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Synthesis, Antimalarial Activities of Secondary Amine-Substituted Eugenol Compounds against *Plasmodium falciparum* and *in silico* Molecular Docking Analysis

(Sintesis, Aktiviti Antimalaria Sebatian Eugenol Gantian-Amina Sekunder terhadap *Plasmodium falciparum* dan Analisis Dok Molekul *in silico*)

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ABSTRACT

Multi-resistance cases with antimalarial drugs had been developed in over the years. One of the ways of developing antimalarial drugs is to focus on searching for the potential antifolate inhibitors against *Plasmodium* sp. from synthetic or natural products. The aims of this research was to synthesis secondary amine-substituted eugenol compounds through the Mannich reaction for antimalarial evaluation using *Plasmodium falciparum* 3D7. The compounds were also evaluated on *Plasmodium falciparum* dihydrofolate reductase-thymidylate synthase (PfDHFR-TS) as a protein target and the compounds' drug-likeness properties were determined. Five secondary amine-substituted eugenol compounds (**1a-e)** were synthesized via substitution of the secondary amine i.e., pyrrolidine, piperidine, methyl piperidine, and morpholine in the eugenol structures. The plasmodium lactate dehydrogenase assay (pLDH) showed that **1a** and **1c** had good antimalarial effects against *P. falciparum* 3D7 with the IC_{50}s values of 0.89 µM and 0.62 µM, respectively. The molecular docking analysis showed that **1a** and **1c** had perfect interaction with PfDHFR-TS (PDB ID: 1J3I) with strong hydrogen bond interactions occurring with PfDHFR-TS protein. The eugenol derivatives **1a** and **1c** exerted CDOCKER binding energies of -6.1407 and -6.6536 kcal/mol, respectively. Based on this research, it was found that PfDHFR-TS is a plausible protein target for the synthesized secondary amine-substituted eugenol in *P. falciparum* infection. The substitution of a secondary amine group for eugenol significantly enhanced the antimalarial properties of the compounds. Thus, eugenol derivatives are potential compounds to be pursued to combat folate resistance in malarial infection.

Keywords: Antimalarial activities; eugenol; Mannich reaction; PfDHFR-TS; *Plasmodium falciparum* 3D7

ABSTRAK

Kes pelbagai rintangan dengan dadah antimalarial telah dibangunkan selama bertahun-tahun. Salah satu cara pembangunan dadah antimalaria adalah dengan memberi tumpuan kepada pencarian dadah antifolat melawan *Plasmodium* sp. yang berpotensi daripada bahan semula jadi dan sintetik. Matlamat penyelidikan ini adalah untuk mensintesis sebatian eugenol gantian-amina sekunder melalui tindak balas Mannich untuk penilaian antimalaria menggunakan *Plasmodium falciparum* 3D7. Sebatian tersebut juga dinilai pada *Plasmodium falciparum* dihydrofolate reductase-thymidylate synthase (PfDHFR-TS) sebagai sasaran protein dan sifat keserupaan dadah untuk sebatian sintesis telah ditentukan. Lima sebatian eugenol gantian- amina sekunder (**1a-e**) telah disintesis melalui penggantian amina sekunder iaitu pirolidin, piperidin, metil piperidin dan morfolin dalam struktur eugenol. Ujian plasmodium laktat dehidrogenase (pLDH) menunjukkan bahawa **1a** dan **1c** mempunyai kesan antimalaria yang baik terhadap *P. falciparum* 3D7 dengan nilai IC_{50} masing-masing adalah 0.89 µM dan 0.62 µM. Analisis dok molekul mendedahkan

bahawa **1a** dan **1c** mempunyai interaksi sempurna dengan PfDHFR-TS (PDB ID: 1J3I) yang mana interaksi ikatan hidrogen yang kuat berlaku dengan protein PfDHFR-TS. Sebatian terbitan eugenol **1a** dan **1c** memberikan tenaga pengikatan CDOCKER masing-masing sebanyak -6.1407 dan -6.6536 kcal/mol. Berdasarkan penyelidikan ini, didapati bahawa PfDHFR-TS adalah sasaran protein yang munasabah untuk eugenol gantina-amina sekunder yang disintesis. Penggantian kumpulan amina sekunder ke atas eugenol telah meningkatkan sifat antimalaria dengan ketara. Oleh itu, derivatif eugenol adalah sebatian yang berpotensi dibangunkan untuk memerangi rintangan folat dalam jangkitan malaria.

Kata kunci: Aktiviti antimalaria; eugenol; *Plasmodium falciparum* 3D7; PfDHFR-TS; reaksi Mannich

Introduction

Malaria is a fatal vector-borne disease caused by parasites of the genus *Plasmodium* sp. *Plasmodium falciparum* is reported to be the most infectious and deadly parasite. According to World Health Organization (WHO), there are around 229 million malaria cases from 87 endemic countries and 409,000 deaths in 2020 (WHO 2021). Indonesia is the second country in Asia with the most significant malaria cases in 2020 and malaria infection in Indonesia accounted for 659,000 cases in 2019 (WHO 2021, 2018). The incidence of malaria in Indonesia is still considered high due to *P. falciparum* transmission. The high incidence of *P. falciparum* infection is thought to be due to one of the important factors which is drug resistance occurred to major antimalarial drugs such as chloroquine, artemisinin, and antifolate drugs such as pyrimethamine, proguanil, sulfonamide, and sulfadoxine (Ashley et al. 2014; Hyde et al. 2002; Shibeshi et al. 2020). Drug resistance occurred soon after their deployment as antimalarials for example, antifolate resistance happened due to a mutation in plasmodial DHFR which resulted in a decrease in the binding strengths of the antimalarial drugs. The rate of antimalarial drug resistance must be countered by increasing the discovery of new antimalarial drugs. If these efforts are not carried out, it is feared that it could lead to uncontrolled cases and higher deaths.

Thus, alternatives effort is needed with minimal cost and low toxicity for the decreased burden of malaria drug resistance worldwide. Natural compounds are examples of affordable and sustainable sources of bioactive constituents which can be used in malaria infection (Ginsburg & Deharo 2011). Natural compounds are useful as starting materials to produce synthetic drugs, or as lead compounds from which a synthetic drug is designed. Both natural and synthetic compounds were reported to possess a similar range of favourable and unfavourable effects based on molecular structure and dosage. Eugenol (4-allyl-2-methoxy phenol) is a naturally occurring compound widely found in clove oil. Eugenol is classified as a phenylpropanoid of the allyl-phenol type. This compound is colorless to pale yellow with a clove odour and spice taste. This natural compound is used as a lead molecule to manufacture synthetic compounds in the pharmaceutical, food, agriculture, and cosmetic industries (Kamatou, Vermaak & Viljoen 2012). Eugenol exhibited antimalarial (Clemente et al. 2022; Pontes et al. 2021), antimicrobial (Silva et al. 2021), antioxidant (Barboza et al. 2018), antiviral (Lane et al. 2019), antifungal (Maximino et al. 2020), anticancer (Zari, Zari & Hakeem 2021), and anti-inflammatory (Barboza et al. 2018). Eugenol affected to *Plasmodium falciparum* intracellular development with IC_{50} of 532.42±29.55 μM in a dose-dependent manner, and treatment of this compound in infected animals reduced blood parasitemia and cerebral edema (Pontes et al. 2021). In other studies, the eugenol derivative, 4-chloroeugenol (4CE) exerted potent antiplasmodial activity against sensitive and resistant strains of *P. falciparum* (IC_{50} values: 1.5–5 μM) (Mina et al. 2021). Moreover, low antileishmania and cytotoxicity qualify eugenol as a safe drug without side effects (Arango et al. 2012; Bachiega et al. 2012). Molecular modification in the structure of eugenol compounds is one of the main strategies to increase the therapeutic properties and reduce side effects. Eugenol compounds were converted by adding functional groups such as carbonate (Clemente et al. 2021), aldehydes, chloro (Mina et al. 2021), hydroxyl (Pereira et al. 2021), methoxy, and amines (Julianto et al. 2018; Syahri et al. 2020).

Based on the Structure-Activity Relationship (SAR) analysis, it is known that most of the antimalarial drugs in the market contain an amine (N) group. This fact indicated that amine has a vital role in the antimalarial activity. The amines can form electrostatic interactions with essential amino acids of the *Plasmodium falciparum*

(Suwito et al. 2014). The presence of an amine attached by carbon atoms can cause antimalarial activity on eugenol (Julianto et al. 2018). It is estimated that eugenol compounds substituted for amines can increase the antimalarial activity of eugenol. This research designed and synthesized a secondary amine substituted eugenol **(1a-e)** from a Mannich reaction. The antimalarial activity was assessed by *in vitro* and *in silico* studies. The secondary amines used were pyrrolidine, piperidine, methyl piperidine, and morpholine. As well as the linking carbonyl compounds used are formaldehyde and benzaldehyde. This research aimed to produce new drug compounds with good antimalarial activity by substituting secondary amines on the aromatic ring of eugenol.

Materials and Methods

Materials

All chemicals used are from Sigma-Aldrich and Merck© without purification, such as eugenol, ethanol, hexane, ethyl acetate, formaldehyde, benzaldehyde, pyrrolidine, piperidine, methyl piperidine, and morpholine. The solvent used in H-NMR and C-NMR analysis was chloroform-d_6 ($CDCl_3$). The silica gel 60 F254 TLC aluminium sheet was utilized in monitoring compounds. The protein used in *in silico* study was a crystalline protein from the CQ-sensitive *Plasmodium falciparum* dihydrofolate reductases-thymidylate synthase (PfDHFR-TS) (PDB ID: 1J3I). *In vitro* analysis was carried out using the *Plasmodium falciparum* 3D7 (*Pf*3D7).

Instrumentals

The spectroscopic equipment including H-NMR and C-NMR was measured using TMS as the internal standard on JEOL JNMECA. This study used a PC with an Intel® Core™ i7 M 350 4.54 GHz CPU; 8.00 GB RAM. Molecular docking was performed using MOE 2022.0901 (Chemical Computing Group) software package.

General Synthesis of **1a-e** Compounds

The synthesis of the **1a-c** compound was carried out by dissolving 20 mmol of eugenol in 65 mL of ethanol. About 40 mmol formaldehyde was added to secondary amine groups (pyrrolidine for **1a**, piperidine for **1b**, and methyl piperidine for **1c**) were added. The **1d-e** compound was carried out with dissolving of 10 mmol eugenol in 20 mL of ethanol. After that, 20 mmol of benzaldehyde and secondary amine (morpholine for **1d** and methyl piperidine for **1e**) were added. Each mixture was refluxed for 30 h with stirring. Each reaction was monitored using TLC until one spot is obtained using UV lamps at 366 nm and 254 nm. When the reaction is complete, each of mixture reaction is evaporated using a rotary evaporator. Compounds (**1a-e**) were characterized using 1H-NMR and 13C-NMR to get the molecular structure.

In Vitro Antimalarial Assay

An antimalarial activity assay was carried out on *Pf*3D7 strain (chloroquine-sensitive) according to the Rieckmann et al. (1978) method with slight modification. The antimalarial assessment was conducted by serially diluting the test compounds (**1a-e**) in the RPMI-1640 media to achieve a range of final concentrations of 100 - 0.01 µg/mL. A parasite culture at a parasitemia level of 1% and hematocrit of 5% was added into the plates containing different with variation concentrations for the test compounds. The mixture culture and test compounds were then incubated at 37 °C for 48 h. The parasitemia level was determined using a thin layer of blood with 20% Giemsa stain. The percentage of parasitemia was calculated by counting the number of infected erythrocytes for every 1,000 erythrocytes. The IC_{50} values were determined from a sigmoidal curve generated from percentage inhibition data (y-axis) and the concentration of the test compound (x-axis) by performing Probit log analysis. The antimalarial activity was categorized based on the IC_{50} values; excellent (< 1 µM); good (1 - 20 µM); moderate (20 - 100 µM); low (100 - 200 µM); and inactive (> 200 µM) (Batista et al. 2009).

Molecular Docking

Chemdraw Professional 15.0 was used to create the molecular structure of ligands (compounds **1a** and **1c**). In this study, Chloroquine was used as a positive control. Furthermore, the 3D molecular structure was then refined using the Molecular Operating Environment (MOE) 2022.0901 software package (Chemical Computing Group) with the MMFF94x force field and 0.0001 gradient. Using all the molecular structures, a *mdb database of ligands was subsequently created.

The molecular structure of the protein was retrieved from the protein data bank with PDB ID IJ31 (i.e., wwww.rcsb.org). MOE 2022.0901 (Chemical computing group) and Discovery Studio Visualizer (DSV, Biovia) software packages were used to construct the protein's crystal

structure. Energy minimization of the protein was applied using CHARMM27 force field with RMS gradient of 0.01 kcal/mol. Hence, this protein is allowed to be utilized as a receptor.

Oriented molecular docking was performed by identifying the active site of this protein. Site finder was used to identify the active site of the protein for then it will be set up as a dummy atom. Next, the posture and refinement are set to 50 and 10, respectively and the triangle placement is determined. For then docking can be constructed immediately.

ADMET AND DRUG-LIKENESS ANALYSIS

Physicochemical and pharmacokinetic analysis like absorption, distribution, metabolism, excretion, and toxicology (ADMET) parameters were determined using the online webserver of admetSAR 2.0 (http://lmmd.ecust.edu.cn/admetsar2/admetopt/, accessed on 1 January 2022) (Jia et al. 2020) and ADMETlab 2.0 (https://admetmesh.scbdd.com/service/evaluation/index, assessed on 12 December 2022) (Xiong et al. 2021). Based on Lipinski's rule, a compound is acceptable as a drug, and orally bioavailable if it follows the 'rule of 5' including molecular weight (MW) < 500 g/mol, number of hydrogen bond acceptors (nHA) ≤10, number of hydrogen bond donors (nHD) ≤5, and the logarithm of the n-octanol/water distribution (logP) ≤5 (Lipinski et al. 2001). The parameters of the ADMET properties were presented in Table 3.

Results and Discussion

SYNTHESIS AND CHARACTERIZATION OF SECONDARY AMINO-SUBSTITUTED EUGENOL DERIVATIVES

Synthesis of secondary amino-substituted eugenol was performed through Mannich reaction. Mannich reaction is an amino alkylation reaction of a nucleophilic substrate. This reaction consists of three components; nucleophiles, reactive aldehydes, and primer or secondary amino. The general synthesis reaction of secondary amino-substituted eugenol is depicted in Figure 1. Reactive aldehydes (formaldehyde and benzaldehyde) will interact with secondary amino and as a linker to eugenol. During the reaction, the hydroxyl group and the aromatic ring on eugenol become active and accept secondary amino as a side group at the ortho position (Abdou et al. 2021; Biersack et al. 2018). The structural elucidation of compounds **1a-e** are presented in H-NMR and C-NMR results;

4-allyl-2-methoxy-6-(pyrrolidin-1-ylmethyl)phenol **(1a)** $C_{15}H_{21}NO_2$, molecular weight 247.33. Dark brown oil. Yield 40.68%. H-NMR ($CDCl_3$, 500 MHz) δ (ppm): 6.38 (H-3,s, 1H); 6.72 (H-5, d, 1H); 3.36/3.35 (H-7, m, 2H); 5.98 (H-8, m, 1H); 5.11/5.05 (H-9, m, 2H); 4.33 (H-1', s, 2H); 3.74/3.72 (H-2'/H-5', m, 2H); 3.20/3.19 (H-3/H-4', m, 2H); 7.43 (-OH, s, 1H); 3.86 (-OCH_3, s, 3H). C-NMR ($CDCl_3$, 125 MHz) δ (ppm): 145.3 (C-1); 147.3 (C-2); 110.7 (C-3); 132.0 (C-4); 123.1 (C-5); 128.5 (C-6); 40.0 (C-7); 137.7 (C-8); 115.8 (C-9); 68.2 (C-1'); 66.7 (C-2'/C-5'); 53.0 (C-3'/C-4'); 56.3 (OCH_3).

4-allyl-2-methoxy-6-(piperidin-1-ylmethyl)phenol **(1b)** $C_{16}H_{23}NO_2$, molecular weight 261.36. Light brown oil. Yield 47.86%. H-NMR ($CDCl_3$, 500 MHz) δ (ppm): 6.47 (H-3, d, 1H); 6.66 (H-5, d, 1H); 2.96/2.96 (H-7, d, 2H); 5.91 (H-8, m, 1H); 5.89/4.98 (H-8, m, 2H); 3.78 (H-1', s, 1H); 2.15/2.15 (H-2'/H-6', m, 2H); 1.66/1.66 (H-3'/H-5', d, 2H); 1.23 (H-4', d, 2H); 7.43 (-OH, s, 1H); 3.82 (-OCH_3, s, 3H). C-NMR ($CDCl_3$, 125 MHz) δ (ppm): 142.2 (C-1); 149.7 (C-2); 113.0 (C-3); 134.7 (C-4); 124.4 (C-5); 123.7 (C-6); 40.1 (C-7); 136.5 (C-8); 115.9 (C-9); 58.8 (C-1'); 56.7 (C-2'/C-6'); 25.9 (C-3'/C-5'); 24.5 (C-4'); 56.1 (OCH_3).

4-allyl-2-methoxy-6-(4-methylpiperidin-1-ylmethyl)phenol **(1c)** $C_{17}H_{25}NO_2$, molecular weight 275.39. Brown oil. Yield 68.98%. H-NMR ($CDCl_3$, 500 MHz) δ (ppm): 6.58 (H-3, m, 1H); 6.67 (H-5, m, 1H); 3.24 (H-7, m, 2H); 5.89 (H-8, m, 1H); 4.98 (H-9, m, 2H); 3.56 (H-1', s, 2H); 2.94/2.16 (H-2'/H-6', m, 2H); 1.68/1.23 (H-3'/H-5', m, 2H); 1.20 (H-4', m, 2H); 7.43 (-OH, s, 1H); 3.78 (-OCH_3, s, 3H); 1.14 (-CH_3, d, 3H). C-NMR ($CDCl_3$, 125 MHz) δ (ppm): 144.7 (C-1); 147.5 (C-2); 111.3 (C-3); 131.5 (C-4); 121.2 (C-5); 130.6 (C-6); 39.4 (C-7); 138.1 (C-8); 114.2 (C-9); 59.8 (C-1'); 52.8 (C-2'/C-6'); 33.7 (C-3'/C-5') 30.2 (C-4'); 57.0 (OCH_3); 20.7 (-CH_3).

4-allyl-2-methoxy-6-(morpholino(phenyl)methyl) phenol **(1d)** $C_{21}H_{25}NO_3$, molecular weight 339.43. Darkest brown oil. Yield 34.94%. H-NMR ($CDCl_3$, 500 MHz) δ (ppm): 6.38 (H-3, s, 1H); 6.72 (H-5, d, 1H); 3.36/3.35 (H-7, m, 2H); 5.98 (H-8, m, 1H); 5.11/5.05 (H-9, m, 2H); 4.33 (H-1', s, 2H); 3.20/3.19 (H-2'/H-5', m, 2H); 3.74/3.72 (H-3'/H-4', m, 2H); 7.39 (H-2"/H-6", m, 2H); 7.30 (H-3"/H-5", m, 2H); 7.27 (H-4", m, 2H); 7.43 (-OH, s, 1H); 3.86 (OCH_3, s, 3H). C-NMR ($CDCl_3$, 125 MHz) δ (ppm): 145.3 (C-1); 147.3 (C-2); 110.7 (C-3); 132.0 (C-4); 123.1 (C-5); 128.5 (C-6); 40.0 (C-7); 137.7 (C-8); 115.8 (C-9); 68.2 (C-1'); 53.0 (C-2'/C-5'); 66.7 (C-3'/C-4'); 140.9 (C-1"); 129.0 (C-2"/C-6"); 124.4 (C-3"/C-5"); 127.7 (C-4"); 56.3 (OCH_3).

4-allyl-2-methoxy-6-((4-methylpiperidin-1-yl) (phenyl)methyl)phenol **(1e)** $C_{23}H_{29}NO_2$, molecular weight 351.48. Brown oil. Yield 88.54%. H-NMR ($CDCl_3$, 500 MHz) δ (ppm): 6.61 (H-3, s, 1H); 6.98 (H-5, s, 1H); 3.33 (H-7, m, 2H); 5.92 (H-8, m, 1H); 5.04/4.79 (H-9, m, 2H); 5.14 (H-1', s, 1H); 2.51/2.41 (H-2'/H-6', d, 2H); 1.56/1.31 (H-3'/H-5', m, 2H); 1.60 (H-4', m, 1H); 7.54 (H-2"/H-6", m, 2H); 7.23 (H-3"/H-5", m, 2H); 7.22 (H-4", m, 2H); 7.43 (-OH, s, 1H); 3.83 (OCH_3, s, 3H); 0.86 ($-CH_3$, s, 3H). C-NMR ($CDCl_3$, 125 MHz) δ (ppm): 141.6 (C-1); 150.5 (C-2); 112.0 (C-3); 135.5 (C-4); 123.8 (C-5); 120.4 (C-6); 40.1 (C-7); 136.5 (C-8); 115.9 (C-9); 76.8 (C-1'); 51.6 (C-2'/C-6'); 34.2 (C-3'/C-5'); 32.0 (C-4'); 142.7 (C-1"); 128.2 (C-2"/C-6"); 129.2 (C-3"/C-5"); 126.2 (C-4"); 56.1 (OCH_3); 20.4 ($-CH_3$).

EUGENOL DERIVATIVES EXERTED A GOOD INHIBITORY EFFECT AGAINST *P. falciparum* 3D7

An *in vitro* assay of **1a-e** compounds was performed against the chloroquine-sensitive *Plasmodium falciparum* (*Pf*3D7) strain. Based on Table 1, there is a significantly increased in antimalarial activity with the addition of a secondary amine to eugenol. Eugenol with secondary amine methyl piperidine **(1c)** and pyrrolidine **(1a)** showed good antimalarial activity with IC_{50} of 0.62 µM and 0.89 µM, respectively. Eugenol methyl piperidine with benzaldehyde linker **(1e)** showed an increased IC_{50} of 10.15 µM but decreased antimalarial activity. The presence of formaldehyde and benzaldehyde as linkers also give affected to antimalarial activity. Overall **1a-e** compounds have good antimalarial activity with $IC_{50} \leq$ 10 µM, based on the category by Batista et al. (2009).

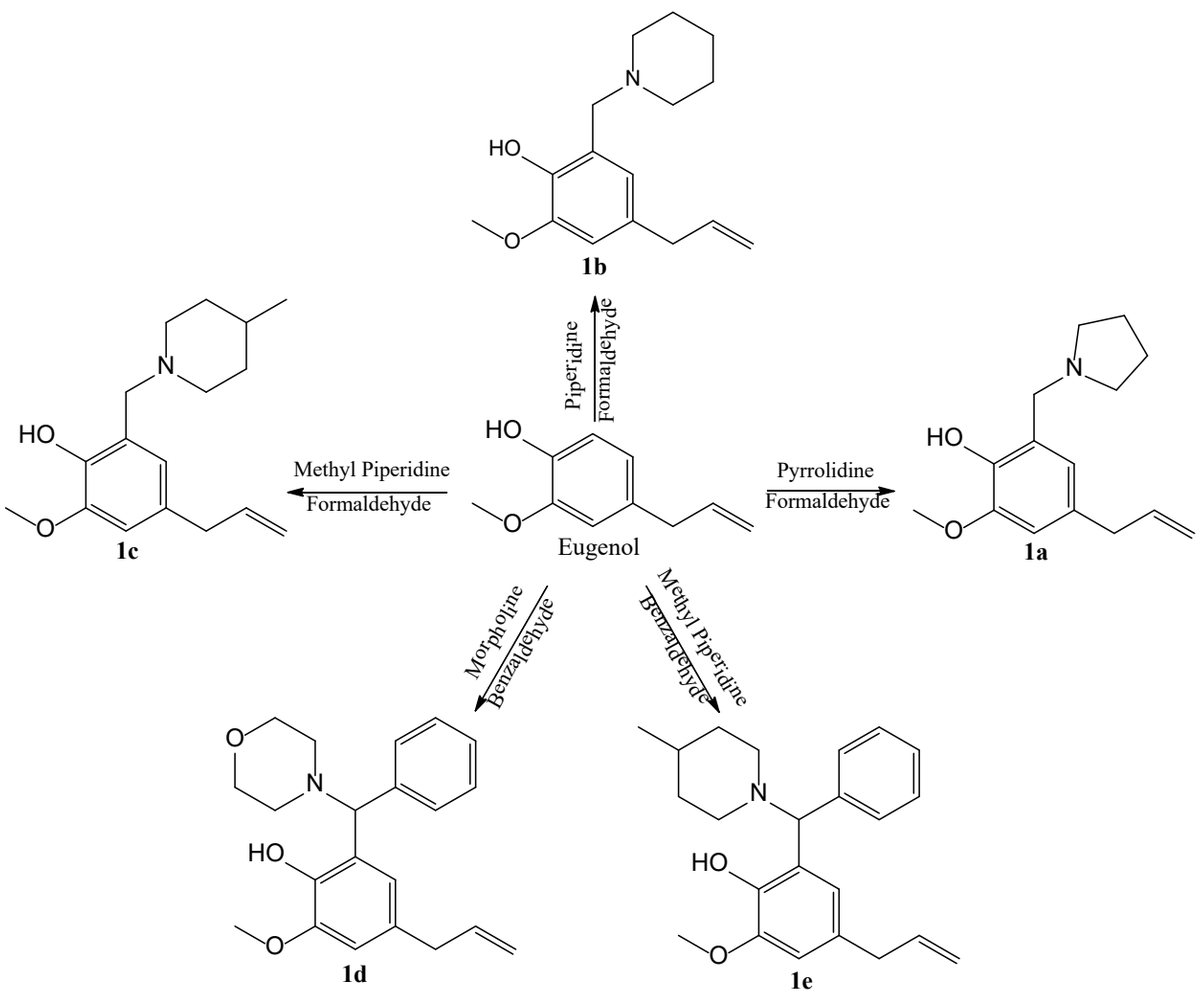

FIGURE 1. Mannich reaction of the secondary amino-substituted eugenol by reflux 30 h

TABLE 1. An *in vitro* antimalarial activity (IC_{50}) of eugenol derivatives against *Pf*3D7

Compounds	In vitro assay against *P. falciparum* 3D7, IC_{50} (μM)	Drug potency
1a	0.89	Excellent
1b	4.11	Good
1c	0.62	Excellent
1d	3.79	Good
1e	10.15	Good
Eugenol (parent compound)	109.00	Low
Chloroquine (an antimalarial drug)	0.03	Excellent

Eugenol is proven to have antimalarial activity like other natural ingredients unfortunately, it has low antimalarial activity compared to another compound. Based on the previous research about the biological activity of eugenol on *Plasmodium falciparum,* the prominent effect of eugenol was disrupted *Plasmodium falciparum* intracellular development during the erythrocytic cycle (Pontes et al. 2021). The mechanism is eugenol interacted with Fe in ferroprotoporphyrin (FP IX) or protein in DNA parasites through hydrogen bonds, it may be caused disturbances in the metabolism of parasite cells thus the *Plasmodium falciparum* will die (Julianto et al. 2018; Syahri et al. 2020).

This result proposed the vital role of the adding amine group in antimalarial activity. The addition of amine to eugenol increased this activity. Suwito et al. (2014) stated that the presence of nitrogen atoms from amines could form electrostatic interaction with carbonyl groups from the protein of *Plasmodium falciparum*. The company of amine and hydroxyl groups will improve the performance of eugenol by creating strong hydrogen bonds with DNA parasites (Abdou et al. 2021; Julianto et al. 2018; Syahri et al. 2020; Suwito et al. 2014). The **1c** compound is easier to bind to parasite amino acids than the **1e** compound. This is due to the presence of phenyl from benzaldehyde at **1e** to have a steric effect. The interaction is depicted in Figure 2.

This study showed that, the –OH group plays in the formation of interaction via hydrogen bonds. The presence of benzene rings will result in π-bonds with amino acids in the PfDHFR-TS protein (Clemente et al. 2022). Meanwhile, the presence of an amino group leads to structural stability as evidenced by the lower energy (Table 2) and the position of the ligand with the target protein becomes stable (Syahri et al. 2020; Suwito et al. 2014).

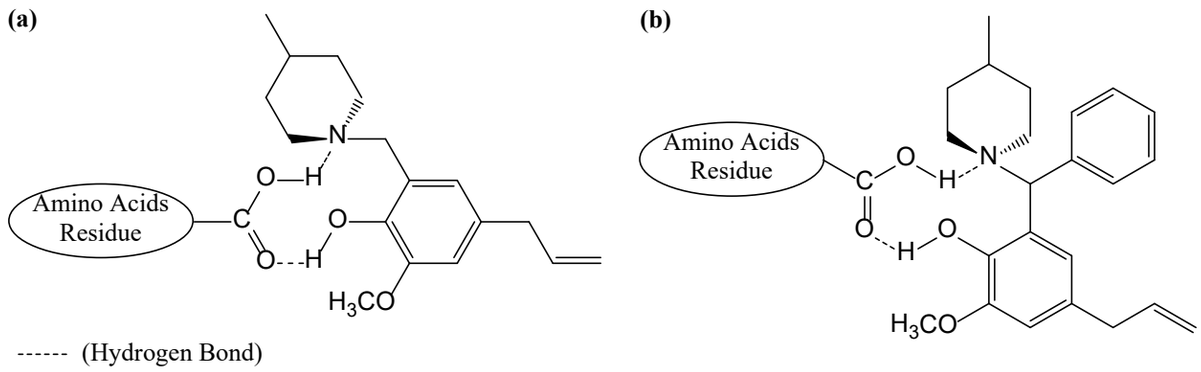

FIGURE 2. The prediction of interaction hydrogen bond on (a) **1c** and **1e** compounds with amino acid residue from *Plasmodium*

EUGENOL DERIVATIVES INHIBITED PfDHFR

In this work, molecular docking was performed in **1a** and **1c** as the best *in vitro* antimalarial activity. The molecular docking was performed to predict the interaction of the secondary amine functional group in **1a** and **1c** to the amino acid of the PfDHFR-TS protein. The molecular docking results are shown in Table 2.

TABLE 2. Energy and binding interaction of PfDHFR-TS with compounds **1a** and **1c**

Compound	Binding free energy (kcal/mol)	RMSD	H bond	Hydrophobic	Van der Walls	The other interactions	Binding factor
Chloroquine	-7.1328	1.2558	Phe58	Ile164	Asp54	Ala16, Ile14, Thr185, Tyr57, Cys15, Tyr170, Met55, Gly165, Gly166, Ser108 Ile112 Ser111 Gly44, Val45 Leu46	-
1a	-6.1407	0.8839	**Ser111**	Lys43	-	**Leu46, Gly44, Val45, Gly166, Gly165, Ile112,** Thr107, Ser167, **Ser108, Ile164,** Val168,	9
1c	-6.6536	1.5902	**Leu46**	-	-	Thr107, **Ser108, Ile164, Ser111, Met55, Ile112, Ile14,** Phe116, **Phe58, Cys15, Ala16,** Leu40, **Val45, Tyr170, Gly44, Gly166, Gly165**	15

Based on the docking results, it is seemed that compound **1a** has a potentiality become an antimalarial drug with a binding free energy of -6.1407 kcal/mol. Compound **1a** was performed a hydrogen bond with amino acid residue Ser111 (green dash line) and it also showed an interaction with Lys43 through hydrophobic interaction. In addition, compound **1a** was also able to make any interaction with amino acid residue as listed in Table 2. The spatial arrangement of compound **1a** is depicted in Figure 3.

Compound **1c** has binding free energy of -6.6536 kcal/mol, it is lower than binding free energy of compound **1a**. Compound **1c** has one hydrogen bonding with Leu46 (green dash line) and also build interaction with some amino acids as presented in Table 2. The spatial arrangement of compound **1c** is presented in Figure 4. This confirms that the methyl piperidine amino group with formaldehyde as a linker makes an excellent contribution as an antimalarial. DHFR in *P. falciparum* existed as a bifunctional enzyme together with thymidylate synthase (TS) (Yuthavong et al. 2005). The PfDHFR-TS is sensitive to the inhibition by the antifolate antimalarials such as pyrimethamine, 2,4-diaminopyrimidines, cycloguanil, and 2,3-dihydrotriazines. They inhibited PfDHFR, thereby depriving the parasite of essential folate cofactors. In this study, the antimalarial effect shown by eugenol derivatives **1a** and **1c** potentially involved the inhibition of PfDHFR.

Physicochemical properties of the eugenol derivatives were revealed by following the Lipinski rule based on the molecular weight (MW), number of hydrogen bond acceptors (nHA), number of hydrogen bond donors (nHD), and the logarithm of the n-octanol/

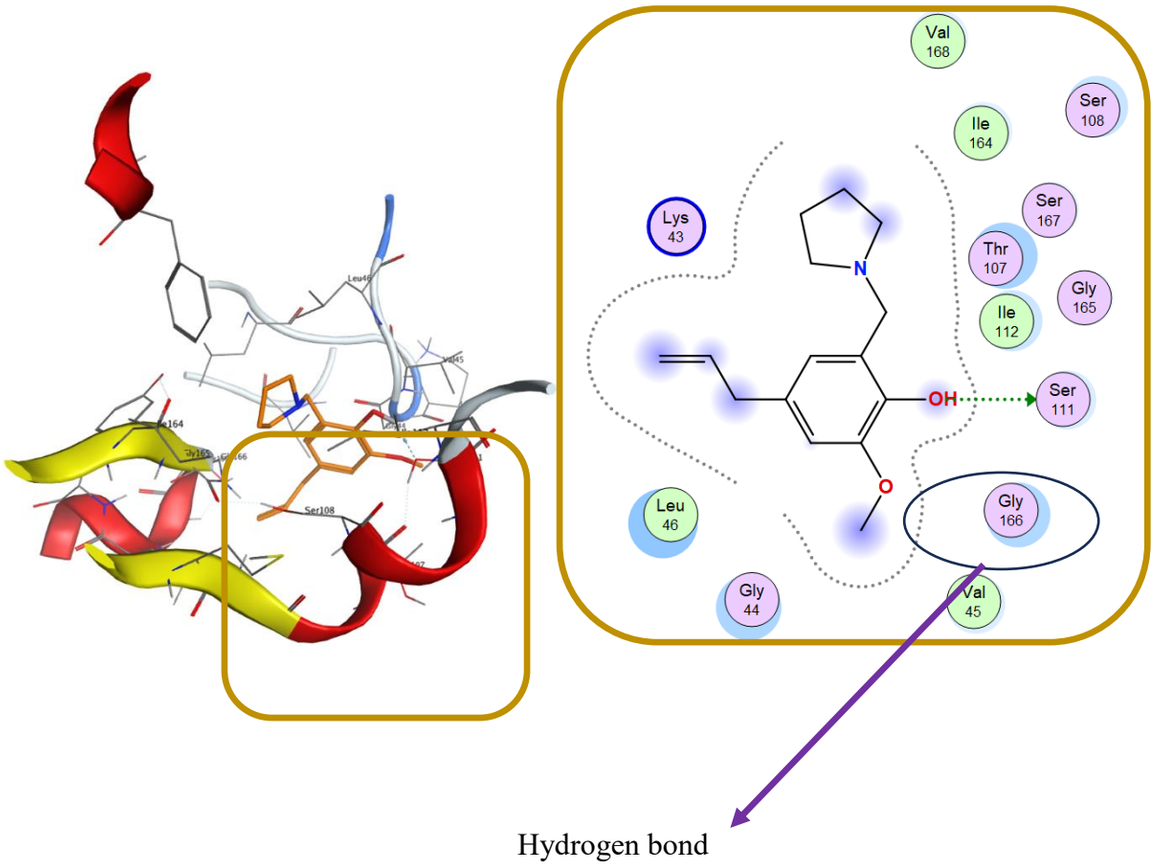

FIGURE 3. Spatial arrangement of compound **1a**

water distribution (logP). Therefore, without any violations of the Lipinski rule, eugenol derivatives **1a** and **1c** were predicted to be orally bioavailable. However, logP values that showed lipophilicity parameters were considered to be higher in **1c** (>3) compared than **1a** (0-3). The absorption of drugs showed good absorption which can be seen by the human intestinal absorption (HIA) value with a prediction score of 0.01 (0 to 0.3). The human colon adenocarcinoma cell lines (Caco-2) have been used to predict drug permeability. In addition, the synthesized eugenol derivatives **1a** and **1c** were considered to have a proper Caco- permeability, as they have a predictive value of ≥5.15 log cm/s. The eugenol derivatives **1a** and **1c** were predicted to be a non-substrate of the P-glycoprotein (P-gp). Eugenol derivatives **1a** and **1c** were predicted to have good bioavailability with predictive values in the range of 0-0.3 (≥ 20 and 30% bioavailability). The synthesized derivatives **1a** and **1c** were classified to have good PPB properties as they have a predicted value of <90%. The ADMET analysis showed that eugenol derivatives **1a**, and **1c**, were classified as good BBB penetration with an empirical value between 0.9-1.0. Meanwhile, the fraction unbound in plasma (Fu) parameter of eugenol derivatives **1a** and **1c** resulted in more than 5%, indicating a good unbound state of drugs in plasma. As reported in Table 3, eugenol derivatives **1a** and **1c,** had lower toxicity based on the ADMET parameter and hERG blockers parameter. Eugenol derivatives **1a** and **1c** were found to become substrates to CYP1A2 and CYP2D6. These findings suggested that both secondary amino-substituted eugenol derivatives are a potential drug to be pursued as a new template for an antifolate inhibitor in malarial infection.

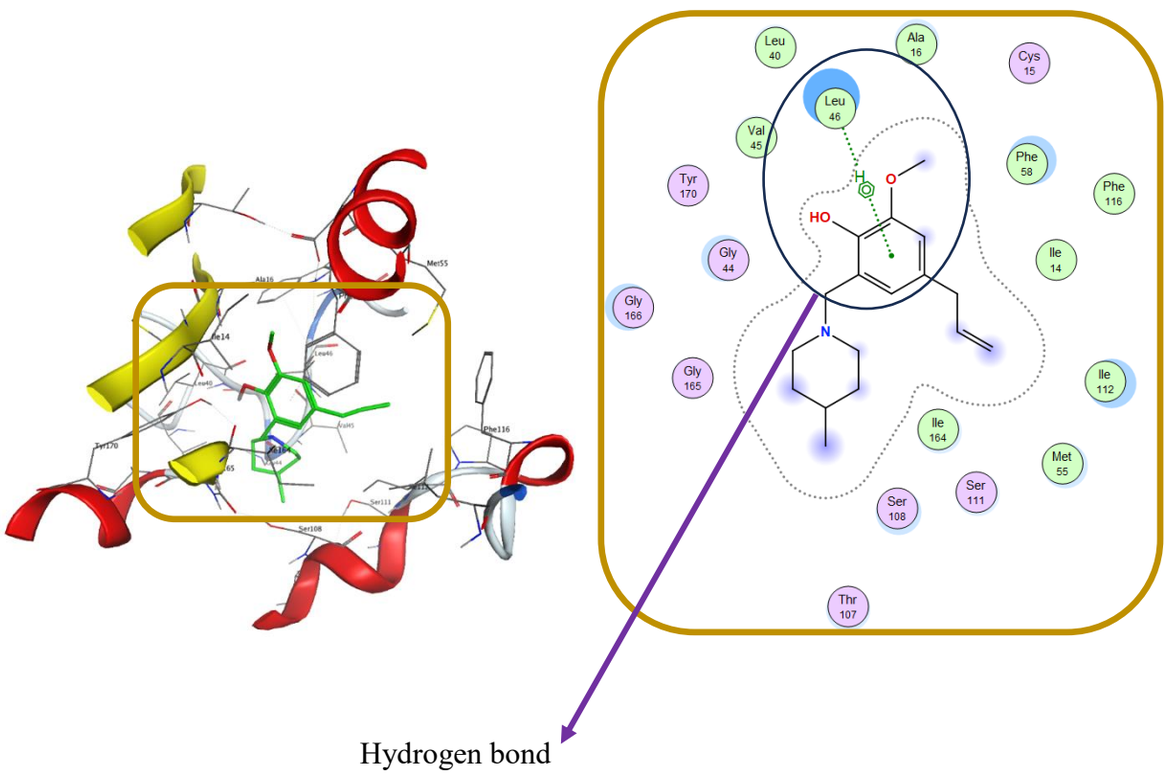

FIGURE 4. Spatial arrangement of compound **1c**

TABLE 3. Prediction of ADMET parameters of eugenol derivatives

Parameters	1a	1b	1c	1d	1e
Drug-likeness					
• Molecular weight	247.33	261.36	275.39	339.43	351.48
• H-bond acceptor	3	3	3	4	3
• H-bond donor	1	1	1	1	1
• LogP	2.816	3.288	3.681	3.560	4.829
• TPSA	32.7	32.7	32.7	41.93	32.7
A (Absorption)					
• Human intestinal absorption (HIA)	0.01	0.01	0.01	0.01	0.01
• Caco-2 permeability (log cm/s)	-4.513	-4.546	-4.553	-4.452	-4.617
• P-glycoprotein inhibitor	0.932	0.932	0.932	0.932	0.932
• P-glycoprotein substrate	0.001	0.001	0.001	0.001	0.001
• $F_{20\%}$	0.862	0.862	0.862	0.862	0.862
• $F_{30\%}$	0.628	0.628	0.628	0.628	0.628
D (Distribution)					
• Plasma protein binding (PPB) (%)	81.64	85.697	89.370	96.616	97.288
• Blood-brain barrier penetration (BBB) (cm/s)	0.966	0.966	0.966	0.966	0.966
• Volume distribution (L/kg)	1.687	1.685	1.763	1.004	1.714
• Fu (%)	12.30	9.224	6.654	3.309	2.901
M (Metabolism)					
• CYP1A2 substrate	0.868	0.900	0.868	0.500	0.900
• CYP1A2 inhibitor	0.138	0.138	0.138	0.138	0.138
• CYP2C19 substrate	0.845	0.845	0.845	0.845	0.845
• CYP2C19 inhibitor	0.04	0.040	0.040	0.900	0.700
• CYP2C9 substrate	0.072	0.040	0.10	0.500	0.700
• CYP2C9 inhibitor	0.06	0.040	0.040	0.700	0.500
• CYP2D6 substrate	0.91	0.900	0.90	0.700	0.700
• CYP2D6 inhibitor	0.958	0.900	0.90	0.700	0.900
• CYP3A4 substrate	0.56	0.500	0.50	0.900	0.900
• CYP3A4 inhibitor	0.02	0.040	0.040	0.700	0.500
E (Excretion)					
• Half time ($T_{1/2}$)	0.849	0.819	0.718	0.621	0.300
• Clearance (mL/min/kg)	14.705	14.099	13.854	12.764	9.987
T (Toxicity)					
• Human hepatotoxicity (H-HT)	0.214	0.100	0.100	0.040	0.040
• hERG blockers	0.058	0.040	0.100	0.040	0.100
• Rat oral acute toxicity	0.831	0.700	0.500	0.300	0.100
• AMES toxicity	0.023	0.040	0.040	0.040	0.040
• Drug induced liver injury (DILI)	0.125	0.100	0.100	0.040	0.040
• Carcinogenicity	0.249	0.100	0.100	0.040	0.040

Conclusions

The results of this study indicated that with addition of secondary amines to eugenol derivatives can increase antimalarial activity. Compounds **1a** and **1c** are showed best antimalarial activity, it parallel with the computational analysis against PfDHFR, suggesting that the eugenol derivatives targeted PfDHFR. Thus, the eugenol derivatives were essential for developing of new candidate antimalarial drugs, especially in preventing folate resistance in malarial infection.

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References

Abdou, A., Elmakssoudi, A., El Amrani, A., JamalEddine, J. & Dakir, M. 2021. Recent advances in chemical reactivity and biological activities of eugenol derivatives. *Medicinal Chemistry Research* 30(5): 1011-1030.

Arango, V., Domínguez, J.J., Cardona, W., Robledo, S.M., Muñoz, D.L., Figadere, B. & Sáez, J. 2012. Synthesis and leishmanicidal activity of quinoline-triclosan and quinoline-eugenol hybrids. *Medicinal Chemistry Research* 21(11): 3445-3454.

Ashley, E.A., Dhorda, M., Fairhurst, R.M., Amaratunga, C., Lim, P., Suon, S., Sreng, S., Anderson, J.M., Mao, S., Sam, B. & Sopha, C. 2014. Spread of artemisinin resistance in *Plasmodium falciparum* malaria. *New England Journal of Medicine* 371(5): 411-423.

Bachiega, T.F., De Sousa, J.P.B., Bastos, J.K. & Sforcin, J.M. 2012. Clove and eugenol in noncytotoxic concentrations exert immunomodulatory/anti-inflammatory action on cytokine production by murine macrophages. *The Journal of Pharmacy and Pharmacology* 64(4): 610-616.

Barboza, J.N., da Silva Maia Bezerra Filho, C., Silva, R.O., Medeiros, J.V.R. & de Sousa, D.P. 2018. An overview on the anti-inflammatory potential and antioxidant profile of eugenol. *Oxidative Medicine and Cellular Longevity* 2018: 3957262.

Batista, R., de Jesus Silva Jr., A. & de Oliveira, A.B. 2009. Plant-derived antimalarial agents: new leads and efficient phytomedicines. Part II. Non-alkaloidal natural products. *Molecules* 14(8): 3037-3072.

Biersack, B., Ahmed, K., Padhye, S. & Schobert, R. 2018, Recent developments concerning the application of the Mannich reaction for drug design. *Expert Opinion on Drug Discovery* 13(1): 39-49.

Clemente, C.M., Pineda, T., Yepes, L.M., Upegui, Y., Allemandi, D.A., Robledo, S.M. & Ravetti, S. 2022. Eugenol carbonate activity against *Plasmodium falciparum*, *Leishmania braziliensis*, and *Trypanosoma cruzi*. *Archiv der Pharmazie* 355(3): 1-11.

Ginsburg, H. & Deharo, E. 2011. A call for using natural compounds in the development of new antimalarial treatments – An introduction. *Malaria Journal* 10(1): 2011.

Hyde, J.E. 2002. Mechanisms of resistance of *Plasmodium falciparum* to antimalarial drugs. *Microbes and Infection* 4(2): 165-174.

Jia, C.Y., Li, J.Y., Hao, G.F. & Yang, G.F. 2020. A drug-likeness toolbox facilitates ADMET study in drug discovery. *Drug Discovery Today* 768(25): 248-258.

Julianto, T.S., Jumina, J., Sastrohamidjojo, H. & Mustofa, M. 2018. Synthesis and heme polymerization inhibitory assay of a new arylamino alcohol derivative compound from methyl eugenol and aniline. *AIP Conference Proceedings* 2026: 1-7.

Kamatou, G.P., Vermaak, I. & Viljoen, A.M. 2012. Eugenol - From the remote Maluku Islands to the international market place: A review of a remarkable and versatile molecule. *Molecules* 17(6): 6953-6981.

Lane, T., Anantpadma, M., Freundlich, J.S., Davey, R.A., Madrid, P.B. & Ekins, S. 2019. The natural product eugenol is an inhibitor of the Ebola virus *in vitro*. *Pharmaceutical Research* 36(7): 1-11.

Lipinski, C.A., Lombardo, F., Dominy, B.W. & Feeney, P.J. 2001. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced Drug Delivery Reviews* 46: 3-26.

Maximino, S.C., Dutra, J.A., Rodrigues, R.P., Gonçalves, R.C., Morais, P.A., Ventura, J.A., Schuenck, R.P., Júnior, V.L., Kitagawa, R.R. & Borges, W.S. 2020. Synthesis of eugenol derivatives and evaluation of their antifungal activity against *Fusarium solani* f. sp. *piperis*. *Current Pharmaceutical Design* 26(14): 1532-1542.

Mina, P.R., Kumar, S., Agarwal, K., Kumar, R., Pal, A., Tandon, S., Yadav, S.K., Yadav, S. & Darokar, M.P. 2021. 4-chloro eugenol interacts synergistically with artesunate against drug resistant *P. falciparum* inducing oxidative stress. *Biomedicine and Pharmacotherapy* 137: 111311.

Ministry of Health, Indonesia (MOH). 2018. Report on the Latest Situational Developments in Indonesia's Malaria Control Programme. https://www.malaria.id/laporan (Accessed 1 December 2022).

Pereira, R.B., Pinto, N.F.S., Fernandes, M.J.G., Vieira, T.F., Rodrigues, A.R.O., Pereira, D.M., Sousa, S.F., Castanheira, E.M.S., Gil Fortes, A. & Gonçalves, M.S.T. 2021. Amino alcohols from eugenol as potential semisynthetic insecticides: Chemical, biological, and computational insights. *Molecules* 26(21): 1-20.

Pontes, K.A.O., Silva, L.S., Santos, E.C., Pinheiro, A.S., Teixeira, D.E., Peruchetti, D.B., Silva-Aguiar, R.P., Wendt, C.H.C., Miranda, K.R., Coelho-de-Souza, A.N., Leal-Cardoso, J.H., Caruso-Neves, C. & Pinheiro, A.A.S. 2021. Eugenol disrupts *Plasmodium falciparum* intracellular development during the erythrocytic cycle and protects against cerebral malaria. *Biochimica et Biophysica Acta* 1865(3): 129813.

Rieckmann, K.H., Sax, L.J., Campbell, G.H., Mrema, J.E. 1978. Drug sensitivity of plasmodium falciparum. An in vitro microtechnique. *The Lancet* 311(8054): 22-23.

Shibeshi, M.A., Kifle, Z.D. & Atnafie, S.A. 2020. Antimalarial drug resistance and novel targets for antimalarial drug discovery. *Infection and Drug Resistance* 13: 4047-4060.

Silva, C.G., Yudice, E.D.C., Campini, P.A.L. & Rosa, D.S. 2021. The performance evaluation of Eugenol and Linalool microencapsulated by PLA on their activities against pathogenic bacteria. *Materials Today Chemistry* 21: 100493.

Suwito, H., Jumina, Mustofa, Pudjiastuti, P., Fanani, M.Z., Kimata-Ariga, Y., Katahira, R., Kawakami, T., Fujiwara, T., Hase, T., Sirat, H.M. & Puspaningsih, N.N.T. 2014. Design and synthesis of chalcone derivatives as inhibitors of the ferredoxin - Ferredoxin-NADP+ reductase interaction of *Plasmodium falciparum*: Pursuing new antimalarial agents. *Molecules* 19(12): 21473-21488.

Syahri, J., Nasution, H., Nurohmah, B.A., Purwono, B. & Yuanita, E. 2020. Novel aminoalkylated chalcone: Synthesis, biological evaluation, and docking simulation as potent antimalarial agents. *Journal of Applied Pharmaceutical Science* 10(6): 001-005.

World Health Organization. 2021. *World Malaria Report, 2021.* Geneva, Switzerland: WHO Press.

Xiong, G., Wu, Z., Yi, J., Fu, L., Yang, Z., Hsieh, C., Yin, M., Zeng, X., Wu, C., Lu, A., Chen, X., Hou, T. & Cao, D. 2021. ADMETlab 2.0: 770 an integrated online platform for accurate and comprehensive predictions of ADMET properties. *Nucleic Acids Research* 49(771): w5-w14.

Yuthavong, Y., Yuvaniyama, J., Chitnumsub, P., Vanichtanankul, J., Chusacultanachai, S., Tarnchompoo, B., Vilaivan, T. & Kamchonwongpaisan, S. 2005. Malarial (*Plasmodium falciparum*) dihydrofolate reductase-thymidylate synthase: Structural basis for antifolate resistance and development of effective inhibitors. *Parasitology* 130(3): 249-259.

Zari, A.T., Zari, T.A. & Hakeem, K.R. 2021. Anticancer properties of eugenol: A review. *Molecules* 26(23): 7407.

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