Dihydroeponemycin inhibits ubiquitin proteasome system of *Plasmodium falciparum* in silico

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1. Introduction

Malaria is a disease caused by a parasite. People with malaria often experience fever, chills, and flu-like illness. They may develop severe complications and die if untreated. Million cases of malaria occurred worldwide and people died, mostly children in the African Region. Many cases of malaria are diagnosed in the United States each year. The vast majority of cases in the United States are in
travelers and immigrants returning from countries where malaria transmission occurs, many from sub-Saharan Africa and South Asia [1].

*Plasmodium falciparum* is the most prevalent malaria parasite in the WHO African Region, accounting for 99.7% of estimated malaria cases in 2017, as well as in the WHO regions of South-East Asia (62.8%), the Eastern Mediterranean (69%) and the Western Pacific (71.9%). *P. vivax* is the predominant parasite in the WHO Region of the Americas, representing 74.1% of malaria cases [1].

*Plasmodium falciparum* strains have acquired resistance to Artemisinin (ART)-based combination therapies across much of Southeast Asia. ART creates widespread protein and lipid damage inside intraerythrocytic parasites, necessitating macromolecule degradation. The proteasome is the main engine of Plasmodium protein degradation. Proteasome inhibition and ART have shown synergy in ART-resistant parasites. Ubiquitin modification is associated with altered parasite susceptibility to multiple antimalarials. Targeting the ubiquitin-proteasome system (UPS), is an attractive avenue to combat drug resistance [2]. Previous study proved that secondary metabolite of *Streptomyces hygroscopicus* Hygroscopicus containing eponemycin significantly inhibits Ubiquitin Proteasome System (UPS) of *Plasmodium* in vivo [3].

2. Methods
The methods of this study were ADMET SAR Toxicity (ACD/LABS), Protein Modelling (SWISS), and Molecular Docking (AUTODOCK VINA PYRX).

3. Results and Discussion
The result of Absorption, Distribution, Metabolism, Excretion and Toxicity profiles (ADMET) of dihydroeponemycin was absorbed 83.5% via intestinal route and belonged to Category 4 of toxicity. Smaller toxicity category means the compound has higher toxicity. The Lipinski-type properties of dihydroeponemycin showed that it can be ligand to bind with E2 and E3. This is based on Lipinski's rule of five [4].

| Properties                      | Value          |
|---------------------------------|----------------|
| Molecular weight                | 400.5 g/mol    |
| No. of Hydrogen Bond Donors     | 4              |
| No. of Hydrogen Bond Acceptors  | 6              |
| TPSA                            | 128 Å²         |
| No of Rotatable Bonds           | 14             |

Several possible drug targets within *Plasmodium* UPS were 26S Proteasome, PfhsIu/pfhsIV proteasome complex, E3 ligases, E3 target substrates, and DUBs. E3 is a potential drug target because of its different structure to that of the human. The E1 and E2 are proteins that conserved in eukaryotic. E3 is important to maturation of schizont, so that the development of Plasmodium was restricted [5][6].
Figure 1. Molecular docking. A. Interaction of E2 and dihydroeponemycin. B. Interaction of E3 and dihydroeponemycin.

Table 2. Binding affinity score of dihydroeponemycin with E2 compared with other proteasome inhibitor.

| Macromolecule | Ligand       | Binding Affinity (kcal/mol) |
|---------------|--------------|-----------------------------|
| E2            | Epoxomicin   | -4.9                        |
|               | MG123        | -5.3                        |
|               | Lactacyin    | -5.5                        |
|               | Dihydroeponemycin | -4.9                      |
|               | Chloroquine  | -4.7                        |

Table 3. Binding affinity score of dihydroeponemycin with E2 compared with other proteasome inhibitor.

| Macromolecule | Ligand       | Binding Affinity (kcal/mol) |
|---------------|--------------|-----------------------------|
| E3            | Epoxomicin   | -5.1                        |
|               | MG123        | -5.4                        |
|               | Lactacyin    | -5                          |
|               | Dihydroeponemycin | -4.8                      |
|               | Chloroquine  | -4.7                        |

The C-score (confidence score of the prediction) of the active side E3 was 0.06 and E2 was 0.45 (COACH ZHANG LAB). Dihydroeponemycin had the ability to bind to E3 with an affinity score binding of -4.8. Dihydroeponemycin also had the ability to bind to E2 with an affinity score binding of -4.9. The more negative the affinity binding value the easier the compound binds to the target protein.

Through the complex life cycle in the hosts, Plasmodium invades and replicates in totally different cells thus making the study of the biology of the parasite and the identification of targets for drug development affecting all stages very difficult. It was shown that host molecules, have a role in the progression and regulation of the parasite cell cycle. When the parasite is exposed to host molecule there is an increase in transcription levels of genes encoding for proteins related to the Ubiquitin Proteasome (UPS) System. This system is essential for the survival of the parasite. The Plasmodium UPS shows low similarity to the ubiquitin proteasome system in Humans; the identification of unique targets to be used for therapeutic molecules development increases the importance of UPS studies in malaria challenging. The study of the mechanism of action of the UPS and the identification of
potential targets for new drugs development are promising alternative strategies to fight the drug-resistance problem in malaria parasites [7].

4. Conclusion
Based on this in silico study, dihydroeponemycin has potential effect as antimalaria by inhibiting Ubiquitin Proteasome System of *Plasmodium falciparum*.

5. Conflicts of interest
The authors declared that there was no potential conflict of interest relevant to this article.

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