Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Original article http://dx.doi.org/10.1016/j.apjtb.2017.07.004

Chalcone analogue as potent anti-malarial compounds against *Plasmodium falciparum*: Synthesis, biological evaluation, and docking simulation study

Jufrizal Syahri[1,2*], Emmy Yuanita[2,3], Beta Achromi Nurohmah[2], Ria Armunanto[2], Bambang Purwono[2*]

[1]*Department of Chemistry, Universitas Muhammadiyah Riau, Jalan Tuanku Tambusai Ujung, Pekanbaru, Indonesia*

[2]*Department of Chemistry, Universitas Gadjah Mada, Jalan Kaliurang Sekip Utara Bulaksumur 21, 55281, Yogyakarta, Indonesia*

[3]*Department of Chemistry, Universitas Mataram, Jalan Majapahit 62A, Mataram, Indonesia*

ARTICLE INFO

Article history:
Received 18 May 2017
Received in revised form 28 May 2017
Accepted 26 Jul 2017
Available online 1 Aug 2017

Keywords:
Antimalarial
Chalcone
Docking
Plasmodium falciparum 3D7

ABSTRACT

Objective: To investigate *in vitro* antimalarial activity of chalcone derivative compounds against *Plasmodium falciparum* 3D7 (*Pf*3D7) strain and *in silico* antimalarial activity.
Methods: Synthesis of the chalcone derivatives was conducted via Claisen-Schmidt method using NaOH 60% base as catalyst. An *in vitro* antimalarial activity assay was carried out according to the Rieckmann method against the chloroquine-sensitive *Pf*3D7 strain. Molecular docking studies of the prepared compounds were performed using Discovery Studio 3.1 (Accelrys, Inc., San Diego, USA) software to dihydrofolate reductases–thymidylate synthase (*Pf*DHFR-TS) protein with Protein Data Bank ID of 1J3I.pdb (sensitive-protein) and ID: 4DP3.pdb (resistance-protein).
Results: This work has successfully synthesized seven chalcone derivatives with a great antimalarial activity. It has been revealed that allyloxy, hydroxy and alkoxy functional groups could increase the antimalarial activity of the chalcone derivatives. The best antimalarial activity of the prepared compounds was possessed by 3b with an IC_{50} value of 0.59 μM and categorized as an excellent antiplasmodial. Molecular docking studies of 3b showed binding interaction with the amino acid residues such as Ala16, Ile164, Phe58, Tyr170 of the 1J3I.pdb protein and also Ala16, Phe58, Ile112, Met55 of the 4DP3.pdb protein.
Conclusions: An *in vitro* antimalarial assay of the prepared chalcone derivative (3a–g) showed an excellent and good antiplasmodial activity against the chloroquine-sensitive Pf3D7 strain. *In silico* antimalarial studies revealed that 3a–g made binding interaction with both sensitive-protein (1J3I.pdb) and resistance-protein (4DP3.pdb), which means that they were both active against chloroquine-sensitive and resistant plasmodium strain.

1. Introduction

Eradication of malaria is one of the aims that were included in the Millennium Development Goals (MGDs) program of World Health Organization (WHO), followed by the Sustainable Development Goals (SDGs), alongside with other diseases such as HIV/AIDS and tuberculosis. In 2015, there were about 212 million malaria cases globally and 429 000 fatalities with most of the cases occurred in the African region (92%), followed by South-East Asia Region (6%) and the Eastern Mediterranean Region (2%) [1]. Hanindita and Tampubolon have stated that Papua is at a higher risk of infection of malaria [2]. In 2016, Data Center and Information of the Ministry of Health of the Republic of Indonesia also reported the most malaria cases occurred in East region of Indonesia, with Annual Parasite Incidence (API) index in Papua being 31.93, West Papua 31.29, East Nusa Tenggara (NTT) 7.04 and Maluku 5.81 (nationwide API was 0.85) [3].

*Corresponding authors: Jufrizal Syahri, Department of Chemistry, Universitas Muhammadiyah Riau, Jalan Tuanku Tambusai Ujung, Pekanbaru, Indonesia.
Bambang Purwono, Department of Chemistry, Universitas Gadjah Mada, Jalan Kaliurang Sekip Utara Bulaksumur 21, 55281, Yogyakarta, Indonesia.
E-mails: jsyachri@umri.ac.id (J. Syahri), purwono.bambang@ugm.ac.id (B. Purwono).

Peer review under responsibility of Hainan Medical University. The journal implements double-blind peer review practiced by specially invited international editorial board members.

Malaria is still considered as the main cause of the high number of deaths. Moreover, it has been reported the occurrence of parasite resistance not only on chloroquine but also on artemisinin [4,5]. Therefore, it is important to find new antimalarial drug candidates that are effective and efficient for therapy of malaria [6,7].

Chalcone is one of secondary metabolites that has been proved to have some biological activities such as anticancer [8–10], anti-inflammatory [11–13], anti-HIV [14], anti-diabetic [15,16], anti-proliferative [17], anti-microbial and anti-oxidant [18–20], and anti-malarial [21–23]. Chalcone derivate with a substituent of prenyl and allyl has been reported to have a good antimalarial activity. Theoretically, prenyl, allyl, alkoxy and the hydroxyl group could increase the lipophilicity of a compound, as important properties in antimalarial activity [24,25]. Furthermore, enone group in chalcone, which is positioned in between phenyl rings, is also reported to be an essential functional group that responsible for antimalarial activity because it binds better to the active site of the parasite. This study was aimed to synthesize some chalcone derivatives, which have an enone group and also to modify the functional group of allyl, hydroxy and alkoxy. Meanwhile, an *in vitro* antimalarial activity was conducted against *Plasmodium falciparum* 3D7 strain.

2. Materials and methods

2.1. Material

The melting point of the synthesized compounds was determined by Melting Point apparatus (Electrothermal 9100) at 10 °C/min temperature gradient. MS spectra were recorded on Shimadzu-QP 2010S. ^{1}H and ^{13}C NMR, spectra were recorded on a JEOL 500 MHz spectrometer with TMS as an internal standard. All reagents were purchased from Aldrich, Acros, and Merck and were used without further purification. All the solvents used in the syntheses were analysis and synthesis grade. The solvents used in spectroscopic measurements were spectroscopic grade. An *in vitro* antimalarial activity assay was conducted against chloroquine-sensitive of *Plasmodium falciparum* 3D7 strain. Molecular docking simulation methods were performed using Discovery Studio® 3.1 (Accelrys, Inc., San Diego, CA, USA) on an Intel® (TM)2 Quad CPU Q8200 @2.33 GHz running under a Windows XP Professional operating system. Other molecular modeling software used throughout this study including CHIMERA 1.9 and ChemOffice® 2015.

2.2. General procedure for the synthesis chalcone derivates (3a–g)

The prepared chalcone (3a–g) was afforded by reaction of substituted acetophenone (10 mmol) and substituted benzaldehyde (10 mmoL) in ethanol (25 mL). The mixture was dropwise added with 60% NaOH (15 mL) and stirred at room temperature overnight. The reaction was monitored by thin layer chromatography. After the completion of the reaction, the mixture was then poured onto the crushed ice and neutralized with 2 M of HCl solution. The precipitate obtained was filtered, washed with aquadest and dried over, then purified by chromatography with *n*-hexane/ethyl acetate (gradient 10%–50% ethyl acetate) to afford the desired product.

2.3. Antimalarial activity

Antimalarial activity assay was conducted against chloroquine-sensitive *Plasmodium falciparum* 3D7 strain. This work was done according to the method of Rieckmann *et al.* and the previous work [26,27].

2.4. Molecular docking

Molecular docking was carried out to dihydrofolate reductases–thymidylate synthase (*Pf*DHFR-TS) protein which retrieved from Protein Data Bank with the code of sensitive-protein of 1J3I (2.33 Å) and 4DP3 (2.40 Å) for the resistant-protein. The cDOKER docking was performed according to the standard protocol implemented in the Discovery Studio® 3.1 (Accelrys, San Diego, USA). The docking procedure was conducted according to previous work [22,27].

3. Results

3.1. Synthesis of chalcone derivates (3a–g)

(E)-3-(4-(diethylamino)phenyl)-1-(2-hydroxy-4-methoxyphenyl) prop-2-en-1-one (3a). Yellow crystals, yield 60%, m.p. 175–177 °C; MS ($C_{20}H_{23}NO_3$) $[M]^+$ 326. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 13.32 (s, 1H, OH); 7.86 (d, J = 15.5 Hz, 1H); 7.82 (d, J = 8.4 Hz, 1H); 7.76 (d, J = 7.5 Hz, 2H); 7.60 (d, J = 15.5, 1H); 6.52 (dd, 2H); 6.48 (d, J = 2.5 Hz, 2H); 3.86 (s, 3H); 3.48 (m, 4H); 1.30 (t, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 191.18; 166.84; 166.52; 145.05; 145.01; 131.26; 130.17; 125.00; 120.01; 113.98; 112.78; 108.01; 108.00; 101.15; 55.67; 47.19; 47.17; 10.62; 10.60.

(E)-3-(3,4-dihydroxyphenyl)-1-(2-hydroxy-4-methoxyphenyl) prop-2-en-1-one (3b). Yellow crystals, yield 60%, m.p. 169–171 °C; MS ($C_{16}H_{14}O_5$) $[M]^+$ 287. ^{1}H NMR (500 MHz, methanol-d4) δ (ppm): 8.00 (d, J = 9.0 Hz, 1H); 7.75 (d, J = 15.3 Hz, 1H); 7.55 (d, J = 15.3 Hz, 1H); 7.18 (d, J = 2.1 Hz, 1H); 7.11 (dd, J = 2.1; 8.3 Hz, 1H); 6.82 (d, J = 8.2 Hz, 1H); 6.53 (dd, J = 2.5; 9.0 Hz, 1H); 6.44 (d, J = 2.5 Hz, 1H); 3.84 (s, 3H). ^{13}C NMR (125 MHz, methanol-d_4) δ (ppm): 193.72; 167.65; 167.45; 150.07; 146.88; 146.48; 132.84; 128.38; 123.72; 118.21; 116.64; 115.90; 115.40; 108.35; 102.02; 56.13.

(E)-1-(4-chlorophenyl)-3-(4-hydroxy-3-methoxyphenyl) prop-2-en-1-one (3c). Yellow crystals, yield 60%, m.p. 110–102 °C; MS ($C_{16}H_{13}ClO_3$) $[M]^+$ 288. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.98 (d, J = 8.50 Hz, 2H); 7.79 (d, J = 15.5 Hz, 1H); 7.49 (d, J = 8.50 Hz, 2H); 7.36 (d, J = 15.57 Hz, 1H); 7.24 (dd, J = 1.30; 1.27 Hz, 1H); 7.13 (s, 1H); 6.99 (d, J = 8.20 Hz, 1H); 3.97 (s, 3H, OCH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 189.7; 148.9; 147.3; 146.2; 139.4; 137.2; 130.2; 129.3; 127.7; 123.9; 119.6; 115.4; 110.5; 56.4.

(E)-3-(4-(allyloxy)-3-methoxyphenyl)-1-(4-chlorophenyl) prop-2-en-1-one (3d). Yellow crystals, yield 80%, m.p. 90–91 °C; MS ($C_{19}H_{17}ClO_3$) $[M]^+$ 328. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 8.11 (d, J = 8.4 Hz, 2H); 7.76 (d, J = 15.5 Hz, 1H); 7.73 (d, J = 15.5 Hz, 1H); 7.55 (d, J = 9.1 Hz, 2H); 7.50 (s, 1H); 7.34 (dd, J = 1.9; 8.4 Hz, 1H); 7.03 (d, J = 8.4 Hz, 1H); 6.11–6.05 (m, 1H); 5.42 (dd, J = 18.8; 10.3 Hz, 2H); 4.64 (d, J = 5.2 Hz, 2H); 3.89 (s, 3H, OCH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 187.9; 151.0; 150.0; 144.9; 138.2; 137.1; 133.6; 130.1; 128.8; 128.2; 123.5; 119.3; 116.9; 113.2; 111.2; 69.2; 55.4.

(E)-1-(4-chlorophenyl)-3-(3,4-dimethoxyphenyl) prop-2-en-1-one (3e). Yellow crystals, yield 85%, m.p. 103–105 °C; MS ($C_{17}H_{15}ClO_3$) $[M]^+$ 302. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 8.12 (d, J = 8.4 Hz, 2H); 7.78 (d, J = 15.5 Hz, 1H); 7.74 (d, J = 15.5 Hz, 1H); 7.58 (d, J = 8.4 Hz, 2H); 7.50 (s, 1H); 7.35 (dd, J = 1.9; 8.4 Hz, 1H); 7.03 (d, J = 8.4 Hz, 1H); 3.88 (s, 3H); 3.86 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 188.8; 153.08; 150.72; 145.93; 139.15; 138.09; 131.03; 129.70; 128.81; 124.64; 120.13; 112.50; 111.77; 56.29; 56.20.

(E)-1,3-bis(3,4-dimethoxyphenyl) prop-2-en-1-one (3f). Yellow crystals, yield 75%, m.p. 101–102 °C; MS ($C_{19}H_{20}O_5$) $[M]^+$ 328. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.86 (d, J = 8.5 Hz, 1H); 7.80 (d, J = 15.5 Hz, 1H); 7.74 (d, J = 15.5 Hz, 1H); 7.67 (s, 1H); 7.50 (s, 1H); 7.37 (d, J = 8.0 Hz, 1H); 7.10 (d, J = 8.5 Hz, 1H); 7.05 (d, J = 8.0 Hz, 1H), 3.93 (s, 4 × OCH_3, 12H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 188.66; 153.13; 151.26; 149.18; 144.21; 131.45; 127.99; 122.96; 119.52; 111.11; 110.76; 110.19; 109.94; 55.95.

(E)-3-(3-chloro-4,5-dimethoxyphenyl)-1-(3,4-dimethoxyphenyl) prop-2-en-1-one (3g). Yellow crystals, yield 65%, m.p. (164–165) °C; MS ($C_{19}H_{19}ClO_5$) $[M]^+$ 362. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 8.12 (d, J = 15.5 Hz, 1H); 7.83 (d, J = 8.5 Hz, 1H); 7.64 (d, J = 15.5 Hz, 1H); 7.35 (s, 1H); 7.10 (d, J = 8.4 Hz, 1H); 7.08 (s, 1H); 7.07 (s, 1H), 3.93 (s, 4 × OCH_3, 12H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 189.15; 153.41; 151.57; 149.42; 148.54; 140.14; 131.42; 128.49; 125.42; 123.28; 122.65; 112.91; 111.12; 110.11; 109.53; 61.25; 56.45.

3.2. Antimalarial activity

The synthesized chalcone derivatives (3a–g) were prepared through base catalyzed Claisen-Schmidt condensation reaction of substituted acetophenone and substituted benzaldehyde (Figure 1). As the results have shown, all of the prepared chalcone (3a–g) were obtained in trans conformation, proved by the ^{1}H NMR spectra that showed doublet signal with coupling constant (J) of H-α and H-β were ± 15.5 Hz. The antimalarial activity assay showed that the presence of functional groups such as allyl, alkoxy, and hydroxy could influence the antimalarial activity as seen in Table 1.

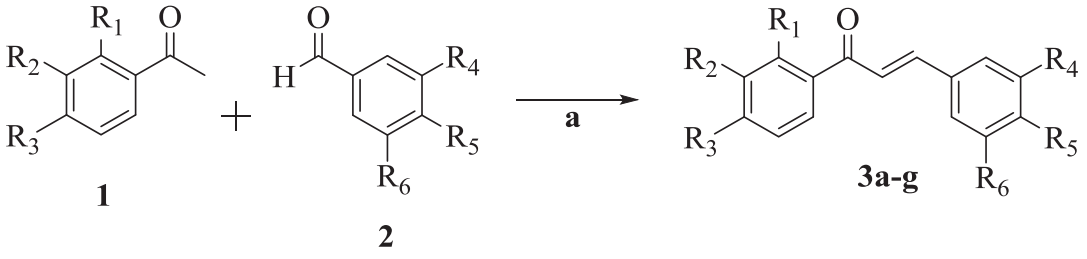
Table 1
An *in-vitro* antimalarial activity (IC_{50}) of chalcone 3a–g against *Pf*3D7.

Compound	IC_{50} (μM)
3a	7.280 ± 0.270
3b	0.590 ± 0.249
3c	25.840 ± 0.412
3d	5.260 ± 0.291
3e	7.300 ± 0.294
3f	5.360 ± 0.230
3g	11.380 ± 0.718
Chloroquine	0.060 ± 0.387

3.3. Molecular docking

Molecular docking of 3b to 1J3I.pdb and 4DP3.pdb generated cDOCKER interaction energy of −46.57 and −40.73 kcal/moL. This result revealed it was much lower than the energy produced by each co-crystallized ligands WR99210 and P218 with −54.32 and −55.89 kcal/moL respectively. Nevertheless, an *in vitro* antimalarial activity of 3b was categorized as excellent antiplasmodial, caused by the binding interaction with the amino acid residue of *Pf*DHFR-TS protein which showed similar result by the each co-crystallized ligands (Figure 2). This result proved the antimalarial activity of 3b toward both chloroquine-sensitive and resistant strain.

Molecular docking studies revealed the binding interaction of 3b with amino acid residues Ala16, Ile164, Phe58, and Tyr170 of 1J3I.pdb protein and also amino acid residues Ala16, Phe58, Ile112, and Met55 of 4DP3.pdb protein. Meanwhile, co-crystallized ligands WR99210 showed binding interaction with Ala16, Ile164, Phe58, Tyr170, Ile14, Asp54, Cys15 and co-crystallized ligands P218 with amino acid residues Ala16, Phe58, Ile112, Met55, Pro113, Leu164, Asp54 (Table 2). This result means binding pocket formed by chalcone 3b was similar to the co-crystallized ligands, so it can be concluded that antimalarial activity from the experimental result could be proved through *in silico* molecular docking studies.



3a R_1 = OH; R_2 = R_4 = R_6 = H; R_3 = OCH_3; R_5 = Diethylamine
3b R_1 = OH; R_2 = R_6 = H; R_3 = OCH_3; R_4 = R_5 = OH
3c R_1 = R_2 = R_4 = H; R_3 = Cl; R_5 = OH; R_6 = OCH_3
3d R_1 = R_2 = R_4 = H; R_3 = Cl; R_5 = O-Allyl; R_6 = OCH_3
3e R_1 = R_2 = R_4 = H; R_3 = Cl; R_5 = R_6 = OCH_3
3f R_1 = R_6 = H; R_2 = R_3 = R_4 = R_5 = OCH_3
3g R_1 = H; R_2 = R_3 = R_4 = R_5 = OCH_3; R_6 = Cl

Figure 1. Reagents and conditions of synthesis: (a) 60% NaOH; EtOH, stir at rt overnight.

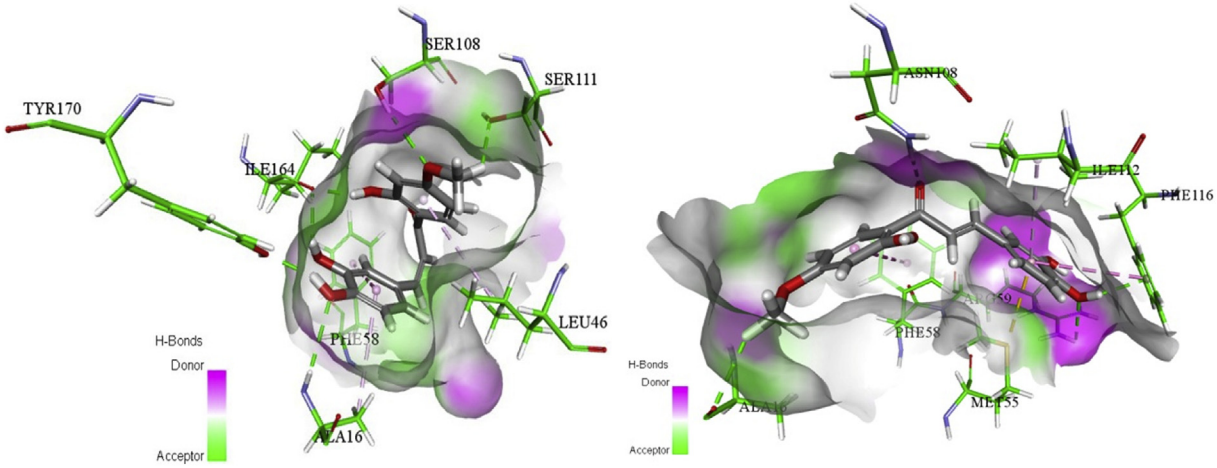


Figure 2. Binding interaction from docking simulation of 3b into the active site of *Plasmodium falciparum* DHFR-TS protein: (A) PDB ID: 1J3I; (B). PDB ID: 4DP3.

The coloring atom for the compound is in order as follows: carbons in black, oxygen in red, and hydrogen in white. The green line indicates hydrogen-bonding interaction with distance ascribed in angstroms, Å.

Table 2

Energy and binding interaction of 3b and co-crystallized ligands.

Compound	cDOCKER energy (kcal/moL)		Binding interaction (amino acid residue)	
	1J3I.pdb	4DP3.pdb	1J3I.pdb	4DP3.pdb
3b	−46.57	−40.73	Ala16, Ile164, Phe58, Tyr170	Ala16, Phe58, Ile112, Met55
WR99210	−54.32	–	Ala16, Ile164, Phe58, Tyr170, Ile14, Asp54, Cys15	–
P218	–	−55.89	–	Ala16, Phe58, Ile112, Met55, Pro113, Leu164, Asp54

4. Discussion

The best antimalarial activity was possessed by 3b, owning three hydroxy and a methoxy group, with IC_{50} of 0.59 μM. Modification of two hydroxyl groups with diethylamine (3a) showed a significant decreasing of the IC_{50} to 7.28 μM. This result showed the importance of the hydroxyl group to determine the antimalarial activity of chalcone derivatives. Hydroxy, allyloxy and methoxy also positively affected the antimalarial activity of the chalcone derivatives. Table 1 showed the increasing activity from 25 μM to 5.26 μM after changing one of the hydroxyl groups of 3c to allyloxy (3d). Furthermore, modification of hydroxy (3c) to methoxy (3e) also gave a better activity with IC_{50} of 7.30 μM. The enhancement of the antimalarial activity of the prepared chalcone compounds by the presence of the hydroxy, allyloxy, and methoxy caused by the ability of the functional groups to do an electron transfer to the protein of the plasmodium parasite that led to the destruction and death of the parasite [25,28]. Meanwhile, increasing the length of the carbon chain could increase the antimalarial activity even though it is not always in the cases because it also would cause decreasing of the activity if it pasts the maximum chain length [29].

Generally, antimalarial activity could be categorized into five groups *i.e.* excellent (IC_{50} < 1 μM); good (IC_{50} 1–20 μM); moderate (IC_{50} 20–100 μM); low (IC_{50} 100–200 μM); and inactive (IC_{50} > 200 μM) [30]. According to that category, the prepared chalcone 3b could be labeled as an excellent antiplasmodial with IC_{50} less than 1 μM, while chalcone 3a and 3d–g as good antimalarial and 3c as moderate. In advance, chalcone 3b could be developed to be a new antimalarial drug candidate, hopefully would overcome the infection of the plasmodium effectively and efficiently.

Molecular docking study was performed toward dihydrofolate reductases–thymidylate synthase (*Pf*DHFR-TS) protein, an essential substrate in the biosynthesis of folate and it has been the main target of the developing of antimalarial drugs. This enzyme could inhibit the production of deoxythymidine monophosphate (dTMP), so it prevents the synthesis of DNA and cell division of parasite in thymidylate cycles [31]. Mechanism of action (MoA) of the prepared compound toward *Pf*DHFR-TS could be seen from the molecular docking. In this work, molecular docking was performed to chalcone 3b, with the best antimalarial activity, toward 1J3I.pdb and 4DP3.pdb protein.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

Authors showed gratitude to Ministry of Research, Technology, and Higher Education-Indonesia for the financial support of this work through Hibah Penelitian Disertasi Doktor (PDD) T.A 2017. Gratitude also delivered for CADD Laboratory, Faculty of Pharmacy, Universiti Kebangsaan Malaysia who provided the Discovery Studio 3.1 licenses.

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