobotanical plant *Cassia fistula*. *Natural Product Communications*, 7(10), 1263-1266.
- [22]. Kopec, R. E., Cooperstone, J. L., Schweiggert, R. M., Young, G. S., Harrison, E. H., Francis, D. M., Clinton, S. K. and Schwartz, S. J. (2014). Avocado consumption enhances human postprandial provitamin A absorption and conversion from a novel high- β -carotene tomato sauce and from carrots. *The Journal of Nutrition*, 144(8), 1158-1166.
- [23]. Nussenblatt, V., Mukasa, G., Metzger, A., Ndeezi, G., Eisinger, W. and Semba, R. D. (2002). Relationship between carotenoids and anaemia during acute uncomplicated *Plasmodium falciparum* malaria in children. *Journal of Health, Population and Nutrition*, 1, 205-214.
- [24]. Baldwin, M. R., Li, X., Hanada, T., Liu, S. C. and Chishti, A. H. (2015). Merozoite surface protein 1 recognition of host glycophorin A mediates malaria parasite invasion of red blood cells. *Blood, the Journal of the American Society of Haematology*, 125(17), 2704-2711.
- [25]. Goel, V. K., Li, X., Chen, H., Liu, S. C., Chishti, A. H. and Oh, S. S. (2003). Band 3 is a host receptor binding merozoite surface protein 1 during the *Plasmodium falciparum* invasion of erythrocytes. *Proceedings of the National Academy of Sciences*, 100(9), 5164-5169.
- [26]. Li, X., Chen, H., Oo, T. H., Daly, T. M., Bergman, L. W., Liu, S. C., Chishti, A. H. and Oh, S. S. (2004). A co-ligand complex anchors *Plasmodium falciparum* merozoites to the erythrocyte invasion receptor band 3. *Journal of Biological Chemistry*, 279(7), 5765-5771.
- [27]. Zhang, Y., Jiang, N., Lu, H., Hou, N., Piao, X., Cai, P., Yin, J., Wahlgren, M. and Chen, Q. (2013). Proteomic analysis of *Plasmodium falciparum* schizonts reveals heparin-binding merozoite proteins. *Journal of proteome research*, 12(5), 2185-2193.
- [28]. Gerold, P., Schofield, L., Blackman, M. J., Holder, A. A. and Schwarz, R. T. (1996). Structural analysis of the glycosyl-phosphatidylinositol membrane anchor of the merozoite surface proteins-1 and-2 of *Plasmodium falciparum*. *Molecular and biochemical parasitology*, 75(2), 131-143.
- [29]. Savage, C.R., Inagami, T. and Cohen, S. (1972). The primary structure of epidermal growth factor. *Journal of Biological Chemistry*, 247(23), 7612-7621.
- [30]. Ishino, T., Chinzei, Y. and Yuda, M. (2005). Two proteins with 6-cys motifs are required for malarial parasites to commit to infection of the hepatocyte. *Molecular Microbiology*, 58(5), 1264-1275.
- [31]. Sanders, P.R., Gilson, P.R., Cantin, G.T., Greenbaum, D.C., Nebl, T., Carucci, D.J., McConville, M.J., Schofield, L., Hodder, A.N., Yates, J. R. and Crabb, B. S. (2005). Distinct protein classes including novel merozoite surface antigens in Raft-like membranes of *Plasmodium falciparum*. *Journal of Biological Chemistry*, 280(48), 40169-40176.
- [32]. Haynes, J.D., Dalton, J. P., Klotz, F. W., McGinniss, M. H., Hadley, T. J., Hudson, D. E. and Miller, L. H. (1988). Receptor-like specificity of a *Plasmodium knowlesi* malarial protein that binds to Duffy antigen ligands on erythrocytes. *The Journal of Experimental Medicine*, 167(6), 1873-1881.
- [33]. Adams, J.H., Sim, B. K., Dolan, S. A., Fang, X., Kaslow, D. C. and Miller, L.H. (1992). A family of erythrocyte binding proteins of malaria parasites. *Proceedings of the National Academy of Sciences*, 89(15), 7085-7089.
- [34]. Su, X. Z., Kirkman, L.A., Fujioka, H. and Wellems, T. E. (1997). Complex polymorphisms in an approximate 330-kDa protein are linked to chloroquine-resistant *P. falciparum* in Southeast Asia and Africa. *Cell*, 91(5), 593-603.