



Novel Cinchona Alkaloid Derivatives as Potential Antimalarial Agents Through Receptor–Inhibitor Interaction Fingerprint and Biosynthesis Design

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ABSTRACT

Malaria parasites have become the major health threat in increasing resistance toward common antimalarial drugs and become prime factors causing the strength of the disease. The objective of this study was investigating novel cinchona alkaloid derivatives (CADs) as potential antimalarial agents through molecular docking, pharmacophore modeling and biosynthesis design. Protein structure and cinchona alkaloid derivative structures were taken and performed for molecular interaction studies, pharmacophore modeling and mapping the binding modes of receptor-inhibitors which may increase the possibility of success rate in finding potential antimalarial candidates. Here, we report the greatest prospective inhibitor of *Plasmodium falciparum* (Pf falcipain-2, PDB ID code 2ghu) falcipain-2 is cinchonidine salicylate (-9.1 kcal/mol) through molecular docking approach. This compound exhibited distortion free of Lipinski's rule. Hence, cinchonidine salicylate showed the most potential compound as antimalarial inhibitor over other cinchona alkaloid derivatives. Eventually, we construct biosynthesis pathways by using iron oxide nanoparticle (IONP) that could act as a coated nanoparticle to the natural bioactives to acquire optimum yield of the product by making coated nanoparticle with CADs which are powerful biosynthesis application in green environment of aqueous solution.

Keywords: Cinchona alkaloid derivatives, Antimalaria, Molecular docking, Biosynthesis.

INTRODUCTION

Malaria is an endemic disease caused by parasitic infection which influencing over than 224 million people and effecting mortality approximately 700,000 every year in the world¹. According to 41% of total world's population, malaria is spreading in

some areas of Africa, Asia, America, Hispaniola, and Oceania²⁻⁴, where Africa is the highest malarial prevalent area of the world annually⁵⁻⁷. Malaria is known as a vector born disease transmitted by female *Anopheles* mosquito and parasitic infection mostly caused by five species, including *Plasmodium ovale*, *Plasmodium vivax*, *Plasmodium falciparum*, *Plasmodium malariae* and *Plasmodium knowlesi*⁸.



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There are three families of drugs currently used for eradicating malaria disease, such as antifolates (pyrimethamine, sulfadoxine), quinolones (chloroquine, primaquine, quinine, mefloquine) and artemisinin derivatives. However, these drugs have limitation due to high cost and high resistance rate to malaria endemic causing frequent chemotherapy failure⁹⁻¹². Understanding the resistance mechanisms could enhance new therapies¹³⁻¹⁵. Thus, drug resistances are becoming key concerns in eradicating malaria. Various promising antimalarial agents have already been developed. Unfortunately, only few promising antimalarial agents contribute sufficient efficacy and small effect toxicity for antimalarial therapies¹⁶⁻¹⁸. Therefore, several attempts have been started to identify clinical targets for healing human malarial disease. Molecular modeling¹⁹⁻²² and database formation approaches as new techniques are currently in the innovation development and execution stages²³⁻²⁶ to enhance potential antimalarial targets²⁷⁻³¹.

In this work, we brought out quantitative structure activity relationship (QSAR) analysis of cinchona alkaloid derivatives as falcipain-2 inhibitors using molecular docking and pharmacophore analysis methods. Moreover, we explored the prime interactions of intermolecular between cinchona alkaloid derivatives and Pf falcipain-2 utilizing molecular docking approach and used this datasets to improve rational drug design methods. Thus, simultaneous QSAR and virtual screening through docking could give benefits to understand the structure–activity relationships of falcipain-2 inhibitors and the design of new potent malarial inhibitors.

MATERIALS AND METHODS

Preparation of crystallographic protein structure

The crystallographic structure of falcipain-2 protein of *Plasmodium falciparum* (Pf falcipain-2, PDB ID code 2ghu) was retrieved from Protein Database (PDB). The removal of ligand binding and all crystallographic water molecules of falcipain-2 structure were done. The final falcipain-2 structure was saved in pdb format for further in silico analysis.

Preparation of CAD datasets

Cinchona alkaloid derivative 3D structures were generated by ChemDraw Ultra 12.0^{32,33} and saved in pdb formats. The atomic spatial arrangement

energies of all the 46 CADs were minimized by using MMFF94 force field. The 46 molecules of CADs were designed by substituting –R group positions of CADs on the side chains. The structures were calculated based on physicochemical properties using Chemicalize (ChemAxon)³⁴ and Molsoft³⁵ platforms. These physicochemical properties are important for evaluating drug candidate in drug discovery from initial design to in vitro and in vivo studies.

The drug-like properties analysis of CADs

Molsoft Drug – Likeness Platform were used in analyzing physicochemical properties of all the 46 CADs 3D structures. The physicochemical properties were identified including molecular weight, Log P, hydrogen bond donor (HBD), hydrogen bond acceptor, and total polar surface of CADs compounds were studied. The drug-likeness were evaluated following Lipinski's rule of five.

Molecular docking analysis of Pf falcipain-2 and CADs

All the 46 CAD compounds were docked into Pf falcipain-2 protein using Autodock Vina program (Vina, The Scripps Institute). The partial charges of the ligands and the polar hydrogens in the protein were set, separately using the Gasteiger method in AutoDock tools (ADT). The flexibility of ligand torsion angles were assigned at all rotatable bonds and the protein structure was prepared as rigid form. protein is prepared as a rigid structure. Protein and ligands were saved as pdbqt format and ready for docking analysis. The expected binding energy values should be the lowest value which indicated the most favorable binding interaction between protein and ligand.

RESULTS

In order to investigate the antimalaria potency of cinchona alkaloid derivatives, crystallographic structure of falcipain-2 protein of *Plasmodium falciparum* was obtained and the structure was refined by removing bound-ligand and all crystallographic water structures. The standalone structure of receptor-inhibitors were focused on molecular docking analysis using Autodock Vina. This docking program is a well-known practical function to use algorithm which is according to steered stochastic parallel direct search evolution strategy optimization method that is adequately rapid and moderately

robust, which is known using an algorithm that incorporates the optimization of distinctive evolution technique and binding pocket prediction. The automated binding pocket prediction in conjunction with self-activating protein and ligand preparation fully executes overall measurement process.

Table 1 : The designed cinchona alkaloid derivatives and parental cinchona compound

No.	Compound Name	Substitution of -R group	Molecular Weight
1	Quinine (Q)	HO-	324
2	Q-butanoate	CH ₃ CH ₂ CH ₂ OC(O)-	364
3	Q-isobutanoate	(CH ₃) ₂ CH-OC(O)-	394
4	Q-isovalerate	(CH ₃) ₂ CHCH ₂ OC(O)-	408
5	Q-tiglate	(CH ₃)(CHCH ₂)OC(O)C-	406
6	Quinidine (Qd)	HO-	326
7	Qd-butanoate	CH ₃ CH ₂ CH ₂ (O)CO-	394
8	Qd-isovalerate	(CH ₃) ₂ CH(O)CO-	408
9	Qd-tiglate	(CH ₃) ₂ CHCH ₂ (O)CO-	406
10	Cinchonidine (C)	HO-	294
11	C-butanoate	CH ₃ CH ₂ CH ₂ (O)CO-	364
12	C-isobutanoate	(CH ₃) ₂ CH(O)CO-	364
13	C-isovalerate	(CH ₃)(CH ₂ CH)C(O)CO-	340
14	C-tiglate	(CH ₃) ₂ CHCH ₂ (O)CO-	376
15	Cinchonine (Cn)	HO-	337
16	Cn-butanoate	CH ₃ CH ₂ CH ₂ (O)CO-	364
17	Cn-isovalerate	(CH ₃) ₂ CH(O)CO-	357
18	Cn-tiglate	(CH ₃) ₂ CHCH ₂ (O)CO-	376
19	Hexyl-Q- ether	H ₁₃ C ₆ O-	408
20	Hexyl-Qd-ether	H ₁₃ C ₆ O-	408
21	Hexyl-C-ether	H ₁₃ C ₆ O-	378
22	Hexyl-Cn-ether	H ₁₃ C ₆ O-	378
23	Isopropyl-Q-ether	(CH ₃) ₂ HCO-	366
24	Isopropyl-Qd-ether	(CH ₃) ₂ HCO-	366
25	Isopropyl-Cn-ether	(CH ₃) ₂ HCO-	336
26	Isopropyl-C-ether	(CH ₃) ₂ HCO-	336
27	Buthyl quinine ether	-OC ₄ H ₉	380
28	Buthyl quinidine ether	-OC ₄ H ₉	380
29	Buthyl cinchonine ether	-OC ₄ H ₉	350
30	Buthyl cinchonidine ether	-OC ₄ H ₉	350
31	Cinchonidine 4-hydroxybenzoate	-O ₃ C ₇ H ₅	414
32	Cinchonidine octanoate	-OC ₈ H ₁₅	420
33	Cinchonidine salicylate	-O ₃ C ₇ H ₅	414
34	Cinchonidine succinate	-O ₄ C ₄ H ₄	394
35	Cinchonine octanoate	-O ₃ C ₇ H ₅	414
36	Cinchonine salicylate	-OC ₈ H ₁₅	420
37	Cinchonine succinate	-O ₃ C ₇ H ₅	414
38	Cinchonine 4-hydroxybenzoate	-O ₄ C ₄ H ₄	394
39	Quinidine 4-hydroxybenzoate	-O ₃ C ₇ H ₅	444
40	Quinidine octanoate	-OC ₈ H ₁₅	450
41	Quinidine salicylate	-O ₃ C ₇ H ₅	444
42	Quinidine succinate	-O ₄ C ₄ H ₄	424
43	Quinine 4-hydroxybenzoate	-O ₃ C ₇ H ₅	430
44	Quinine octanoate	-OC ₈ H ₁₅	450
45	Quinine salicylate	-O ₃ C ₇ H ₅	444
46	Quinine succinate	-O ₄ C ₄ H ₄	414

From all the 46 CADs tested, the variation of R-substitutions gave different molecular weights ranging from 294.17 to 450.29 (Table 1). The molecular interactions found in molecular docking analysis were shown by the presence of cinchona alkaloid derivatives in various affinity energies interacting with Pf Falcipain-2 (Table 2). It showed the best inhibition activity in terms of affinity energy is cinchonidine salicylate (-9.1 kcal/mol). All CADs also showed followed Lipinski's law and only 4 out of 46 that have 1 violation. According to the lowest affinity energy values of all CADs, only Cinchonidine salicylate was selected for further bioactivity analyses among others.

CADs have various inhibition activities, with inhibitor constant (K_i) values ranging from 0.2 to 109 μM . It indicates the potent of Pf falcipain-2 inhibitor which is a number of concentration required to produce half maximum inhibition, as depicted in Fig.1. Molecular docking interaction between Pf falcipain-2 and cinchonidine salicylate is described in Fig. 2. The chemical interaction between cinchonidine salicylate and Pf falcipain-2 resulted nine hydrophobic interactions to stabilize the binding mode of cinchonidine salicylate in cavity site of Pf falcipain-2. Two hydrogen bonds also contributed in stabilizing complex interaction Figure 3.

It has been known that physical and chemical nanomaterial synthesis procedures are either complicated, time consuming or expensive. Sometimes, the unwanted toxic chemicals could stay adsorbed to the surface of nanomaterials and ultimately prevent its wide range therapeutic applications³⁶⁻³⁸. A mild, less costly, fast and simple method for the biosynthesis IONPs has been successfully exhibited at the first time by using aqueous leaf extracts of *Sageretia thea*³⁹. Biomimetic synthesis is more suitable due to it has no obstacle relative to the physical or chemical methods. Hence, biomimetic synthesis could be effectively used for biological applications. Here, we propose deionized water technique in aqueous extraction of CA natural resources and coating with iron oxide nanoparticle to produce IONP coated with CAD, as we describe on Figure 4.

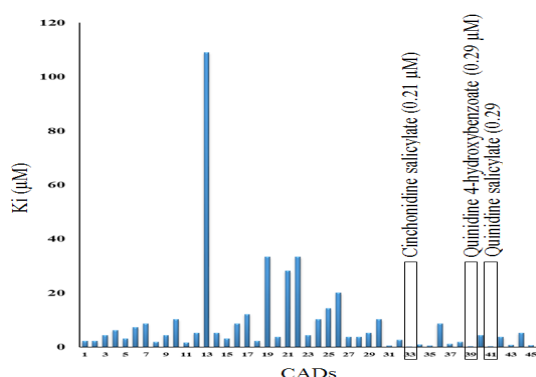


Fig. 1. Inhibition constant (K_i) values of CADs

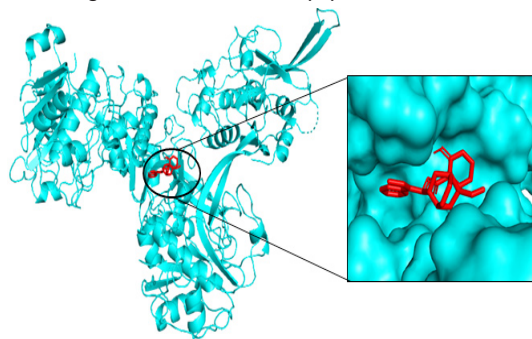


Fig. 2. Binding pose of cinchonidine salicylate (red) in interaction to the various subsites of Pf Falcipain-2 (cyan)

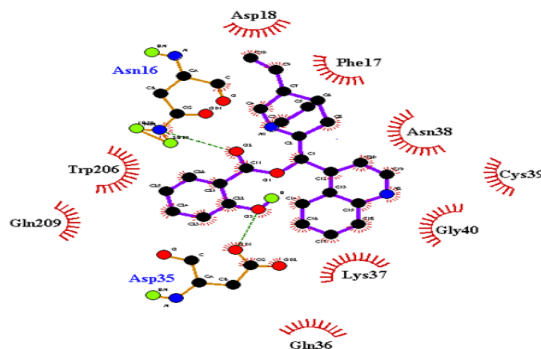


Fig. 3. Chemical complex interaction of Pf Falcipain-2 and cinchonidine salicylate

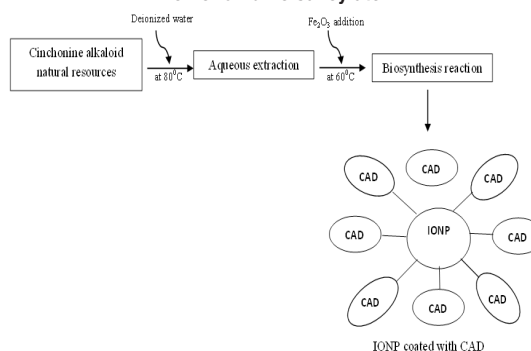


Fig. 4. Schematic biosynthesis design of cinchona alkaloids derivatives (CAD) with iron oxide nanoparticle (IONP)

DISCUSSION

Previous experiments targeting Pf falcipain-2 using two inhibitors, cystatin⁴⁰ and chagasin⁴¹ have failed due to their poor pharmacological profiles besides being prone to degradation by host enzymes. Thus, we drive to investigate new potential antimalarial drugs from native plant sources of Indonesia. The compounds are mainly alkaloids and phenolics, which have antimalarial activities such as cinchona alkaloids⁴²⁻⁴⁴. Some of these compounds have known biological activities. For instance, quinine, quinidine, cinchonine and cinchonidine are identified as antiparasitic malaria converting enzyme inhibitors⁴⁵⁻⁴⁷. Docking results showed that inhibition activities of CAD compounds exhibited poor to excellent binding affinities to targeted Pf falcipain-2 (Table 2). In most cases, most of these poor binders had a long carbon chain or an attached nucleus hence could not fit in the "ditch-like" binding pocket, the characteristic of the falcipains. These results increase our understanding of the structure-function of complex interactions. Thus, based on the binding energy and hydrogen bond interaction, it can be confirmed that cinchonidine salicylate is the best inhibitor to Pf falcipain-2.

Moreover, we analyze the molecular interactions through chemical bonds that are found in docking interaction between cinchonidine salicylate and Pf falcipain-2. The result showed that the best affinity energy of cinchonidine salicylate and falcipain-2 interaction was -9.1 kcal/mol as depicted in Fig. 2. The complex interaction was stabilized by nine residues of Pf falcipain-2 present in binding pocket, such as Asp-18, Phe-17, Gln-36, Asn-38, Cys-39, Gly-40, Lys-37, Gln-209 and Trp-206 (Fig. 3). Non-ligand residues involved in two hydrogen bonds are Asn-16 and Asp-35. A correlation coefficient of CADs were calculated to obtain Ki values of the inhibitors against Pf falcipain-2 and the corresponding interaction energies from docking results Figure 1.

Based on molecular docking result, cinchonidine salicylate is the best candidate as antimalaria from all the 46 constructed molecules of CADs. This dataset will be used as a candidate for molecule organic synthesis and bioactive validation into *in-vivo* and *in-vitro* experiments.

Furthermore, we propose the design of green synthesis of CAD which is non toxic and more effective through the interface of eco-friendly and nanomaterial technology application allows a great line to synthesize CADs, for instance cinchonidine salicylate with eco-friendly and safe all-around functional nanoparticles of metal oxide. Multifunctional iron oxide nanoparticles (IONPs) have arose as an expectant material for various applications, such as cosmeceutical, biomedicine, bioremediation and material engineering purposes³⁹. Surprisingly, IONPs utilization in biomedical field are in high demand and growth exponentially^{48, 49}. The capability of IONPs in the restraint detach of drugs including for tissue restoration, medical treatment and diagnostic has previously been proven^{50, 51}. The wide range of IONP applications can be assigned by ideal properties such as high surface area, small band gap and stability. This proposed schematic biosynthesis with IONPs would be very beneficial in complete environmental friendly process utilizing the aqueous plant part extracts of CADs. The phytochemical resources is an effective reducing toxic effects as well as capping agent without adding any chemical reagents.

The development of protease inhibitors become a promising approach in antimalarial drug discovery, particularly in interfering organisms with capability in metabolizing human hemoglobin as main nutrient sources. A great relationship between the affinity energies for docked CAD compounds and their bound Ki values assured to be an acceptance for the protein target. Moreover, our study also presented the insight of the nature of ligands binding to the receptor. Pf falcipain-2 crystallographic structure serves a great feature to construct and develop novel antimalarial agents that could combat one of the major factors of unveiling conformation of hemoglobin. In contrast, the effective block of parasite growth by parasite suppression in hemoglobin hydrolysis needs a potent inhibition of Pf falcipain-2. In addition, the proposed cinchonidine salicylate could be used as structure-based drug design reference studies which supporting chemo-treatment development to eradicate malaria. In this study, cinchonidine salicylate is proposed for further lead synthesis by using pure iron oxide maghemite (Fe₂O₃) phase nanoparticles as an environment-friendly, uncomplicated, fast and powerful method through green chemistry ways to obtain high yield of CAD product in non-toxic aqueous solution.

Table 2 : Involved binding energy values and CADs properties

No.	Compound Name	Consensus Log P	Binding Energy (kcal/mol)	Violation of Lipinski's Rule
1	Quinine (Q)	2.41	-7.7	0
2	Q-butanoate	3.76	-7.7	0
3	Q-isobutanoate	3.59	-7.3	0
4	Q-isovalerate	4.18	-7.1	0
5	Q-tiglate	4.22	-7.5	0
6	Quinidine (Qd)	1.96	-7.0	0
7	Qd-butanoate	3.85	-6.9	0
8	Qd-isovalerate	4.18	-7.8	0
9	Qd-tiglate	4.22	-7.3	0
10	Cinchonidine (C)	2.32	-6.8	0
11	C-butanoate	3.76	-7.9	0
12	C-isobutanoate	3.50	-7.2	0
13	C-isovalerate	4.10	-5.4	0
14	C-tiglate	4.13	-7.2	0
15	Cinchonine (Cn)	2.41	-7.5	0
16	Cn-butanoate	3.76	-6.9	0
17	Cn-isovalerate	4.68	-6.7	0
18	Cn-tiglate	4.13	-7.7	0
19	Hexyl-Q- ether	5.27	-6.1	1
20	Hexyl-Qd-ether	5.27	-7.4	1
21	Hexyl-C-ether	5.18	-6.2	1
22	Hexyl-Cn-ether	5.18	-6.1	1
23	Isopropyl-Q-ether	3.66	-7.3	0
24	Isopropyl-Qd-ether	3.66	-6.8	0
25	Isopropyl-Cn-ether	3.57	-6.6	0
26	Isopropyl-C-ether	3.57	-6.4	0
27	Buthyl quinine ether	4.30	-7.4	0
28	Buthyl quinidine ether	4.30	-7.4	0
29	Buthyl cinchonine ether	4.21	-7.2	0
30	Buthyl cinchonidine ether	4.21	-6.8	0
31	Cinchonidine 4-hydroxybenzoate	4.20	-8.6	0
32	Cinchonidine octanoate	5.69	-7.6	0
33	Cinchonidine salicylate	4.08	-9.1	0
34	Cinchonidine succinate	2.36	-8.2	0
35	Cinchonine octanoate	4.20	-8.6	0
36	Cinchonine salicylate	5.69	-6.9	0
37	Cinchonine succinate	4.08	-8.1	0
38	Cinchonine 4-hydroxybenzoate	2.36	-7.8	0
39	Quinidine 4-hydroxybenzoate	4.29	-8.8	0
40	Quinidine octanoate	5.78	-7.3	0
41	Quinidine salicylate	4.17	-8.9	0
42	Quinidine succinate	2.45	-7.4	0
43	Quinine 4-hydroxybenzoate	3.94	-8.3	0
44	Quinine octanoate	5.78	-7.2	0
45	Quinine salicylate	4.17	-8.4	0
46	Quinine succinate	2.16	-7.1	0

CONCLUSION

One of the promising approaches in antimalarial drug discovery is the development of protease inhibitors for interfering malaria parasites ability to metabolize human hemoglobin as a source of nutrients. An excellent correlation between the interaction energies for various docked CAD compounds and their respective K_i values proven to be an endorsement for the protein target. In addition, these studies also provided insight into the nature of binding and interaction of ligands with the enzyme. Falcipain-2 crystallographic structure offers a promising opportunity to design and develop novel inhibitors that could block one of the major mediators of hemoglobin denaturation. However, effective blockade of parasite development by suppression of parasite hemoglobin hydrolysis would

likely require a potent inhibition of falcipain-2. In any event, the proposed cinchonidine salicylate could be used to direct structure-based drug design studies leading to development of chemotherapeutic agents to eradicate malaria. In this study, cinchonidine salicylate is proposed for further lead synthesis by using pure iron oxide maghemite (Fe_2O_3) phase nanoparticles as an eco-friendly, economical, simple, rapid and robust method based on green chemistry approach, to obtain high yield of CAD product in non-toxic aqueous environment.

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