PHARMACIA

Pharmacia 70(4): 1305–1313
DOI 10.3897/pharmacia.70.e107406

Research Article

Synthesis of chalcone derivatives with methoxybenzene and pyridine moieties as potential antimalarial agents

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Received 5 June 2023 ♦ **Accepted** 23 September 2023 ♦ **Published** 7 November 2023

Citation: Mulyana FE, Waskitha SSW, Pranowo D, Khairuddean M, Wahyumingsih TD (2023) Synthesis of chalcone derivatives with methoxybenzene and pyridine moieties as potential antimalarial agents. Pharmacia 70(4): 1305–1313. https://doi.org/10.3897/pharmacia.70.e107406

Abstract

Malaria remains an endemic disease in tropical regions, urgently needed the search for effective antimalarial agents due to resistance against existing drugs. This study investigated the potential antimalarial activity of pyridine-based chalcone derivatives against *P. falciparum* 3D7 and FCR3 strains. The chalcones were synthesized through a one-pot method using various pyridine carbaldehyde, resulting in yields ranging from 53.74 to 86.37%, and all products were characterized using FTIR, GC-MS, and NMR spectroscopies. Among the six chalcones tested, chalcone **A** [1-(2-methoxyphenyl)-3-(pyridin-2-yl)prop-2-en-1-one] displayed the highest antimalarial activity with IC_{50} values of 0.48 and 0.31 µg/mL against *P. falciparum* 3D7 and FCR3 strains, respectively, and a resistance index of 0.65. Molecular docking studies highlighted the interaction of the carbonyl group of all chalcones with Asn108 amino acid residue in the P*f*DHFR-TS active site via hydrogen bonding, demonstrating their potential as the antimalarial agent. Notably, the positioning of methoxy and pyridine substituents significantly influenced the antimalarial activity of the chalcones.

Keywords

antimalarial, chalcone, *P. falciparum*, pyridine

Introduction

The World Health Organization has observed a rise in malaria cases in developing countries. Globally, a total of 229 million malaria cases were reported in 2019, resulting in approximately 558 thousand deaths annually. There were 241 million malaria cases and 627,000 deaths due to the disease in 2020 (WHO 2021), representing a 12% increase in cases and deaths within one year. Indonesia, as one of the developing countries, reported 1.8 million malaria cases, mostly found in Papua (85.35%), West Nusa Tenggara (6.02%), and West Papua (3.92%). The primary reason for this situation is the resistance exhibited by the Plasmodium parasite towards widely used antimalarial drugs such as artemisinin, mefloquine, chloroquine, and piperaquine (Veiga et al. 2016; Kim et al. 2019; Pannu 2019; Talapko et al. 2019). As a result, there is an urgent requirement for research focused on developing novel antimalarial drugs (Tse et al. 2019; Belete 2020).

Chalcone has emerged as a promising candidate for a new antimalarial drug due to its simple structure, ease of synthesis, and potent activity against both the normal 3D7 strain and the resistant FCR3 strain of *P. falciparum*

(Cheng et al. 2020; Qin et al. 2020). Chalcones demonstrated antimalarial activity, exhibiting IC_{50} values of up to 11 μg/mL (Wanare et al. 2010). The antimalarial activity against *P. falciparum* 3D7 is influenced by the methoxy groups on the acetophenone and benzaldehyde aromatic rings of chalcones (Kumar et al. 2010). When the methoxy group is positioned on the benzaldehyde aromatic ring, the *in vitro* IC_{50} values exceed 20 μg/mL. Conversely, when the methoxy group is on the acetophenone aromatic ring, the IC_{50} values range from 7.8 to 11.5 μg/mL (Kumar et al. 2010). Furthermore, the presence of a methoxy substituent significantly influences the antimalarial activity of chalcones, with tetramethoxychalcone demonstrating an IC_{50} value of 5.36 μg /mL against *P. falciparum* (Syahri et al. 2017).

Hybridizing organic structures in drug design has attracted significant attention from researchers due to their enhanced effectiveness against drug-resistant parasites (Muregi and Ishih 2010). The pyridine moiety is commonly present in conventional antimalarial drugs like chloroquine, piperaquine, mefloquine, and amodiaquine (Bekhit et al. 2012). By hybridizing pyridine with fosmidomycin, the resulting compound exhibits increased antimalarial activity, with an IC_{50} range of 0.18–0.63 μg/mL against *P. falciparum* 3D7 and Dd2 strains. However, replacing the pyridine ring with benzene significantly decreases fosmidomycin's antimalarial activity, with an IC_{50} range of 1.17–3.50 μg/mL (Xue et al. 2013).

A preliminary screening in a search of the antimalarial agent could be conducted by using a specific receptor in Plasmodium parasites. Among the protein receptors located inside the Plasmodium parasites, *P. falciparum* dihydrofolate reductase-thymidylate synthase (P*f*DHFR-TS) is a well-defined receptor target for antimalarial agents as this receptor plays a pivotal role in the production of folates and thymidylates which are necessary for the DNA synthesis process (Dasgupta and Anderson 2008). Approved antimalarial drugs such as chloroquine, pyrimethamine and cycloguanil act as antimalarial agents through P*f*DHFR-TS inhibition as their mechanism of action (Chaianantakul et al. 2013). Bekhit et al. (2012) and Rajendran et al. (2022) reported that some chalcone derivatives exhibited antimalarial activity through P*f*DHFR-TS inhibition. On the other hand, the mechanism of action of pyridines is similar to chloroquine as chloroquine also contains a pyridine moiety on its chemical structure (Bekhit et al. 2012). Limited research has explored the combination of methoxychalcone with a pyridine structure to create novel antimalarial agents. This study synthesized a series of chalcones containing methoxybenzene and pyridine moieties through a one-pot process using respective methoxyacetophenone and pyridine-carbaldehydes precursors. The *in vitro* antimalarial activity of the six synthesized chalcones was then evaluated against *P. falciparum* 3D7 and FCR3 strains. The effect of the positioning of the methoxy and pyridine moieties was investigated and discussed using both experimental *in vitro* and computational *in silico* approaches.

Experimental part

Materials

All the materials employed in this study were procured from E. Merck in pro analysis grade, i.e., 2-methoxyacetophenone, 3-methoxyacetophenone, 4-methoxyacetophenone, 2-pyridinecarbaldehyde, 3-pyridinecarbaldehyde, 4-pyridinecarbaldehyde, methanol, and NaOH. Furthermore, chemicals such as Roswell Park memorial institute (RPMI) solution, DMSO, inoculum solution, human serum, red blood cells, methanol, Giemsa coloring solution, *P. falciparum* strain 3D7 and FCR3 were used for the *in vitro* antimalarial assay.

Instrumentations

Thin-layer chromatography using Merck pre-coated aluminum F_{254} plates was employed to monitor the progress of the synthesis reactions. The melting point of the synthesized compounds was measured using the Electrothermal 9100 equipment. Analysis of the infrared spectrum of the products was conducted using the Shimadzu Prestige-21 Fourier transform infrared (FTIR), while the purity and mass spectrum of the products were recorded using the Shimadzu QP2010S gas chromatography-mass spectrometry (GC-MS). The nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) spectra were analyzed on JEOL JNMECA with a frequency of 500 and 125 MHz, respectively. The antimalarial assay was carried out using equipment, including laboratory glassware and 96-well microplates, micropipette, microtube, microcentrifuge, ELISA reader (Microplate Reader, Benchmark), CO_2 incubator, object-glass, and microscope (Euromex iScope IS.1152-PLPHi). Molecular docking simulations were performed using a laptop with specifications of a central processing unit (CPU) of AMD Ryzen 7 4800H, a graphic processing unit (GPU) of NVIDIA GTX 1650 Ti, 16 GB of RAM, and a Windows 11 operating system.

Procedures

The chalcones were synthesized in a one-pot synthesis method. A mixture of 3 mmol pyridinecarbaldehyde derivative in 15 mL methanol and 15 mL 10% (w/v) NaOH solution was added into a round-bottom flask placed in an ice bath with a temperature range of 0–10 °C. The next step involved the addition of methoxyacetophenone derivatives (3 mmol in 5 mL methanol) dropwise for 1 h, and then it was stirred for 5 h with a temperature range of 10±1 °C. The resulting solid was filtered, washed with water, and recrystallized with ethanol. The structure elucidation was performed using FTIR, GC-MS, ^{1}H- and ^{13}C-NMR spectrometers.

1-(2-methoxyphenyl)-3-(pyridin-2-yl) prop-2-en-1-one (chalcone A).

Chalcone **A** (0.39 g) was yielded as a yellowish solid in 53.74%. m.p 60–61 °C. FT-IR (KBr): 3070, 2947, 1658,

1604, 1597, 1473, 1319, 1249, 1018, 763 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 3.87 (s, 3H, -OCH_3), 6.96 (d, *J* 7.4 Hz, 1H, H_{Ar-Ph}), 7.00 (tod, *J* 1.1, 7.5 Hz, 1H, H_{Ar-Py}), 7.24 (dodod, *J* 1.1, 4.6, 7.5 Hz, 1H, H_{Ar-Py}), 7.44 (tod, *J* 1.7, 7.4 Hz, 1H, H_{Ar-Ph}), 7.46 (dod, *J* 1.1, 7.5 Hz, 1H, H_{Ar-Py}), 7.59 (d, *J* 15.5 Hz, 1H, =CH_α,), 7.61 (dod, *J* 1.7, 7.4 Hz, 1H, H_{Ar-Ph}), 7.69 (tod, *J* 1.7 & 7.4 Hz, 1H, H_{Ar-Ph}), 7.79 (d, *J* 15.5 Hz, 1H, =CH_β), 8.63 (dod, *J* 1.1, 4.6 Hz, 1H, H_{Ar-Py}). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 55.86 (-OCH_3), 111.68, 120.77, 124.21, 124.72, 129.07, 130.53, 133.27, 136.84, 150.22, 153.77, 158.41 (C_{Ar}), 130.47, 141.67 (C_{alkene}), 193.27 (C=O). Mass spectrum (EI, m/z) = 239 (M^+, 5), 180 (50), 135 (40), 119 (38), 118 (38), 104 (40), 92 (45), 77 (100), 51 (90).

1-(2-methoxyphenyl)-3-(pyridin-3-yl) prop-2-en-1-one (chalcone B).

Chalcone **B** (0.56 g) was produced in 78.01% as a yellowish solid. m.p. 63–65 °C. IR (KBr): 3039, 2947, 1658, 1604, 1597, 1473, 1327, 1242, 1018, 756 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 3.92 (s, 3H, -OCH_3), 7.02 (d, *J* 8.0 Hz, 1H, H_{Ar-Ph}), 7.06 (t, *J* 8.0 Hz, 1H, H_{Ar-Ph}), 7.34 (dod, *J* 4.8, 7.8 Hz, 1H, H_{Ar-Py}), 7.49 (d, *J* 15.9 Hz, 1H, =CH_α), 7.51 (tod, *J* 2.0, 8.0 Hz, 1H, H_{Ar-Ph}), 7.62 (d, *J* 15.9 Hz, 1H, =CH_β), 7.67 (dod, *J* 1.9, 7.8 Hz, 1H, H_{Ar-Py}), 7.90 (dot, *J* 2.0, 8.0 Hz, 1H, H_{Ar-Ph}), 8.60 (dod, *J* 1.9, 4.8 Hz, 1H, H_{Ar-Py}), 8.81 (d, *J* 1.9 Hz, 1H, H_{Ar-Py}). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 55.84 (-OCH_3), 111.74, 120.96, 123.85, 128.77, 130.67, 131.07, 133.54, 134.75, 149.94, 150.86, 158.41 (C_{Ar}), 128.86, 138.94 (C_{Alkene}), 192.15 (C=O). Mass spectrum (EI, m/z) = 239 (M^+, 3), 135 (45), 120 (20),104 (20), 92 (40), 77 (100), 51 (75).

1-(3-methoxyphenyl)-3-(pyridin-3-yl) prop-2-en-1-one (chalcone C).

Chalcone **C** (0.41 g) was yielded in 57.12% as a yellowish solid. m.p 87–88 °C. IR (KBr): 3070, 2947, 1666, 1604, 1581, 1481, 1303, 1249, 1018, 784, 694 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 3.89 (s, 3H, OCH_3), 7.15 (dod, *J* 2.0, 7.9 Hz, 1H, H_{Ar-Py}), 7.37 (dod, *J* 4.7, 7.9 Hz 1H, H_{Ar-Py}), 7.43 (t, *J* 8.0 Hz 1H, H_{Ar-Ph}), 7.55 (dod, *J* 1.4, 2.3 Hz, 1H, H_{Ar-Ph}), 7.59 (d, *J* 15.8 Hz, 1H, =CH_α), 7.61 (dot, *J* 1.4, 8.0 Hz, 1H, H_{Ar-Ph}), 7.79 (d, *J* 15.8 Hz, 1H, =CH_β), 7.96 (dot, *J* 2.3, 8.0 Hz, 1H, H_{Ar-Ph}), 8.63 (dod, *J* 2.0, 4.7 Hz, 1H, H_{Ar-Py}), 8.86 (d, *J* 2.0 Hz, 1H, H_{Ar-Py}). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 55.60 (OCH_3), 112.96, 119.75, 121.19, 123.95, 129.80, 130.75, 134.73, 139.16, 150.07, 151.18, 160.05 (C_{Ar}), 123.92, 141.03 (C_{Alkene}), 189.68 (C=O). Mass spectrum (EI, m/z) = 239 (M^+, 30), 210 (20), 135 (30), 132 (40), 104 (40), 92 (45), 77 (100), 51 (90).

1-(4-methoxyphenyl)-3-(pyridin-2-yl) prop-2-en-1-one (chalcone D).

Chalcone **D** (0.52 g) was yielded in 71.65% as a yellowish solid. m.p 76–78 °C. IR (KBr): 3055, 2970, 1658, 1604, 1597, 1465, 1327, 1257, 1018, 833 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 3.85 (s, 3H, OCH_3), 6.95 (dot, *J* 2.3, 9.1 Hz, 2H, H_{Ar-Ph},), 7.25 (dodod, *J* 1.2, 5.2, 7.5 Hz, 1H, H_{Ar-Py}), 7.43 (d, *J* 7.5 Hz, 1H, H_{Ar-Py}), 7.70 (tod, *J* 1.2, 7.5 Hz, 1H, H_{Ar-Py}), 7.73 (d, *J* 15.0 Hz, 1H, =CH_α), 8.09 (dot, *J* 2.3, 9.1 Hz, 2H, H_{Ar-Ph}), 8.10 (d, *J* 15.0 Hz, 1H, =CH_β), 8.65 (dod, *J* 1.2, 5.2 Hz, 1H, H_{Ar-Py}). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 55.60 (OCH_3), 113.95, 124.40, 125.48, 130.47, 131.20, 137.00, 150.21, 153.43, 163.74 (C_{Ar}), 125.51, 142.06 (C_{Alkene}), 188.74 (C=O). Mass spectrum (EI, m/z) = 239 (M^+, 40), 210 (100), 135 (60), 104 (50), 92 (40), 77 (80), 51 (50).

1-(4-methoxyphenyl)-3-(pyridin-3-yl) prop-2-en-1-one (chalcone E).

Chalcone **E** (0.54 g) was produced as a yellowish solid with a 75.23% yield. m.p 99–100 °C. IR (KBr): 3039, 2947, 1666, 1604, 1597, 1465, 1311, 1257, 1026, 840 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 3.89 (s, 3H, OCH_3), 6.98 (dot, *J* 2.7, 9.0 Hz, 2H, H_{Ar-Ph}), 7.35 (dod, *J* 4.6, 8.0 Hz, 1H, H_{Ar-Py}), 7.60 (d, *J* 15.8 Hz, 1H, =CH_α), 7.76 (d, *J* 15.8 Hz, 1H, =CH_β), 7.93 (dot, *J* 2.0, 8.0 Hz, 1H, H_{Ar-Py}), 8.03 (dot, *J* 2.7, 9.0 Hz, 2H, H_{Ar-Ph}), 8.61 (dod, *J* 2.2, 4.6 Hz, 2H, H_{Ar-Py}), 8.84 (d, *J* 2.0 Hz, 1H, H_{Ar-Py}). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 55.65 (OCH_3), 114.04, 123.79, 123.88, 128.58, 130.34, 131.03, 148.31, 149.39, 163.67 (C_{Ar}), 123.41, 135.54 (C_{Alkene}), 195.65 (C=O). Mass spectrum (EI, m/z) = 239 (M^+, 40), 210 (20), 135 (70), 119 (38), 107 (20), 92 (50), 77 (100), 51 (80).

1-(4-methoxyphenyl)-3-(pyridin-4-yl) prop-2-en-1-one (chalcone F).

Chalcone **F** (0.62 g) was yielded as a yellowish solid in 86.37%. m.p 128–130 °C. IR (KBr): 3032, 2970, 1666, 1604, 1597, 1465, 1311, 1234, 1018, 810 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 3.80 (s, 3H, OCH_3), 6.83 (d, *J* 8.6 Hz, 2H, H_{Ar-Ph}), 6.93 (d, *J* 5.7 Hz, 2H, H_{Ar-Py}), 6.99 (d, *J* 9.2 Hz, 1H, =CH_α), 7.73 (d, *J* 8.6 Hz, 2H, H_{Ar-Ph}), 8.03 (d, *J* 9.2 Hz, 1H, =CH_β), 8.40 (d, *J* 5.7 Hz, 2H, H_{Ar-Py}). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 55.67 (OCH_3), 114.07, 123.04, 128.44, 130.35, 140.80, 149.97, 163.75 (C_{Ar}), 126.06, 147.95 (C_{Alkene}), 195.35 (C=O). Mass spectrum (EI, m/z) = 239 (M^+, 40), 210 (10), 135 (100), 107 (20), 92 (40), 77 (80), 51 (70).

In vitro antimalarial assay

The *in vitro* antimalarial assays were performed using the technique described by Zakiah et al. (2021) with a slight modification. The synthesized product (10 mg) was dissolved in 1 mL DMSO. Then 100 µL of this solution was diluted using 900 µL RPMI solution to obtain a stock solution with a final concentration of 1000 µg/mL. A series of diluted solutions (1, 2, 10, and 20 µg/mL) was prepared by utilizing the stock solution. Next, each diluted solution (100 µL) and inoculum solution (100 µL) with

P. falciparum parasites were added into a 96-well microplate. The negative control was prepared in the absence of the sample solution. The 96-well microplate was incubated at 37 °C for 72 h. Afterward, the cultures were used to produce blood smears and stained for 10 min using a 15% Giemsa solution.

The inhibition percentage of *P. falciparum* was determined by analyzing each group of 1000 erythrocyte cells infected with *P. falciparum* using a microscope with a magnification of 1000×. The IC_{50} value was calculated through probit analysis. Triplicate analyses were performed for each sample.

Molecular docking study

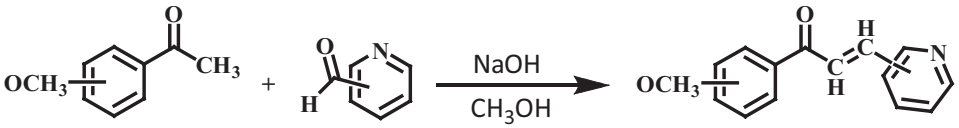
In this study, we investigated the mechanism of action of all synthesized chalcones, chalcone A–F, in inhibiting *P. falciparum* dihydrofolate reductase-thymidylate synthase (P*f*DHFR-TS) receptor. In addition, we also investigated the mechanism of a well-recognized antimalarial agent, chloroquine, in inhibiting the same receptor as a comparison. AutoDock Vina 1.1.2 software was used for all molecular docking simulations (Trott and Olson 2010). The crystal structure of P*f*DHFR-TS receptor, complexed with WR99210, NADPH, and dUMP, was retrieved from Protein Data Bank (PDB ID: 1J3K) with the chain A as the model receptor. To prepare the receptor, all solvent molecules and complexed ligands were removed using Discovery Studio Visualizer software and then appended hydrogen atoms and Kollman charges with AutoDock Tools 1.5.6. The active site of PfDHFR-TS was identified by the amino acid residues interacting with the native ligand, WR99210. The molecular docking grid box was focused on the native ligand and sized 12 Å × 12 Å × 12 Å with 1.00 Å grid spacing. The exhaustiveness of the molecular docking protocol was set to 16. To validate the molecular docking protocol, the native ligand was redocked, and the root-mean-square deviation (RMSD) of the docked pose relative to the native ligand crystal structure was calculated using PyMOL software (Schrödinger and DeLano 2020). The molecular docking protocol was considered valid if the RMSD was ≤ 2.00 Å (Diallo et al. 2021).

The preparation of the three-dimensional structure of all chalcones and chloroquine was carried out prior to the molecular docking simulation. The process involved building and geometrically optimizing their structure using GaussView 5.0 and Gaussian 09 package (Frisch et al. 2016) with the DFT/B3LYP method and 6–31G basis set similar to our previous works (Suma et al. 2019; Waskitha et al. 2021). AutoDock Vina was used to carry out the molecular docking simulation of all chalcones and chloroquine, following the same method as the native ligand. The conformation with the least binding affinity was selected as the best orientation, indicating the most stable interaction with the active site (Minnelli et al. 2020).

Results and discussion

Synthesis of chalcones

Six chalcone derivatives have been synthesized from the methoxy-substituted acetophenone and various pyridinecarbaldehyde using a base-catalyzed Claisen-Schmidt condensation reaction. The synthesis scheme is illustrated in Scheme 1. The resulting products were recrystallized from ethanol to obtain yellowish solids with yields ranging from 53.74 to 86.37%. Purity analysis was performed using GC, which showed a single peak at a retention time range of 24–27 min for all products, indicating their high purity level (approximately 100%). In the MS spectra, all chalcones displayed a molecular ion at m/z = 239, corresponding to each chalcone's molecular mass. FTIR analysis revealed the presence of characteristic enone functional groups at 1604–1666 cm^{-1} (HC=CH-C=O) and these findings were consistent with previous reports (Joseph et al. 2014). Both protons of enone were observed in the ^{1}H-NMR spectra of all products at 6.99–7.73 and 7.62–8.10 ppm, respectively. Furthermore, the HC=CH- and C=O carbons were detected



Chalcone A-F

Chalcones	R_1	Pyridine
A	2-OCH_3	pyridine-2-yl
B	2-OCH_3	pyridine-3-yl
C	3-OCH_3	pyridine-3-yl
D	4-OCH_3	pyridine-2-yl
E	4-OCH_3	pyridine-3-yl
F	4-OCH_3	pyridine-4-yl

Scheme 1. The synthesis scheme of chalcones A–F.

at 123.41–147.95 and 188.74–195.65 ppm, respectively, in the ^{13}C-NMR spectra of all products (Marcovicz et al. 2022).

In vitro antimalarial assay

All synthesized compounds have the same molecular formula, i.e., $C_{15}H_{13}NO_{2,}$ and were examined to assess the effect of methoxy groups and nitrogen atoms on the antimalarial activity of chalcones. The results of the antimalarial activities of chalcones A–F against *P. falciparum* 3D7 and FCR3 strains are listed in Table 1. According to Basco et al. (1994), the *in vitro* antimalarial activity has good activity with IC_{50} < 10 μg/mL, moderate activity with IC_{50} 10–50 μg/mL, low activity with IC_{50} 50–100 μg/mL, and inactive with IC_{50} > 100 μg/mL, so all chalcones could be classified as highly effective antimalarial agents, as their IC_{50} values were below 5 μg/mL. Even though antimalarial activities from chloroquine against *P. falciparum* 3D7 and FCR3, respectively, have IC_{50} of 0.0032 μg/mL and 0.0352 μg/mL (Zakiah et al. 2021).

Table 1. The antimalarial activity of chalcones A–F against *Plasmodium falciparum*.

Chalcones	$IC_{50,}$ 3D7 strain (μg/mL)	IC_{50} FCR3 strain (μg/mL)	Resistance index
A	0.48	0.31	0.65
B	4.16	2.68	0.65
C	0.98	1.03	1.05
D	4.46	2.17	0.49
E	1.07	1.13	1.06
F	1.11	1.02	0.92

In terms of antimalarial activity against *P. falciparum* 3D7, ortho-methoxy groups exhibited an IC_{50} value range of 0.48–4.16 μg/mL, while meta- and para-methoxy groups demonstrated IC_{50} value of 0.98 and 1.07–4.46 μg/mL, respectively. These results suggest that ortho-methoxy groups have greater antimalarial activity compared to meta-methoxy groups and are significantly more active than para-methoxy groups of the chalcone's chemical structure as a *P. falciparum* 3D7 antimalarial agent. A similar trend was observed for *P. falciparum* FCR3, with ortho-methoxy groups displaying an IC_{50} range of 0.31–2.68 μg/mL, whereas meta- and para-methoxy groups exhibited IC_{50} values of 1.03 and 1.02–2.17 μg/mL, respectively.

Regarding the nitrogen atoms, the 2-pyridine moiety demonstrated an IC_{50} value range of 0.48–4.46 μg/mL against *P. falciparum* 3D7, while the 3- and 4-pyridine moieties exhibited IC_{50} values of 0.98–4.16 and 1.11 μg/mL, respectively. These results indicate that the 2-pyridine moiety possesses higher antimalarial activity than 3-pyridine and is significantly more active than 4-pyridine within the chalcone chemical structure as a *P. falciparum* 3D7 antimalarial agent. Similar observations were made for *P. falciparum* FCR3, with the IC_{50} range for the 2-pyridine moiety being 0.31–2.17 μg/mL, while the IC_{50} values for 3- and 4-pyridine moieties were 1.03–2.68 and 1.02 μg/mL, respectively. These findings indicate that the presence of 2-methoxy and 2-pyridine substituents play a crucial role in the antimalarial activity of chalcones against both *P. falciparum* 3D7 and FCR3.

Furthermore, all chalcones had a low resistance index (0.49–1.06) compared with chloroquine, which has a resistance index of 11 (Zakiah et al. 2021), thus demonstrating no-cross resistance with compounds like chloroquine, which share a similar mechanism of action (Sinha et al. 2019). These findings emphasize the potential of these chalcones as antimalarial agents.

Molecular docking study

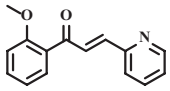
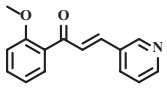
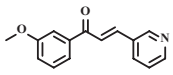
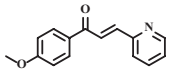
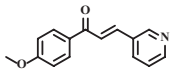
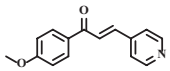
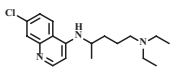
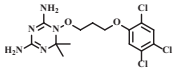
A molecular docking study was conducted on the P*f*-DHFR-TS receptor as the target, which is one of a kind important target of antimalarial drugs, to investigate the possibility of the inhibitory mechanism of chalcone **A**–**F** and chloroquine as antimalarial drugs. The redocking results revealed that the native ligand had a binding affinity of -6.8 kcal/mol and closely matched the X-ray crystallographic conformation, with an RMSD value of 1.828 Å. This demonstrates the molecular docking protocol's reliability and validity (Diallo et al. 2021).

The molecular docking results of chalcone A–F, chloroquine, as well as the native ligand are tabulated in Table 2. The binding affinity of all chalcones ranged from -6.9 to -7.3 kcal/mol, showing their relatively strong interactions with the active site of P*f*DHFR-TS whereas the binding affinity of chloroquine was -7.4 kcal/mol. Notably, even though chloroquine had the lowest binding affinity, all chalcones exhibited lower binding affinity than the native ligand, indicating higher stability of the ligand-receptor complex within the active site of the P*f*DHFR-TS receptor.

The findings indicated that chloroquine was stabilized by a hydrogen bond with one of the crucial amino acid residues, Asn108, and mostly by hydrophobic interactions along with van der Waals interactions. We also found that all chalcones established interaction with Asn108 through the formation of a hydrogen bond as well. The carbonyl group of the chalcones played a crucial role in binding to P*f*DHFR active site, and there were no steric clashes with Asn108, which is associated with resistance (Adane et al. 2010). A previous study has also highlighted the significance of the carbonyl group of chalcones in P*f*DHFR binding (Ahmed and Abdullah 2019). Additionally, interactions with hydrophobic amino acid residues such as Met55, Phe58, and Ile112 contributed to the stability of ligand-receptor interactions (Kyei et al. 2022). These hydrophobic interactions were reinforced by the phenyl and pyridine group, enhancing the overall stability of the interactions in the active site of P*f*DHFR-TS. Fig. 1 illustrates the two-dimensional molecular interactions of chalcones A–F, chloroquine, and the native ligand within the active site of P*f*DHFR.

The molecular docking study of chloroquine against P*f*DHFR-TS has been also reported (Melaku et al. 2022). Similar to the chalcone-pyridine hybrid compound, chloroquine also interacted with Ala16, Leu46, Phe58, Asn108,

Table 2. The molecular docking results of the native ligand, chalcones A–F, and chloroquine against P*f*DHFR-TS receptor.

Ligand	Binding affinity (kcal/mol)	Interactions	
 Chalcone **A**	-7.0	H-Bond	: Asn108 (2.322Å)
		van der Waals	: Ile14, Cys15, Leu40, Gly41, Val45, Asp54, Ser111, Leu164, Tyr170
		Carbon H-bond	: Gly44
		Pi-Pi Stacked	: Phe58
		Alkyl	: Met55
		Pi-Alkyl	: Ala16, Phe58
		Pi-Sigma	: Leu46
 Chalcone **B**	-6.9	H-Bond	: Asn108 (2.469 Å)
		van der Waals	: Cys15, Met55, Ser111, Pro113, Phe116, Leu119, Leu164
		Unfavorable Acceptor-Acceptor	: Asp54
		Pi-Pi Stacked	: Phe58
		Pi-Alkyl	: Ile14, Ala16, Ile112
		Alkyl	: Leu46
 Chalcone **C**	-7.2	H-Bond	: Asn108 (2.266 Å)
		van der Waals	: Cys15, Leu40, Leu46, Met55, Phe58, Pro113, Leu119, Leu164
		Pi-Donor H-Bond	: Tyr170
		Carbon H-bond	: Ala16, Ser111
		Pi-Sigma	: Ile112
		Alkyl	: Ile112
		Pi-Alkyl	: Ala16
 Chalcone **D**	-7.2	H-Bond	: Asn108 (2.103 Å)
		van der Waals	: Cys15, Asp54, Tyr57, Pro113, Phe116, Leu119, Leu164, Tyr170, Thr185
		Pi-Sigma	: Ile112
		Pi-Pi Stacked	: Phe58
		Alkyl	: Ile14, Ala16
		Pi-Alkyl	: Ala16, Met55, Phe58
 Chalcone **E**	-7.3	H-Bond	: Asn108 (2.393 Å)
		van der Waals	: Leu40, Leu46, Phe58, Leu119, Leu164
		Carbon H-Bond	: Cys15, Ala16
		Pi-Donor H-bond	: Tyr170
		Pi-Sigma	: Ile112
		Alkyl	: Met55, Pro113
		Pi-Alkyl	: Ala16, Phe116
 Chalcone **F**	-7.1	H-Bond	: Asn108 (2.004 Å)
		van der Waals	: Cys15, Asp54, Tyr57, Pro113, Phe116, Leu119, Leu164, Tyr170, Thr185
		Pi-Sigma	: Ile112
		Pi-Pi Stacked	: Phe58
		Alkyl	: Ile14, Ala16
		Pi-Alkyl	: Ala16, Met55, Phe58
 Chloroquine	-7.4	H-Bond	: Asn108 (2.286 Å)
		van der Waals	: Cys15, Trp48, Met55, Leu164, Tyr170
		Carbon H-Bond	: Asp54, Ser111, Pro113
		Pi-Sigma	: Ile112
		Alkyl	: Ile14, Ala16, Leu40, Leu46, Leu119
		Pi-Alkyl	: Phe58, Ile112, Phe116
 Native ligand (WR99210)	-6.8	H-Bond	: Asp54 (3.331 Å), Leu164 (3.200 Å)
		van der Waals	: Ile14, Cys15, Ala16, Met55, Asn108, Ile112, Thr185
		Unfavorable Donor-Donor	: Tyr170
		Pi-Sigma	: Phe58
		Amide-Pi Stacked	: Ser111
		Alkyl	: Val45, Leu46
		Pi-Alkyl	: Leu46, Pro113

and Ser111 in the active site of P*f*DHFR-TS since both chloroquine and our synthesized compounds contain pyridine moiety. This result also agreed with the molecular docking result of pyridine derivatives that interacted with Ala16, Leu46, Phe58, Asn108, and Ser111 key amino acid residues on P*f*DHFR-TS receptor (Bekhit et al. 2012). These findings show that the antimalarial mechanism of action of the chalcone-pyridine hybrid compounds is similar to the chloroquine, as well as to the reported chalcones and pyridine derivatives.

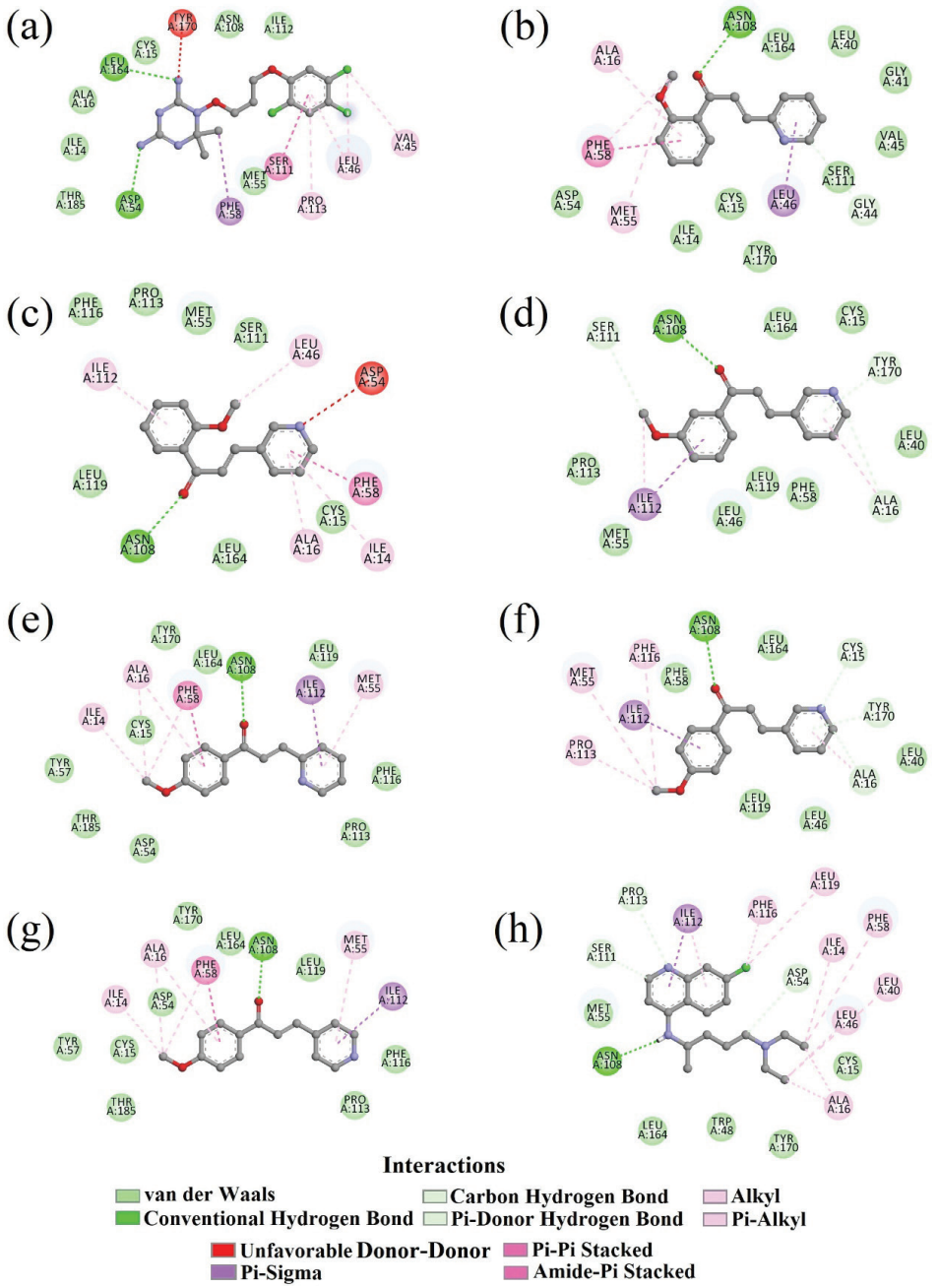


Figure 1. The two-dimensional molecular interactions of **a.** Native ligand; **b–g.** Chalcones A–F, and **h.** Chloroquine in the active site of P*f*DHFR-TS receptor.

Conclusion

Chalcone derivatives bearing methoxy and pyridine moieties were successfully synthesized via a one-pot method using NaOH as a base catalyst, yielding between 53.74 to 86.37%. The structures of all synthesized compounds were confirmed to be highly pure through FTIR, MS, and NMR spectroscopies. *In vitro* assays were performed to examine the antimalarial activity of the synthesized chalcones against *P. falciparum* 3D7 and FCR3 strains. The results indicated that 2-methoxy and 2-pyridine substituents significantly contributed to the antimalarial activity, yielding IC_{50} values of 0.48 and 0.31 µg/mL, respectively. Molecular docking studies revealed that the carbonyl group in chalcones formed a hydrogen bond with the crucial amino acid residue Asn108 in the PfDHFR-TS active site. Furthermore, other interactions such as van der Waals, pi-pi stacking, carbon-H bond, alkyl, and pi-alkyl further stabilized the ligand-receptor interaction.

Acknowledgments

We would like to express our gratitude to the Austrian-Indonesian Centre for Computational Chemistry (AIC) for their generous provision of GaussView 5.0 and Gaussian 09, enabling us to conduct this study.

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