© 2025 Journal of Pharmacy & Pharmacognosy Research, 13 (2), 416-430, 2025
ISSN 0719-4250
https://jppres.com

DOI: https://doi.org/10.56499/jppres24.2097_13.2.416

Original Article

Synthesis and docking studies of the functionalized dibenzylideneacetones as promising antimalarial agents

[Estudios de síntesis y acoplamiento de dibencilidenoacetonas funcionalizadas como agentes antipalúdicos prometedores]

Melanny Ika Sulistyowaty[1,2*], Galih Satrio Putra[3], Tutuk Budiati[4], Anastasia Wheni Indrianingsih[5], Yunita Wahyu Renawanti[1], Kholis Amalia Nofianti[1], Juni Ekowati[1], Neny Purwitasari[1], Charinrat Saechan[6], Sabry A. H. Zidan[7], Takayasu Yamauchi[2]

[1]Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia.
[2]School of Pharmacy and Pharmaceutical Sciences, Hoshi University, Tokyo, Japan.
[3]Department of Chemistry, Faculty of Mathematics and Natural Sciences, State University of Malang, Malang, Indonesia.
[4]Faculty of Pharmacy, Widya Mandala Catholic University, Surabaya, Indonesia.
[5]Research Center for Food Technology and Processing, National Research Innovation and Agency (BRIN), Gunung Kidul, Yogyakarta, Indonesia.
[6]Faculty of Medical Technology; Prince of Songkla University, Songkhla, Thailand.
[7]Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Assiut, Egypt.
*E-mail: melanny-i-s@ff.unair.ac.id

Abstract

Context: First-line therapy using artemisinin-based combination therapy (ACT) and chloroquine in cases of malaria infection has developed resistance worldwide.

Aims: To synthesize functionalized dibenzylideneacetones that are similar to curcumin and to perform the *in silico* evaluations on *Plasmodium falciparum* enoyl acyl carrier protein reductase (PfENR) and through human dihydrofolate reductase (hDHFR) as targets for antimalarial agents.

Methods: The functionalized dibenzylideneacetones were synthesized through a reaction of acetone with benzaldehydes in a base or acid catalyst. The resulting solids were purified by recrystallization to conduct UV/Vis, FTIR, and NMR tests. The confirmed products underwent an *in silico* molecular docking test on PfENR (PDB ID: 1NHG) and hDHFR (PDB ID: 1MVT). The products were obtained in good yields.

Results: The confirmed products underwent *in silico* molecular docking test on PfENR (PDB ID: 1NHG) and hDHFR (PDB ID: 1MVT). The products were obtained with good yields. Based on *in silico* results, the dibenzylideneacetone was predicted to have a strong potential to inhibit the growth of malaria parasites through inhibit malaria growth through the PfENR pathway more than through the hDHFR pathway because its moldock score was lower than its native ligand.

Conclusions: This study showed a promising pathway to obtain antimalarial agents for further application in the medical and pharmaceutical industries.

Keywords: antimalarial agent; chemical synthesis; dibenzylideneacetones; molecular docking; tropical disease.

Resumen

Contexto: La terapia de primera línea con terapia combinada basada en artemisinina (ACT) y cloroquina en casos de infección por malaria ha desarrollado resistencia en todo el mundo.

Objetivos: Sintetizar dibencilidenoacetonas funcionalizadas que sean similares a la curcumina y realizar evaluaciones *in silico* en la proteína transportadora de enoil acilo reductasa (PfENR) de *Plasmodium falciparum* y a través de la dihidrofolato reductasa humana (hDHFR) como objetivos para agentes antimaláricos.

Métodos: Las dibencilidenoacetonas funcionalizadas se sintetizaron a través de una reacción de acetona con benzaldehídos en un catalizador básico o ácido. Los sólidos resultantes se purificaron por recristalización para realizar pruebas de UV/Vis, FTIR y RMN. Los productos confirmados se sometieron a una prueba de acoplamiento molecular in silico en PfENR (PDB ID: 1NHG) y hDHFR (PDB ID: 1MVT). Los productos se obtuvieron con buenos rendimientos.

Resultados: Los productos confirmados se sometieron a pruebas de acoplamiento molecular *in silico* en PfENR (PDB ID: 1NHG) y hDHFR (PDB ID: 1MVT). Los productos se obtuvieron con buenos rendimientos. Con base en los resultados *in silico*, se predijo que la dibencilidenoacetona tenía un fuerte potencial para inhibir el crecimiento de los parásitos de la malaria a través de la vía PfENR más que a través de la vía hDHFR porque su puntaje de moldock era menor que su ligando nativo.

Conclusiones: Este estudio mostró una vía prometedora para obtener agentes antipalúdicos para su posterior aplicación en las industrias médica y farmacéutica.

Palabras Clave: acoplamiento molecular; agente antipalúdico; dibencilidenoacetonas; enfermedad tropical; síntesis química.

ARTICLE INFO
Received: May 22, 2024.
Accepted: October 7, 2024.
Available Online: October 19, 2024.

AUTHOR INFO
ORCID:
0000-0002-9510-6822 (MIS)
0000-0003-4192-3621 (GSP)
0000-0002-7073-2421 (AWI)
0000-0002-9398-3063 (KAN)
0000-0002-4402-2039 (JE)
0000-0003-0817-7065 (NP)
0000-0001-5537-0488 (CS)
0000-0003-4975-5933 (SAHZ)
0009-0002-1948-9726 (TY)

INTRODUCTION

Curcumin is one of the natural products that are interesting to be developed as an antimalarial agent. It was reported as a natural product that possessed antimalarial activity (Franco et al., 2012). An *in vitro* study on the human malaria parasite *Plasmodium falciparum* resulted in the IC_{50} value of 5 µM, which was classified as very potential activity (Chakrabarti et al., 2013; Indrayanto et al., 2021). It also has a high potential for antimalarial activity by *in vivo* study. The oral administration of curcumin to mice with the malaria parasite (*Plasmodium berghei*) reduced blood parasitemia by 80-90% with a value of IC_{50} < 5 µM (Reddy et al., 2005).

First-line therapy using artemisinin-based combination therapy (ACT) and chloroquine in cases of malaria infection has resistance (Widyawaruyanti et al., 2007). The use of curcumin-artemisinin combination therapy (CACT) is an alternative solution to overcome malaria resistance (Khairani et al., 2021; Nandakumar et al., 2006; Padmanaban et al., 2012). However, curcumin is easily degraded due to the presence of a dual ketone H alpha group that can undergo keto-enol tautomerism and then autoxidation into the bicyclopentadione compound shown in Fig. 1 (Gordon et al., 2015; Schneider et al., 2015).

One of the efforts made by the researchers is to synthesize dibenzylideneacetones, which is a mimicry of curcumin, by removing one of the carbonyl groups so that the reactivity of H-alpha from two ketones is reduced, which causes the keto-enol tautomerization process and autoxidation to be reduced or not occur (Franco et al., 2012). Our previous study had been successful in the synthesis of chalcone and its two methoxy derivatives (Sulistyowaty et al., 2013). Several dibenzylideneacetones tested for *in silico* and *in vitro* activities as antimalarial compounds gave significant results with a value of $IC_{50} \leq 5$ µM (Franco et al., 2012). Another study also revealed that the antioxidant properties of dibenzylideneacetones were as potent as curcumin (Budiati et al., 2019; Handayani et al., 2008). Several previous studies have studied compounds and their potential malaria activity (Ngoro et al., 2017; Tiwari et al., 2021; Uzor, 2020). However, no one has studied comprehensively the compound dibenzylideneacetones **1-7** as in this study. The novelty of this research is that all compounds **1-7** have never been tested for absorption–distribution–metabolism–excretion–toxicity (ADMET).

The functionalized dibenzylideneacetones **1-7** can be synthesized by reacting acetone with a base (NaOH or KOH) and then adding benzaldehydes that can be refluxed or microwaved (Budiati et al., 2019; Franco et al., 2012; Oliveira et al., 2020). In this study, we synthesized seven dibenzylideneacetones (Fig. 2). After the compound was synthesized, an *in silico* test was run on (PfENR) with PDB ID code 1NHG (Perozzo et al., 2022) and through hDHFR with PDB ID 1MVT (Aroonsri et al., 2016; Cody et al., 2003; Yuthavong et al., 2012). To our best understanding, this is the first report about the synthesis of dibenzylideneacetones and their *in silico* studies to 1NHG and IMVT.

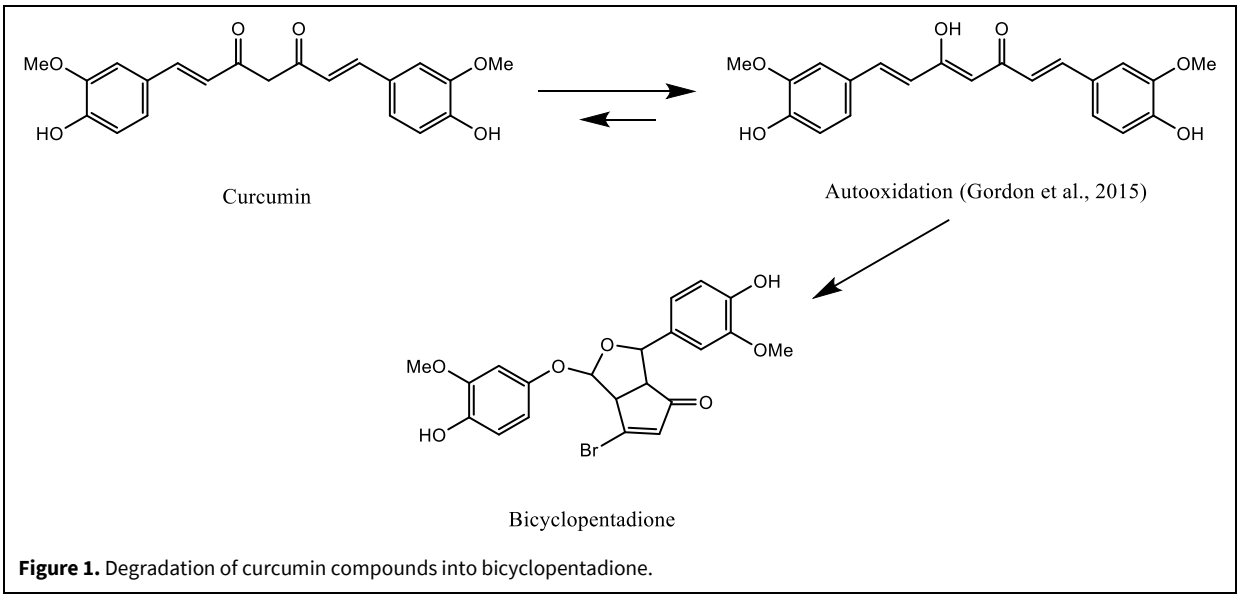
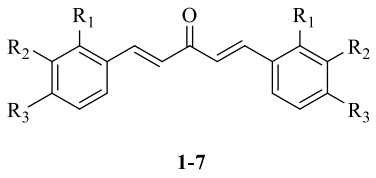
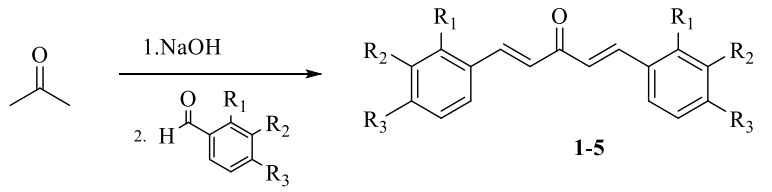


Figure 1. Degradation of curcumin compounds into bicyclopentadione.



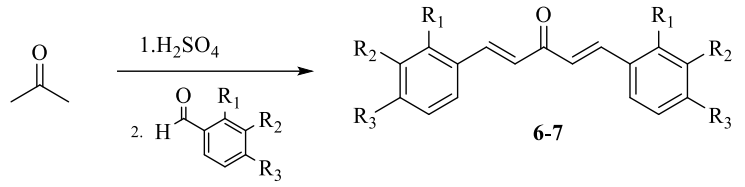
1.R_1=H; R_2=H; R_3=H
2.R_1=H; R_2=H; R_3=NO_2
3.R_1=H; R_2=NO_2; R_3=H
4.R_1=OMe; R_2=H; R_3=H
5.R_1=H; R_2=H; R_3=OMe
6.R_1=H; R_2=H; R_3=OH
7.R_1=H; R_2=OMe; R_3=OH

Figure 2. The functionalized dibenzylideneacetones.



1. R_1=H; R_2=H; R_3=H (yield=85 %)
2. R_1=H; R_2=H; R_3=NO_2 (yield=80 %)
3. R_1=H; R_2=NO_2; R_3=H (yield=74 %)
4. R_1=OMe; R_2=H; R_3=H (yield=70 %)
5. R_1=H; R_2=H; R_3=OMe (yield=80 %)

Figure 3. Synthesis of dibenzylideneacetones **1**-**5**.



6. R_1=H; R_2=H; R_3=OH (yield=90 %)
7. R_1=H; R_2=OMe; R_3=OH (yield=90 %)

Figure 4. Synthesis of dibenzylideneacetones **6**-**7**.

MATERIAL AND METHODS

Chemicals and instruments

All chemicals were analytical grade and used as received without further purification. Characterizations of the functionalized dibenzylideneacetones were performed using various spectroscopic methods. ^{1}H-NMR and ^{13}C-NMR spectra measurements were carried out using JEOL 400 spectrometer and were recorded at 400 MHz for ^{1}H and 100 MHz for ^{13}C using TMS (tetramethylsilane) as the internal standard. Chemical shift values (δ) in parts per million (μg/mL) and coupling constant (*J*) in Hz. The symbols s, d, t, dd, and ddd stand for singlet, doublet, triplet, double doublet, and double double doublet. FT-IR spectra were recorded by Jasco FT-IR 5300. Ultraviolet spectra were analyzed using Shimadzu UV–Vis Spectrophotometer 1800. The melting point of the products was determined using Fisher-John Electrothermal MelTemp without correction.

Synthesis procedure

Synthesis of dibenzylideneacetones ***(1-5)***

Benzaldehydes (5 mmol) were dissolved in ethanol 5 mL, and then 2.5 mmol acetone was added prior to stirring at room temperature. To the mixture, 20% NaOH (1.25 mL) was slowly added and then stirred for 1-3 hours. When the reaction was completed, the precipitate was filtered and washed with the solution of saturated NaHCO3. The products were purified by recrystallization using suitable solvents. (Fig. 3).

Synthesis of dibenzylideneacetones ***(6-7)***

Benzaldehydes (2 mmol) were dissolved in methanol 2 mL, and acetone (1 mmol) was added and stirred at room temperature. The mixture was placed on an ice bath, then slowly added concentrated H_2SO_4 (0.2 mL) and stirred overnight at room temperature. When the reaction was completed, then add distiller water to wash the precipitate. The precipitate was filtered and recrystallized with methanol (Fig. 4).

Molecular docking studies

The *in silico* studies were conducted using MVD software (Molegro ® Virtual Docker version 5.5). The compounds to be docked were stored in 3D geometry with the lowest energy set of MMFF94 mode (Halgren, 1996). The validation of the experiment was performed through PfENR by redocking the native ligand (triclosan) on the active side of this enzyme (Perozzo et al., 2022). Meanwhile, *in silico* antimalarial study through hDHFR, the validation of the assay by redocking of the native ligand (2,4-diamino-6- [N-(3

',4',5'-trimethoxybenzyl)-N-methylamino] pyrido[2,3-D] pyrimidine) on the active side of the enzyme (Cody et al., 2003). The acceptance criteria both were set with the value of RMSD ≤ 2.0 Å.

The data resulted from this experiment was the moldock scores. The smaller the moldock score, the more stable the binding between ligand and receptor. The moldock score is the total energy from external and internal ligand interaction. External ligand interaction is a sum of the energy consistency of protein-ligand interaction and cofactor-ligand interaction. Internal energy interaction is a sum of energy dependent on the chemical structures of the ligand, such as torsional strain, torsional strain sp^2-sp^2 steric, and electrostatic (Thomsen and Christensen, 2006). The results of *in silico* tests from molecular docking are also visualized and interpreted to provide an overview of ligand-receptor binding interactions, which include hydrogen bonds, hydrophobic interactions, and electronic interactions.

ADME and toxicity

To predict the pharmacokinetic profile of seven dibenzylideneacetones derivatives compounds, we used the online pkCSM application (https://biosig.lab.uq.edu.au/pkcsm/) and Swiss ADME (http://www.swissadme.ch/). The prediction of the absorption phase is made by paying attention to the Caco 2 permeability and the logarithm value of solubility (Log S values). By considering these two values, you can classify compounds in the Biopharmaceutical Classification System (BCS) I-IV. The prediction of the distribution phase is to pay attention to the volume distribution (Vd) and logarithmic value of the blood-brain barrier (BBB log) values. By considering these two values, we can classify compounds that have a large distribution volume, which means the compound is easily distributed in body cells or stored in adipose cells, or a low distribution volume, which means the compound is more widely distributed in the bloodstream. Apart from that, the BBB log shows the meaning that a compound has characteristics that are easy or difficult to distribute in brain cells.

Prediction of metabolic phases by focusing on dibenzylideneacetones derivatives compounds inhibiting drug metabolizing enzymes such as cytochrome P450 (CPY), namely CYP2D6; CYP3A4; CYP1A2; CYP2C9; and CYP2C19. Each CYP subunit metabolizes different drug compounds. The more dibenzylideneacetones derivatives there are, the more drugs there are that interact with various drugs. Predictions in the excretion phase or elimination phase consist of three important points, namely total clearance (total Cl), elimination rate constant (k), and half-time ($t_{1/2}$). Total Cl is a prediction of the rate of drug clearance in the body, which is a combination of hepatic clearance (liver metabolism and biliary clearance) and renal clearance. Total Cl can be calculated using the following equation [1].

$$Cl = k.\mathrm{Vd} \qquad [1]$$

Where Cl is the total clearance, k was the rate of elimination constant, and Vd was the volume of distribution of the compound.

To obtain k value, the Cl value was divided by the Vd of the compound. Most drugs have an elimination rate constant of order 1, which means the value of the half time (t1/2) can be calculated using the following equation [2].

$$t_{1/2} = \frac{\ln 2}{k} \qquad [2]$$

Where $t_{1/2}$ was the half-time of the compound, ln 2 has a value of 0.693, and k was the rate constant of compound elimination.

In predicting toxicity, there are two important parameters, namely the LD_{50} prediction value for oral rat acute toxicity, which shows the predicted lethal dose of oral administration so that it can kill 50% of the rats' population. The smaller the LD_{50} value, the more toxic the compound is. The second parameter, namely hepatotoxicity, is an important parameter in drug toxicity because as a drug compound that is absorbed through the gastrointestinal tract (GIT), it must be carried through the hepatic portal vein to the liver before being distributed throughout the body, so it is very important to know whether the compound being developed has hepatotoxicity in liver cells or not.

RESULTS AND DISCUSSION

Results of synthesis

Compounds **1-7** have been successfully synthesized with good yield (70-90%). Dibenzylideneacetones **1-5** were successfully yielded using base catalysts (NaOH), while dibenzylideneacetones **6** and **7** were using acid catalysts (H_2SO_4), employing aldol condensation.

Compounds **1-7** have been successfully synthesized based on the results of ^{1}H-NMR and ^{13}C-NMR analysis along with FT-IR and UV-vis spectrometry as the following data:

(1E,4E)-1,5-diphenylpenta-1,4-dien-3-one ***(1)***

It was obtained as a pale-yellow powder with a yield of 85%, melting point (mp): 109-111°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.75 (d, *J* = 16.0 Hz), 7.63 (dd, *J* = 6.7, 2.8 Hz), 7.42 (dd, *J* = 5.0, 1.7 Hz), 7.36 – 7.31 (m), 7.10 (d, *J* = 16.0 Hz). ^{13}C-NMR (101 MHz, $CDCl_3$)

δ 189.1, 143.5, 134.9, 130.7, 129.1, 128.6, 125.5. FT-IR (KBr) cm^{-1}: 1598 (C=C), 1651 (C=O), 3026 (C-H alkene), 982 (trans alkene). UV-vis (λ_{max}): 330 nm.

(1E,4E)-1,5-bis (4-nitrophenyl) penta-1,4-dien-3-one ***(2)***

It was obtained as a beige powder with a yield of 80%, mp: 246-248°C. ^{1}H-NMR (400 MHz, DMSO-d6) δ 8.32 (d, *J* = 8.8 Hz), 8.08 (d, *J* = 8.8 Hz), 7.94 (d, *J* = 16.2 Hz), 7.57 (d, *J* = 16.2 Hz). ^{13}C-NMR (101 MHz, DMSO-d6) δ 188.5, 148.2, 141.2, 140.9, 129.7, 129.1, 124.2. FT-IR (KBr) cm^{-1}: 1492 (C=C), 1651 (C=O), 3108 (C-H alkene), 988 (trans alkene), 1535 (-NO_2). UV-vis (λ_{max}): 334 nm.

(1E,4E)-1,5-bis (3-nitrophenyl) penta-1,4-dien-3-one ***(3)***

It was obtained as pale-beige powder with a yield of 74%, mp: 200-202°C. ^{1}H-NMR (400 MHz, DMSO-d6) δ 8.64 (s), 8.32-8.24 (t-like), 7.97 (d, *J* = 15.4 Hz), 7.77 (t, *J* = 7.2 Hz), 7.58 (d, *J* = 16.2 Hz). ^{13}C-NMR (101 MHz, DMSO-d6) δ 190.9, 148.4, 140.9, 136.6, 134.6, 130.6, 127.9, 124.8, 123. FT-IR (KBr) cm^{-1}: 1493 (C=C), 1651 (C=O), 3108 (C-H alkene), 988 (trans alkene), 1535 (-NO_2 group). UV-vis (λ_{max}): 314 nm.

(1E,4E)-1.5-bis (2-methoxyphenyl) penta-1,4-dien-3-one ***(4)***

It was obtained as a bright yellow powder with a yield of 70%, mp: 136-138°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.07 (d, *J* = 16.1 Hz), 7.63 (dd, *J* = 7.7, 1.3 Hz), 7.42 – 7.30 (m), 7.18 (d, *J* = 16.1 Hz), 6.99 (t, *J* = 7.5 Hz), 6.94 (d, *J* = 8.3 Hz), 3.92 (s). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 190.2, 158.7, 138.4, 131.7, 128.8, 126.4, 124.1, 120.9, 111.3, 55.7. FT-IR (KBr) cm^{-1}: 1598 (C=C), 1651 (C=O), 3026 (C-H alkene), 982 (trans alkene). UV-vis (λ_{max}): 360 nm.

(1E,4E)-1.5-bis (4-methoxyphenyl) penta-1,4-dien-3-one ***(5)***

It was obtained as a white powder with a yield of 80%, mp: 123-124°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.70 (d, *J* = 15.9 Hz), 7.58 (d, *J* = 8.8 Hz), 6.96 (d, *J* = 16.0 Hz), 6.93 (d, *J* = 9.2 Hz), 3.86 (s). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 189.0, 161.7, 142.8, 130.2, 127.7, 123.6, 114.5, 55.6. FT-IR (KBr) cm^{-1}: 1598 (C=C), 1651 (C=O), 3026 (C-H alkene), 982 (trans alkene). UV-vis (λ_{max}): 362 nm.

(1E,4E)-1,5-bis (4-hydroxyphenyl) penta-1,4-dien-3-one ***(6)***

It was obtained as red-orange powder with a yield of 90%, mp: 239-240°C. ^{1}H-NMR (400 MHz, CD_3OD) δ 7.67 (d, *J* = 15.8 Hz), 7.54 (d, *J* = 8.6 Hz), 7.02 (d, *J* = 15.8 Hz), 6.81 (d, *J* = 8.6 Hz). ^{13}C-NMR (101 MHz, CD_3OD) δ 190.5, 160.2, 144.0, 130.4, 126.4, 122.1, 115.7.

(1E,4E)-1,5-bis (4-hydroxy-3-methoxyphenyl) penta-1,4-dien-3-one ***(7)***

It was obtained as a dark purple powder with a yield of 90%. mp: 160-161°C. ^{1}H-NMR (400 MHz, CD_3OD) δ 7.74 (d, *J* = 15.8 Hz), 7.32 (d, *J* = 1.9 Hz), 7.20 (dd, *J* = 8.3, 1.9 Hz), 7.12 (d, *J* = 15.8 Hz), 6.85 (d, *J* = 8.2 Hz), 3.93 (s). ^{13}C-NMR (101 MHz, CD_3OD) δ 189.6, 149.6, 148.1, 142.9, 127.0, 123.7, 122.3, 115.3, 110.7, 55.3.

The NMR spectrum of **1** was similar to that reported by Dohutia et al. (2017), Jessica et al. (2021), and Aher et al. (2011). Jessica et al. (2021) also reported a matching IR of **2**. The NMR spectra of **3**, **4,** and **5** were identical, as described by Aher et al. (2011). C. Dohutia et al. (2017) accordingly wrote the identical of **6** as well. At the same time, Sidarthan and Peter Amaladhas (2017) stated the same NMR spectrum of **7.**

Although these compounds had been synthesized previously by other researchers, there is no studies have been reported yet that examine the *in silico* activity of the compounds against the selected enzyme that plays a role in the development of the malaria parasite (PfENR and hDHFR) and test their ADMET predictions.

Results of molecular docking studies

Antimalarial docking studies through PfENR

The grid box binding site was set X= 51.57 Å; Y = 93.29 Å; Z= 34.31 Å with a cavity surrounded by 17 amino acids, namely Leu 216; Ala 217; Asn 218; Ala 219; Val 222; Tyr 267; Tyr 277; Met 281; Lys 284; Pro 314; Leu 315; Ser 317; Ala 319; Ala 320; Ile 323; Phe 368; Ile 369. The result of the re-docking validation process with RMSD 0.64 Å is shown in Fig. 5. Hence, these results indicate that the method is valid for the docking test of the tested compound since the RMSD obtained is less than 2 Å.

The results of molecular docking on PfENR can all be seen in Table 1 and Figs. 6 and 7.

Based on the results of molecular docking, all dibenzylideneacetones had lower moldock scores than the native ligand triclosan (-84.21 Kcal/mol). Curcumin had the lowest moldock score (-131.15 Kcal/mol), indicating that curcumin was able to inhibit the growth of *P. falciparum* with an IC_{50} value of 5 μM through the *P. falciparum* enoyl acyl carrier protein reductase pathway. The presence of hydrogen bond interactions (in amino acids Thr 266; Leu 315; Ser 317) and steric interactions (in amino acids Asn 218; Thr 266; Tyr 267; Met 281; Leu 315; Ser 317; Ala 319) that occur in curcumin against active site PfENR which is not owned by its native ligand makes the value of curcumin's moldock score lower. The polari-

ty of the hydroxy group can be replaced by a nitro group in dibenzalcetones **2** and **3** because it has a moldock value similar to dibenzalcetones **6**. The loss of the methoxy group in compound **6** (-111.56 Kcal/mol) will reduce the bond interaction at the active site PfENR. This is the case with the loss of hydroxy groups in dibenzalcetones **4** and **5**. Dibenzylideneacetones that have the same moldock score as curcumin are compound **7** (-131.53 kcal/mol) because it has similarity with curcumin in the presence of hydroxyl group at *para* position and methoxy group at *meta* position.

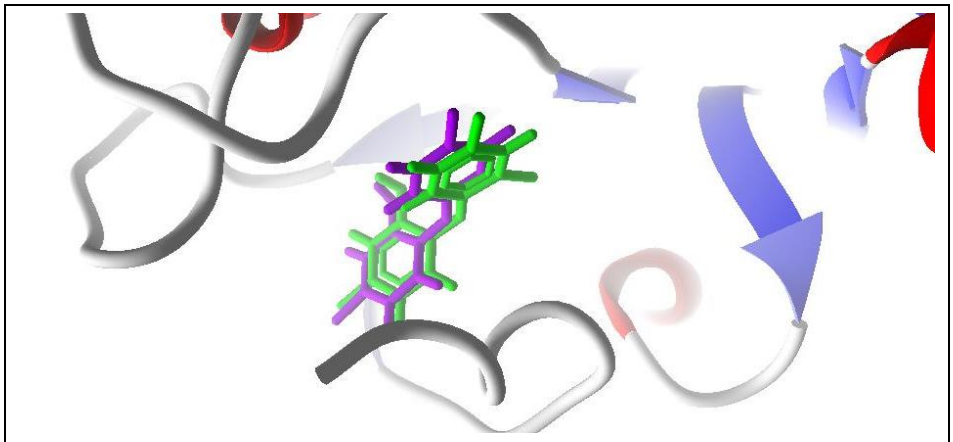


Figure 5. Comparison of the native ligand (triclosan) (green) with the docking result simulation (purple) by Molegro Virtual Docker (MVD) Ver.5.5 with RMSD was 0.64 Å.

Table 1. The Molecular docking result of triclosan, curcumin, and compounds 1-5 into active site *P. falciparum* enoyl acyl carrier protein reductase (PfENR).

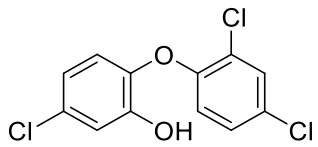
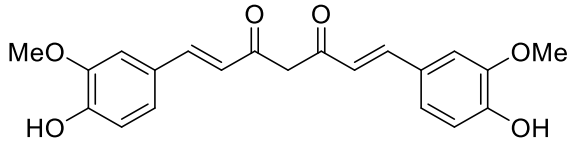
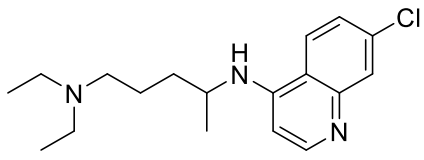
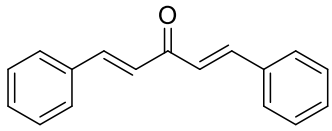
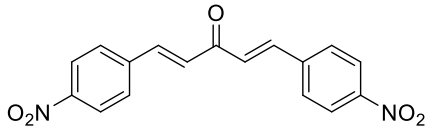
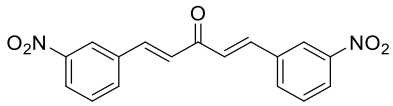
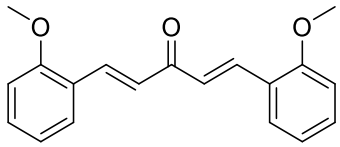
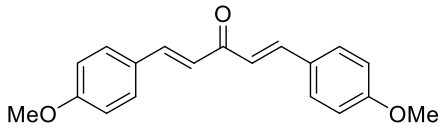
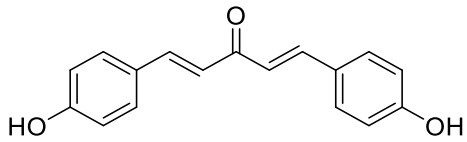
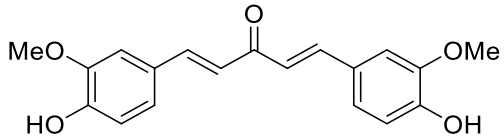
Compound	Moldock score (kcal/mol)	H-bond interaction	Steric interaction
 (Triclosan)	-84.21	Tyr 277	-
 (Curcumin)	-131,15	Thr 266 Leu 315 Ser 317	Asn 218 Thr 266 Tyr 267 Met 281 Leu 315 Ser 317 Ala 319
 (Chloquine)	-88.92	Tyr 277	Ile 369

Table 1. The Molecular docking result of triclosan, curcumin, and compounds 1-5 into active site *P. falciparum* enoyl acyl carrier protein reductase (PfENR) (continued...)

Compound	Moldock score (kcal/mol)	H-bond interaction	Steric interaction
 (**1**)	-97.90	Ser 215	-
 (**2**)	-114.65	Gly 112 Tyr 267	Tyr 111 Gly 112 Ser 215 Thr 266 Tyr 267 Lys 285 Pro 314 Ile 369
 (**3**)	-112.64	Tyr 111 Ser 112 Ser 215 ASN 218 Tyr 277	Ser 112 Ser 215 ASN 218 Tyr 277 Met 281
 (**4**)	-115.45	Lys 285	Val 222 Met 281 Lys 285 Ile 323
 (**5**)	-115.41	ASN 218 Ala 219 Tyr 267 Lys 285	ASN 218 Ala 219 Try 267 Lys 285 Ala 372
 (**6**)	-111.56	Leu 216 Tyr 267	Leu 216 Leu 265 Tyr 267 Ser 317
 (**7**)	-131.53	Gly 106 Ser 215 Ala 312 Leu 315	Gly 106 Gly 104 Asp 107 Tyr 111 Ser 215 Tyr 267 Ala 312 Gly 313 Leu 315

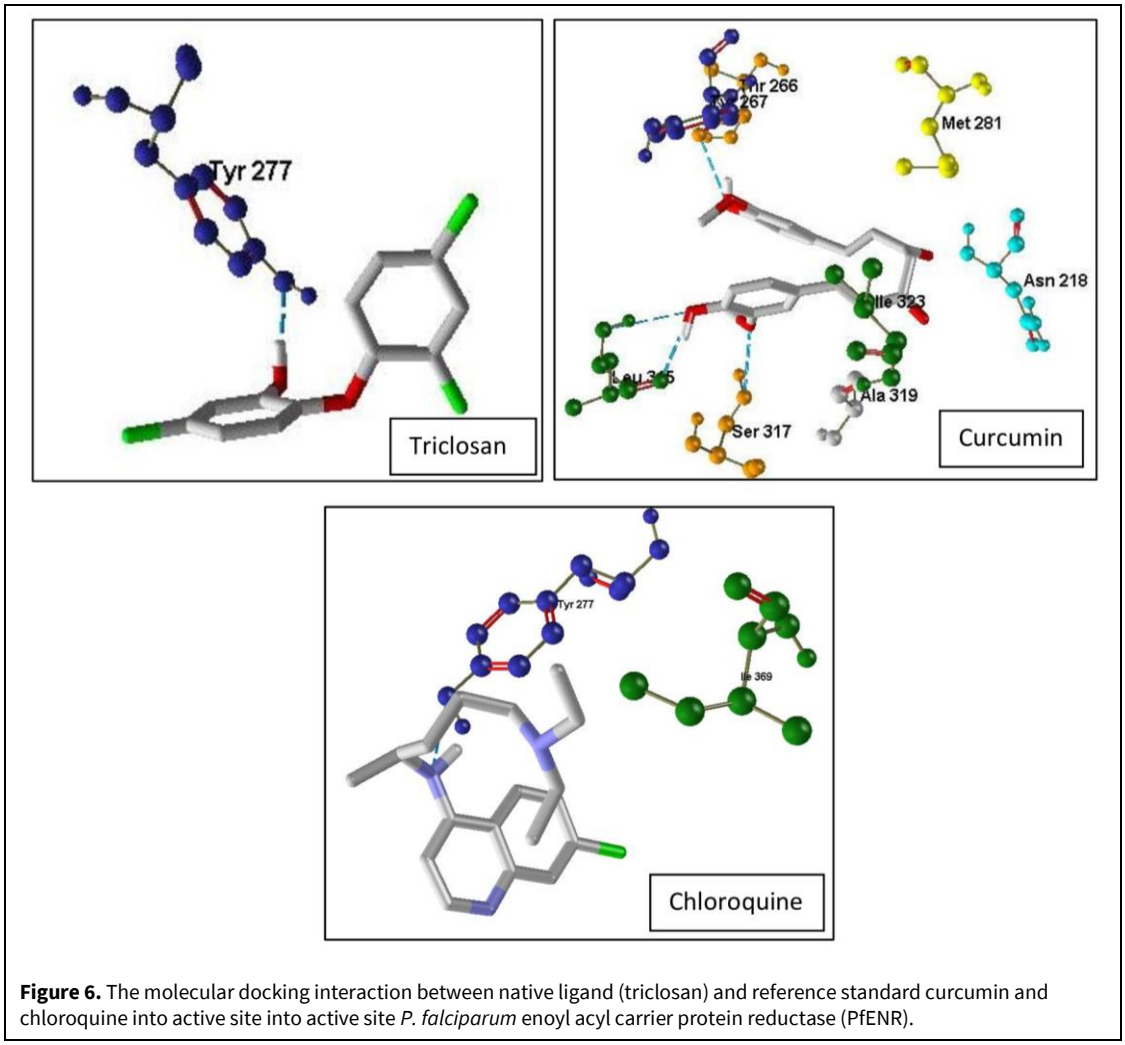


Figure 6. The molecular docking interaction between native ligand (triclosan) and reference standard curcumin and chloroquine into active site into active site *P. falciparum* enoyl acyl carrier protein reductase (PfENR).

Antimalarial docking studies through hDHFR

The grid box binding site was set X= -2.92 Å; Y = 27.88Å; Z= 3.57 Å with a cavity surrounded by 19 amino acids, namely Ile 7; Val 8; Ala 9; Asp 21; Leu 22; Glu 30; Phe 31; Tyr 33; Phe 34; Gln 35; Thr 56; Ser 59; Ile 60; Pro 61; Asn 64; Leu 67; Val 115; Gly 116; Tyr 121. The result of the redocking validation process with RMSD 1.12 Å is shown in Fig. 8. Hence, these results indicate that the method is valid for the docking test of the tested compound since the RMSD obtained is less than 2 Å.

Based on the results of molecular docking study, the functionalized dibenzylideneacetones had a larger moldock score (-92.60 to -119.53 Kcal/mol) than the native ligand, DTM_187 (-134.76 Kcal/mol). This indicates that dibenzylideneacetones have antimalarial activity, not through the mechanism of inhibiting site of hDHFR. The data showed that curcumin had the lower moldock score (-147.04 Kcal/mol) than its native ligand. Some similarities between curcumin and native ligands DTM_187 are the interactions on amino acids Val 8, Val 115, and Tyr 121. Compound **7** has the lowest moldock score (-119.53 Kcal/mol) compared to other dibenzylideneacetones because the substituents in compound **7**, methoxy and hydroxyl groups, were also owned by curcumin. The results of molecular docking on hDHFR can be seen in Table 2 and Figs. 9 and 10.

Results of ADMET prediction

Based on the results of absorption predictions using SCFBIO software, compounds **1-7** fulfill Lipinski's 5th law, which includes LogP < 5, molar refractivity (MR) 40-130 Å, molecular weight (Mr) ≤ 500, hydrogen bond acceptor (HBA) ≤ 10, and hydrogen bond donors (HBD) ≤ 5. Therefore, compounds **1-7** are predicted to be absorbed through the GIT (Paniker and Gosh, 2013). Curcumin and chloroquine also meet the requirements of Lipinski's rule of five criteria, so chloroquine is available as a tablet for oral administration.

Compounds **1-7** are predicted to have excellent high permeability in the intestine. Because it has a Caco-2 permeability value > 8.10-6 cm/s (Pires et al., 2015). Meanwhile, the water solubility prediction results for compounds **1-7** have a Log S value range of between -5 and -4, which is included in the sparingly

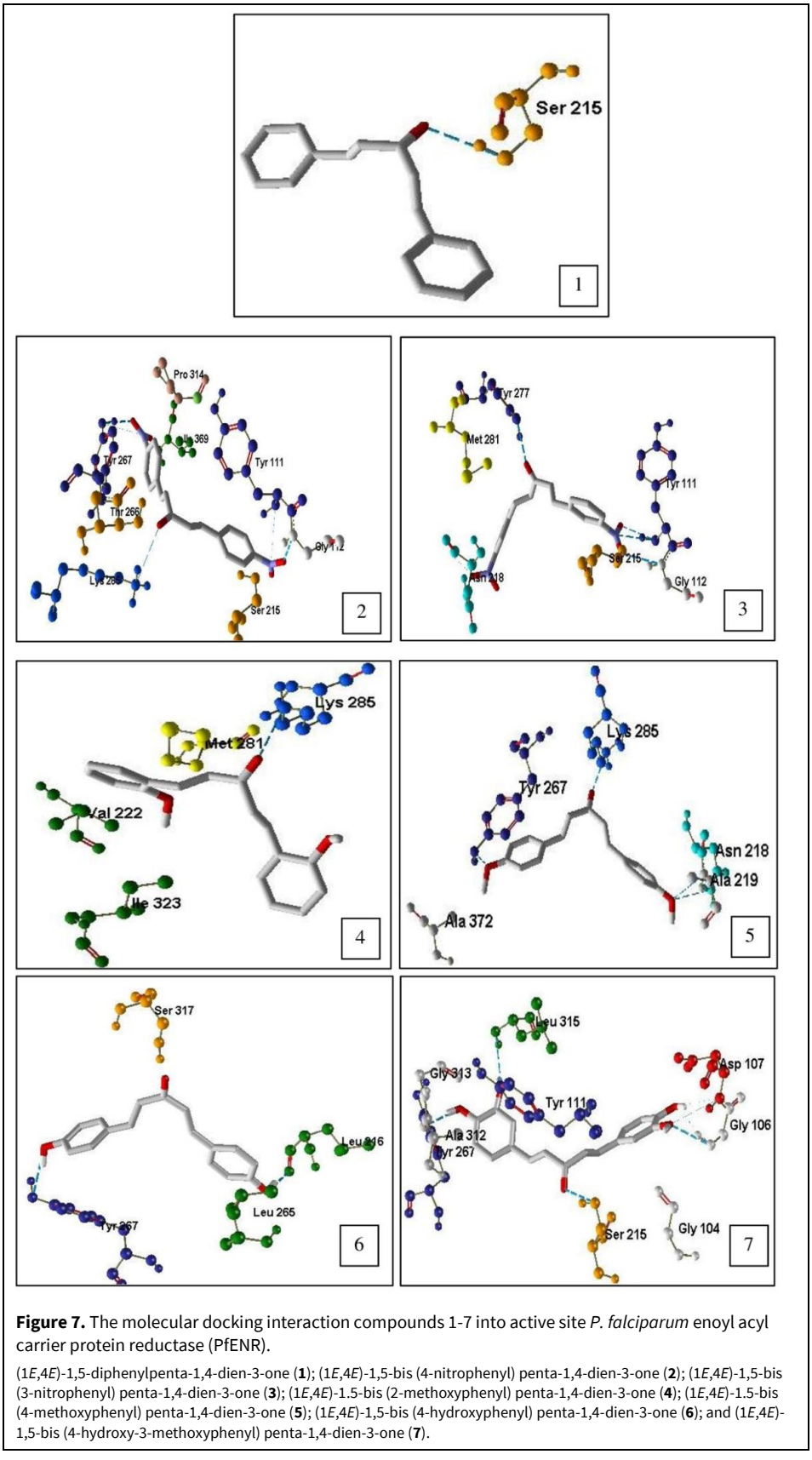
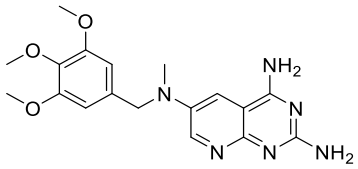
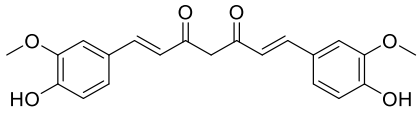
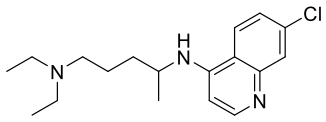
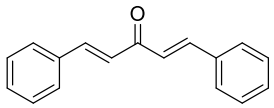
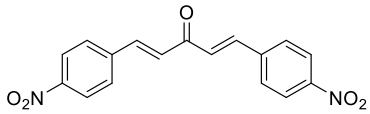
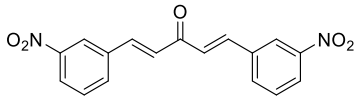
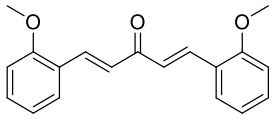
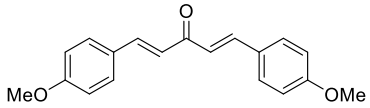
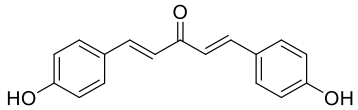
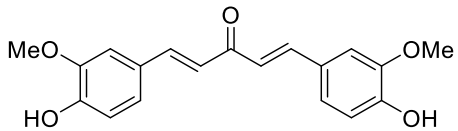


Figure 7. The molecular docking interaction compounds 1-7 into active site *P. falciparum* enoyl acyl carrier protein reductase (PfENR).

(1*E*,4*E*)-1,5-diphenylpenta-1,4-dien-3-one (**1**); (1*E*,4*E*)-1,5-bis (4-nitrophenyl) penta-1,4-dien-3-one (**2**); (1*E*,4*E*)-1,5-bis (3-nitrophenyl) penta-1,4-dien-3-one (**3**); (1*E*,4*E*)-1.5-bis (2-methoxyphenyl) penta-1,4-dien-3-one (**4**); (1*E*,4*E*)-1.5-bis (4-methoxyphenyl) penta-1,4-dien-3-one (**5**); (1*E*,4*E*)-1,5-bis (4-hydroxyphenyl) penta-1,4-dien-3-one (**6**); and (1*E*,4*E*)-1,5-bis (4-hydroxy-3-methoxyphenyl) penta-1,4-dien-3-one (**7**).

Table 2. The molecular docking result of native ligand, curcumin, and dibenzylideneacetones **1**-**7** into the active site of hDHFR.

Compound	Moldock score (kcal/mol)	Hydrogen bond interaction	Steric interaction
 (DTM_187)	-134.76	Ile 7 Glu 30 Val 115	Ile7 Val 8 Glu 30 ASN 64 Val 115 Tyr 121
 (Curcumin)	-147.04	Ala 9 Val 115 Tyr 121	Val 8 Ala 9 Leu 22 Val 115 Tyr 121
 (Chloquine)	-98.43	Asn 64	Asn 64
 (**1**)	-92.60	-	Thr 56
 (**2**)	-114.85	Thr 56 ASN 64	Phe 31 Phe 34 Thr 56 ASN 64
 (**3**)	-111.69	Thr 56 Ser 59	Ala 9 Ile 16 Thr 56 Ser 59
 (**4**)	-115.15	Ser 59	Ser 59 Phe 31
 (**5**)	-111.86	Ser 59	Ile 7 Val 8 Ala 9 Ser 59
 (**6**)	-111.79	Asp 21 Glu 30 Thr 136	Asp 21 Glu 30 Ser 59 Thr 136
 (**7**)	-119.53	Ala 9 ASN 64 Tyr 121	Ala 9 Ile 60 ASN 64 Tyr 121 Val 115

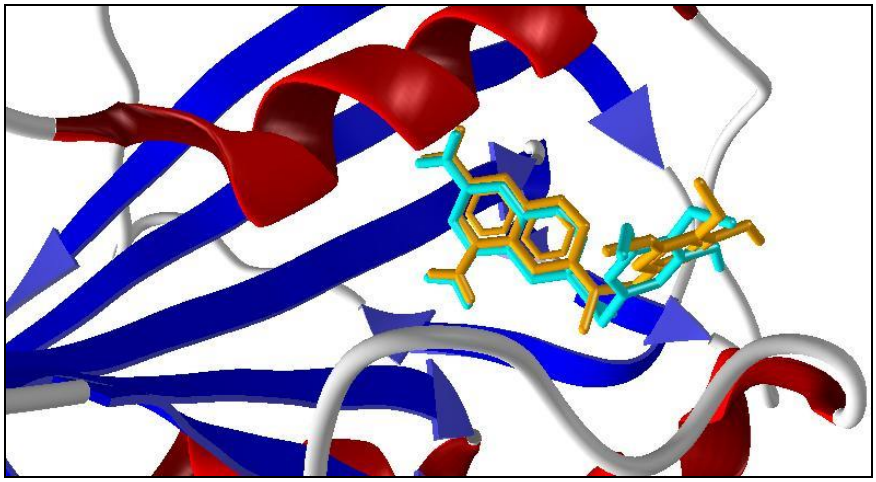


Figure 8. Comparison of the native ligand DTM_187 (orange) with the docking result simulation (blue pale) by Molegro Virtual Docker (MVD) Ver.5.5 with RMSD is 1.12 Å

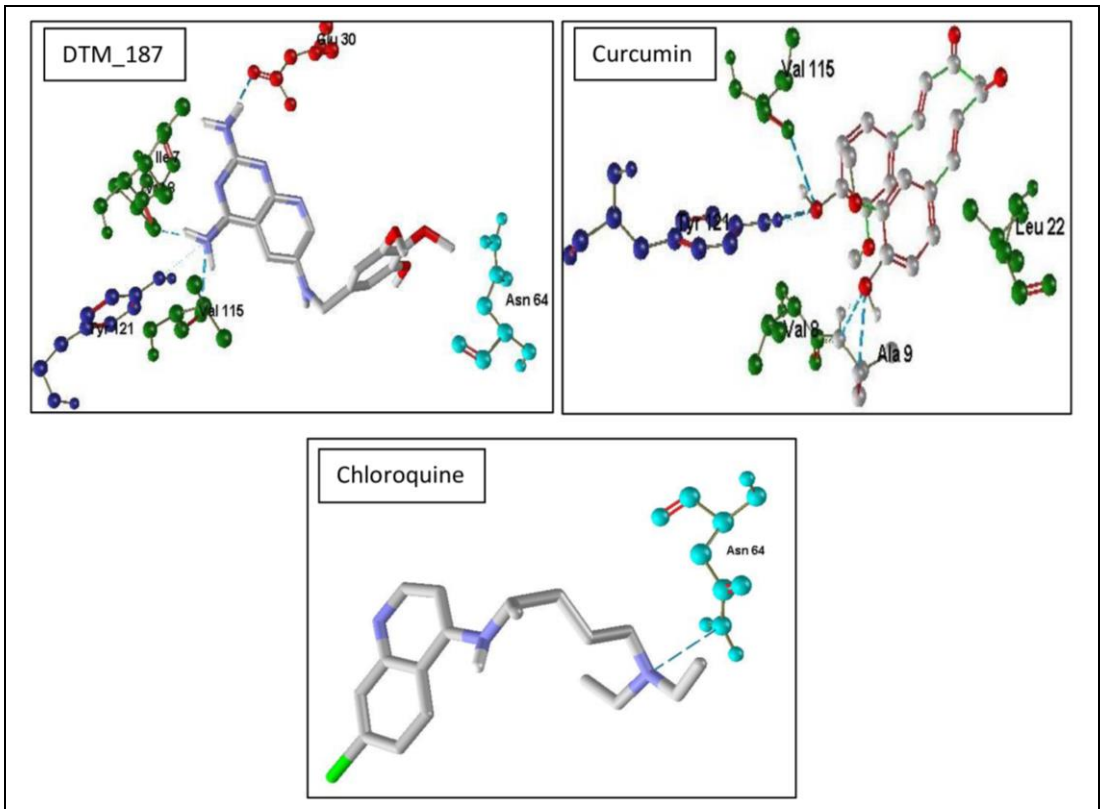


Figure 9. The molecular docking interaction between native ligand (DTM_187) and reference standard curcumin and chloroquine into active site hDHFR.

soluble category. Based on Caco-2 permeability and water solubility data, compounds **1-7** can be grouped into the BCS I Class because they have high permeability and high solubility properties.

Distribution volume is a theoretical volume that states the total homogeneous concentration of a drug circulating in blood plasma. If the distribution volume is large, the drug concentration in the tissue is greater than in the plasma, whereas if the distribution volume is small, the drug concentration in the plasma is greater than in the tissue. The distribution volume is small if < 0.71 L/kg, and the distribution volume is large if > 2.81 L/kg. If the value is in the range of 0.71-2.81 L/kg, it is included in the moderate distribution volume category (Pires et al., 2015). The predicted distribution volume of compounds **1-7** is included in the moderate distribution volume category.

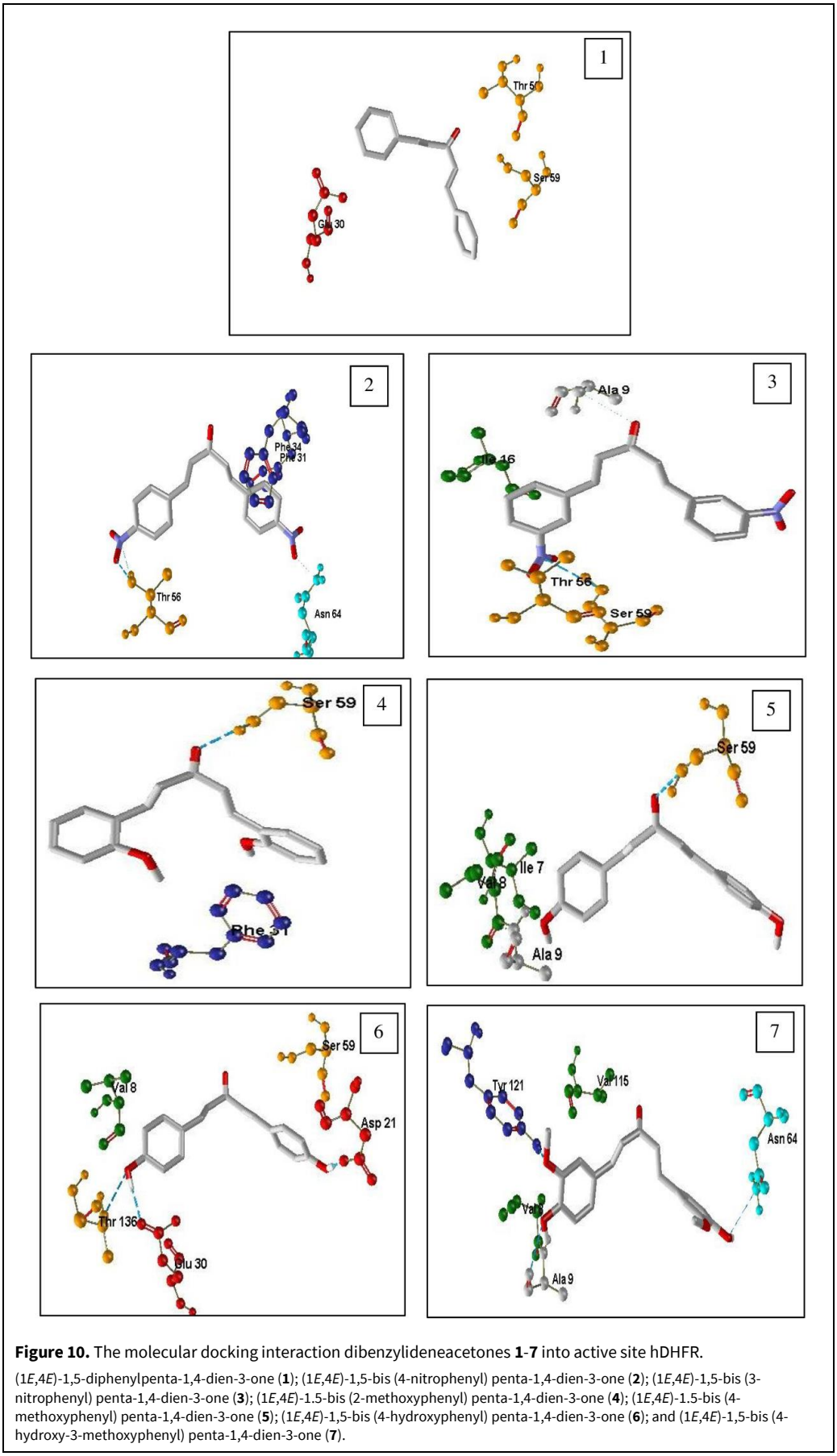


Figure 10. The molecular docking interaction dibenzylideneacetones **1**-**7** into active site hDHFR.

(1*E*,4*E*)-1,5-diphenylpenta-1,4-dien-3-one (**1**); (1*E*,4*E*)-1,5-bis (4-nitrophenyl) penta-1,4-dien-3-one (**2**); (1*E*,4*E*)-1,5-bis (3-nitrophenyl) penta-1,4-dien-3-one (**3**); (1*E*,4*E*)-1.5-bis (2-methoxyphenyl) penta-1,4-dien-3-one (**4**); (1*E*,4*E*)-1.5-bis (4-methoxyphenyl) penta-1,4-dien-3-one (**5**); (1*E*,4*E*)-1,5-bis (4-hydroxyphenyl) penta-1,4-dien-3-one (**6**); and (1*E*,4*E*)-1,5-bis (4-hydroxy-3-methoxyphenyl) penta-1,4-dien-3-one (**7**).

Table 3. The result of ADMET prediction of dibenzylideneacetones.

Compound	Absorption		Distribution		Metabolism (CYP inhibitor)					Excretion			Toxicity	
	Caco-2 Perm. (10^{-6} cm/s)	Log S	Vd (L/kg)	Log BB	2D6	3A4	1A2	2C9	2C19	Total Cl (L/hour/kg)	k (1/hour)	$t_{½}$ (hour)	ORAT mol/kg (LD_{50})	Hepato-toxicity
Chloroquine	30.13	-4.55	22.284	0.146	Yes	No	No	No	No	0.742	0.0333	20.81	2.766	Yes
Curcumin	4.71	-3.94	0.957	-0.298	No	Yes	No	Yes	Yes	0.074	0.0773	8.96	2.045	No
Compound **1**	62.37	-4.12	4.977	0.633	No	No	Yes	No	Yes	0.080	0.0161	43.00	1.946	No
Compound **2**	8.73	-4.22	2.698	-0.659	No	No	Yes	No	Yes	0.087	0.0322	21.49	2.416	No
Compound **3**	8.71	-4.21	3.090	-0.628	No	No	Yes	Yes	Yes	0.091	0.0294	23.58	2.416	No
Compound **4**	34.59	-4.49	2.432	-0.051	No	No	Yes	Yes	Yes	0.129	0.0529	13.11	2.135	No
Compound **5**	19.95	-4.39	2.244	-0.011	No	Yes	Yes	Yes	Yes	0.089	0.0396	17.47	2.186	No
Compound **6**	16.90	-3.93	1.242	-0.199	No	No	Yes	Yes	Yes	0.069	0.0552	12.55	1.742	No
Compound **7**	10.28	-3.95	1.019	-0.221	No	Yes	Yes	Yes	Yes	0.074	0.0726	9.55	1.792	No

(1*E*,4*E*)-1,5-diphenylpenta-1,4-dien-3-one (**1**); (1*E*,4*E*)-1,5-bis (4-nitrophenyl) penta-1,4-dien-3-one (**2**); (1*E*,4*E*)-1,5-bis (3-nitrophenyl) penta-1,4-dien-3-one (**3**); (1*E*,4*E*)-1.5-bis (2-methoxyphenyl) penta-1,4-dien-3-one (**4**); (1*E*,4*E*)-1.5-bis (4-methoxyphenyl) penta-1,4-dien-3-one (**5**); (1*E*,4*E*)-1,5-bis (4-hydroxyphenyl) penta-1,4-dien-3-one (**6**); and (1*E*,4*E*)-1,5-bis (4-hydroxy-3-methoxyphenyl) penta-1,4-dien-3-one (**7**).

Compounds **1-7** are also predicted to be unable to penetrate the BBB because they have a Log BBB value < 0.3. The BBB is a single protective layer of the brain so that chemical compounds cannot easily enter the nerve cells in the brain. Drugs are estimated to be included in the category of easily crossing the BBB if log BBB > 0.3. This is expected because compounds that can penetrate the BBB can certainly affect the central nervous system and patient consciousness. There are several drugs designed to penetrate the BBB, such as antibiotics for meningitis, Parkinson's type drugs, and general anesthetic drugs. Compounds **1-7** are designed for malaria infections, so it is not expected to penetrate the BBB.

The predicted results of inhibition of drug-metabolizing enzymes for compounds **5** and **7** are predicted to have interactions with many drugs because they inhibit CYP3A4, CYP1A2, CYP2C9, and CYP2C19. Moreover, CYP3A4 is an enzyme that metabolizes almost 90% of drugs. If this enzyme is inhibited, it will increase the concentration of free drug in the plasma, resulting in a state of toxicity. Some drugs that can increase due to the presence of 3A4 inhibitors are antidepressants, azole antifungals, benzodiazepines, calcium channel blockers, proton pump inhibitors, selective serotonin reuptake inhibitors, HIV protease inhibitors, antiarrhythmics, HMG-CoA reductase inhibitors.

Total clearance is the rate of drug clearance in the body, which is a combination of hepatic clearance (liver metabolism and biliary clearance) and renal clearance (Shargel and Yu, 2016). Total clearance is directly proportional to the values of Vd and k (constant elimination), so it can be formulated as Cl = Vd.k (Shargel et al., 2016). Therefore, the k value is directly proportional to the Cl value and inversely proportional to the Vd value. The greater the value of k, the smaller the half-time ($t_{½}$) of a compound, which means the bioavailability of the compound in the body takes longer to be excreted. Conversely, the smaller the k value, the greater the $t_{½}$ of a compound, which means the compound's bioavailability in the body is excreted more quickly.

Classification of drug half-lives may vary depending on the literature and clinical context. The following are some general groupings based on half-life: The $t_{½}$ < 1 hour falls into the Ultra Short Half-Life category, namely for drugs that require frequent administration in the form of IV drip administration. The value of $t_{½}$ = 1-4 hours is in the short half-life category, namely for drugs that usually require dosing several times a day to maintain therapeutic levels in the blood. The value of $t_{½}$ = 4-12 hours is in the Intermediate half-life category, namely for drugs that are usually given 1-2 times a day to maintain therapeutic levels in the blood. The value of $t_{½}$ = 12-24 hours is in the long half-life category, namely for drugs that usually require a once-daily dose to maintain therapeutic levels in the blood. The $t_{½}$ > 24 hours is included in the ultra-long half-life category, namely for drugs that usually require a dose once a day or every few days to maintain therapeutic levels in the blood. Based on the elimination prediction results, compound **1** is included in the ultra-long half-life category, compounds **2-6** are included in the long half-life category, and compound **7** is included in the intermediate half-life category.

Based on the results of the toxicity predictions, compounds **1-7** are not hepatotoxic, whereas chloroquine, which is the first-line therapy for malaria infection, is indeed hepatotoxic. Meanwhile, curcumin does have hepatoprotection properties. Based on oral rat acute toxicity (ORAT), compound **1** has LD_{50} = 1.946 mol/kg, which is the most toxic com-

pared to other compounds. Overall results of pharmacokinetic predictions or ADMET predictions for compounds **1-7** using pkCSM and SIB online software can be seen in Table 3.

CONCLUSION

The functionalized dibenzylideneacetones could be easily synthesized with good results through the reaction of acetone with benzaldehydes in a base or acid catalyst. The *in silico* result showed they have a strong potential to inhibit the growth of malaria parasites through the *P. falciparum* enoyl acyl carrier protein reductase (PfENR) pathway.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGMENTS

The authors would like to express our gratitude to Prof Siswandono of Airlangga University for MVD license and to Hoshi University for conducting the NMR spectroscopic instruments. The authors also thank Airlangga University, Indonesia, for financial support through Airlangga Basic Research Grant Program (No. 745/UN3.1.5/PT/2023).

REFERENCES

Aher RB, Wanare G, Kawathekar N, Kumar RR, Kaushik NK, Sahal D, Chauhan VS (2011) Dibenzylideneacetone analogues as novel *Plasmodium falciparum* inhibitors. Bioorg Med Chem Lett 21(10): 3034–3036. https://doi.org/10.1016/j.bmcl.2011.03.037

Aroonsri A, Akinola O, Posayapisit N, Songsungthong W, Uthaipibull C, Kamchonwongpaisan S, Gbotosho GO, Yuthavong Y, Shaw PJ (2016) Identifying antimalarial compounds targeting dihydrofolate reductase-thymidylate synthase (DHFR-TS) by chemogenomic profiling. Int J Parasitol 46(8): 527–535. https://doi.org/10.1016/j.ijpara.2016.04.002

Budiati T, Soewandi A, Soegianto L (2019) Microwave-assisted synthesis of dibenzylideneacetones derivatives and study of their potential antioxidant activities. J Chem Pharm Res 11(9): 11–16.

Chakrabarti R, Rawat PS, Cooke BM, Coppel RL, Patankar S (2013) Cellular effects of curcumin on *Plasmodium falciparum* include disruption of microtubules. PLoS ONE 8(3): e57302. https://doi.org/10.1371/journal.pone.0057302

Cody V, Galitsky N, Luft JR, Pangborna W, Gangjee A (2003) Analysis of two polymorphic forms of a ppyrido[2,3-d]pyrimidine N9—C10 reversed-bridge antifolate binary complex with human dihydrofolate reductase. Acta Cryst D59(4): 654–661. https://doi.org/10.1107/s0907444903001951

Dohutia C, Chetia D, Gogoi K, Sarma K (2017) Design, *in silico* and *in vitro* evaluation of curcumin analogues against *Plasmodium falciparum*. Exp Parasitol 175: 51–58. https://doi.org/10.1016/j.exppara.2017.02.006

Franco LL, de Almeida MV, E Silva LF, Vieira PP, Pohlit AM, Valle MS (2012) Synthesis and antimalarial activity of dihydroperoxides and tetraoxanes conjugated with bis(benzyl)acetone derivatives. Chem Biol Drug Des 79(5): 790–797. https://doi.org/10.1111/j.1747-0285.2012.01345.x

Gordon ON, Luis PB, Sintim HO, Schneider C (2015) Unraveling curcumin degradation: autoxidation proceeds through spiroepoxide and vinylether intermediates en route to the main bicyclopentadione. J Biol Chem 290(8): 4817–4828. https://doi.org/10.1074/jbc.m114.618785

Halgren TA (1996) Merck molecular force field. V. extension of MMFF94 using experimental data, additional computational data, and empirical rules. J Comput Chem 17 (5–6): 616–641. https://doi.org/10.1002/(SICI)1096-987X(199604)17:5/6%3C616::AID-JCC5%3E3.0.CO;2-X

Handayani L, Pebrorizal, Soeyoko (2008) The risk factors of malaria vivax transmition. BKM Public Health Comm Med 24(1): 38–43. https://doi.org/10.22146/bkm.3606

Indrayanto G, Putra GS, Suhud F (2021) Validation of *in-vitro* bioassay methods: Application in herbal drug research. In: Profiles of Drug Substances Excipients and Related Methodology 46: 273–307. https://doi.org/10.1016/bs.podrm.2020.07.005

Jessica J, Budiati T, Caroline C (2021) Synthesis and antioxidant activity of 4,4'-dinitrodibenzalacetone by DPPH method. J Pharm Sci Pract 8(2): 62–68. https://doi.org/10.33508/jfst.v8i2.2761

Khairani S, Fauziah N, Wiraswati HL, Panigoro R, Setyowati EY, Berbudi A (2021) The potential use of a curcumin-piperine combination as an antimalarial agent: A systematic review. J Trop Med 2021: 9135617. https://doi.org/10.1155/2021/9135617

Nandakumar DN, Nagaraj VA, Vathsala PG, Rangarajan P, Padmanaban G (2006) Curcumin-artemisinin combination therapy for malaria. Antimicrob Agents Chemother 50(5): 1859–1860. https://doi.org/10.1128/aac.50.5.1859-1860.2006

Nqoro X, Tobeka N, Aderibigbe BA (2017) Quinoline-based hybrid compounds with antimalarial activity. Molecules 22(12): 2268. https://doi.org/10.3390/molecules22122268

Oliveira NKA, Lima-Cury L, de Abreu-Fernandes, Lavigne AR, Pina-Costa A, Perce-da-Silva DS, Catanho M, Brasil P, Daniel-Ribeiro CT, Ferreira-da-Cruz MF (2020) Extensive genetic diversity of *Plasmodium vivax dbp*-II in Rio de Janeiro Atlantic Forest and Brazilian Amazon Basin: Evidence of positive selection. Malar J 19: 81. https://doi.org/10.1186/s12936-020-03159-y

Padmanabha H, Durham D, Correa F, Diuk-Wasser M, Galvani A (2012) The interactive roles of Aedes aegypti super-production and human density in dengue transmission. PLoS Negl Trop Dis 6(8): e1799. https://doi.org/10.1371/journal.pntd.0001799

Paniker CKJ, Ghosh S (2013) Paniker's textbook of medical parasitology (Seventh edition). New Delhi: Jaypee Brothers Medical Publishers (P) Ltd. 266 pp.

Perozzo R, Kuo M, Sidhu, AbS, Valiyaveettil JT, Bittman R, Jacobs WR, Fidock DA, Sacchettini JC (2002) Structural elucidation of the specificity of the antibacterial agent triclosan for malarial enoyl acyl carrier protein reductase. J Biol Chem 277(15): 13106–13114. https://doi.org/10.1074/jbc.M112000200

Pires DE, Blundell TL, Ascher DB (2015) pkCSM: Predicting small-molecule pharmacokinetic and toxicity properties using graph-based signatures. J Med Chem 58(9): 4066–4072. https://doi.org/10.1021/acs.jmedchem.5b00104

Reddy RC, Vatsala PG, Keshamouni VG, Padmanaban G, Rangarajan PN (2005) Curcumin for malaria therapy. Biochem Biophys Res Commun 326(2): 472–474. https://doi.org/10.1016/j.bbrc.2004.11.051

Schneider C, Gordon ON, Edwards RL, Luis PB (2015) Degradation of curcumin: from mechanism to biological implications. J Agric Food Chem 63(35): 7606–7614. https://doi.org/10.1021/acs.jafc.5b00244

Shargel L, Yu A (2016) Applied Biopharmaceutics & Pharmacokinetics. Seventh ed. New York: McGraw-Hill Education.

Sidharthan J, Peter Amaladhas T (2017) Synthesis and characterization of photo-crosslinkable liquid crystalline copolyesters containing arylidene-keto and chalcone moieties. J Polym Res 24: 53. https://doi.org/10.1007/s10965-017-1206-7

Sulistyowaty MI, Nofianti KA, Budiati T (2013) Synthesis and brine shrimp bioassay of chalcone and its two methoxy derivatives. Int J Pharm Chem Sci 2(2): 719–724.

Thomsen R, Christensen MH (2006) MolDock: a new technique for high-accuracy molecular docking. J Med Chem 49: 3315–3321. https://doi.org/10.1021/jm051197e

Tiwari VS, Joshi P, Yadav K, Sharma A, Chowdhury S, Manhas A, Kumar N, Tripathi R, Haq W (2021) Synthesis and antimalarial activity of 4-methylaminoquinoline compounds against drug-resistant parasite. ACS Omega 6(20): 12984–12994. https://doi.org/10.1021/acsomega.0c06053

Uzor PF (2020) Alkaloids from plants with antimalarial activity: A review of recent studies. Evid Based Complement Alternat Med 2020: 8749083. https://doi.org/10.1155/2020/8749083

Widyawaruyanti A, Subehan, Kalauni SK, Awale S, Nindatu M, Zaini NC, Syafruddin D, Asih PBS, Tezuka Y, Kadota S (2007) New prenylated flavones from *Artocarpus champeden*, and their antimalarial activity *in vitro*. J Nat Med 61: 410–413. https://doi.org/10.1007/s11418-007-0153-8

Yuthavong Y, Tarnchompoo B, Vilaivan T, Chitnumsub P, Kamchonwongpaisan S, Charman SA, McLennan DN, White KL, Vivas L, Bongard E, Thongphanchang C, Taweechai S, Vanichtanankul J, Rattanajak R, Arwon U, Fantauzzi P, Yuvaniyama J, Charman WN, Matthews D (2012) Malarial dihydrofolate reductase as a paradigm for drug development against a resistance-compromised target. Proc Natl Acad Sci USA 109(42): 16823–16828. https://doi.org/10.1073/pnas.1204556109

AUTHOR CONTRIBUTION:

Contribution	Sulistyowaty MI	Putra GS	Budiati T	Indrianingsih AW	Renawanti YW	Nofianti KA	Ekowati J	Purwitasari N	Saechan C	Zidan SAH	Yamauchi T
Concepts or ideas	x										
Design	x										
Definition of intellectual content	x	x	x		x						
Literature search	x		x	x					x	x	x
Experimental studies	x	x	x			x	x	x			
Data acquisition	x	x	x			x	x	x			
Data analysis	x	x		x					x	x	x
Statistical analysis	x										
Manuscript preparation	x	x	x			x	x	x			
Manuscript editing				x					x	x	x
Manuscript review	x	x	x	x	x	x	x	x	x	x	x

Citation Format: Sulistyowaty MI, Putra GS, Budiati T, Indrianingsih AW, Renawanti YW, Nofianti KA, Ekowati J, Purwitasari N, Saechan C, Zidan SAH, Yamauchi T (2025) Synthesis and docking studies of the functionalized dibenzylideneacetones as promising antimalarial agents. J Pharm Pharmacogn Res 13(2): 416–430. https://doi.org/10.56499/jppres24.2097_13.2.416

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