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In-silico discovery of potential anti-malarial drugs targeting pfATP6 using Artemisinin as a model

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Abstract

Background: Malaria is one among the major reasons responsible for deaths worldwide than any other parasitic disease. **Objective**: 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. **Methodology:** In this study, pfATP6 is used as a target to come up with different compounds that have high binding affinity to it. Artemesinin constitutes the frontline treatment to aid rapid clearance of parasitaemia and quick resolution of malarial symptoms. Artemesinin, which is the current anti-malarial, has some drawbacks like low bioavailability which makes it even more necessary to develop more effective anti-malarial drugs. **Results:** After performing docking studies, it was seen that compound-3 has low binding energy. Further analysis of the compound can be done to check for it's effectiveness as anti-malarial drug.

Keywords: Malaria, pfATP6, artemesinin, docking studies

1. Introduction

Malaria is a mosquito-borne infectious disease affecting humans and other animals caused by parasitic protozoans (a group of single-celled microorganisms) belonging to the Plasmodium type. In the last decade, an observable decrease has been seen in malaria cases leading to a decrease of 60% in the death rate. By 2015, almost half of the world was at risk of malaria. Roughly 250 cases were registered [1].

A combination of antimalarial medications which include artemisinin is considered a recommended treatment for malaria. Artemisinin contains a peroxide bridge which is considered to be important for action in the sesquiterpene lactone endoperoxide.

Mechanism of action of artemisinin is by heme-dependent activation of endoperoxide bridge which occurs in the parasite's food vacuole, as suggested by research. A different mode of action was proposed which was based on the structural resemblance to thapsigargin, which is considered a selective inhibitor of Sarco/ endoplasmic reticulum Ca2+-ATPase (SERCA). The only SERCA- type Ca2+-ATPase enzyme in the malarial parasite if PfATP6, a suitable target for artemisinin. Facts like the lack of information of the acute mechanism of artemisinin, poor bioavailability and limited effectiveness are the driving factors for the development of different compounds[2].

In this study, using PfATP6 as the target and artemesinin as the skeleton, binding affinity of different compounds was analyzed. Protozoan parasites of Plasmodium genus are responsible for causing malaria, where Plasmodium falciparum is responsible for 90% of the deaths[3]. Artemisinin-derived free radicals chemically modify and inhibit a variety of parasite molecules, ultimately resulting in the death of the parasite. Haem, essential component of haemoglobin is a high source of intracellular Fe21. The parasite is rich in haem iron which is derived from the degradation of host cell haemoglobin. Fe21-haem is suspected to activate artemisinin inside the parasite. Around 80% of the host-cell haemoglobin is degraded by the parasite by ingestion, in the food vacuole. Artemisinin is activated

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by free iron neighbor PfATP6 in the endoplasmic reticulum in the parasite. Large number of ferrous ions in the vacuole catalyze the cleavage of peroxide bridge leading to a free radical which is highly reactive[4].

SERCA is important for the calcium signalling process in the parasites, where the signalling is associated with the regulation of many processes during the life cycle of the parasite. PfATP6 is one of the proteins that controls molecular mechanisms that sustain the calcium homeostasis in the parasite[5]. The protein sequence of PfATP6, as obtained from the UniProt database is composed of 1228 amino acids[6,7]. 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.

2. Materials and methods

2.1 Homology modeling of malarial PfATP6 protein

The PfATP6 sequence was retrieved from UniProt database id-Q5R2K6_PLAFA. As no structure was available in the PDB database, homology modeling was done using Swiss-PDB by using 3TLM as the template. It was seen that the 3TLM sequence of the Bovine muscle had the maximum identity to the target sequence. The model was validated using Ramachandran Plot for accuracy between PfATP6 and the template obtained using RAMPAGE to calculate the accuracy.

2.2 Virtual Screening and docking analysis

Compounds similar to artemesinin were obtained from PubChem with ID- CID53323323, CID70680624, CID107770 and CID68827. Using molinspiration tool, properties of the compounds were obtained. The SMILES format was converted to PDB file format using Open Babel software. Each of the compounds were saved as a PDB file and docked with the target PfATP6 using Hex software. The energy values are obtained. The compound with the least amount of binding energy can be considered as an alternative to artemesinin. .

3. Results and discussion

3.1 Sequence identity

The BLAST results obtained showed that endoplasmic recticulum Ca2+ from Bovine Muscle (3TLM)- A chain has the highest identity with Plasmodium falciparum ATP6 with coverage of 82% and identity 45% . In order to select the template among 3TLM, 4BEW and 3BA6 phylogenetic analysis was done. From the phylogenetic analysis it was found out that the malarial PfATP6 and the 3TLM bovine muscle of Bos taurus is having evolutionarily close relationship as shown in Fig. 1

Figure-1: Phylogenetic analysis

3.2 Homology modelling

Modelling of PfATP6 was done using the template 3TLM-A bovine muscle of Bos taurus shown in Fig 2a. and the 3D structure for PfATP6 using swiss PDB software was obtained as shown in Fig 2b. Ramchandran plot analysis was done to check the accuracy and quality of the protein model. The distribution of the Psi/Phi torsion angles of the best model is represented by a Ramachandran plot Fig 3 & 4, which shows 83.4% of

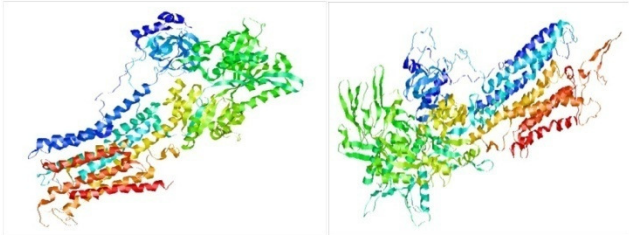


Figure-2(a): 3D structure of 3TLM 2(b). Homology modelled 3D

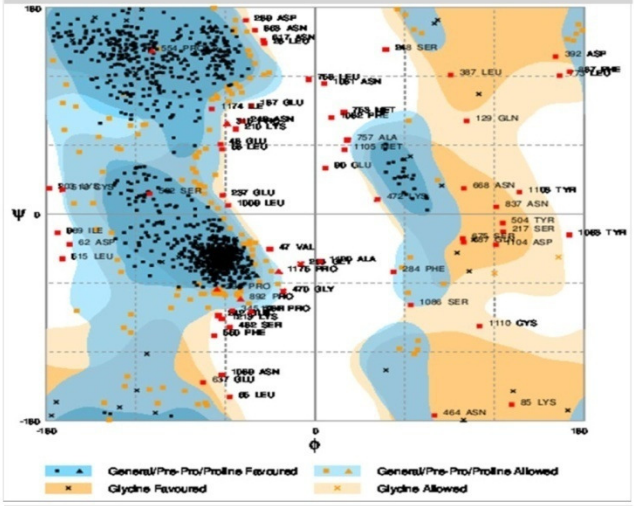


Figure-3: RAMPAGE result (general)

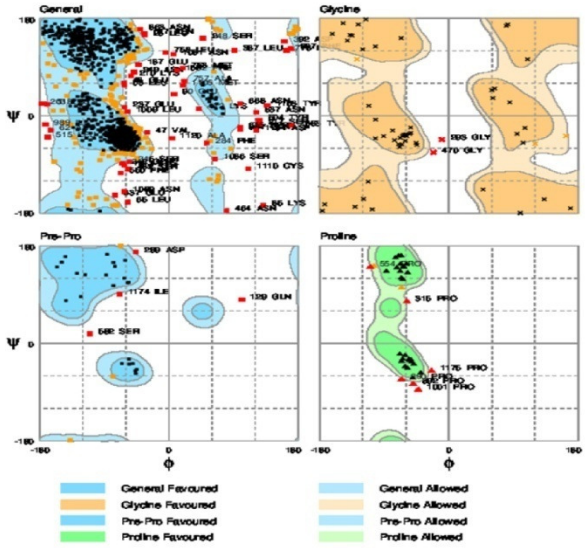


Figure-4: RAMPAGE result (glycine & proline)

residues are in most favoured regions, 11.4% in generously allowed regions and 5.5% in disallowed regions. The calculated Ramachandran plot showed that there is a good agreement between the PfATP6 model and the SERCA template.

3.3 Drug likeliness

Molinspiration tool was used to obtain the molecular properties, drug-likeness and the bioactivity of the compounds. The compounds obtained by changing the functional groups in artemisinin along with the properties (LogP, molecular weight, etc..) is as shown in Fig 5, the compounds with their SMILES format and bioactivity prediction of the molecules is shown in as shown in TABLE 1 and TABLE 2 respectively.

3.4 Docking

Hex software was used to dock the three compounds obtained and artemisinin. The docking studies of compound 1 showed the energy value of -2.848920e+002. The docking studies of compound 2 showed the energy of -2.931258e+002. The docking studies of compound 3 showed the energy of -2.243476e+002. The docking studies of artemisinin showed energy value of -2.324981e+002.The energy values of these three compounds was compared with the energy value of artemisinin. The docking studies is as shown in Fig 6.

4. Conclusion

With docking studies minimum energy more is the binding affinity of the ligand to the receptor, hence compound 1 and 2 is the best suited structure when it is also compared with artemisinin. With the LogP values obtained it can be seen that compound 2 and 3 satisfies the LIPNISKI'S RULE compared to artemisinin. The larger the value of the bioactivity score, the higher is the probability that the particular molecule will be active. This can be obtained using the bioactivity prediction from which it can be concluded that compound 1, 2 and 3 is more active when compared to artemisinin. Compound 3 satisfies the minimum energy, Lipniski's rule and also the bioactivity when compared with artemisinin.The compounds can be further selected based on which one has the true binding site .

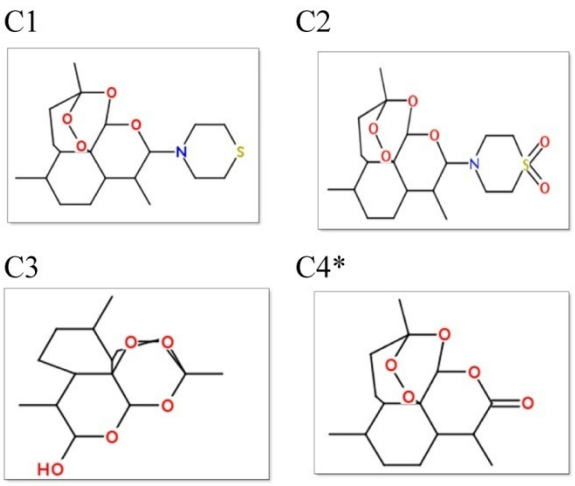


Figure 5: 2D representation of artemisinin like molecules obtained from pubchem

Table 1: Properties of the compounds

	Properties ⇨ / Compound in smiles ⇩	miLogP	TPSA	Natoms	MW	nON	nOHNH	nviola-tions	nrtb	Volume
C1	CC1CCC2C(C(OC3C24C1CCC(O3)(OO4)C)N5CCSCC5)C	3.83	40.17	25	369.53	5	0	0	1	343.35
C2	CC1CCC2C(C(OC3C24C1CCC(O3)(OO4)C)N5CCS(=O)(=O)CC5)C	2.71	74.32	27	401.52	7	0	0	1	356.65
C3	CC1CCC2C(C(OC3C24C1CCC(O3)(OO4)C)O)C	2.78	57.16	20	284.35	5	1	0	0	264.09
C4*	CC1CCC2C(C(=O)OC3C24C1CCC(O3)(OO4)C)C	3.32	54.01	20	282.34	5	0	0	0	258.23

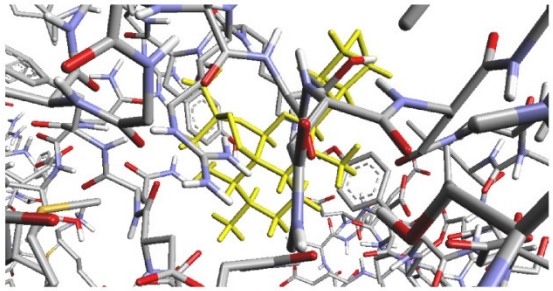
Legends : miLogP: Molinspiration logP value , TPSA: Total Polsar Surface Area, Natoms: Number of Atoms, MW: Molecular Weight, nON: hydrogen bonds, nOHNH: hydrogen bonds donors, nviolations: number of Rule of 5 violations, nrtb: Number of rotatable bonds

Table-2: The bioactivity prediction of the compounds

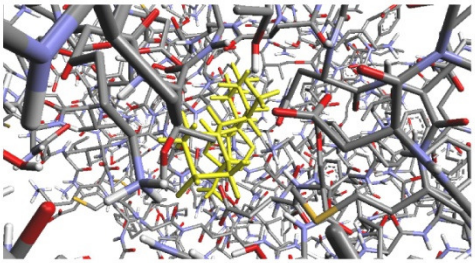
Compounds	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
C1	0.09	-0.19	-0.31	0.04	0.06	0.41
C2	0.10	-0.39	-0.22	0.03	0.12	0.35
C3	0.05	-0.12	-0.39	0.18	0.12	0.59
C4*	-0.17	-0.31	-0.65	-0.00	-0.19	0.39

Fig 6: Molecular docking studies

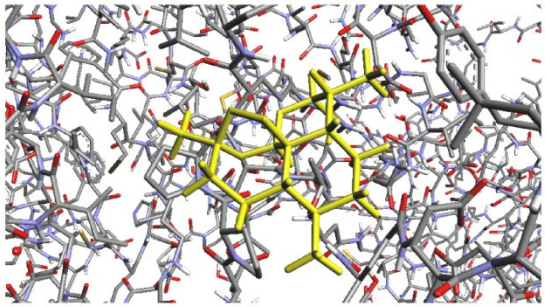
C1



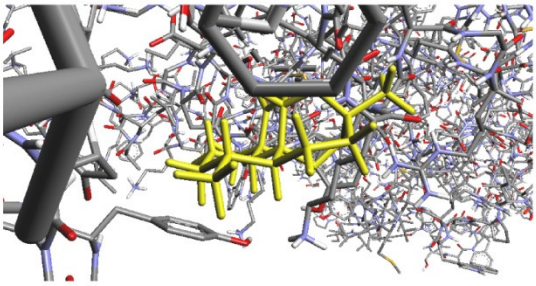
C2



C3



C4*



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Conflict of interest

The author's declares none.

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