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Synthesis, biological evaluation, QSAR analysis, and molecular docking of chalcone derivatives for antimalarial activity

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ABSTRACT

Objective: To synthesize chalcone derivatives and investigate their antimalarial activity toward chloroquine-sensitive *Plasmodium falciparum* 3D7 (Pf3D7) strain; to develop quantitative structure-activity relationships (QSAR) model to estimate IC_{50} values for biological activity of antimalarial and compared to experimental measurement; and to determine the binding interactions of the most active compounds with targeting *P. falciparum* dihydrofolate reductase-thymidylate synthase using molecular docking simulation.

Methods: Seven chalcone derivatives have been synthesized from substituted acetophenone and substituted benzaldehyde in ethanol with the presence of bases catalysis at reflux condition. The QSAR analysis was conducted by using Gaussian 09 software to predict IC_{50} value for antimalarial activity. The *in vitro* test was evaluated against the chloroquine-sensitive Pf3D7 strain. Finally, the docking studies were performed with the CDOCKER protocol under the receptor-ligand interaction section in Discovery Studio® 3.1 (Accelrys, Inc., San Diego, USA).

Results: Among the synthesized chalcone, a prenylated chalcone 5c and an allylated chalcones 10a showed the best IC_{50} values of 1.08 and 1.73 μg/mL respectively against Pf3D7 strain (1.37 and 2.33 μg/mL based on QSAR analysis). Comparison between the prediction of IC_{50} value generated from the QSAR and the outcome from an *in vitro* assay showed a similar result as seen from the r^2 value (r^2 = 0.99). The most active compound 5c was employed in the docking simulation to determine the potential binding interactions with active sites of *P. falciparum* dihydrofolate reductase-thymidylate synthase (protein data bank ID: 1J3I). The docking simulation study showed 5c bind well with Ala16, Ser108, Ile164, Trp48, and Phe58 which are the crucial interactions that could possibly interrupt the sequential catalysis reactions in the thymidylate cycle and subsequently prevent deoxythymidine monophosphate production and DNA synthesis. The formed binding interaction (H-bond) toward residues of Ala16, Ser108, and Ile164 also indicate the activity of 5c against chloroquine-resistance *P. falciparum* strain.

Conclusions: We have successfully determined the effects of some chalcone derivatives on antimalarial activity against the chloroquine-sensitive Pf3D7 strain. Compound 5c and 10a were described a good antiplasmodial compounds. Interestingly, these *in vitro* results relevance with IC_{50} predicted QSAR studies. Moreover, molecular docking simulation provided insight into the binding modes of 5c into the anti-folate resistance from malarial *P. falciparum*.

1. Introduction

Malaria disease is one of the world's most attention and serious tropical diseases. In 2015, the World Health Organization reported that there were 214 million cases of malaria globally and 438 thousand among of them deaths, most of which were children under five years old, pregnant women and patients with HIV/AIDS. Moreover, the huge cases and deaths to be found to occurred in around African region (88%), South East Asia (10%) and East Mediterranean (2%). Parasite of *Plasmodium falciparum* (*P. falciparum*) is the most prevalent species responsible for causing malaria disease in humans. This parasite believed as the largest cause of deaths for most malaria-related deaths worldwide[1]. For several decades, chloroquine and other quinine have been the most frequently used drugs for the first-line treatment of malaria because

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its relatively inexpensive, non-toxic, and also active toward all of the parasite strain. However, resistance of the *P. falciparum* parasite toward chloroquine and artemisinin is a major obstacle to the control of malaria disease. That resistance has accelerated the morbidity and mortality in malaria case[2,3]. Thus, it is very important to discovery a new drug for malaria disease with better activity against the parasite[4-8].

First reported chalcone derivatives with antimalarial activity was licochalcone A, which has prenyl group in aromatic rings[9]. Since that several chalcone derivatives bearing prenyl and allyl group have been reported to have some bioactivity such as anticancer[10-12], antioxidant[13], anti-inflammatory[14], anti-tubulin[15], inhibitor of prostaglandin E_2 production[16], anti-tumor[17], anti-leishmanial[18], anti-trypanosomal[18] and also antimalarial[19,20]. Nevertheless, these compounds have a unique and defined chemical structure, which are limited abundance in natural sources. Therefore, the synthesis route was needed in order to scale up the amount of compound and to improve the bioactivity through structure modification.

Previously, our group has established a quantitative structure-activity relationships (QSAR) study of chalcone derivatives as antimalarial agents[21]. The descriptor from chalcone derivatives, which affected the antimalarial activity were net charges from C6, C7, C8, O16 and E_{LUMO} with r^2 values of 0.937. The QSAR model equation afforded from the calculation was used to predict the antimalarial activity of the synthesized chalcone derivatives.

In the present study, we have successfully synthesized a series of prenyl substituted-chalcone derivatives and investigated their antimalarial activity. Antimalarial activity (presented in IC_{50}) was afforded in two ways, firstly was prediction using QSAR methods and the second was an *in vitro* test against *P. falciparum* 3D7 strain. Furthermore, docking simulation was performed to understand the binding interaction between the active compound with the active site of *P. falciparum* dihydrofolate reductase-thymidylate synthase.

2. Materials and methods

2.1. Material

The melting point of the synthesized compounds was determined using electro-thermal 9100 with temperature gradient 10 ˚C/min. High resolution electrospray ionisation-mass spectrometry (HRESI-MS) spectra were recorded on a Bruker micro time of flight mass spectrometer. Meanwhile, ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a JEOL 500 MHz spectrometer with tetramethylsilane 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.

All calculation the mechanical quantum of QSAR analysis was conducted using Gaussian 09 software. Molecular docking simulation methods were performed using Discovery Studio® 3.1 (Accelrys Inc., San Diego, USA) on an Intel® (TM)2 Quad CPU Q8200 @2.33GHz 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 of chalcone

An intermediate compound (2, 3, and 8) and a series of prenylated chalcones 5(a-d) have been synthesized according to the previuos report[16]. Meanwhile, 10(a-c) were prepared through the same method with modification on the base catalyst used (40% KOH to 60% NaOH) (Figure 1).

2.3. In vitro antimalarial activity assay

Antimalarial assay was conducted in 96 well microtitre plates against *P. falciparum* 3D7 strain which sensitive to chloroquine according to the microassay by Rieckmann *et al.* with slight modifications[22,23]. For this assay, the chalcone compounds were dissolved in dimethylsulfoxide and being prepared in a series of concentration, *i.e.* 100.00, 10.00, 1.00, 0.10 and 0.01 μg/mL in Roswell Park Memorial Institute-1640 media. About ± 1% parasitemia and 5% hematocrit were added into the sample respectively. The culture was incubated for 48 h at 37 ˚C, treated with 20% Giemsa dyes and made it as thin blood layer. Determination of the percent of parasitemia and also the inhibition percentage of *P. falciparum* growth were calculated from the number of infected erythrocytes for every 1 000 erythrocytes. Based on the inhibition percentage data, analysis of the correlation between concentrations of the compound with the inhibition percentage was conducted using probit log analysis in order to determine the IC_{50}.

2.4. QSAR analysis

QSAR studies of chalcone as antimalarial compounds have been reported before[21]. In the previous study, all of the mechanical quantum was calculated using Gaussian 09 software with DFT/B3LYP method. Correlation models were evaluated by multiple linear regression analysis using SPSS 18.0 statistics. From the studies, they found the descriptor which influences the antimalarial activity, *i.e.* the atomic net charge of C6, C7, C8, O16, and E_{LUMO}. Those descriptors were obtained from the best QSAR equation (equation 1). Furthermore, this equation was used to predict the IC_{50} value of compounds 5(a-d) and 10(a-c).

Log pIC_{50} (predicted) = 30.719 (qC6) – 44.913 (qC7) – 101.702 (qC8) – 89.497 (qO16) – 37.408 (E_{LUMO}) – 67.188------ (Equation 1)

The equation has 95% conviction level with statistical parameters: n = 31, r = 0968, r^2 = 0937, the adjusted r^2 = 0.920, the standard error of estimate = 0.096, F_{calc}/F_{table} = 21.712 and predicted residual sum of square = 0.174.

2.5. Docking studies

The protein crystal structure of the *P. falciparum* dihydrofolate reductase-thymidylate synthase with inhibitor was retrieved from the Brookhaven protein data bank (PDB ID: 1J3I, 2.33Å). The CDOCKER docking was performed according to the standard protocol implemented in the Discovery Studio® 3.1 (Accelrys, San Diego, USA). The protein crystal structure was pre-treated before docking process. Hydrogen atoms were added to the protein structure, and all ionizable residues were set at their default protonation at pH 7.4 while the ligands were prepared and minimized. During the docking process, the receptor was held rigid while the ligands were

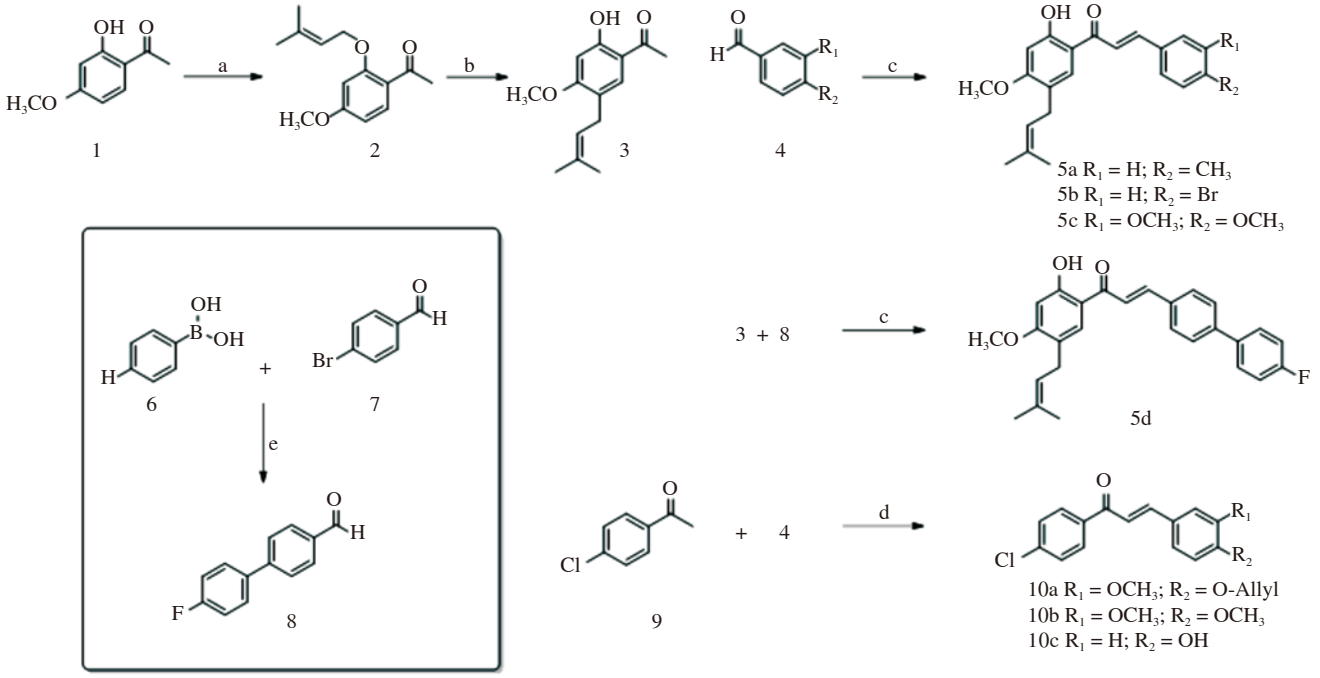

Figure 1. Reagents and conditions of synthesis.
a: Prenyl bromide, K_2CO_3, acetone, reflux for 20 h; b: N, N-dimethylaniline, reflux at 200 °C under N_2 atmosphere; c: 40% KOH, EtOH, stir at rt for 17 h; d: 60% NaOH, EtOH, stir at rt for 17 h; e: Pd(OAc)2, PEG-400, K_2CO_3 stir at 60 °C for 20 h.

allowed to flex during the refinement. A number of polar or nonpolar receptor hotspots for conformer matching starting were set at 500 with the docking tolerance 0.25 Å. The conformations generated of the ligands was set at 500 within this relative energy threshold 20 kcal/mol.

3. Results

3.1. Synthesis

4'-Methoxy-2'-prenyloxy-acetophenone (2). Yield 90%, yellow oil; ^{1}H-NMR (500 MHz, $CDCl_3$); δ (ppm): 1.78 (3H, s, CH_3), 1.82 (3H, s, CH_3), 2.60 (3H, s, -$COCH_3$), 3.87 (3H, s, OCH_3), 4.62 (2H, d, J = 6.6 Hz, CH_2), 5.51 (^{1}H, t, CH-aliphatic), 6.48 (^{1}H, s, CH-aromatic), 6.54 (^{1}H, d, J = 8.7 Hz, CH-aromatic), 7.84 (^{1}H, d, J = 8.7 Hz, CH-aromatic). ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): 18.26, 25.72, 32.01, 55.49, 65.42, 99.33, 100.86, 105.08, 119.06, 132.62, 138.34, 160.44, 164.38, 198.04.

2'-Hydroxy-4'-methoxy-5'-prenyl-acetophenone (3). Yield 35%, yellow oil; ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 1.73 (3H, s, CH_3), 1.78 (3H, s, CH_3), 2.56 (3H, s, -$COCH_3$), 3.25 (2H, d, J = 7.2 Hz, CH_2), 3.88 (3H, s, OCH_3), 5.26 (^{1}H, t, CH-aliphatic), 6.40 (^{1}H, s, CH-aromatic), 7.42 (^{1}H, s, CH-aromatic), 12.72 (^{1}H, s, OH). ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): 17.76, 25.76, 26.17, 27.77, 55.68, 99.05, 113.18, 121.68, 122.03, 130.73, 133.05, 163.89, 164.07, 202.52.

(*E*)-3-(4-methylphenyl)-1-(2-hydroxy-4-methoxy-5-(prenyl) phenyl) prop-2-en-1-one (5a). Yield 65%, needle-yellow crystals, m.p. 119–120 °C; ^{1}H-NMR (500 MHz, $CDCl_3$) (ppm): 1.76 (3H, s), 1.80 (3H, s, CH_3), 2.43 (3H, s, CH_3), 3.28 (2H, d, J = 7.1 Hz), 3.90 (3H, s, OCH_3), 5.32 (^{1}H, t), 6.47 (^{1}H, s), 7.28 (2H, d, J = 8.0 Hz), 7.55 (2H, d, J = 8.0 Hz), 7.58 (^{1}H, d, J = 15,5 Hz), 7.62 (^{1}H, s), 7.89 (^{1}H, d, J = 15.5 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): 18.00, 21.69, 25.91, 28.15, 55.86, 99.53, 113.58, 119.76, 121.79, 122.43, 128.66, 129.90, 132.41, 133.14, 141.27, 144.24, 164.36, 165.54, 192.05. HRESI-MS calcd. for $C_{22}H_{24}O_3$, $[M+H]^+$ 337.1804, found 337.1811.

(*E*)-3-(4-bromophenyl)-1-(2-hydroxy-4-methoxy-5-(prenyl) phenyl)-prop-2-en-1-one (5b). Yield 55%, needle-yellow crystals, m.p. 138–140 °C; ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 1.76 (3H, s, CH_3), 1.80 (3H, s, CH_3), 3.29 (2H, d, J = 7.1 Hz), 3.91 (3H, s, OCH_3), 5.29 (^{1}H, t), 6.47 (^{1}H, s), 7.54 (2H, d, J = 8.5 Hz), 7.58 (^{1}H, d, J = 15.5 Hz), 7.59 (2H, d, J = 2.7 Hz), 7.61 (^{1}H, s), 7.83 (^{1}H, d, J = 15.5 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): 18.01, 25.92, 28.17, 55.90, 99.57, 100.15, 113.47, 121.41, 122.01, 122.35, 124.95, 129.84, 129.94, 131.82, 132.41, 133.20, 134.06, 142.68, 164.60, 165.68, 191.59. HRESI-MS calcd. for $C_{21}H_{21}BrO_3$, $[M+H]^+$ 401.0753, found 401.0735.

(*E*)-3-(3,4-dimethoxyphenyl)-1-(2-hydroxy-4-methoxy-5-(prenyl) phenyl)-prop-2-en-1-one (5c). Yield 60%, needle-yellow crystals, m.p. 132–133 °C; ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 1.76 (3H, s, CH_3), 1.80 (3H, s, CH_3), 3.28 (2H, d, J = 7.1 Hz), 3.90 (3H, s, OCH_3), 3.97 (3H, s, OCH_3), 3.99 (3H, s, OCH_3), 5.33 (^{1}H, t), 6.47 (^{1}H, s), 6.95 (^{1}H, d, J = 8.4 Hz), 7.19 (^{1}H, s), 7.28 (^{1}H, d, J = 8.4 Hz), 7.47 (^{1}H, d, J = 15.5 Hz), 7.62 (^{1}H, s), 7.87 (^{1}H, d, J = 15.5 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): 17.98, 25.90, 27.99, 55.85, 56.08, 56.17, 99.51, 110.38, 111.39, 113.54, 118.52, 121.62, 122.41, 123.35, 128.12, 129.71, 133.18, 144.23, 149.47, 151.68, 164.26, 165.51, 191.83. HRESI-MS calcd. for $C_{23}H_{26}O_5$, $[M+H]^+$ 383.1859, found 383.1856.

(*E*)-3-(4'-fluorobiphenyl-4-yl)-1-(2-hydroxy-4-methoxy-5-(prenyl)phenyl)-prop-2-en-1-one (5d). Yield 36%, yellow solid, m.p. 150–151 °C; ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 1.77 (3H, s, CH_3), 1.81 (3H, s, CH_3), 3.30 (2H, d, J = 7.2 Hz), 3.91 (3H, s, OCH_3), 5.32 (^{1}H, t), 6.48 (^{1}H, s), 7.20–7.16 (2H, m), 7.64–7.61 (6H, m), 7.66 (^{1}H, d, J = 15.5 Hz), 7.75 (^{1}H, s), 7.94 (^{1}H, d, J = 15.5 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): 18.02, 25.93, 28.17, 55.89, 68.00, 99.57, 113.58, 115.94, 116.11, 120.75, 121.92, 122.41, 127.64, 128.81,

128.87, 129.19, 129.89, 130.89, 133.18, 134.11, 136.43, 142.36, 143.53, 164.50, 165.65, 191.82. HRESI-MS calcd. for $C_{27}H_{25}FO_3$, $[M+H]^+$ 417.1867, found 417.1856.

(*E*)-3-(4-(allyloxy)-3-methoxyphenyl)-1-(4-chlorophenyl)prop-2-en-1-one (10a). Yield 85%, yellow crystals, m.p. 89–91 °C; ^{1}H-NMR (500 MHz, Acetone-d6) δ 3.90 (3H, s, OCH_3), 4.66 (2H, d, *J* = 5.2 Hz), 5.47 (2H, dd, *J* = 18.8; 10.3 Hz),), 6.13–6.07 (^{1}H, m), 7.05 (^{1}H, d, *J* = 8.4 Hz), 7.36 (^{1}H, dd, *J* = 1.9; 8.4 Hz), 7.52 (^{1}H, d, *J* = 1.9 Hz), 7.58 (2H, d, *J* = 9.1 Hz), 7.75 (^{1}H, d, *J* = 15.5 Hz), 7.78 (^{1}H, d, *J* = 15.5 Hz), 8.13 (2H, d, *J* = 8.4 Hz). ^{13}C-NMR (125 MHz, Acetone-d_6) δ 55.4, 69.2, 111.2, 113.2, 116.9, 119.3, 123.5, 128.2, 128.8, 130.1, 133.6, 137.1, 138.2, 144.9, 150.0, 151.0, 187.9. HRESI-MS calcd for $C_{19}H_{17}O_3Cl$, $[M+H]^+$ 329.094 1, found 329.093 2.

(*E*)-1-(4-chlorophenyl)-3-(3,4-dimethoxyphenyl)prop-2-en-1-one (10b). Yield 85%, yellow crystals, m.p. 105–106 °C; ^{1}H-NMR (500 MHz, Acetone-d6) δ 3.88 (3H, s, OCH_3), 3.89 (3H, s, OCH_3), 7.05 (^{1}H, d, *J* = 8.4 Hz), 7.38 (^{1}H, dd, *J* = 1.9; 8.4 Hz), 7.50 (^{1}H, d, *J* = 1.9 Hz), 7.58 (2H, d, *J* = 8.4 Hz), 7.75 (^{1}H, d, *J* = 15.5 Hz), 7.78 (^{1}H, d, *J* = 15.5 Hz), 8.14 (2H, d, *J* = 8.4 Hz). ^{13}C-NMR (125 MHz, Acetone-d_6) δ 56.20, 56.29, 111.77, 112.50, 120.13, 124.64, 128.81, 129.70, 131.03, 138.09, 139.15, 145.93, 150.72, 153.08, 188.8. HRESI-MS calcd for $C_{17}H_{15}O_3Cl$, $[M+H]^+$ 303.072 3, found 303.071 1.

(*E*)-1-(4-chlorophenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one (10c). Yield 65%, yellow crystals, m.p. 175–176 °C; ^{1}H-NMR (500 MHz, Acetone-d_6) δ 6.93 (2H, d, *J* = 9.0 Hz), 7.58 (2H, d, *J* = 8.4 Hz), 7.68 (^{1}H, d, *J* = 15.5 Hz), 7.72 (2H, d, *J* = 9.0 Hz), 7.78 (1H, d, *J* = 15.5 Hz), 8.14 (2H, d, *J* = 8.4 Hz). ^{13}C-NMR (125 MHz, Acetone-d_6) δ 116.86, 119.44, 127.64, 129.71, 131.04, 131.77, 138.19, 139.13, 145.80, 161.08, 188.83. HRESI-MS calcd for $C_{15}H_{11}O_2Cl$, $[M+H]^+$ 259.046 9, found 259.045 6.

3.2. Antimalarial activity

The antimalarial activity (IC_{50}) of 5(a-d) and 10(a-c) compared to chloroquine as a common antimalarial drug was presented in Table 1. Compound 5a, 5b, and 5d revealed to have IC_{50} greater than 30 μg/mL, so far than chloroquine with only 0.02 μg/mL. On the other hand, 5c, 10(a-c) showed IC_{50} less than 3 μg/mL. This result has similar tendencies with QSAR analysis as could also see in Table 1. Furthermore, r^2 value as a result of plotting curves between experimental antimalarial activity (IC_{50}) versus prediction from QSAR analysis (pIC_{50}) showed satisfied linearity 0.998 (Figure 2). This value indicated that the QSAR equation being used has a great accuracy in predicting antimalarial activity. DFT/B3LYP methods were used in the QSAR calculation method and the descriptor which influencing the antimalarial activity was an atomic net charge of C6, C7, C8, O16 and E_{LUMO} as could be seen in Table 2.

Table 1
Comparison of antimalarial activity (IC_{50}) of chalcone between *in vitro* assay (3D7 strain) and QSAR analysis.

Compound	IC_{50} (μg/mL)	
	In vitro (3D7)	QSAR analysis
5a	30.85 ± 0.698	31.31
5b	37.05 ± 0.581	38.32
5c	1.08 ± 0.405	1.37
5d	30.09 ± 0.313	32.80
10a	1.73 ± 0.291	2.33
10b	2.21 ± 0.294	1.58
10c	2.44 ± 0.184	1.89
Chloroquine	0.02 ± 0.692	-

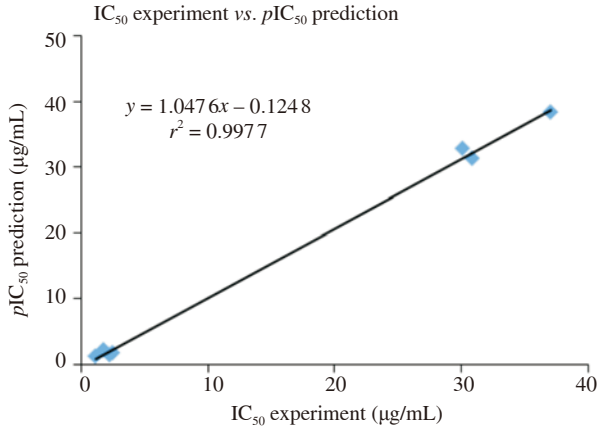

Figure 2. The plot of experimental antimalarial activity (IC_{50}) against 3D7 versus prediction from QSAR analysis (pIC_{50}).

Table 2
Atomic net charge of C6, C7, C8, O16 and E_{LUMO} calculated with DFT/B3LYP method from synthesized chalcone.

Compound	qC6	qC7	qC8	qO16	E_{LUMO}
5a	–0.186	–0.165	–0.216	–0.478	–0.060
5b	–0.184	–0.167	–0.214	–0.477	–0.068
5c	–0.192	–0.124	–0.222	–0.483	–0.052
5d	–0.186	–0.159	–0.218	–0.477	–0.067
10a	–0.236	–0.172	–0.223	–0.467	–0.070
10b	–0.240	–0.173	–0.223	–0.465	–0.072
10c	–0.233	–0.175	–0.224	–0.463	–0.068

3.3. Molecular docking studies

Molecular docking studies showed compound 5c has an almost same binding pocket as co-crystallized ligands on protein crystal structure PDB ID 1J3I. The similar binding pocket of 5c indicates that the compound has excellent antimalarial activity. As seen in Figure 3, the binding site is formed between 5c with Ala16, Ser108, Ile164, Trp48 and Phe58 which is the crucial interactions that determine the antimalarial activity according to the binding interactions displayed by the ligand co-crystal of WR99210.

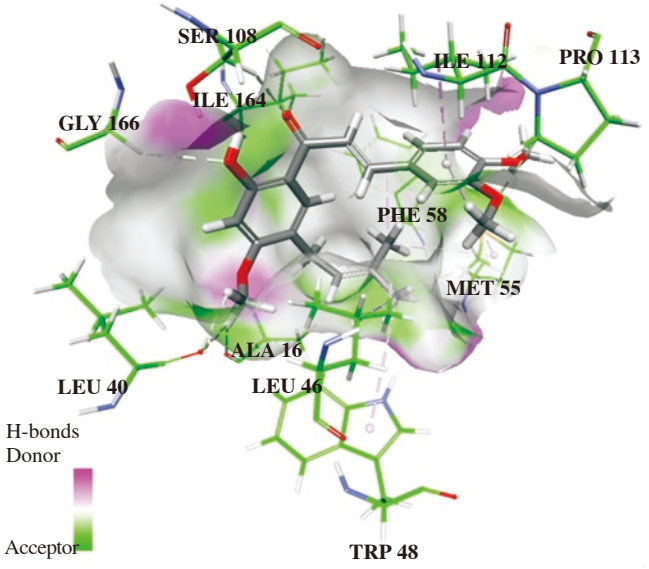

Figure 3. Predicted binding mode from docking simulation of 5c into active site of *P. falciparum* dihydrofolate reductase-thymidylate synthase (PDB ID: 1J3I). The coloring atom for the compound is in order as follows: carbons in gray, oxygen in red, nitrogen in blue, and hydrogen in white. The green line indicates hydrogen-bonding interaction with distance ascribed in angstroms, Å.

4. Discussion

Briefly, synthesis of 5(a-d) and 10(a-c) compounds were carried out through a series of reactions, including prenyl-sigmatropic rearrangement of prenyl group, *O*-alkylation, Suzuki coupling and Claisen-Schmidt condensation. To synthesize compounds 5(a-d), respective acetophenone 3 was first prepared according to the previously reported method[16]. The 2-hydroxy-4-methoxyacetophenone 1 was reacted with prenyl bromide in the presence of anhydrous K_2CO_3 in acetone under reflux condition for 20 h to afford respective intermediate 1-(4-methoxy-2-(prenyl)-phenyl)ethanone (2). To facilitate the migration, a prenyl-sigmatropic rearrangement approach was then adopted by refluxing intermediate *O*-prenylated product 2 in *N*, *N*-dimethylaniline at 200 ˚C under a nitrogen atmosphere to afford the desired C-prenylated product 3 in moderate yield. The rearrangement happened due to the steric hindrance at ortho position which allows the migration to a para position which is less steric. The Claisen-Schmidt condensation reaction between 3 with respective benzaldehyde derivatives 4 was performed in the presence of KOH to give corresponding C-prenylated chalcone 5(a-c) in satisfactory yields. Meanwhile, to prepare intermediate compound 8, Suzuki-Miyaura coupling reactions was employed utilizing the carbon-carbon single bond formation between 4-bromobenzaldehyde and 4-fluorophenylboronic acid (7) to afford 4-fluorophenyl benzaldehyde (8) according to the previous method[16,24]. The obtained Suzuki product was then coupled with 3 via Claisen-Schmidt condensation reaction to afford C-prenylated chalcone 5d.

On the other hand, vanillin was reacted with allyl bromide in the presence of K_2CO_3 under reflux condition for 6 h to prepare 10a. The reaction of 4-chloroacetophenone with purified allylated vanillin and other respective benzaldehyde 4 could give 10(a-c), which were conducted by the similar method as for 5(a-d) but with the different base catalyst (replaced with 60% NaOH). All of the synthesized chalcone compounds were afforded in trans conformation proven by ^{1}H-NMR spectra which showed the formation of doublet signal with a coupling constant (*J*) between H-α and H-β is 15.5 Hz.

The antimalarial activity of the synthesized compounds was evaluated using chloroquine-sensitive of *P. falciparum* 3D7 strain. The result showed that the synthesized compounds exhibited moderate to strong of antimalarial activity. Among of these tested compound, 5c and 10a displayed to be potential as a candidate for antimalarial compounds with excellent inhibition value (IC_{50}) of 1.08 and 1.73 μg/mL respectively. As comparison, Tadigoppula and co-worker have classified the activity profile of some compounds as defined on basis of their minimal inhibitory concentrations values: $\geqslant$ 155 μmol/L was inactive; between 50 and 155 μmol/L showed poor activity; between 6 and 50 μmol/L showed moderate activity; between 0.25 and 6 μmol/L, was strong activity)[19]. Based on these preliminary results, the prenylated chalcones 5c and allylated chalcone 10c were found to be highly potential as antimalarial compounds.

It is important to note that the experimental results from the *in vitro* studies (IC_{50}) were in good agreement with the predicted IC_{50} obtained from QSAR study (pIC_{50}). This tendency could be explained by the correlation between pIC_{50} and IC_{50} (3D7), which give the slope and correlation coefficient (r^2) 1.048 and 0.998 respectively. The QSAR analysis was carried out by entering atomic net charge of atom C6, C7, C8, O16, and E_{LUMO} into QSAR equation in order to predict the antimalarial activity (pIC_{50}).

Inhibition mechanism of the most active compound 5c was studied through molecular docking simulation. Compound 5c was docked to the active site of the *P. falciparum* dihydrofolate reductase-thymidylate synthase crystal structure, which was retrieved from Brookhaven PDB (ID: 1J3I). Actually, The *P. falciparum* dihydrofolate reductase-thymidylate synthase is an important target of antimalarial drugs[25]. The inhibition of this enzyme could prevent the deoxythymidine monophosphate production and DNA synthesis of parasite since it involved in the catalysis sequential reactions in the thymidylate cycle[26,27]. From the docking results, 5c displayed favorable binding affinity towards *P. falciparum* dihydrofolate reductase-thymidylate synthase with -CDOCKER interaction energy of 52.6392 kcal/mol, which closely resembled the -CDOCKER interaction energy of the co-crystallized ligands, 46.6183 kcal/mol. On the active site of *P. falciparum* dihydrofolate reductase-thymidylate synthase, compound 5c interacts with the side chains of Ala16, Ser108, Ile164, Trp48 and Phe58 which is the crucial amino acid for antimalarial activity based on the protein-ligand co-crystal structure of WR99210 (1,3,5-triazine, a pre-clinical molecule as *P. falciparum* dihydrofolate reductase-thymidylate synthase inhibitor). On the other hand, 5c is well-positioned in the *P. falciparum* dihydrofolate reductase-thymidylate synthase active site, constituting the residues of Ile14, Ala16, Trp48, Asp54, Phe58, Ser108, Ile164, and Thr185. Residues of Ala16, Ser108, and Ile164 are located in the active site of the mutant protein, so 5c was also expected to have antimalarial activity against chloroquine-resistance strain. The residues involved in the active site of mutant protein were Ala16, Cys50, Asn51, Cys59, Ser108 and Ile164[26-28].

In summary, we have successfully determined the effects of some chalcone and its prenylated analogs on antimalarial activity against of *P. falciparum* (3D7). Compound 5c and 10a were found to exhibited a great antimalarial activity with IC_{50} of 1.08 and 1.73 μg/mL against the chloroquine-sensitive *P. falciparum* 3D7 strain, which was described as good antiplasmodial compounds. An *in vitro* results verified the relevancies of the antimalarial activity prediction from the QSAR studies. Detailed binding mode from docking simulations revealed that 5c was well-positioned in the active site of *P. falciparum* dihydrofolate reductase-thymidylate synthase and was found hydrogen bond interaction with Ala16, Ser108, Ile164 and π-bond interaction with Trp48 and Phe58 which is the crucial amino acid that could possibly interrupt the sequential catalysis reactions in the thymidylate cycle, subsequently prevents deoxythymidine monophosphate production and DNA synthesis. Compound 5c also displayed to engage a hydrogen bond interaction with the active site of the mutant protein such as Ala16, Ser108, and Ile164. This result justified the reason for the good antimalarial activity demonstrated by 5c. Further studies are required to identify the real molecular target(s) and also the signaling pathway that might contribute to the antimalarial activity.

Conflict of interest statement

We declare that we have no conflict of interest.

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