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In silico study on the apicoplast L4 ribosomal protein and three domains from 23S rRNA from *Plasmodium falciparum* and comparison with the existing co-crystal structures

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ABSTRACT

We performed preliminary computational studies on the construction of a segment of ribosomal protein L4 from the apicoplast ribosome of *Plasmodium falciparum*. With a Z-score of -3.404, it is arguably the best constructed model of this drug target so far. Three domains from 23S rRNA were made from scratch using the software RNA2D3D: domain II, IV and V. They were not validated but show reasonable similarity with bacterial 23S rRNA. This model has technical limitations but is a starting point; refined models are expected to find use in antimalarial drug design.

Keywords: *in sillico*, *Plasmodium falciparum*, ribosome

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Introduction

Malaria is a disease caused by several strains of protozoa from the *Plasmodium* genus. Many attempts have been made to control the disease including vaccination, vector control and parasiticidal drugs (Ralph et al., 2001).Currently, parasiticides constitute the most routinely applied approach in combating malaria, complementing the widely popular use of insecticide-treated nets (World Health Organization, 2019). However, there is widespread resistance to existing anti-malarial drugs (World Health Organization, 2019). Also, many studies have been conducted into the development of prophylactic treatments (especially vaccines) (World Health Organization, 2019). The most promising vaccine to date is a recombinant protein-basedRTS, S/AS01 vaccine.One of the drawbacks of this vaccine is that children treated with this medicine show an elevated risk of meningitis infection (Moorthy and Okwo-Bele, 2015).

Plastid can be defined as any organelle that is the site of manufacture and storage of important chemical compounds used by the cell. The apicoplast originates from the same endosymbiosis as other plastids (Gleeson, 2000), so it can be regarded as a mini-bacterium living inside the malaria parasite. All familiar processes happening inside apicoplast are bacterial in nature (DNA replication, transcription, translation, post-translational modification, catabolism, and anabolism), and can be potential drug targets.

In addition to the standard treatments, there are several families of drugs in experimental use or under investigation as potential medicines against the malaria parasite. Some of them act on metabolic targets such as DNA replication (Gozalbes et al., 2000), RNA transcription (Pukrittayakamee et al., 1994), protein translation (Clough et al., 1999; McConkey et al., 1997; Pfefferkorn and Borotz, 1994; Rogers et al., 1998; Woods et al., 1996), amino acid biosynthesis (Roberts et al., 1998), isopentenyl diphosphate biosynthesis (Clastre et al., 2007; Jomaa et al., 1999) and fatty acid biosynthesis (Surolia and Surolia, 2001). However, only a few of them show significant inhibitory activity against *P. falciparum* (rifampicin, azithromycin, thiostrepton, tetracycline, amythiamicin and fosmidomycin). Rifampicin inhibits transcription from the 35kb apicoplast genome by targeting the plastid-encoded RNA polymerase (McConkey et al., 1997; Uddin et al., 2018). Azithromycin has activity on plastid 23S rRNA, but nevertheless there is no substantial evidence of its mode of action (Dahl and Rosenthal, 2007; Sidhu et al., 2007; Yeo and Rieckmann, 1995).

Azithromycin is a potent anti-bacterial agent that exhibits mild anti-malarial activity (Arsic et al., 2014). Schlunzen et al. (2003) reported the crystal structure of azithromycin bound to the ribosome of *D. radiodurans* and postulated two binding sites. It was found that the first azithromycin molecule has interactions mostly with domains IV and V of 23S rRNA,while the second azithromycin molecule interacts with the ribosomal proteins L4,L22and domain II of 23S rRNA. We wanted to investigate whether other macrolide antibiotics possess anti-malarial activity but we were hindered by the lack of a

crystal structure of the exit tunnel of the apicoplast ribosomal exit tunnel from *P. falciparum* (caused in part by the difficulty of separating apicoplasts from mitochondria). We therefore explored the use of the bacterial crystal structure of *D. radiodurans* as a template for superpositions of the modelled *P. falciparum* exit tunnel and *D. radiodurans*(Figure 1).

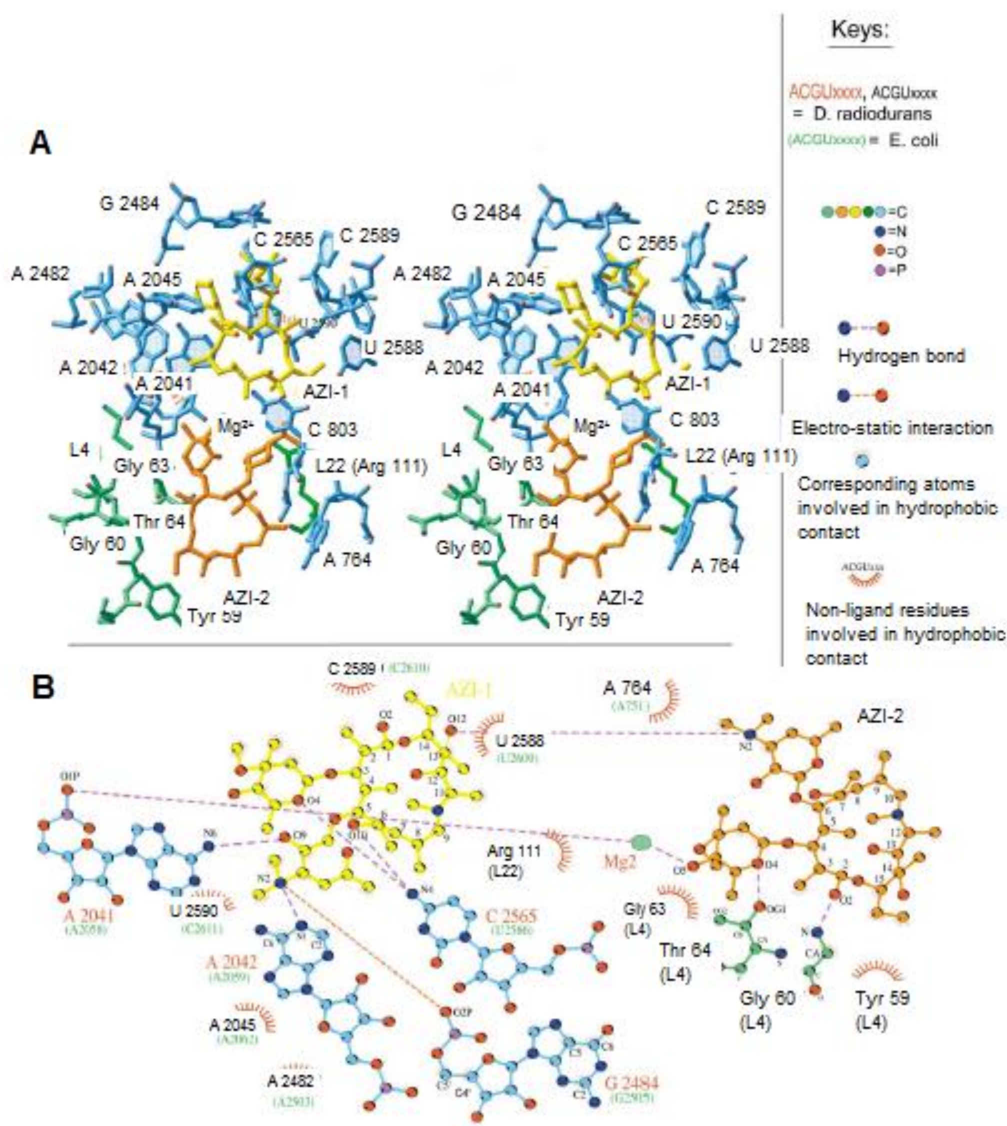


Figure 1. (**A**) Stereoview of the local environment around the two azithromycin molecules. (**B**) Two-dimensional sketch of interactions between the two azithromycin molecules, 23S rRNA, and the ribosomal proteins L4 and L22. Nucleotides or amino acids contributing to hydrophobic interactions are indicated; those contributing to hydrogen bonds or electrostatic interactions are represented by their structures (Schlunzen et al., 2003)

Sidhu et al.(2007)modelled the L4 segment from *P. falciparum* (Lys^{57} to Pro^{97}) using MODELLER and *E. coli* and *D. radiodurans* as templates. However, no quantitative scores of the quality of the model were provided, so its precision cannot be estimated (Sidhu et al., 2007).

Experimental

Homology modelling using the SWISS Model server (Arnold et al., 2006)

Homology modelling using the SWISS Model server was performed by submitting the sequence of the target sequence of ribosomal protein L4 from *P. falciparum*, and template pdb files of L4 from *D. radiodurans* and *E. coli* extracted using Molecular Viewer.

Ab initio molecular modelling using I-TASSER server (Roy et al., 2010; Roy et al., 2011;Zhang, 2008)

Ab initio molecular modelling using I-TASSER server of L4 ribosomal protein from *P. falciparum* was performed by submitting the sequence of this protein and using the option of modelling without the template.

Modelling of 23S rRNA using RNA2D3D (http://www-lmmb.ncifcrf.gov/~bshapiro/software.html)

The molecular modelling of 23S rRNA from *P. falciparum* was performed making two separate input files containing the RNA sequences of 5' and 3'-half of 23S rRNA and information of the base pairs obtained from the secondary structure of 23S rRNA. The obtained crude models were refined according to the manual given by the creators of the software RNA2D3D (http://www-lmmb.ncifcrf.gov/~bshapiro/software.html). Energy refinement was performed using the TINKER software available in RNA_2D3D, and two separate models were generated.

PyMOL (https://pymol.org/2/)superposition of 23S rRNA of *D. radiodurans* domains and constructed domains of 23S rRNA from *P. falciparum*

L4 and 23S rRNA were modelled separately as described in previous sections above. These were now aligned with the corresponding *D. radiodurans* moieties using Pymol. Three domains of 23S rRNA (II, IV, and V) are important for macrolide binding, and each was aligned separately with the

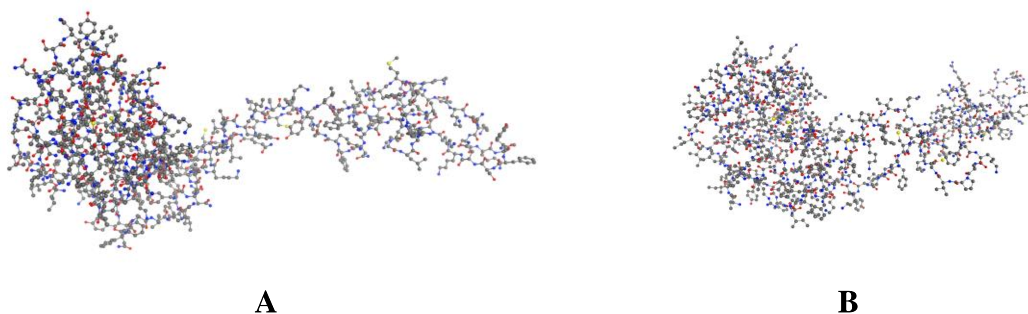
corresponding domains from *D. radiodurans*. The smallest RMS was shown with the alignment of domain IV (RMS=14.528). Domain IV was now superimposed on the *D. radiodurans*RNA structure, and the *D. radiodurans* domain IV deleted. This procedure was repeated sequentially with domains II and V to give a hybrid 23S rRNA.

Results and Discussion

Modelling of apicoplast-encoded L4

Ribosomal protein L4 from the *P. falciparum* apicoplast (PfRpl4) shows only modest similarity in primary sequence with reported crystal structures of bacterial L4, even in the protein region responsible for macrolide activity against bacteria (Sidhu et al., 2007). It was reported that *E.coli* and *D.radiodurans*L4 proteins, share 39% and 32% sequence identity with PfRpl4 in this loop region (Sidhu et al., 2007).

In our study, we used SWISS Model (Arnold et al., 2006), and *E. coli*(Mitra et al., 2006) and *D. radiodurans*(Harms et al., 2008)as templates, and the whole L4 protein from *P. falciparum* was constructed from each template separately. The two models show similar structural characteristics. The model obtained using *D. radiodurans* as a template analyzed by YASARA (www.yasara.org) yielded 29.9% Helix, 11.4% Sheet, 9.6% Turn, 41.2% Coil, 0.0% 3-10 Helix, and 7.9% of the model was not organized into the common motifs. Whilst, the model obtained using *E. coli* as a template analyzed by YASARA gave 24.2% Helix, 5.2% Sheet, 13.4% Turn, 50.5% Coil, and 6.7% 3-10 Helix. Unfortunately, the Ramachandran Z-scores (Hooft et al., 1997) obtained from these two models were low (model obtained using *D. radiodurans* as a template has the value of -7.192 and model using *E. coli* as a template has the value of -5.982) showing that they are not satisfactory models (Figure 2).



A **B**

Figure 2. A An apicoplast ribosomal L4 protein of *Plasmodium falciparum* obtained using SWISS model and *D. radiodurans* as a template; **B** An apicoplast ribosomal L4 protein of *Plasmodium falciparum* obtained using SWISS model and *E. coli* as a template

Ab initio calculations were performed using an I-TASSER server (Roy et al., 2010; Roy et al., 2011; Zhang, 2008) by submitting the sequence of the protein to be modelled in FASTA format and using the option for modelling without the template (*ab initio*). The whole sequence of L4 ribosomal protein from *P. falciparum* apicoplast was used. The obtained model (Figure 3) shows c-score=-2.26 and Ramachandran Z-score=-4.081. Again, the model was not satisfactory because the Z-score was on the border of acceptability.

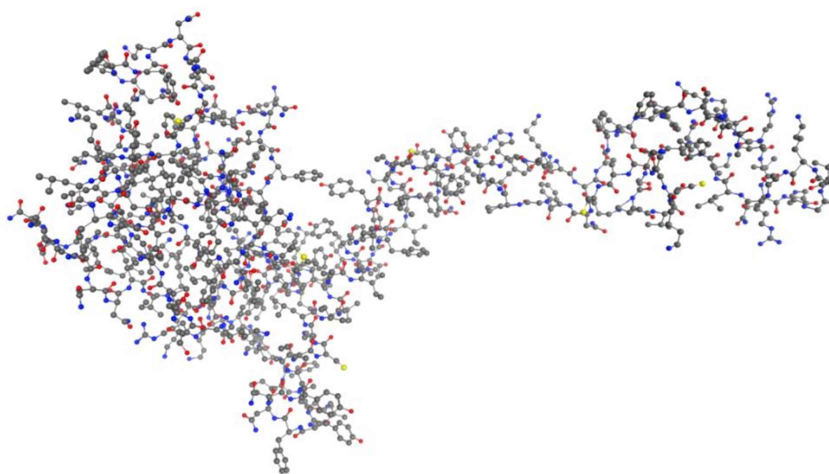


Figure 3. L4 protein from *P. falciparum* obtained using *ab initio* method on I-TASSER

In order to improve the modelling, *ab initio* modelling was used on the I-TASSER server to design the L4 ribosomal protein segment (Lys^{57} to Pro^{97}) also previously modelled by Sidhu et al. (2007). A model was obtained with a corresponding Ramachandran Z-score of -3.404, which is satisfactory (Figure 4).

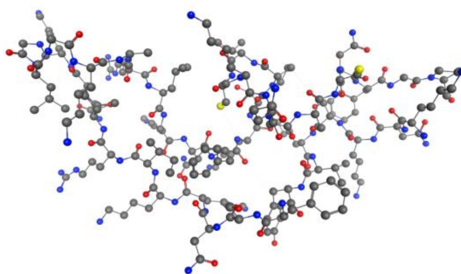


Figure 4. A segment of L4 ribosomal protein from *P. falciparum* obtained using *ab initio* method on I-TASSER

The results demonstrated that the homology modelling on the apicoplast proteins from *P. falciparum* had little similarity to the templates (*D. radiodurans* and *E. coli*) and all known and available crystal structures of L4 protein was less successful than *ab initio* modelling of the same structure. The modelled segment of L4 apicoplast ribosomal protein (Lys^{57} to Pro^{97} in *D. radiodurans*) from *P. falciparum* using *ab initio* method represents a reliable model on the basis of its Ramachandran Z-score and could be useful for the construction of the exit tunnel of the apicoplast ribosome from *P. falciparum*.

Modelling on 23S rRNA from P. falciparum

Modelling of the 23S rRNA presented an additional challenge. In order to model the sequence, due to its length the sequence had to be divided into two halves. Modelling was subsequently performed, and refinement and energy minimization of the two halves was performed separately. The division of the RNA into two halves was somewhat arbitrary and it is difficult to judge whether this necessary simplification could be justified. Unfortunately, there is no method similar to protein methods for checking the quality of generated structures. However, there was high similarity between the *P. falciparum* apicoplast LSU rRNA and the *E. coli* 23 S rRNA (they have 70% sequence identity (Sidhu et al., 2007)), particularly in hairpins; therefore, we are quite confident that the separation into two halves did not affect significantly the conformations of the hairpin regions involved in the binding to macrolides.

Comparison of the sequence and secondary structure of *P. falciparum* and organisms with known crystal structures yielded a significant similarity in domain V, but very small similarity in sequence and base pairs of domains II and IV of 23S rRNA. The final models

(Figure 5) appeared to be satisfactory, but there were concerns about the folding because of the mutual influence of one modelled 23S rRNA residue on other.

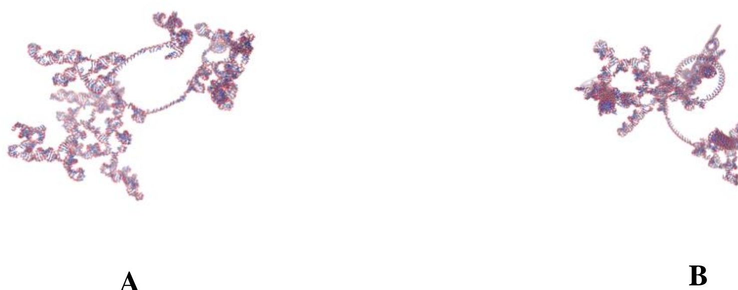


Figure 5. A Model of large subunit ribosomal RNA-3' half of *P. falciparum*; **B** Model of large subunit ribosomal RNA-5' half of *P. falciparum*

Conclusion

We have made a first attempt to model the apicoplast ribosome exit tunnel, which may be an important drug target in the fight against malaria. *Ab initio* modelling of apicoplast ribosomal L4 gave models with acceptable Ramachandran Z-scores, whereas homology modelling did not. The challenging task of modelling the long sequence of RNA was performed by splitting the RNA into two fragments *in silico*. Comparison of the sequence and secondary structure of *P. falciparum* and organisms with known crystal structures yielded a significant similarity in domain V, but very small similarity in sequence and base pairs of domains II and IV of 23S rRNA. The obtained modelscouldbe good starting point for docking drugs.

Acknowledgment

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Conflict-of-Interest Statement

There is no conflict of interest.

References

Arnold, K., Bordoli, L., Kopp, J., &Schwede, T. (2006). The SWISS-MODEL workspace: a web-based environment for protein structure homology modelling. Bioinformatics, 22,195-201.

Arsic, B., Awan, A., Brennan, R., Aguilar, J., Ledder, R., McBain, A. J., Regan, A. C., & Barber, J. (2014). Theoretical and experimental investigation on clarithromycin, erythromycin A and azithromycin and descladinosyl derivatives of clarithromycin and azithromycin with 3-*O*-substitution as anti-bacterial agents. MedChemComm, 5(9), 1347-1354.

Clastre, M., Goubard, A., Prel, A., Mincheva, Z., Viaud-Massuart, M.-C., Bout, D., Rideau, M., Velge-Roussel, F., &Laurent, F. (2007). The methylerythritol phosphate pathway for isoprenoid biosynthesis in coccidia: Presence and sensitivity to fosmidomycin. Experimental Parasitology, 116, 375-384.

Clough, B., Rangachari, K., Strath, M., Preiser, P. R., & Wilson, R. (1999). Antibiotic inhibitors of organellar protein synthesis in *Plasmodium falciparum*. Protist, 150, 189-195.

Dahl, E. L., & Rosenthal, P. J. (2007). Multiple antibiotics exert delayed effects against the *Plasmodium falciparum* apicoplast. Antimicrobial Agents and Chemotherapy, 51, 3485-3490.

Gleeson, M. T. (2000). The plastid in Apicomplexa: what use is it?International Journal of Parasitology, 30, 1053-1070.

Gozalbes, R., Brun-Pascaud, M., Garcia-Domenech, R., Galvez, J., Girard, P.-M., Doucet, J.-P., & Derouin, F. (2000). Anti-*Toxoplasma* activities of 24 quinolones and fluoroquinolones in vitro: prediction of activity by molecular topology and virtual computational techniques. Antimicrobial Agents and Chemotherapy, 44, 2771-2776.

Harms, J. M., Wilson, D. N., Schluenzen, F., Connell, S. R., Stachelhaus, T., Zaborowska, Z., Spahn, C. M. T, &Fucini P. (2008). Translational regulation *via* L11: Molecular switches on the ribosome turned on and off by Thiostrepton and Micrococcin. Molecular Cell, 30, 26-38.

Hooft, R. W., Sander, C., &Vriend, G. (1997). Objectively judging the quality of a protein structure from a Ramachandran plot. Computer Applications in the Biosciences, 13, 425-430.

http://www-lmmb.ncifcrf.gov/~bshapiro/software.html, accessed 19/11/2018

https://pymol.org/2/, accessed 19/11/2018

https://www.who.int/malaria/en/, accessed 24/09/2019

Jomaa, H., Wiesner, J., Sanderbrand, S., Altincicek, B., Weidemeyer, C., Hintz, M., Turbachova, I., Eberl, M., Zeidler, J., Lichtenthaler, H. K., Soldati, D., & Beck, E. (1999). Inhibitors of the nonmevalonatepathway of isoprenoid biosynthesis as antimalarial drugs. Science, 285, 1573-1576.

McConkey, G.A., Rogers, M. J., &McCutchan, T. F. (1997). Inhibition of *Plasmodium falciparum* protein synthesis. Targeting the plastid-like organelle with thiostrepton. The Journal of Biological Chemistry, 272, 2046-2049.

McConkey,G. A., Rogers, M. J., &McCutchan, T. F. (1997). Inhibition of *Plasmodium falciparum* protein synthesis. The Journal of Biological Chemistry, 272, 2046-2049.

Mitra, K.,Schaffitzel, C., Fabiola, F., Chapman, M. S., Ban, N., & Frank, J. (2006). Elongation arrest by SecM*via* a cascade of ribosomal RNA rearrangements. Molecular Cell, 22, 533-543.

Moorthy, V. S., &Okwo-Bele, J-M. (2015). Final results from a pivotal phase 3 malaria vaccine trial. Lancet, 386, 5-7.

Pfefferkorn, E. R., &Borotz, S. E. (1994). Comparison of mutants of *Toxoplasma gondii* selected for resistance to azithromycin, spiramycin, or clindamycin. Antimicrobial Agents and Chemotherapy, 338, 31-37.

Pukrittayakamee, S., Viravan, C., Charoenlarp, P., Yeamput, C., Wilson, R. J. M., & White, N. J. (1994). Antimalarial effects of Rifampin in *Plasmodium vivax*malaria. Antimicrobial Agents and Chemotherapy, 38, 511-514.

Ralph, S. A., D'Ombrain, M. C., & McFadden, G. I. (2001). The apicoplast as an antimalarial drug target.Drug Resistance Update, 4, 145-151.

Roberts, F., Roberts, C. W., Johnson, J. J., Kyle, D. E., Krell, T., Coggins, J. R., Coombs, G. H., Milhous, W. K., Tzipori, S., Ferguson, D. J. P., Chakrabarti, D., &McLeod, R. (1998). Evidence for the shikimate pathway in apicomplexan parasites. Nature, 393, 801-805.

Rogers, M. J., Cundliffe, E., &McCutchan, T. F. (1998). The antibiotic Micrococcinis a potent inhibitor of growth and protein synthesis in the malaria parasite. Antimicrobial Agents and Chemotherapy, 42, 715-716.

Roy, A., Kucukural, A., & Zhang, Y. (2010). I-TASSER: a unified platform for automated protein structure and function prediction. Nature Protocols, 5, 725-738.

Roy, A., Xu, D., Poisson, J., & Zhang, Y. (2011). A protocol for computer-based protein structure and function prediction. Journal of Visualized Experiments, 57, e3259.

RTS,S Clinical Trials Partnership. (2015). Efficacy and safety of RTS,S/AS01 malaria vaccine with or without a booster dose in infants and children in Africa: Final results of a phase 3, individually randomized, controlled trial. The Lancet, 386 (9988), 31-45.

Schlunzen, F., Harms J. M., Franceschi, F., Hansen, H. A. S., Bartels, H., Zarivach, R., &Yonath, A. (2003). Structural basis for the antibiotic activity of ketolides and azalides. Structure, 11, 329-338.

Sidhu, A. B. S., Sun, Q., Nkrumah, L. J., Dunne, M. W., Sacchettini, J. C., & Fidock, D. A. (2007). *In vitro* efficacy, resistance selection, and structural modelling studies implicate the malarial parasite apicoplast as the target of azithromycin. The Journal of Biological Chemistry, 282, 2494-2504.

Surolia, N., & Surolia, A. (2001). Triclosan offers protection against blood stages of malaria byinhibiting enoyl-ACP reductase of *Plasmodium falciparum*. Nature Medicine, 7, 167-173.

Uddin, T., McFadden, G.I., & Goodman, C.D. (2018). Validation of putative apicoplast-targeting drugs using a chemical supplementation assay in cultured human malaria parasites. Antimicrobial Agents and Chemotherapy, 62 (1), e01161-17.

Woods, K. M., Nesterenko, M. V., &Upton, S. J. (1996). Efficacy of 101 antimicrobials and other agents on the development of *Cryptosporidium parvumin vitro*. Annals of Tropical Medicine and Parasitology, 90, 603-615.

World Health Organization. (2019). World malaria Report 2019.

www.yasara.org, accessed 19/11/2018

Yeo, A. E. T., & Rieckmann, K. H. (1995). Increased antimalarial activity of azithromycin during prolonged exposure of *Plasmodium falciparum in vitro.* International Journal of Parasitology, 25, 531-532.

Zhang, Y. (2008). I-TASSER server for protein 3D structure prediction. BMC Bioinformatics, 9, 40.