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Antimalarial activity of HIV-1 protease inhibitor in chromone series

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ABSTRACT

Increasing parasite resistance to nearly all available antimalarial drugs becomes a serious problem to human health and necessitates the need to continue the search for new effective drugs. Recent studies have shown that clinically utilized HIV-1 protease (HIV-1 PR) inhibitors can inhibit the *in vitro* and *in vivo* growth of *Plasmodium falciparum*. In this study, a series of chromone derivatives possessing HIV-1 PR inhibitory activity has been tested for antimalarial activity against *P. falciparum* (K1 multi-drug resistant strain). Chromone **15**, the potent HIV-1 PR inhibitor (IC_{50} = 0.65 μM), was found to be the most potent antimalarial compound with IC_{50} = 0.95 μM while primaquine and tafenoquine showed IC_{50} = 2.41 and 1.95 μM, respectively. Molecular docking study of chromone compounds against plasmepsin II, an aspartic protease enzyme important in hemoglobin degradation, revealed that chromone **15** exhibited the higher binding affinity (binding energy = −13.24 kcal/mol) than the known PM II inhibitors. Thus, HIV-1 PR inhibitor in chromone series has the potential to be a new class of antimalarial agent.

1. Introduction

Malaria is still one of the major causes of ailment and mortality, threatening and killing millions of people annually [1,2]. The causative agents of malaria are four different species of Plasmodium, i.e., *Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae* and *Plasmodium ovale*. However, almost all deaths are due to inflection by *P. falciparum* [3,4]. In recent years, some human cases of malaria have also occurred with *Plasmodium knowlesi*, the fifth species that causes malaria among monkeys and occurs in some rainforest areas of the South-East Asia [5,6]. Malaria has become more difficult to treat because of an increase in multi-drug resistant strains [7,8]. This situation underlines the urgent need for the development of new antimalarial drugs with novel mechanism of action.

The United States Food and Drug Administration (US FDA) approved HIV-1 protease (HIV-1 PR) inhibitors, ritonavir and saquinavir, have been reported to induce CD36 deficiency, resulting in decreased CD36-mediated cytoadherence and phagocytosis of parasite erythrocytes [9]. Recent studies have indicated that saquinavir, ritonavir, indinavir, nelfinavir, amprenavir, lopinavir and atazanavir directly inhibited the growth of both drug-sensitive and drug-resistant *P. falciparum in vitro* at pharmacologically relevant concentrations [10,11]. The most potent compound, lopinavir, was active against parasites (IC_{50} 0.9–2.1 μM) at concentrations well below those achieved by ritonavir-boosted lopinavir therapy. Saquinavir, ritonavir, and lopinavir were evaluated *in vivo* for antimalarial efficacy in mice infected with *P. chabaudi* AS. Ritonavir alone and combined with saquinavir or lopinavir significantly attenuated parasitemia, with the most active regimen being a combination of ritonavir and saquinavir or ritonavir and lopinavir [12]. HIV-1 PR inhibitors also had significant effects on the morphology of *P. falciparum* parasites and their hemoglobin digestion. Lopinavir combined with ritonavir exerted a dose-dependent effect in reducing liver parasite in mice infected with *Plasmodium yoelii* [13]. More recent study has demonstrated the synergistic interactions between indinavir and chloroquine against both the chloroquine sensitive line *Plasmodium chabaudi* ASS and the chloroquine-resistant line *P. chabaudi* ASCQ [14]. These findings suggest that use of HIV-1 PR inhibitors may offer clinically relevant antimalarial activity. Although HIV-1 PR inhibitors are less likely to become first-choice drugs for the treatment of malaria, their antimalarial activity may lead to the development of a new class of antimalarial drugs.

In the previous study, our research group has designed, synthesized a series of chromone derivatives and evaluated for their *in vitro* inhibitory activity against HIV-1 PR (Fig. 1) [15–17]. The results revealed that the studied chromone compounds exhibited promising inhibitory effect on the HIV-1 PR activity with IC_{50} values ranging from 0.34 to 11.50 μM) [15]. In this study, the chromone derivatives showing potent HIV-1 PR inhibitory activity

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(over 70% inhibition) were evaluated for their antimalarial activity against *P. falciparum*. In addition, in order to preliminary investigate the potential of chromone derivatives as plasmepsin (PM) II inhibitor, docking simulation was performed. PM II is one of the four catalytically active plasmepsins (PM I, PM II, PM IV and histoaspartyl protease) that has been identified in the food vacuole of *P. falciparum* [18]. PM I and PM II initiate the degradative process by cleaving the native hemoglobin molecule in a highly conserved hinge region [19–21]. Among several types of plasmepsins, PM II has received considerable attention as a promising target for antimalarial drug design [22–26].

2. Experimental

2.1. Synthesis

Chromones **1–20** were synthesized via one-pot cyclization reaction as shown in pathway a in Fig. 2. More details of the synthesis procedures and spectroscopic data were reported in Ref. [15]. Chromone **21** was prepared via pathway b as follow:

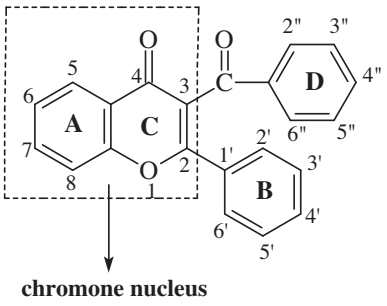


Fig. 1. General structure of synthesized chromone derivatives.

2.1.1. 2,4,5-Trihydroxyacetophenone

A solution of 2,4,5-trimethoxyacetophenone (4.00 g, 19.03 mmol) in chlorobenzene (20 mL) was treated with $AlCl_3$ (6.60 g, 49.47 mmol) at room temperature and then refluxed for 12 h. The solvent was evaporated and the residue was hydrolyzed with cooled 1 M HCl (50 mL) and extracted with ethyl acetate (3 × 50 mL). The organic layer was washed with water (2 × 50 mL), dried over sodium sulfate anhydrous and then filtered. After evaporation, the crude product was purified by column chromatography (methanol/dichloromethane [0.5:9.5]) to provide 2,4,5-trihydroxyacetophenone as the pale yellow solid (2.42 g, 75.87%); m.p. 206–207 °C; FTIR (KBr) (cm^{-1}): 3405, 3238 (O—H st.), 1634 (C=O st.) 1589, 1536 (C=C st.), 1300, 1211, 1135 (C—O st.); ^1H NMR 300 MHz (CD_3OD): δ 2.46 (s, 3H, CH_3), 6.27 (s, 1H, H3), 7.14 (s, 1H, H6); HRMS (ESI) *m/z* calculated for $C_8H_8O_4$, 168.0428 $[M]^+$, 167.0350 $[M-H]^+$; found 167.0356 $[M-H]^+$.

2.1.2. 4,5-Bis(benzyloxy)-2-hydroxyacetophenone

To a solution of 2,4,5-trihydroxyacetophenone (1.50 g, 8.93 mmol) in acetone (24 mL) was added anhydrous potassium carbonate (2.22 g, 16.07 mmol). The mixture was stirred at room temperature for 10 min. Then benzyl bromide (2.75 g, 16.08 mmol) was added and the reaction mixture was refluxed for 5 h. After cooling to room temperature, the solvent was evaporated and water was added to the residue. The aqueous mixture was extracted with ethyl acetate (3 × 40 mL). The combined organic layer was washed with water (2 × 40 mL) and dried over sodium sulfate anhydrous, then filtered. After removing the solvent, the crude product was purified by column chromatography (ethyl acetate/hexane [1:4]) to provide 4,5-bis(benzyloxy)-2-hydroxyacetophenone as the white solid (2.18 g, 70.19%); m.p. 99–100 °C; FTIR (KBr) (cm^{-1}): 3065, 3037 (aromatic C—H st.), 2867 (aliphatic C—H st.), 1633 (C=O st.), 1510, 1455 (C=C st.), 1370 (C—H bending), 1263, 1210, 1166 (C—O st.); ^1H NMR 300 MHz ($CDCl_3$): δ

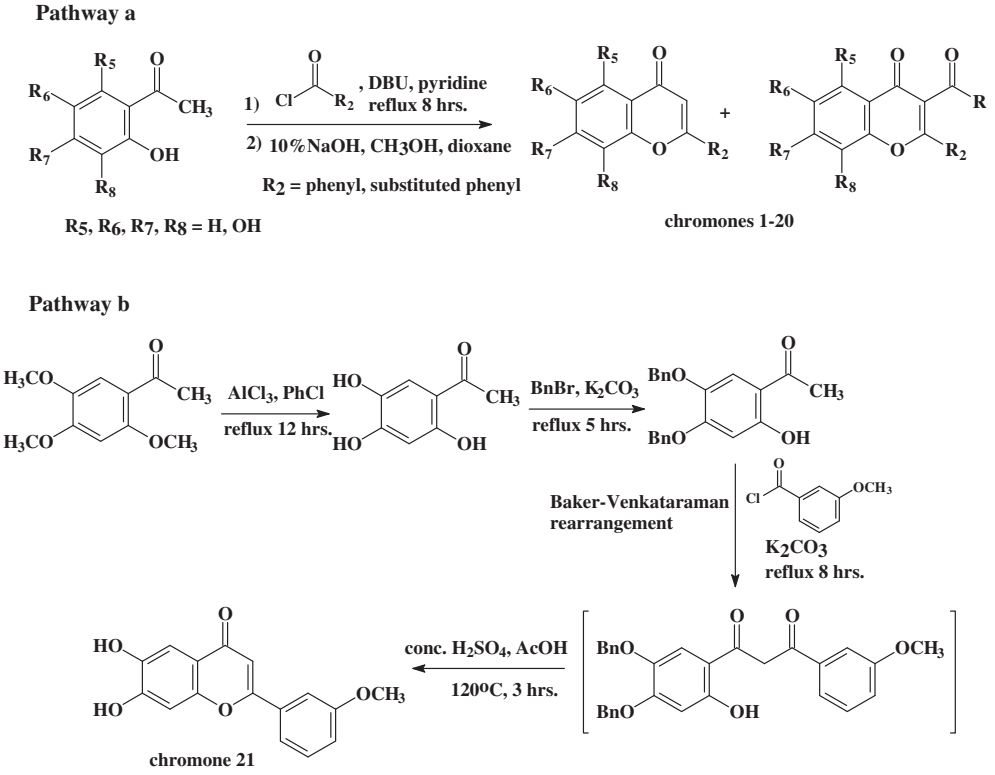


Fig. 2. Synthesis pathways for chromone derivatives **1–21**.

2.48 (s, 3H, CH_3), 5.10 (s, 2H, CH_2-Ph), 5.21 (s, 2H, CH_2-Ph), 6.54 (s, 1H, H3), 7.19 (s, 1H, H6), 7.34–7.48 (m, 10H, H2′, H3′, H4′, H5′, H6′, H2″, H3″, H4″, H5″, H6″); LRMS (ESI) *m/z* $[M+Na]^+$ 371.29 (46.0), 280.20 (100.0), 189.29 (10.0).

2.1.3. 6,7-Dihydroxy-2-(3′-methoxyphenyl) chromone, 21

The Baker-Venkataraman rearrangement was performed by adding potassium carbonate anhydrous (1.90 g, 13.79 mmol) to a solution of 4,5-bis(benzyloxy)-2-hydroxyacetophenone (1.20 g, 3.45 mmol) in acetone (25 mL). The mixture was stirred at room temperature for 20 min then 3-methoxybenzoyl chloride (0.56 mL, 4.14 mmol) was added dropwise. The reaction mixture was refluxed for 24 h. After the reaction mixture was allowed to cool to room temperature, the solvent was evaporated and water was added to the residue. The aqueous mixture was extracted with ethyl acetate (3 × 40 mL). The organic layer was washed with water (2 × 40 mL), dried over anhydrous sodium sulfate and filtered. After removing the solvent, the yellow residue of 1,3-diketone was obtained.

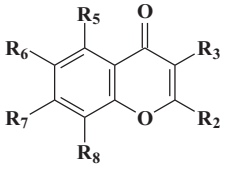
To a mixture of 1,3-diketone in glacial acetic acid (20 mL) was added concentrated sulfuric acid (0.28 mL) and refluxed at 120 °C for 4 h. After cooling to room temperature, the reaction mixture was poured into cool water and extracted with ethyl acetate (3 × 40 mL). The combined organic layer was washed with water (2 × 40 mL), dried over sodium sulfate anhydrous, filtered and solvent was evaporated. The crude product was purified by column chromatography (ethyl acetate/hexane [3:2]) to provide chromone **21** as the pale yellow solid (547.4 mg, 55.89%); m.p. 246–247 °C; FTIR (KBr) (cm^{-1}): 3495 (O—H st.), 3092 (aromatic C—H st.), 1630 (C=O st.), 1602, 1590, 1471 (C=C st.), 1346 (C—H bending), 1293, 1145 (C—O st.); ^1H NMR 300 MHz (DMSO-d6): δ 3.84 (s, 3H, OCH_3), 6.85 (s, 1H, H3), 7.03 (s, 1H, H8), 7.12 (dd, *J* = 8.00, 2.35 Hz, 1H, H4′), 7.28 (s, 1H, H5), 7.45 (t, *J* = 8.00 Hz, 1H, H5′), 7.52 (s, 1H. H2′), 7.59 (d, *J* = 8.00 Hz, 1H, H6′); HRMS (ESI) *m/z* calculated for $C_{16}H_{12}O_5$, 284.0681 $[M]^+$, 285.0759 $[M+H]^+$; found 285.0755 $[M+H]^+$.

2.2. In vitro antimalarial activity assay

In vitro cultivation of *P. falciparum* (K1, multi-drug resistant strain) was performed according to the method previously described by Trager and Jensen [27]. The parasites were cultivated in RPMI 1640 medium containing 25 mM HEPES (N-2-hydroethylpiperazine-N′-2-ethanesulfonic acid), 25 mM $NaHCO_3$, 10% heat-activated human serum and 3% erythrocytes. The culture was incubated at 37 °C in a humidified incubator with 3% CO_2-enriched atmosphere (3% CO_2, 17% O_2 and 80% N_2). Daily passaged to fresh medium containing erythrocyte in order to maintain parasite growth was performed. Before the assay, the parasite at an early ring-stage growth was collected and prepared to a parasite mixture of 1% parasitemia in 1.5% erythrocytes.

In vitro antimalarial activity of chromone derivatives against *P. falciparum* was assessed using microculture radioisotope method described by Desjardins et al. [28]. The assay was performed in duplicate wells in 96-well plate. In each well, 200 μL of parasite mixture (1% parasitemia and 1.5% erythrocytes) was pre-exposed with 25 μL of the medium containing a test sample dissolved in 1% DMSO (0.1% final concentration) for 24 h. Twenty-five μL of medium containing 0.5 μCi $[^3H]$-hypoxanthine (Perkin Elmer, USA) was added to each well. The plates were incubated for an additional 24 h. Levels of incorporated radioactive labeled hypoxanthine indicating parasite growth were determined using the

Table 1
Structures, antimalarial activity and AutoDock binding energy against PM II of chromone derivatives.



Compd	R_2	R_3	R_5	R_6	R_7	R_8	Activity[a]	Antimalarial IC_{50} (μM)	AutoDock Binding energy (kcal/mol)
1	Benzyl	H	H	H	OH	OH	Active	9.43	−8.86
2	Phenyl	H	H	H	OH	OH	Active	19.66	−8.16
3	4′-(t-butyl)-Phenyl	H	H	H	OH	H	Active	11.41	−8.37
4	3′-(CF_3)-Phenyl	H	OH	H	OH	H	Active	11.07	−8.60
5	4′-(F)-Phenyl	H	OH	H	OH	H	Inactive	–	−8.32
6	3′,4′-(diF)-Phenyl	H	OH	H	OH	H	Inactive	–	−8.54
7	4′-(t-butyl)-Phenyl	H	OH	H	OH	H	Active	9.15	−8.87
8	3′-(Cl)-Phenyl	H	OH	H	OH	H	Active	13.83	−8.27
9	3′,4′-(diCl)-Phenyl	H	OH	H	OH	H	Active	11.25	−8.89
10	4′-(OCH_3)-Phenyl	H	OH	H	OH	H	Inactive	–	−8.70
11	3′-(OCH_3)-Phenyl	H	OH	H	OH	H	Inactive	–	−8.93
12	3′-(OCH_3)-Phenyl	H	H	OH	H	H	Active	13.23	−7.95
13	3′-(CF_3)-Phenyl	3″-(CF_3)-Benzoyl	H	H	OH	OH	Inactive	–	−10.53
14	4′-(F)-Phenyl	4″-(F)-Benzoyl	H	H	OH	OH	Inactive	–	−10.83
15	4′-(NO_2)-Phenyl	4″-(NO_2)-Benzoyl	H	H	OH	OH	Active	0.95	−13.24
16	3′,4′-(diF)-Phenyl	3″,4″-(diF)-Benzoyl	H	H	OH	H	Active	12.40	−10.56
17	3′-(CF_3)-Phenyl	3″-(CF_3)-Benzoyl	H	H	OH	H	Active	4.87	−11.84
18	4′-(NO_2)-Phenyl	4″-(NO_2)-Benzoyl	H	H	OH	H	Active	9.85	−11.79
19	4′-(t-butyl)-Phenyl	4″-(t-butyl)-Benzoyl	H	H	OH	H	Active	5.46	−12.21
20	4′-(NO_2)-Phenyl	4″-(NO_2)-Benzoyl	OH	H	OH	H	Active	5.91	−13.03
21	3′-(OCH_3)-Phenyl	H	H	OH	OH	H	Active	13.94	−8.89
DHA								2.02 nM	
Mefloquine								30.1 nM	
Primaquine								2.41	
Tafenoquine								1.95	
Chloroquine								0.42	

[a] Less than 50% inhibition of parasite growth = Inactive. More than 50% inhibition of parasite growth = Active.

TopCount NXT Microplate Scintillation and Luminescence Counters (Perkin Elmer, USA). The percentage of parasite growth was calculated from the signal count per minute of treated (CPM_T) and untreated samples (CPM_U) using the following equation:

$$\%\text{parasite growth} = CPM_T/CPM_U \times 100$$

If % parasite growth was >50%, the antimalarial activity was reported as active and ⩽50%, reported as inactive.

The IC_{50} values (the concentration of sample required to inhibit 50% parasite growth) were calculated by linear interpolation [29–31] as follow:

$$IC_{50} = 10^{\left[\left\{\log\left(\frac{C_1}{C_2}\right)\times\left(\frac{G_2-50}{G_2-G_1}\right)\right\}+\log(C_2)\right]}$$

where

C1 = concentration of sample with parasite growth <50%.
C2 = concentration of sample with parasite growth ⩾50%.
G1 = percentage of parasite growth at C1.
G2 = percentage of parasite growth at C2.

Dihydroartemisinin (DHA) and mefloquine were used as the positive controls. The negative control was 0.1% DMSO. All of chromone compounds were dissolved in 100% of DMSO as the stock solution at concentration 10 mg/mL. The test concentrations of chromone compounds were 10, 1 and 0.1 μg/mL for evaluating the % parasite growth. All assays were performed by Bioassay laboratory of the National Center of Genetic Engineering and Biotechnology (BIOTEC).

2.3. Docking study

The 3-dimensional (3-D) structures of chromone compounds were constructed using the standard parameters of the molecular modeling software package SYBYL 8.0 (Tripos Associates, Saint Louis, MO, USA). Geometrical optimization was performed using Powell method with a root-mean-squared (RMS) energy gradient of 0.05 kcal/mol Å. Tripos force field with Gasteiger-Hückel charges was employed during the energy minimization. These conformations of chromones were then docked into active sites of PM II using AutoDock version 4.2 (The Scripps Research Institute, Molecular Graphics Laboratory, Department of Molecular biology, CA, USA).

The 3-D structures of PM II complexed with inhibitor (pdb code 1SME and 1ME6) were retrieved from the Brookhaven Protein Data Bank (http://www.rcsb.org/pdb). The protein templates were prepared for docking study by removing all the native ligand structures and all water molecules from the complex structures. The polar hydrogen atoms were added and Gasteiger charges were assigned to protein atoms. The optimized run parameters for docking study were as follow: the maximum number of energy evaluations was increased to 2,500,000 per run and the number of GA run was 100. All other parameters were maintained at their default setting. One hundred independent docking runs were carried out for each ligand. The docked poses were clustered using a tolerance of 2.0 Å root-mean-square deviations (RMSD). The docking results, i.e. the docked pose, binding mode, and binding free energy were analyzed to evaluate the interaction between the ligand and the amino acid residues of PM II.

The PM II template validation was performed by re-docking and cross-docking experiments. Each ligand was docked into the native

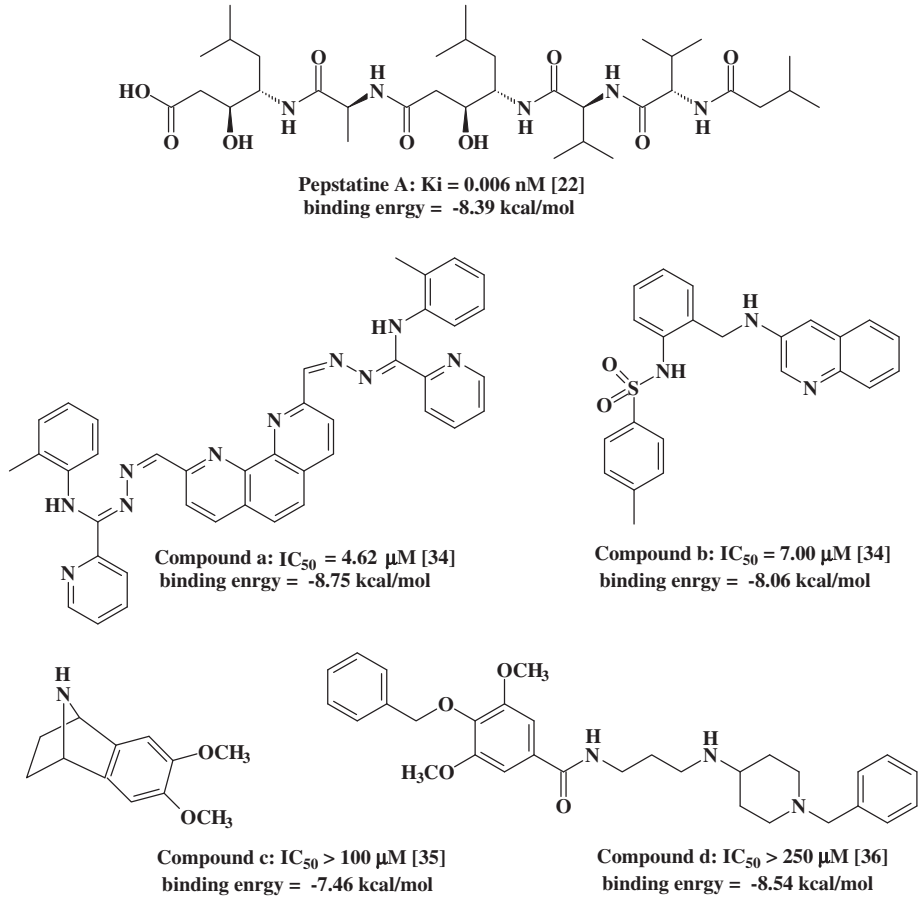


Fig. 3. Structures and binding energies of the reported PM II inhibitors.

protein in order to determine the ability of AutoDock program to reproduce the orientation and position of the ligand observed in the crystal structure. Then, cross-docking of each ligand into the non-native protein was performed. The RMSD values were obtained from the best cluster conformation of the re-docking and cross-docking studies. The PM II template, 1SME, with the lowest RMSD values was used for further docking study.

3. Results and discussion

3.1. Chemical synthesis

Chromones **1–20** were prepared by the one pot cyclization reaction modified from the method of Riva et al. (pathway a, Fig. 2) [32]. More details in synthesis procedures were described in Ref. [15]. Chromone **21** was synthesized according to pathway b (Fig. 2) which required only simple and inexpensive reagents. The Baker-Venkataraman rearrangement and subsequent intramolecular cyclization with a catalytic amount of strong acid were used to provide the desired chromone structure with satisfactory yield higher than 55%.

3.2. Evaluation of antimalarial activity

In this study, chromone derivatives which exhibited potent HIV-1 PR inhibitory activity (higher than 70% inhibition) from the previously study [15] were evaluated for their antimalarial activity against *P. falciparum* (K1 multi-drug resistant strain). The ability of the chromone derivatives to inhibit the malaria parasite growth was determined *in vitro* using microculture radioisotope technique [28]. The amount of [^{3}H]-hypoxanthine taken up by malaria parasite for purine salvage pathway and DNA synthesis was an indicator of parasites growth and their multiplication. Incorporation of [^{3}H]-hypoxanthine was quantified with a liquid β-scintillation counter as the signal count per minute (CPM). The percentage of inhibition of parasite growth and IC_{50} were calculated as described in experimental section and results were summarized in Table 1. As shown in Table 1, most of the test compounds exhibited promising inhibitory activity against *P. falciparum*. The four most potent compounds were chromones **15**, **17, 19** and **20** with IC_{50} = 0.95, 4.87, 5.46 and 5.91 μM, respectively. Although the most active, chromone **15,** showed the lower potency than the positive controls (DHA and mefloquine), it showed the higher potency than the antimalarial drugs currently used in patients, i.e., primaquine and tafenoquine (IC_{50} = 2.41 and 1.95 μM, respectively) [33].

3.3. Molecular modeling

As a preliminary study toward the potential of chromone series as PM II inhibitor, the docking simulation study was performed using AutoDock 4.2. The docking results were reported as binding energy (Table 1), the lower the binding energy the higher the binding affinity. All chromone compounds bearing substituents at C-2 and C-3 as well as 7-OH group displayed strong binding energies (−10.53 to −13.24 kcal/mol). Interestingly, the results from docking indicated that chromone **15**, the experimentally observed most potent antimalarial, showed the highest binding affinity against PM II (binding energy = −13.24 kcal/mol. Chromones **17**,

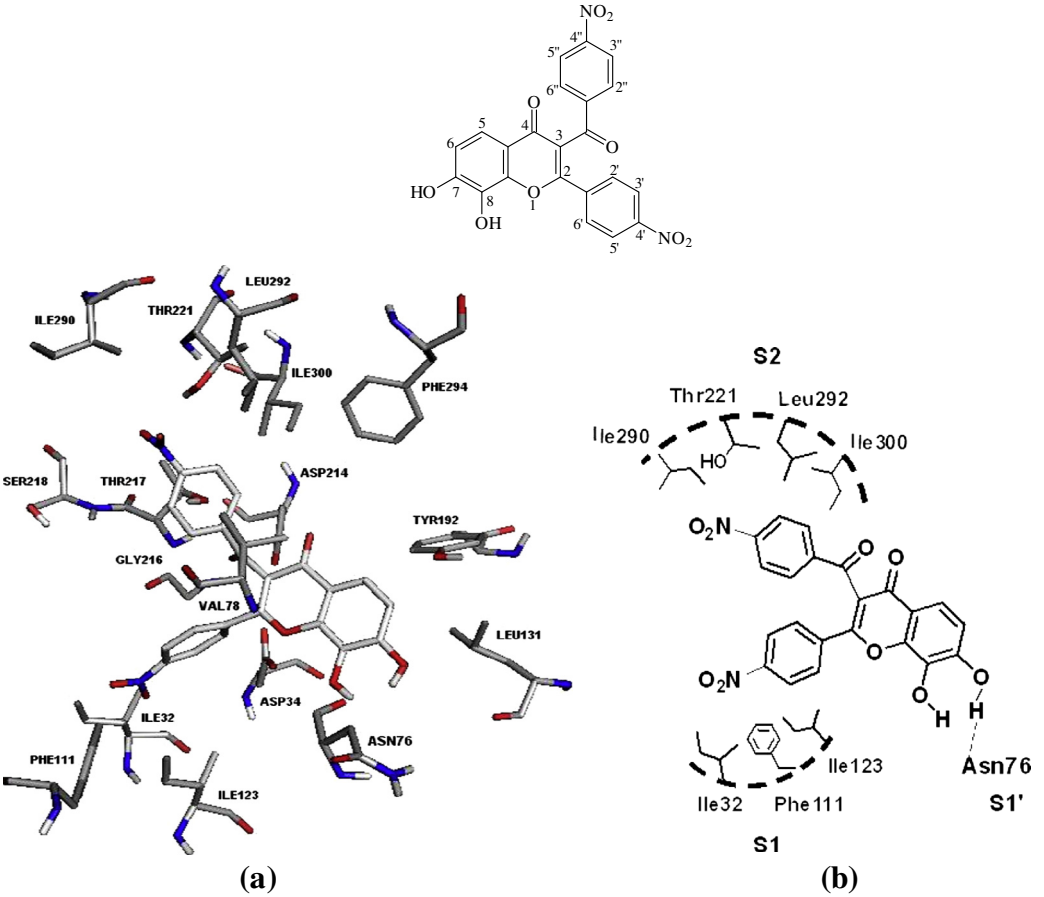


Fig. 4. (a) Binding interaction of chromone **15** against PM II. (b) Schematic view of the binding conformation of chromone **15** in the enzyme active sites.

19 and **20** also showed strong binding energies, −11.84, −12.21 and −13.03 kcal/mol, respectively.

In this study, the known PM II inhibitors were also docked into the PM II active site (Fig. 3). Pepstatin A, a highly potent PM II inhibitor (experimental Ki = 0.006 nM) [22] showed weaker binding affinity (−8.39 kcal/mol) than chromone **15**. Compounds **a** and **b** which were reported as moderate potent PM II inhibitors (IC_{50} = 4.62 and 7.00 μM, respectively) [34] showed binding energy = −8.75 and −8.06 kcal/mol, respectively while the poor inhibitor compounds **c** and **d**, (IC_{50} > 100 and > 250 μM, respectively) [35,36] exerted binding energies = −7.46 and −8.54 kcal/mol, respectively.

The binding interaction of chromone **15** against PM II is depicted in Fig. 4. The 4-nitro-phenyl group (at C-2) and 4-nitrobenzoyl group (at C-3) pointed toward the hydrophobic S1 subsite (Ile32, Phe111 and Ile123) and S2 subsite (Val78, Ile290 Leu292 and Ile300), respectively. The 7-OH formed noticeable hydrogen bonding interaction with the carbonyl oxygen of Asn76 in S1′ subsite.

4. Conclusions

In this study chromone **15**, one of the most potent HIV-1 PR inhibitor, showed high inhibitory activity against malaria parasite, *P. falciparum*. From docking result, chromone **15** also exhibited the strongest binding affinity against PM II. Though the mechanism underlying this activity remains to be fully investigated, the results from this study raise the prospect of chromone series as new antimalarial drug.

Acknowledgments

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