INTRODUCTION

Plasmodium falciparum, a microorganism that belongs to the phylum Apicomplexa, is the most common cause of malaria in Indonesia [1]. The first-line treatment for uncomplicated Plasmodium falciparum malaria in Indonesia is the artesunate-amodiaquine combination [1]. However, a failure rate of over 10% for this treatment, associated with drug resistance, was reported in Indonesia during the period 2000-2007 [1]. The present report, therefore, offers the basis for the development of a new antimalarial drug. Geranylgeranyl pyrophosphate synthase (GGPPS) is a key enzyme in P. falciparum isoprenoid biosynthesis [2]. It catalyzes the synthesis of geranylgeranyl pyrophosphate (GGPP) [2], as well as farnesyl pyrophosphate [2], in the presence of Mg²⁺ cofactors [3]. This enzyme is recommended as an antimalarial drug target due to its essentiality, druggability, and amenability to high-throughput screening [4].

Andrographolide (1) is a major secondary metabolite in the Indonesian herb sambiloto (Andrographis paniculata) [5]. It has been found to have a moderate antiplasmodial activity against the chloroquine-resistant strain of P. falciparum [6], which makes it an interesting lead for the new antimalarial drug. Since it is biosynthesized from GGPP [7], it is reasonable to hypothesize that andrographolide exerts its antimalarial activity through inhibition of GGPPS. To establish this hypothesis by molecular docking, however, the crystal structure of P. falciparum GGPPS is required, which is not yet available.

Meanwhile, high-resolution ligand binding crystal structures of Plasmodium vivax GGPPS are available (PDB 3CC9, 3EZ3, 3LDW, and 3PH7), based on a sequence at UniProt (at http://www.uniprot.org/). A BLAST search on UniProt by for this sequence (ASK4I6) yielded a P. falciparum GGPPS sequence (Q86GK8) that is evidenced from transcript level. A >70% similarity between the two sequences was revealed in our preliminary sequence alignment by ClustalX 2.1 [8]. Based on this result, we conducted a comparative modeling of P. falciparum GGPPS using one of the P. vivax GGPPS crystal structures as a template. The best model from this comparative modeling was then used in a molecular docking to investigate the binding mode of andrographolide in the P. falciparum GGPPS active site.

METHODS

Comparative modeling

The comparative modeling was conducted using EasyModeller 4.0 [9]. A sequence of P. falciparum GGPPS, which had been downloaded from UniProt as a FASTA file (Q86GK8), was employed as the query. A high-resolution ligand binding crystal structure of P. vivax GGPPS, which had been downloaded from the RCSB Protein Data Bank (at http://www.pdb.org/) as a PDB file (PDB 3PH7), was selected as the template since it contains GGPP and is supported by literature [3]. During the comparative modeling, heteroatoms were not included, and loops were not refined. Instead, the model with the lowest DOPE score was optimized by default settings.

Molecular docking

The molecular docking was conducted using an AutoDock Vina module in PyRx 0.8 (At http://pyrx.sourceforge.net/) by default settings. AutoDock Vina automatically prepared protein and ligands. No water molecule was included during the molecular docking.

To investigate the binding mode of 1 in P. falciparum GGPPS, derivatives of 1 (2a and 2b) were employed. All ligand structures were drawn using ACD/ChemSketch 12.01 and then converted into three-dimensional structures, optimized, and saved as mol files using ACD/3D Viewer 12.01 (At http://www.acdlabs.com). The mol files were then converted into mol2 files using OpenBabel 2.3.2 [10]. ACD/ChemSketch was also used to calculate log P of the ligands.
RESULTS AND DISCUSSION

Before the comparative modeling, EasyModeller aligned sequences of the query and the template. For chain A-D of the template, the alignment scores were 265,257.6250, 273,733.8750, 252,205.1562 and 260,121.5312, respectively. Since chain B of the template gained the highest score for the alignment, and no gap presented at predicted secondary structures of the query (Supplementary Fig. 1), the comparative modeling was conducted on that chain.

Supplementary Fig. 1: Sequence alignment between the query (Q86GK8) and template (PDB 3PH7) performed by EasyModeller 4.0 showed 70% similarity

Supplementary Fig. 2: Left: Ramachandran plot of the optimized Model 5. Right: Comparison between optimized Model 5 (red) and template (green); image captured using PyMOL Molecular Graphics System (at http://www.pymol.org/) [12]
The comparative modeling produced seven models with the following DOPE scores: $-45,876.36719$, $-45,724.93750$, $-45,170.59375$, $-45,537.05625$, $-45,908.73047$, $-45,727.52344$, and $-45,883.46484$. Model 5 had the lowest DOPE score, so it was optimized. After optimization of this model, a Ramachandran plot revealed only a few residues outside the allowed regions ($G^\text{a}$, $G^\text{b}$, and $S^\text{a}$; Supplementary Fig. 2). The residues were not parts of the presumed P. falciparum GGPPS active site. A comparison between this model and the template is presented in Fig 2.

The active site of P. vivax GGPPS consists of two pockets: a hydrophobic anchor ($L^{118}$, $Q^{119}$, $A^{120}$, $F^{122}$, $V^{123}$, $V^{124}$, $T^{188}$, and $I^{192}$) and a catalytic pocket, which contains the first aspartate-rich motif (FARM; $^{192}$DDIMD$^{197}$) and second aspartate-rich motif (SARM; $^{210}$DDIYD$^{215}$) [3]. GGPP positions itself in the P. vivax GGPPS active site such that its hydrophobic part is held by the anchor, while its pyrophosphate group interacts with the catalytic pocket, either directly or through Mg$^{2+}$ cofactors and/or water molecules [3]. A similar position and orientation were confirmed for 1 in the P. falciparum GGPPS active site by our molecular docking. The double rings of 1 were pointing toward the hydrophobic pocket, while its lactone group is positioned between FARM and SARM of the catalytic pocket. This finding suggests the double rings of 1 are essential for hydrophobic interactions, while its lactone group is essential for hydrophilic ones.

The log P of 1 is low (1.62) and its bioavailability is also low [11]. Thus, we proposed a more hydrophobic analog of 1 by the elimination of its hydroxyl groups at C-3 and C-14 (2a; log P = 3.70). This analog, when docked into our P. falciparum GGPPS model, left the catalytic pocket and positioned itself close to F$^{122}$, T$^{188}$, and I$^{192}$ of the hydrophobic pocket (Supplementary Fig. 3). However, when this analog was alkylated at the C-19 hydroxyl group (2b; log P = 4.91), the original position and orientation were restored (Supplementary Fig. 3).

Molecule 1 did not fill most of the P. falciparum GGPPS hydrophobic pocket. This explains its moderate inhibitory activity. An alkylation strategy for 1, as demonstrated by 2b, would enhance its coverage on the hydrophobic pocket at the expense of its log P.

CONCLUSION

In the P. falciparum GGPPS active site, andrographolide is situated with its double rings pointing toward the hydrophobic pocket, while its lactone group is positioned between FARM and SARM of the catalytic pocket.

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