DESIGN AND SCREENING OF GALLIC ACID DERIVATIVES AS INHIBITORS OF MALARIAL DIHYDROFOLATE REDUCTASE BY IN SILICO DOCKING

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ABSTRACT

Objective: Malaria is an infection disease caused by Plasmodium parasite with high prevalence in tropic and subtropic countries. The aim of this work was to design and screening of gallic acid derivatives as inhibitors of malarial dihydrofolate reductase (DHFR) by in silico docking.

Methods: The derivatives were designed by expanding the carboxyl group of gallic acid with open-chain moiety of L-threonine-allyl esters, as well as to modify the hydroxyl groups on the aromatic ring of gallic acid with methoxyl group in the derivatives. In silico approach has been utilized in finding the potential antimalaria of gallic acid derivatives. Fourteen gallic acid derivatives (compound 2-15) were modeled into three-dimensional (3D) structures by ACD Labs software. Geometry optimization and minimization of energy 3D structure of gallic acid derivatives as ligands using the MOE software. Docking process and amino acid analysis were executed using MOE software.

Results: In silico docking study resulted in the three top-ranked compounds, namely, compound 5, 8, and 12. Among those three top-ranked compounds, compound 12 (octyl gallate) exhibited the strongest interaction and greatest inhibitory activity against the receptor of malarial DHFR.

Conclusion Our results clearly demonstrated that compound 12 (octyl gallate) could be developed as a promising candidate for the new antimalarial agent.

Keywords: Design, Screening, Gallic acid derivatives, Antimalaria, Dihydrofolate reductase, In silico docking.

INTRODUCTION

Malaria is one of the Plasmodium parasite infections which found in many tropical and subtropical countries. According to the WHO (2010), around 2.3-3.3 billion people suffered in malaria, especially in Africa, Asia, Middle East, Latin America, and some parts of Europe [1]. Particularly, children under age 5 and pregnant women are people with the most severe infection of malaria [2]. In Indonesia, there are 396 districts of malaria endemic and approximately 45% of the population live in areas at risk of malaria [3]. Since 2010, the directorate general of disease control and environmental sanitation, Ministry of health, Republic of Indonesia, has been used annual parasite incidence (API) to express incidence of malaria in Indonesia. Papua, West Papua, and East Nusa Tenggara are the provinces with the highest malarial incidence with API 18.03, 17.86, and 12.14/1000 population, respectively. Strategic Plan of the Ministry of Health Republic of Indonesia in 2010-2014 targeted to reduce morbidity of malaria from 2 to 1/1000 population [4,5]. There are four species of the genus Plasmodium that can cause infections in humans, namely, *Plasmodium falciparum*, *plasmodium vivax*, *Plasmodium malariae*, and *Plasmodium ovale*. Among these four types, the *P. falciparum* that causes malaria falciparum is the most dangerous type because it can cause serious complications which lead to the death [6].

Currently, the treatment of malaria caused by *P. falciparum* parasite relies on the use of chloroquine or synergistic combination of aminoquinoline-antifolate and sulfadoxine-pyrimethamine. However, a recent study reported that antimalarial drugs have shown resistance which leads to the decreasing in its effectiveness [7]. This fact indicated

the new antimalarial drugs that are more potent, more effective, and safer are required. Gallic acid (1) is naturally obtained compound which showed biological activity as an antioxidant, antifungal, antiviral, anti-inflammatory, and anticancer. It has been reported that gallic acid significantly inhibited the growth of viruses and bacteria, as well as induced apoptosis of cancer cells [8-10]. Previous researchers also reported that gallic acid (1) and some of alkyl ester gallate showed antimalarial activity. Gallic acid which is one of the main components in oregano (*Origanum compactum*) was reported to have antimalarial activity [11]. Researcher from Italy, Sannela *et al.,* reported that gallic acid derivatives containing in green tea, namely, epigallocatechin-3-gallate, and epicatechin gallate showed strong antimalarial activity against *P. falciparum* [12]. In 1999, Murphy and Lang-Unnasch reported that the combination of alkyl ester gallate, i.e. propyl gallate with atovaquone has antimalarial activity more powerful than the combination of atovaquone with gallic acid [13]. This result revealed that the alkyl ester derivative of gallic acid (alkyl ester gallate) could be developed as an antimalarial drug. The strong antimalarial activity of alkyl ester gallate prompted us to carry out research that is aimed to design a series of alkyl ester derivative of gallic acid, compound 2-15 (Fig. 1), as well as to study the interaction of the derivatives with receptor target of malarial dihydrofolate reductase (DHFR) by computational in silico molecular docking. The three best compounds which showed the strongest interaction with receptor target of malarial DHFR will be synthesized and will be examined its *in vitro* and *in vivo* antimalarial activity in our next research project.

Many researchers have been completed the study on the interaction of compounds with antimalaria drug targets by in silico molecular
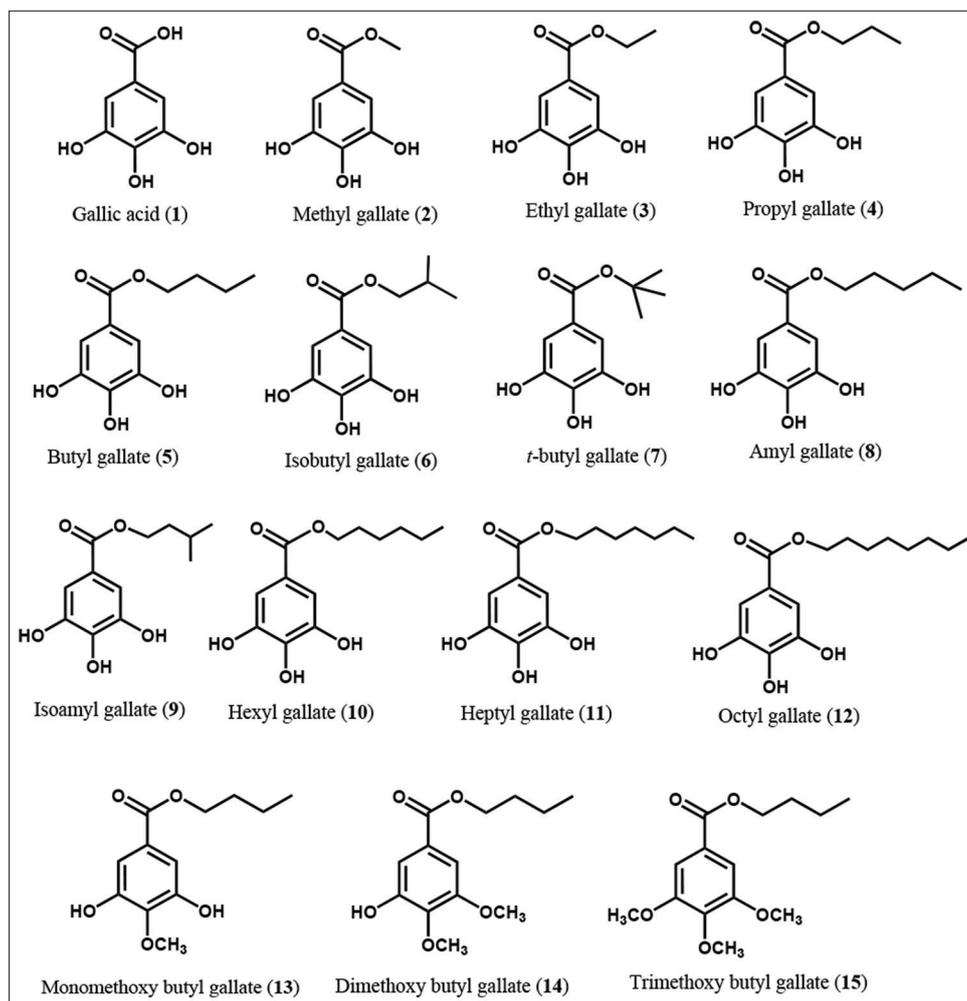
Fig. 1: Chemical structure of gallic acid (1) and its derivatives (2-15)

docking [14,15]. Sharma and Chetia (2013) conducted docking studies on quinine analog for plasmepsin II of malarial parasite [16]. Lulu *et al.* (2015) have been accomplished in silico docking study of 240 antimalarial compounds with active site of *P. falciparum* chorismate synthase protein [17]. Ravi and Ragunathan (2016) conducted in silico analysis of alien and microdontin with *P. falciparum* calcium-dependent protein kinase [18]. In 2014, we conducted in silico molecular docking study on antimycin A analogs as inhibitors of antiapoptotic Bcl-2 of breast cancer [19]. Recently, In 2016, we have reported in silico molecular docking studies of opened-chain analogs of antimycin A_3 as caspases inhibitors of apoptosis in colorectal cancer [20]. In this work, as a part our continuing research to develop computer-aided drug design and screening by docking approach, we conduct in silico study and screening of 14 gallic acid derivatives as inhibitors of malarial DHFR.

METHODS

Design structure of gallic acid derivatives

A series of gallic acid derivatives were designed by modifying the carboxyl group of gallic acid with alkyl ester group which have one to eight linear aliphatic carbon chains in derivative 2, 3, 4, 5, 8, 10, 11, 12, 13, 14, and 15 and have a branched aliphatic carbon chain in derivative 6, 7, and 9. The previous studies showed that the methylation of the hydroxyl group on the aromatic ring of gallic acid will enhance the antiviral activity, so in this study, we modify the hydroxyl groups on the aromatic ring of butyl gallate become monomethoxy butyl gallate in derivative 13, 3,5-Dihydroxy-4-methoxy benzoic acid butyl ester in derivative 14 and trimethoxy butyl gallate in derivative 15.

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These modifications are expected to increase antimalarial activity of derivatives 2-15 as growth inhibitors *Plasmodium falciparum.*

Structure preparation

Gallic acid derivatives were modeled into three-dimensional (3D) structures. The modeling is performed using ACD Labs software.

Geometry optimization and energy minimization 3D structure of ligands

Geometry optimization and minimization of energy 3D structure of gallic acid derivatives (ligands) using the MOE software that runs on a single computer Intel Pentium Dual Core. The algorithm used is the conjugate gradient *Polak–Ribiere* with maximum convergence root mean square gradient of 0.01 kcal/mol and parameters Å molecular mechanics force field MMFFx.

In silico docking of gallic acid derivative on receptor DHFR

The process of in silico docking begins with file preparation using a docking program in MOE software. Both molecules of gallic acid derivative (then called ligand) and the enzyme, polar hydrogen, and Gasteiger charge were added, and nonpolar hydrogen was merged. Ligand and enzyme were stored in molecular format file for later use in the preparation parameters. Grid box used was 60×60×60 with a grid spacing of 0.375 Å. Docking calculations executed by the algorithm parameters Lamarckian genetic algorithm with a population size of 150, 10 million energy evaluation and repetition (engine runs) as much as 100 times. These parameters are stored in a format MOE as a file to be used to carry out the process of docking. Docking process is executed using MOE software.
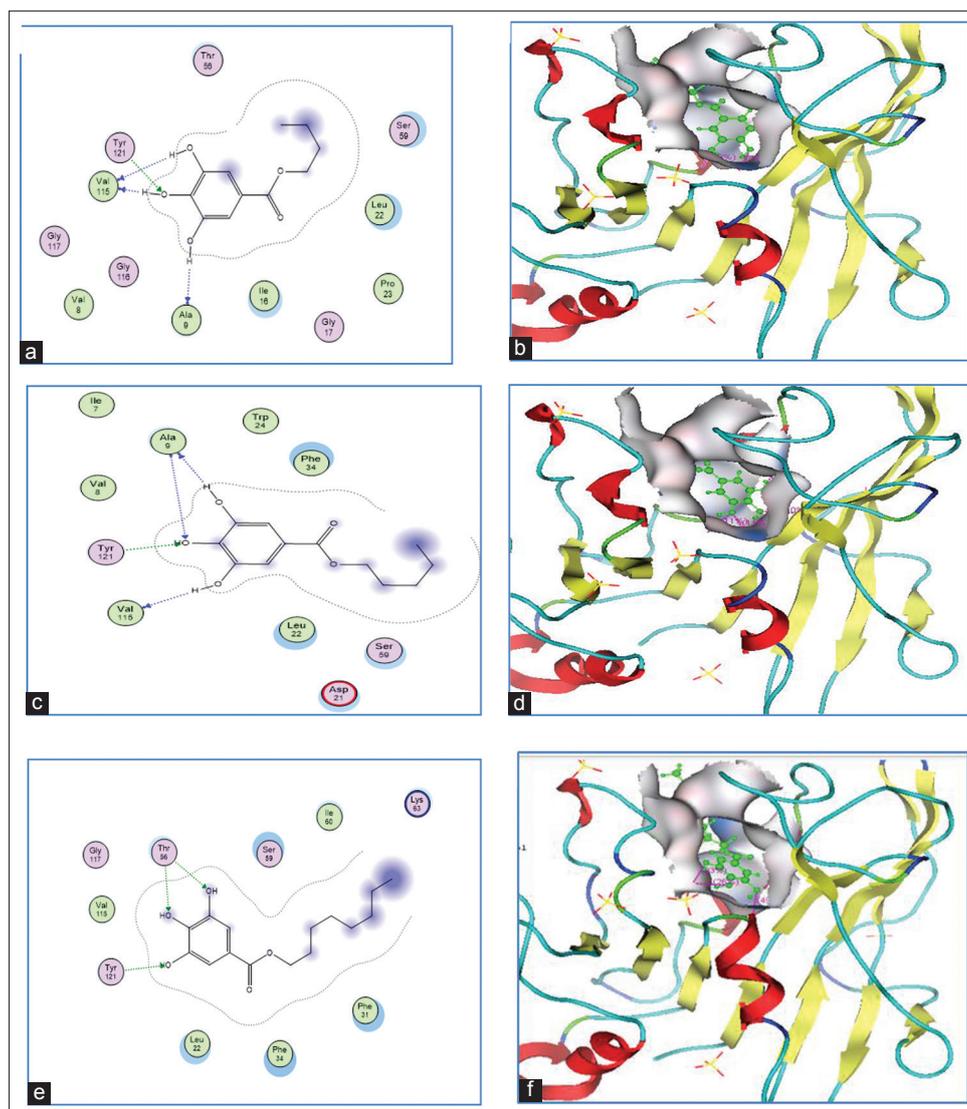
Fig. 2: Two-dimensional and three-dimensional complex interaction of derivative 5 (a, b), 8 (c, d), and 12 (e, f) with dihydrofolate reductase

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