

Original Research Article

Synthesis, antimalarial activity assay and molecular docking study of N-substituted chloro-pyrazolines

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Abstract

Purpose: To synthesize chloro-pyrazolines (A–D), determine their antimalarial activity against *Plasmodium falciparum* strain 3D7 *in vitro*, and understand the interaction between *falcipain-2* active sites and synthesized compounds by molecular docking simulation.

Methods: Chloro-pyrazolines (A–D) were synthesized via cyclo-condensation of 4-chloro chalcone derivatives using several types of hydrazines, i.e., formylhydrazine, benzoylhydrazine, phenylhydrazine and chlorophenylhydrazine. The compounds were analyzed and subjected to antimalarial assay against *P. falciparum* 3D7. Molecular docking was performed using AutoDock Tools and AutoDock Vina, while each docked compound was visualized using Discovery Studio Visualizer.

Results: Pyrazolines A–D yield was 87.09, 61.71, 50.24 and 57.64 %, respectively. Antimalarial assay showed half-maximal inhibitory concentration (IC_{50}) values of 16.46 and 5.55 μ M for pyrazoline A and B, respectively, and ≥ 100 μ M for pyrazoline C and D. Molecular docking study revealed that pyrazolines A–D had good interaction with the active site of *falcipain-2* receptor.

Conclusion: A series of N-substituted chloro-pyrazolines has successfully been synthesized with moderate yield. Pyrazoline B has the highest antimalarial activity against *P. falciparum* 3D7 with IC_{50} of 5.55 μ M. This finding is supported by molecular docking and indicates that the benzoyl substituent increases the antimalarial activity of pyrazoline B. Pyrazoline B has potentials for clinical application as an antimalaria agent.

Keywords: Pyrazoline, Antimalarial, Docking Simulation, *Plasmodium falciparum* 3D7

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INTRODUCTION

Malaria is a disease caused by the *Plasmodium* parasite and transmitted by Anopheles mosquitoes. Globally, it is widespread in regions between longitude 60° in the north and 40° in the south, covering more than 100 tropical and subtropical countries. According to the World

Health Organization (WHO), World Malaria Report in 2018, the number of malaria cases worldwide had reached 219 million in 2017 [1]. The highest number of deaths with 200 million cases was recorded in the African region [1]. In Indonesia, the parasite *P. falciparum* accounts for 21 % of malaria cases, 16 % of which have led to death.

The *Plasmodium* parasite grows and repeatedly develops in regular red blood cells (RBC), thus affecting humans and resulting in disorders such as respiratory problems, kidney failure, anemia, and heart problems [2]. Malaria prevention efforts have been implemented globally, including in Indonesia. This parasite is resistant to some drugs, such as chloroquine [3] and artemisinin, (the first line antimalarial medicine recommended by WHO) [4]. Therefore, developing new antimalarial drugs can be a solution to the problem of antimalarial drug resistance.

Pyrazoline derivatives are heterocyclic organic compounds containing nitrogen atoms. They are of interest to researchers due to their diverse biological activities. Pyrazolines exhibit a wide range of biological activities including antitumor, antimicrobial, and antioxidant properties [5]. It also exhibits antimalarial activity by providing a detoxification pathway from malaria parasites [6]. Reacting aldehydes and unsaturated ketones and then adding hydrazine are often the steps required to synthesize pyrazoline compounds.

A previous study has evaluated the antimalarial activity of pyrazoline derivatives through molecular docking [7]. Pyrazoline derivatives, *i.e.*, coumarin containing pyrazoline inhibits the falcipain-2 enzyme, which degrades red cell hemoglobin to provide free amino acids for parasite protein synthesis [7,8].

In this study, several pyrazoline compounds were synthesized, and their antimalarial activities against *P. falciparum* 3D7 strain was evaluated. Molecular docking was also applied to the target falcipain-2 to understand the possible mechanism of synthesized pyrazolines as antimalarial drug [9].

EXPERIMENTAL

Materials

All reagents were of analytical grade, obtained from Merck, (Germany) and were used as received. They include veratraldehyde, *p*-chloroacetophenone, hydrazine hydrate, phenylhydrazine, *p*-chlorophenyl-hydrazine, benzoyl chloride, glacial acetic acid, formic acid, hydrochloric acid (37 %), absolute ethanol, methanol, *n*-hexane, dichloromethane, and ethyl acetate.

Thin-layer chromatography (20 × 20 cm aluminum plate) coated with silica gel 60 F254 (Merck) was applied to control the reaction. The materials used in the antimalarial test were

Roswell Park Memorial Institute (RPMI) solution, dimethyl sulfoxide (DMSO), inoculum solution containing RPMI solution, human serum, normal and infected RBC, Giemsa dye, methanol, and emulsion oil.

Instrumentation

All melting points were determined using Electrothermal 9100. Infrared spectra were acquired from Shimadzu Prestige-21 using KBr discs. Mass spectra and purity of the products were obtained from Shimadzu QP2010S (EI) GC-MS spectrometer, while the NMR spectra were obtained on JEOL JNM-ECA 500 MHz (¹H) and 125 MHz (¹³C) using an internal standard of tetramethylsilane. Antimalarial assay was carried out using 96 microwell plate (Biologix), micropipette (Gilson and Thermo Scientific), candle jar, microcentrifuge (Thermo Sorvall Legend Micro 17R), and microscope (Nikon Eclipse E100).

Synthesis of chalcone and Pyrazolines A-D

4-chloro-(3',4'-dimethoxy)-chalcone (chalcone) was synthesized following the method of Suma *et al* [10].

1-formyl-(3-(4-chlorophenyl)-5-(3,4-dimethoxy)-4,5-dihydro-2-pyrazoline (pyrazoline A)

Chalcone (0.344 g, 1 mmol) was dissolved in 10 mL of ethanol. This solution was then added to hydrazine hydrate (0.2 mL, 4 mmol), and the mixture was refluxed for 2 h. Afterward, 5 mL of formic acid and two drops of 37 % HCl were consecutively added, and the mixture was continuously refluxed for 3 h. The mixture was poured into ice-cold water and stored in a refrigerator overnight. The precipitate was filtered off, washed with water, and dried in a desiccator. After drying, the solid was recrystallized using methanol to obtain pyrazoline **A**.

1-benzoyl-(3-(4-chlorophenyl)-5-(3,4-dimethoxy)-4,5-dihydro-2-pyrazoline (pyrazoline B)

Chalcone (0.15 g, 0.5 mmol) was dissolved in 10 mL of ethanol and then consecutively added with hydrazine hydrate (0.2 mL, 4 mmol), glacial acetic acid (0.5 mL, 8 mmol), and benzoyl chloride (0.2 mL, 7 mmol). The solution was refluxed for 5 h. After acidification, filtration, washing, and recrystallization using methanol, the sample was dried to yield pyrazoline **B**.

1-phenyl-(3-(4-chlorophenyl)-5-(3,4-dimethoxy)-4,5-dihydro-2-pyrazoline (pyrazoline C)

Pyrazoline **C** was prepared similarly to **B** using a mixture of chalcones (0.15 g, 0.5 mmol), 5 mL of glacial acetic acid, and phenylhydrazine (0.05 mL, 0.5 mmol) in 10 mL of ethanol under reflux for 4 h.

1-chlorophenyl-(3-(4-chlorophenyl)-5-(3,4-dimethoxy)-4,5-dihydro-2-pyrazoline (pyrazoline D)

Pyrazoline **D** was prepared similarly to **B** by reacting chalcone (0.15 g, 0.5 mmol), 5 mL of glacial acetic acid, and chlorophenyl hydrazine (0.05 mL, 0.5 mmol) in 10 mL of ethanol under reflux for 8 h.

Antimalarial activity assay of pyrazolines A–D

Antimalarial test was performed by preparing a stock solution with 1 mg of sample and 100 μ L of DMSO and adding 990 μ L of RPMI. The sample was diluted to several concentrations of 10, 5, 1 and 0.5 μ g/mL and then transferred to microplate 96 wells. Parasite culture with parasitemia \pm 1 % was prepared, and 100 μ L of parasite (inoculum solution) was added to the test solution. The microplate was placed in a candle jar and incubated for 72 h at 37 °C. After incubation, the parasites were harvested and colored by 10 % Giemsa staining. The number of infected RBC against 1000 cells of normal RBC was counted under a microscope, and the percent inhibition was calculated. The data were analyzed statistically using probit analytical methods to calculate the parasitic resistance (IC₅₀) [10].

Molecular docking study of Pyrazolines A–D

The 3D chloro-pyrazoline **A–D** structures were geometrically optimized using DFT/B3LYP with a 6-31G basis set in Gaussian® 09W. As the targeted receptor, the crystal structure of falcipain-2 complexed with inhibitor E64 (PDB ID: 6JW9) was retrieved from Protein Data Bank. Molecular docking was performed using AutoDock Tools and Autodock Vina in the grid box size of 14 Å × 14 Å × 14 Å with a grid spacing of 1.00 Å. Exhaustiveness value was also set to its default value, which was 8. As a standard ligand, redocking against E64 inhibitor was conducted to validate the molecular docking procedure and the root mean square deviation (RMSD) value of redocking was calculated. The docking poses were ranked according to their binding affinity. The most suitable conformation was selected according to the lowest binding

affinity, which represented the most stable and favorable conformation of the docked compound. Each docked compound was visualized using Discovery Studio Visualizer.

RESULTS

Chemistry

1-formyl-(3-(4-chlorophenyl)-5-(3,4-dimethoxy)-4,5-dihydro-2-pyrazoline (pyrazoline A)

Pyrazoline **A** yield was 87.09 % as a white solid with 96.65 % purity. The FTIR spectra (KBr, cm⁻¹) was as follows: 3030 (C_{sp}²-H), 2931 (C_{sp}³-H), 2854 (C-H aldehyde), 1666 (C=O carbonyl), 1597 (C=N), 1512 (C=C aromatic), 1141 (C-N aliphatic), 1234 (C-O-C asymmetric), 1026 (C-O-C symmetric), and 1087 (C-Cl). ¹H-NMR spectrum (500 MHz, CDCl₃, ppm) was as follows: δ 3.10 (*dd*, *J*_{AB} = 18 Hz, *J*_{AX} = 5 Hz, 1H, CH₂), 3.75 (*dd*, *J*_{BA} = 18 Hz, *J*_{BX} = 12 Hz, 1H, CH₂), 3.83 (*s*, 3H, OCH₃), 3.84 (*s*, 3H, OCH₃), 5.47 (*dd*, *J*_{AX} = 5 Hz, *J*_{BX} = 12 Hz, 1H, CH), 6.47 (*d*, *J* = 1.5, 1H, Ar-H), 6.8 (*m*, 2H, Ar-H), 7.40 (*d*, *J* = 8.5 Hz, 2H, Ar-H), 7.60 (*d*, *J* = 8.5 Hz, 2H, Ar-H), and 8.90 (*s*, 1H, CHO). ¹³C-NMR spectrum (125 MHz, CDCl₃, ppm) was as follows: δ 42.54 (CH₂), 55.93 (O-CH₃), 56.55 (O-CH₃), 59.02 (N-CH), 108.93 (Ar-H), 111.53 (Ar-H), 118.86 (Ar-H), 127.90 (2 Ar-H), 129.09 (2 Ar-H), 129.42 (Ar), 133.22 (Ar), 136.62 (Ar), 148.80 (Ar-O), 149.40 (Ar-O), 154.66 (C=N), and 160.09 (C=O). Mass spectrum (EI) was as follows: *m/z* 346 (M+2, ³⁷Cl, 25 %), 344 (M⁺, ³⁵Cl, 35), 315 (10), 207 (5), 176 (68), 163 (48), 151 (28), 121 (30), 91 (70), and 77 (100).

1-benzoyl-(3-(4-chlorophenyl)-5-(3,4-dimethoxy)-4,5-dihydro-2-pyrazoline (pyrazoline B)

Pyrazoline **B** was obtained as a white solid up to 61.71 % yield with 92.57 % purity. The FTIR (KBr, cm⁻¹) spectra shows: 3062 (C_{sp}²-H), 2931 (C_{sp}³-H), 1643 (C=O carbonyl), 1589 (C=N), 1512 (C=C aromatic), 1141 (C-N aliphatic), 1258 (C-O-C asymmetric), 1026 (C-O-C symmetric), and 1088 (C-Cl). ¹H-NMR (500 MHz, CDCl₃, ppm) δ 3.19 (*dd*, *J*_{AB} = 17 Hz, *J*_{AX} = 4.5 Hz, 1H, CH₂), 3.76 (*dd*, *J*_{BA} = 17.8 Hz, *J*_{BX} = 12 Hz, 1H, CH₂), 3.85 (*s*, 3H, OCH₃), 3.86 (*s*, 3H, OCH₃), 5.78 (*d*, *J* = 7 Hz, 1H, CH), 6.82 - 6.87 (*m*, 3H, Ar-H), 7.38 (*d*, *J* = 8 Hz, 2H, Ar-H), 7.45 - 7.50 (*m*, 3H, Ar-H), 7.64 (*d*, *J* = 8 Hz, 2H, Ar-H), and 7.99 (*d*, *J* = 5.5 Hz, 2H, Ar-H). ¹³C-NMR (125 MHz, CDCl₃, ppm): δ 41.55 (CH₂), 55.89 (2 O-CH₃), 61.14 (C-N), 108.98 (Ar-H), 111.46 (Ar-H), 117.57 (Ar-H), 127.69 (2 Ar-H), 127.97 (2 Ar-H), 129.00 (2 Ar-H), 129.83 (Ar-H), 130.00 (Ar), 131.03 (Ar),

134.25 (Ar), 136.34 (Ar), 148.50 (Ar-O), 149.20 (Ar-O), 153.57 (C=N), and 165.50 (C=O). Mass spectrum (EI): m/z 421 (M+2, ³⁷Cl, 5%), 419 (M+, ³⁵Cl, 5), 314 (5), 282 (15), 137 (5), 105 (100), 77 (70), and 51 (35).

1-phenyl-(3-(4-chlorophenyl)-5-(3,4-dimethoxy)-4,5-dihydro-2-pyrazoline (pyrazoline C)

Pyrazoline **C** was produced as a yellowish–white solid and the yield was 50.24 % with 100 % purity. The FTIR (KBr, cm⁻¹) showed: 3062 (C_{sp}²-H), 2931 (C_{sp}³-H), 1597 (C=N), 1496 (C=C aromatic), 1134 (C-N aliphatic), 1234 (C-O-C asymmetric), 1026 (C-O-C symmetric), and 1087 (C-Cl). ¹H-NMR (500 MHz, CDCl₃, ppm): δ 3.10 (dd, J_{AB} = 17.5 Hz, J_{AX} = 7.8 Hz, 1H, CH₂), 3.78 (dd, J_{BA} = 17.5 Hz, J_{BX} = 12 Hz, 1H, CH₂), 3.82 (s, 3H, OCH₃), 3.86 (s, 3H, OCH₃), 5.21 (dd, J_{AX} = 7.8 Hz, J_{BX} = 12 Hz, 1H, CH), 6.81 (m, 3H, Ar-H), 6.87 (dd, J = 8.45 Hz, 1H, Ar-H), 7.08 (d, J = 7.75 Hz, 2H, Ar-H), 7.19 (t, J = 8.1 Hz, 2H, Ar-H), 7.35 (d, J = 8.4 Hz, 2H, Ar-H), and 7.65 (d, J = 8.45 Hz, 2H, Ar-H). ¹³C-NMR (125 MHz, CDCl₃, ppm): δ 43.72 (CH₂), 56.08 (O-CH₃), 56.11 (O-CH₃), 64.92 (C-N), 108.74 (Ar-H), 111.67 (Ar-H), 113.72 (2 Ar-H), 118.20 (Ar-H), 119.63 (Ar-H), 127.08 (2 Ar-H), 128.95 (2 Ar-H), 129.09 (2 Ar-H), 131.46 (Ar), 134.47 (Ar), 135.12 (Ar), 145.06 (Ar), 145.94 (Ar), 148.81 (Ar), and 149.81 (C=N). Mass spectrum (EI): m/z 394 (M+2, ³⁷Cl, 25 %), 392 (M+, ³⁵Cl, 10), 255 (5), 228 (5), 163 (5), 151 (5), 121 (5), 91 (100), 77 (45), 65 (25), and 51 (20).

1-chlorophenyl-(3-(4-chlorophenyl)-5-(3,4-dimethoxy)-4,5-dihydro-2-pyrazoline (pyrazoline D)

Pyrazoline **D** was obtained as a yellow solid with 67.64 % yield and 94.70 % purity. The FTIR (KBr, cm⁻¹) revealed: 3070 (C_{sp}²-H), 2924 (C_{sp}³-H), 1597 (C=N), 1489 (C=C aromatic), 1134 (C-N aliphatic), 1234 (C-O-C asymmetric), 1026 (C-O-C symmetric), and 1087 (C-Cl). ¹H-NMR (500 MHz, CDCl₃, ppm): δ 3.11 (dd, J_{AB} = 17 Hz, J_{AX} = 8 Hz, 1H, CH₂), 3.82 (s, 3H, OCH₃), 3.86 (s, 3H, OCH₃), 3.97 (dd, J_{BA} = 13 Hz, J_{BX} = 5 Hz, 1H, CH₂), 5.17 (dd, J_{AX} = 8 Hz, J_{BX} = 13 Hz, 1H, CH), 6.72 (s, 1H, Ar-H), 6.76 (d, J = 6.5 Hz, 1H, Ar-H), 6.99 (d, J = 9 Hz, 1H, Ar-H), 7.36 (d, J = 9 Hz, 2H, Ar-H), 7.40 (d, J = 9 Hz, 2H, Ar-H), 7.64 (d, J = 8.5 Hz, 2H, Ar-H), and 7.83 (d, J = 8.5 Hz, 2H, Ar-H). ¹³C-NMR (125 MHz, CDCl₃, ppm): δ 43.62 (CH₂), 55.90 (O-CH₃), 55.93 (O-CH₃), 64.65 (C-N), 108.36 (Ar-H), 111.46 (Ar-H), 114.61 (2 Ar-H), 117.96 (Ar-H), 124.16 (Ar), 126.93 (2 Ar-H), 128.77 (2 Ar-H), 128.80 (2 Ar-H), 130.96 (Ar),

134.29 (Ar), 134.53 (Ar), 143.32 (Ar), 146.33 (Ar-O), 148.51 (Ar-O), and 149.65 (C=N). Mass spectrum (EI): m/z 428 (M+2, ³⁷Cl, 10 %), 426 (M+, ³⁵Cl, 10), 289 (5), 262 (5), 178 (5), 163 (10), 125 (100), 90 (60), 77 (30), and 51 (20).

Antimalarial activity of pyrazoline A–D

Pyrazolines **A–D** were tested for their antimalarial activity by their inhibition percentage of parasite growth indicated by the IC₅₀ values from probit analysis, and the results are presented in Table 1. Batista *et al* [12] categorized the antimalarial ability of a compound as either active, moderate, low, or inactive based on IC₅₀ values in μM.

Table 1: Antimalarial activity of pyrazolines against *Plasmodium falciparum* 3D7

Pyrazoline	Substituent	IC ₅₀ (μg/mL)	IC ₅₀ (μM)
A	Formyl	5.68	16.46
B	Benzoyl	2.34	5.55
C	Phenyl	432.19	1100.06
D	Chlorophenyl	427.33	544.10

Molecular docking

Docking study was conducted to determine the interaction and calculate the binding affinity of pyrazolines **A–D** on the active site of falcipain-2. The visualization of their interactions is shown in Figure 1 and Figure 2, and the docking results are tabulated in Table 2. Pyrazolines **A – D** were synthesized through conventional cyclocondensation by reacting some hydrazine derivatives with chalcones. The scheme of pyrazolines **A – D** synthesis is shown in Figure 3.

DISCUSSION

Pyrazolines **A – D** yield was 87.09, 61.47, 50.24, and 67.64 %, respectively, and their structures were identified using FTIR, GC-MS, ¹H-NMR, and ¹³C-NMR spectrometry. The FTIR spectra showed that all products have the same pyrazoline ring characteristics as indicated by the appearance of absorption bands at 1660 cm⁻¹ for the C=N bond and 1134 cm⁻¹ for the C-N bond. Furthermore, the bending absorption band of *trans*-disubstituted alkene disappeared when chalcones were used as reactants. Mass spectra further showed the related molecular ion (M⁺) for each pyrazoline sample, i.e., m/z 344 (A), 419 (B), 392 (C), and 426 (D). The ¹H-NMR spectra supported these assignments by showing pyrazoline's characteristics in the ABX system as three peaks with multiple doublets of doublets at 3.10 - 3.19, 3.75 - 3.97, and 5.17 - 5.78 ppm [13].

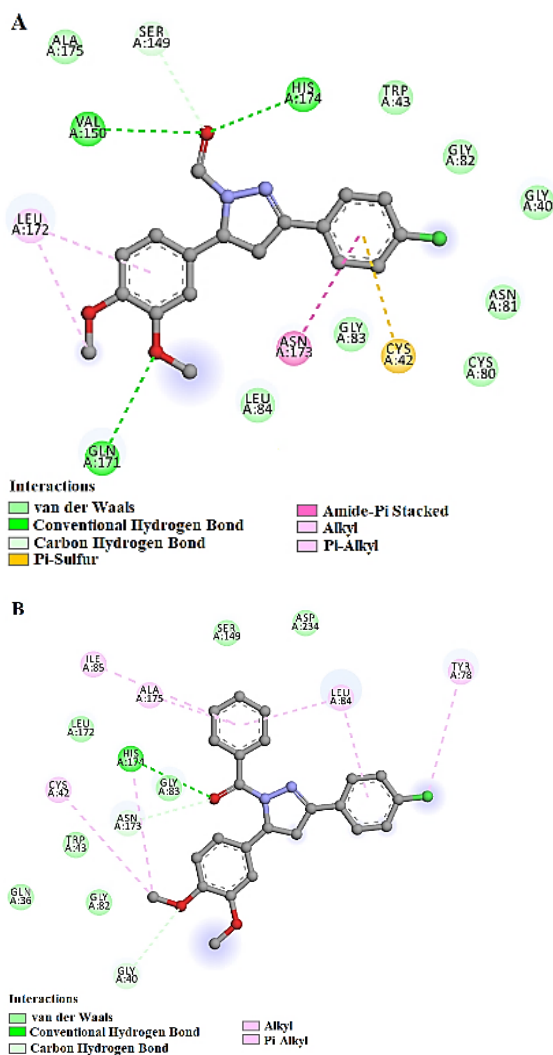


Figure 1: Interaction of falcipain-2 receptor with (A) pyrazoline A, (B) pyrazoline B from molecular docking model

Furthermore, ^{13}C -NMR spectra confirmed the presence of carbon atoms in the pyrazoline ring and revealed the correct number of carbons for the related pyrazoline, i.e., 16-C (A), 18-C (B), 19-C (C), and 19-C (D).

In vitro antimalarial assay revealed that the carbonyl group bound to nitrogen had a higher antimalarial activity than pyrazoline, which bonded to the phenyl group on nitrogen. Pyrazolines A and B are classified as active antimalarial compounds with IC_{50} values of 8.25 and 2.79 $\mu\text{g}/\text{mL}$, respectively, and pyrazolines C and D were considered as inactive antimalarial agents with IC_{50} value greater than 100 $\mu\text{g}/\text{mL}$. The activity loss for pyrazolines C and D is possibly due to the absence of carbonyl groups that bind to nitrogen, which instead is able to bind to *P. falciparum* proteins.

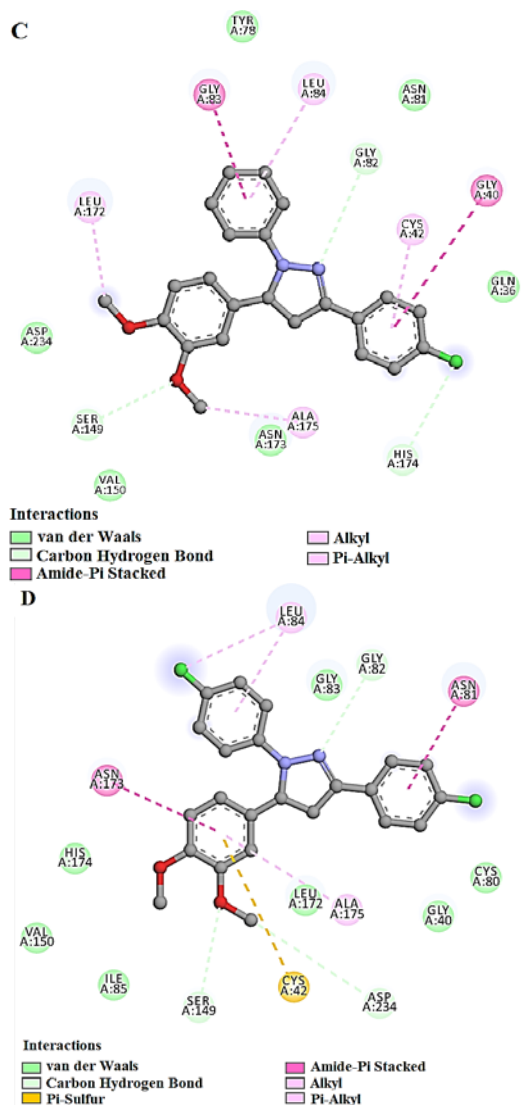


Figure 2: Interaction of falcipain-2 receptor with (C) pyrazoline C, and (D) pyrazoline D from molecular docking model

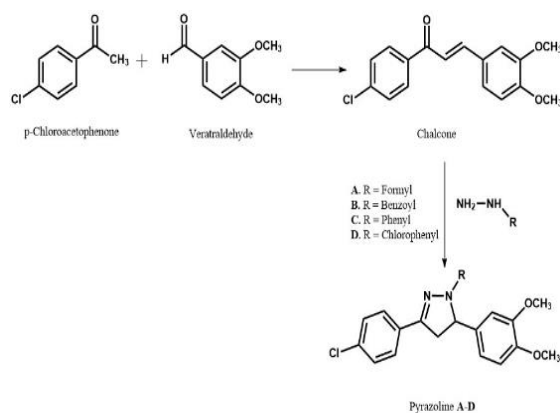


Figure 3: Scheme of synthesis of pyrazolines A – D

Table 2: Molecular docking results of pyrazolines **A–D**

Compound	Binding affinity (kcal/mol)	Interaction
Inhibitor E64 (Standard ligand)	-4.6	H-Bond: ASN173, GLY83, CYS42 Carbon H-Bond: TRP43, GLY82 Alkyl: ALA175 van der Waals: SER41, GLY40, TYR78, LEU84, VAL150, SER149, LEU172, HIS174
Pyrazoline A	-6.3	H-Bond: HIS174, VAL150, GLN171 Pi-Alkyl: LEU172 Alkyl: LEU172 Carbon H-Bond: SER149 van der Waals: ALA175, LEU84, GLY83, ASN81, CYS80, GLY40, GLY82, TRP43 Amide-Pi Stacked: ASN173 Pi-Sulfur: CYS42
Pyrazoline B	-7.7	H-Bond: HIS174 Carbon H-Bond: GLY40, ASN173 Alkyl: CYS42 Pi-Alkyl: HIS174, TYR78, LEU84, ALA175, ILE85 van der Waals: LEU172, TRP43, GLY82, ASP234, SER149, GLY83
Pyrazoline C	-7.2	Carbon H-Bond: SER149, GLY82, HIS174 Alkyl: LEU172, ALA175 Pi-Alkyl: LEU84, CYS42 Amide-Pi Stacked: GLY40, GLY83 van der Waals: ASN81, ASP234, TYR78, GLN36, VAL150, ASN173
Pyrazoline D	-6.7	Carbon H-Bond: SER149, ASP234, GLY82 Pi-Alkyl: LEU84, ALA175 Alkyl: LEU84 Amide-Pi Stacked: ASN81, ASN173 Pi-Sulfur: CYS42 van der Waals: HIS174, VAL150, ILE85, LEU172, GLY40, GLY83, CYS80.

Therefore, the antimalarial activity of pyrazoline as an antimalarial agent is influenced by the type of substituent on the nitrogen atom. Inhibitor (E64) was stable enough to attach to the binding site with a -4.6 kcal/mol binding affinity. The RMSD value of redocking was 2.840 Å, indicating that the RMSD value was acceptable [11]. The redocked inhibitor E64 interacted via hydrogen bonds on GLY83 (2.83 and 2.25 Å), CYS42 (2.32 Å), ASN173 (distance: 2.70 Å), and other interactions such as carbon hydrogen bond, alkyl, and van der Waals interactions.

Pyrazoline **A** has a relatively high binding affinity but shows good antimalarial activity. Unlike the other pyrazolines, pyrazoline **A** forms three hydrogen bonds with HIS174, VAL150, and GLN171. Meanwhile, the amino acid residue of HIS174 is a crucial catalytic residue of falcipain-2 [14]. Therefore, the recorded activity is probably due to the presence of hydrogen bonding of pyrazoline **A** with HIS174 (2.69 Å). Pyrazoline **B** had the lowest binding affinity among the docked compounds and had an excellent IC₅₀ from the antimalarial assays. Pyrazoline **B** forms a hydrogen bond on one of the crucial amino acid residues, *i.e.*, HIS174, which may lower its binding affinity as well as increase its antimalarial

activity. All the docked compounds also interacted with the important amino acid residues of falcipain-2, namely, LEU84, ASN173, HIS174, and ALA175 which are crucial in binding and catalysis [15]. This analysis proved that the antimalarial activity of pyrazoline **B** could be enhanced by the presence of the benzoyl group, which can form hydrogen bond, pi-alkyl, carbon hydrogen bond, and van der Waals interactions on the active site of falcipain-2.

CONCLUSION

A series of high purity *N*-substituted chloro-pyrazolines was successfully synthesized via the cyclo-condensation of some hydrazine derivatives with chalcones. Activity assay revealed that pyrazoline **B** has the best antimalarial activity against *P. falciparum* 3D7. Molecular docking study showed that pyrazolines **A–B** are potential inhibitors of falcipain-2, and the presence of benzoyl substituent on pyrazoline **B** significantly increases the antimalarial activity and lowers the binding affinity of this derivative. Pyrazoline **B** has potentials for further development into antimalarial agent against resistant *P. falciparum* strains.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was accomplished by the authors named in this article, and the authors will bear all liabilities of the claim relating to the content of this article. Wahyuningsih conceived the original idea, supervised the project, and finalized the manuscript. Wiratama carried out the synthesis and bioactivity assay experiments. Waskitha performed the molecular docking while Wiratama and Waskitha wrote the manuscript, and Haryadi helped supervise the project. All authors discussed the results and contributed to the final manuscript.

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