Structure-based drug design studies of the interactions of ent-kaurane diterpenes derived from *Wedelia paludosa* with the *Plasmodium falciparum* sarco/endoplasmic reticulum Ca\(^{2+}\)-ATPase PfATP6

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Malaria is responsible for more deaths around the world than any other parasitic disease. Due to the emergence of strains that are resistant to the current chemotherapeutic antimalarial arsenal, the search for new antimalarial drugs remains urgent though hampered by a lack of knowledge regarding the molecular mechanisms of artemisinin resistance. Semisynthetic compounds derived from diterpenes from the medicinal plant *Wedelia paludosa* were tested in silico against the *Plasmodium falciparum* Ca\(^{2+}\)-ATPase, PfATP6. This protein was constructed by comparative modelling using the three-dimensional structure of a homologous protein, IWO, as a scaffold. Compound 21 showed the best docking scores, indicating a better interaction with PfATP6 than that of thapsigargin, the natural inhibitor. Inhibition of PfATP6 by diterpene compounds could promote a change in calcium homeostasis, leading to parasite death. These data suggest PfATP6 as a potential target for the antimalarial ent-kaurane diterpenes.

Key words: malaria - ent-kaurane diterpenes - PfATP6 - docking - computer aided-drug design

Malaria is the most widespread infectious parasitic disease around the world (WHO 2013). The burden of emerging parasites resistant to artemisinin combination therapies threatens global efforts to control malaria, making the need for new antimalarial drugs urgent (Miller et al. 2013). However, the molecular mechanisms of artemisinin resistance are unknown (Nunes-Alves 2014). Among the numerous genes involved in artemisinin resistance, PfATP6, a sarco/endoplasmic reticulum Ca\(^{2+}\)-ATPase (SERCA) homologue expressed in *Plasmodium falciparum*, has been suggested as a possible target of artemisinin (Valderramos et al. 2010, Cui et al. 2012).

Overcoming the major challenges in the development of new antimalarial candidates relies on efficient candidate synthesis and a better understanding of the mechanism of action of defined targets (Médebielle 2014). In this context, our research group recently demonstrated the in vitro antiplasmodial activity of oxidised/epoxidiased ent-kaurane diterpene derivatives obtained from the naturally occurring ent-kaurenes of *Wedelia paludosa* DC (Batista et al. 2013). Due to the selective antimalarial activity exhibited by these compounds and knowing that diterpenes induce calcium overload in myocytes (Sun et al. 2014) we investigated the interaction between the ent-kaurane diterpenes and PfATP6 (Gardner et al. 2002). SERCA are crucial for calcium signalling in malarial parasites (Furuyama et al. 2014, Krishna et al. 2014). Calcium signalling is associated with the regulation of many processes during the parasite life cycle, including modulation of kinase activity and synchronisation of the intraerythrocytic cycle (Bagnaresi et al. 2012). Molecular mechanisms that maintain parasite calcium homeostasis are controlled by proteins such as PfATP6 (Krishna et al. 2010). Any alteration to calcium homeostasis, triggered by the inhibition of PfATP6, could promote an increase in cytoplasmic calcium, leading to the activation of the metacaspase PfMCA-1 (Meslin et al. 2011). This mode of action could provide a new strategy to design new pro-apoptotic antimalarial drugs.

Thus, the aim of this study was to construct the PfATP6 enzyme model and perform a rigid and flexible molecular docking analysis of synthetic and semisynthetic diterpenes derived from *W. paludosa* DC.

Initially, the primary sequence of the PfATP6 was obtained from the *Plasmodium* genomics Resource (plasmodb.org/plasmo/). Subsequently, a three-dimensional model was built following a comparative modelling approach. The model was constructed using SwissPDB Viewer 3.7 following a standard protocol as previously described (Bordoli et al. 2009): (i) load the primary sequence of PfATP6, (ii) search for templates against Protein Data Bank (Berman et al. 2013) and (iii) perform structural alignment and submit it to the Swiss Model Server. In this process, the atomic coordinates of thapsigargin (TG), the natural SERCA inhibitor, were transferred from IWO (Toyoshima & Nomura 2002)
Flexible PfATP6 2+ in vitro (Batista et al. 2013). Compound PfATP6 1IWO 1IWO (A) and parasite PfATP6 model (B).

![Image](https://via.placeholder.com/150)

Fig. 1: three-dimensional structure of the mammalian Ca2+-ATPase 1IWO (A) and parasite PfATP6 model (B).

**TABLE**

| Compounds | I1WO Rigid | PfATP6 Rigid | I1WO Flexible | PfATP6 Flexible |
|-----------|------------|--------------|---------------|-----------------|
| 8         | -7.6       | -7.7         | -7.1          | -6.6            |
| 9         | -7.6       | -7.8         | -6.6          | -6.3            |
| 21        | -9.5       | -8.4         | -7.4          | -6.8            |
| Thapsigargin | -7.7     | -7.2         | -5.8          | -4.7            |

The binding energies of the enzymes PfATP6 and 1IWO, as well as those of ent-kaurane diterpenes (8, 9 and 21) and TG are shown in Table. The three ent-kaurane diterpenes shown in Table were active against *P. falciparum* with 43%, 49% and 43.5% identity, respectively. All of them were used as a template to build the model (Fig. 1) using Swiss Model Project Mode (Bordoli et al. 2009, Naik et al. 2011).

Sequence alignment between PfATP6 and the templates showed a region with low similarity that was removed from PfATP6 to build the model. The PfATP6 refined model was evaluated based on the root-mean-square deviation (RMSD) value of the Cα coordinates and the Ramachandran Plot generated by PROCHECK software (Laskowski et al. 1993). Both methods compared against 1IWO showed an RMSD value of 1.61 Å and 87% of residues in the allowed regions; the corresponding percentage for the 1IWO crystal structure was 77.3%. This model is composed of three cytoplasmic domains, denoted as the actuator, phosphorylation and nucleotide binding domains; in addition, there are 10 transmembrane segments denoted as M1 to M10. Initially, the re-docking process showed the structural differences in the natural ligand before and after the molecular docking. The RMSD score was 1.12 Å, indicating that the structures of the molecules are similar to each other. The limit value accepted by the docking approach is 2.0 Å (Trott & Olson 2010). As a result, AutoDock Vina software was used to generate the binding energies of diterpenes and the two enzymes.

The binding energies of the enzymes PfATP6 and 1IWO, as well as those of ent-kaurane diterpenes (8, 9 and 21) and TG are shown in Table. The three ent-kaurane diterpenes shown in Table were active against *P. falciparum* in vitro (Batista et al. 2013). Compound 21 was the most active, with a 50% inhibitory concentration (IC50) value of 5.4 μM. Compounds 8 and 9 displayed the highest selective antimalarial activity, with selective index values of 1,238.5 and 810.2, respectively. Compounds 8 and 9 exhibited similar binding energies to those of TG. Compound 21 showed the best docking results against PfATP6 and 1IWO, indicating a stronger interaction with the targets than that of TG, the natural inhibitor. Fig. 2 shows the interaction of TG and compound 21 with the PfATP6 binding site.

These data suggest PfATP6 as a potential target for the semisynthetic antimalarial diterpene epoxides derived from naturally occurring ent-kauranes from *W. paludosa*. The three ent-kaurane diterpenes presented similar binding energy values to those obtained with mammalian and parasite enzyme models. Further optimisation of these lead compounds may provide a more potent and selective antimalarial drug.
Fig. 2: interactions of thapsigargin (A) and ent-kaurane diterpene 21 (B) with the PfATP6 binding site.

REFERENCES

Bagnaresi P, Nakabashi M, Thomas AP, Reiter RJ, Garcia CRS 2012. The role of melatonin in parasite biology. Mol Biochem Parasitol 181: 1-6.

Batista R, Garcia PA, Castro MA, del Corral JMM, Speziali NL, Varotti FP, de Paula RC, Garcia-Fernández LF, Francesch A, San Feliciano A, de Oliveira AB 2013. Synthesis, cytotoxicity and antiplasmodial activity of novel ent-kaurane derivatives. Eur J Med Chem 62: 168-176.

Berman HM, Kleywegt GJ, Nakamura H, Markley JL 2013. The future of the protein data bank. Biopolymers 99: 218-222.

Bikadi Z, Hazai E 2009. Application of the PM6 semi-empirical method to modeling proteins enhances docking accuracy of AutoDock. J Cheminform 1: 15.

Bordoli L, Kiefer F, Arnold K, Benkert P, Battey J, Schwede T 2009. Protein structure homology modeling using SWISS-MODEL workspace. Nat Protoc 4: 1-13.

Case D, Darden T, Cheatham TI, Simmerling C, Wang I, Duke R, Luo R, Walker R, Zhang W, Merz K, Roberts B, Wang B, Hayik S, Roitberg A, Seabra G, Kolossvai I, Wong K, Paesani F, Vanicek J, Liu J, Wu X, Brozell S, Steinbrecher T, Gohlke H, Cai Q, Ye X, Hsieh M-J, Cui G, Roe D, Mathews D, Seetin M, Sagui C, Pomelli C, Ochterski JW, Martin RL, Morokuma K, Zakrewski VG, Voth GA, Salvador P, Dannenberg JJ, Dapprich S, Daniels AD, Farkas O, Foresman JB, Ortiz JV, Cioslowski J, Fox DJ 2009. Gaussian 09, Gaussian Inc, Wallingford CT.

Furuyama W, Enomoto M, Mossaad E, Kawai S, Mikohisa K, Kawazu S 2014. An interplay between 2 signaling pathways: melatonin-cAMP and IP3-Ca2+ signaling pathways control intraerythrocytic development of the malaria parasite Plasmodium falciparum. Biochem Biophys Res Commun 446: 125-131.

Gardner MJ, Hall N, Fung E, White O, Berriman M, Hyman RW, Carlton JM, Pain A, Nelson KE, Bowman S, Paulsen JT, James K, Eisen JA, Rutherford K, Salzberg SL, Craig A, Kyes S, Chan M-S, Nene V, Shlomot SJ, Suh B, Peterson J, Anguilo S, Perta M, Allen J, Selengut J, Haft D, Mather MW, Vaidya AB, Martin DMA, Fairlamb AH, Fraunholz MJ, Roos DS, Ralph SA, McFadden GI, Cummings LM, Subramanian GM, Mungall C, Venter JC, Carucci DJ, Hoffman SL, Newbold C, Davis RW, Fraser CM, Barrell B 2002. Genome sequence of the human malaria parasite Plasmodium falciparum. Nature 419: 498-511.

Krishna S, Pulcini S, Fatih F, Staines H 2010. Artemisinins and the biological basis for the PfATP6/SECA hypothesis. Trends Parasitol 26: 517-523.

Krishna S, Pulcini S, Moore CM, Teo BH-Y, Staines HM 2014. Pumped up: reflections on PfATP6 as the target for artemisinins. Trends Pharmacol Sci 35: 4-11.

Laskowski RA, MacArthur MW, Moss DS, Thornton JM 1993. PROCHECK: a program to check the stereochemical quality of protein structures. J Appl Crystallogr 26: 283-291.

Lee MC, Duan Y 2004. Distinguish protein decoys by using a scoring function based on a new AMBER force field, short molecular dynamics simulations and the generalized born solvent model. Proteins 55: 620-634.

Leite FHA, da Fonseca AL, Nunes RR, Comar Jr M, Varotti FP, Taranto AG 2013. Malária: dos velhos fármacos aos novos alvos moleculares. Biomed Rep 2: 59-76.

Médébielle M 2014. The medicinal chemistry and drug development of novel antimalarials. Curr Top Med Chem 14: 1635-1636.

Meslin B, Beavogui AH, Fasel N, Picot S 2011. Plasmodium falciparum metabolism PFMCA-1 triggers a z-VAD-fmk inhibitable protease to promote cell death. PLoS ONE 6: 1-11.

Miller LH, Ackerman HC, Su X, Wellems TE 2013. Malaria biology and disease pathogenesis: insights for new treatments. Nat Med 19: 156-167.

Naik PK, Srivastava M, Bajaj P, Jain S, Dubey A, Ranjan P, Kumar R, Singh H 2011. The binding modes and binding affinities of artemisinin derivatives with Plasmodium falciparum Ca2+-ATPase (PfATP6). J Mol Model 17: 333-337.

Nunes-Alves C 2014. Propelling artemisinin resistance. Nat Rev Microbiol 13: 1.

Salomon-Ferrer R, Case DA, Walker RC 2013. An overview of the amber biomolecular simulation package. Wiley Interdiscip Rev Comput Mol Sci 3: 198-210.

Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, Scalmani G, Barone V, Mennucci B, Petersson GA, Nakatsuji H, Caricato M, Li X, Hratchian HP, Izmaylov AF, Bloino J, Zheng C, Sonnenberg JL, Hada M, Ehara M, Toyota K, Fukuda R, Hasegawa J, Ishida M, Nakajima T, Honda Y, Kitao O, Nakai H, Vreven T, Montgomery JA, Peralta JE, Ogliaro F, Bearpark M, Heyd JJ, Brothers E, Kudin KN, Staroverov VN, Kobayashi R, Normand J, Raghavachari K, Rendell A, Burant JC, Iyengar SS, Tomasi J, Cossi M, Rega N, Millam MJ, Klene M, Knox JE, Cross JB, Bakken V, Adamo C, Jaramillo J, Gomperts R, Stratmann RE, Yazyev O, Austin AJ, Cammi R, Pomelli C, Ochterski JW, Martin RL, Morokuma K, Zakrewski VG, Voth GA, Salvador P, Dannenberg JJ, Dapprich S, Daniels AD, Farkas O, Foresman JB, Ortiz JV, Cioslowski J, Fox DJ 2009. Gaussian 09, Gaussian Inc, Wallingford CT.
Stewart JJP 2007. Optimization of parameters for semiempirical methods. V. Modification of NDDO approximations and application to 70 elements. *J Mol Model* 13: 1173-1213.

Sun G-B, Sun H, Meng X-B, Hu J, Zhang Q, Liu B, Wang M, Xu H-B, Sun X-B 2014. Aconitine-induced Ca$^{2+}$ overload causes arrhythmia and triggers apoptosis through p38 MAPK signaling pathway in rats. *Toxicol Appl Pharmacol* 279: 8-22.

Toyoshima C, Nomura H 2002. Structural changes in the calcium pump accompanying the dissociation of calcium. *Nature* 418: 605-611.

Valderramos SG, Scanfeld D, Uhlemann A, Fidock DA, Krishna S 2010. Investigations into the role of the *Plasmodium falciparum* SERCA (PfATP6) L263E mutation in artemisinin action and resistance. *Antimicrob Agents Chemother* 54: 3842-3852.

WHO - World Health Organization 2013. World Malaria Report 2013. Available from: who.int/malaria/publications/world_malaria_report_2013/en/.

Trott O, Olson AJ 2010. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. *J Comput Chem* 31: 455-461.