Therapeutic Potency of Mono- and Diprenylated Acetophenones: A Case Study of *In-Vivo* Antimalarial Evaluation

Muhammad H. Yahya[1]* Sodeeq A. Babalola[1]* Abdullahi Y. Idris[1] Asmau N. Hamza[1] Nosakhare Igie[2] Isaiah Odeyemi[2] Aliyu M. Musa[1] Amina B. Olorukooba[3]

[1] Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria
[2] Department of Chemistry and Biochemistry, The University of Dallas, Richardson, Texas, United States
[3] Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria

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Address for correspondence Muhammad H. Yahya, MSc, Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Kaduna State 801129, Nigeria (e-mail: yahyahayatudeen@gmail.com).

Sodeeq A. Babalola, MSc, Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Kaduna State 801129, Nigeria (e-mail: wale.babalola91@gmail.com).

Abstract

Malaria remains a febrile infection of public health concern in many countries especially tropical countries in Africa and certain countries in Southern and North America such as Brazil, Costa Rica, Mexico, Dominican Republic, Colombia, and Ecuador. Hence this has made research into this area paramount. Acetophenones are active fragments in many compounds with promising antimalarial activity, such as chalcones. The aim of the present study was to investigate antimalarial activity of 3,5-diprenyl acetophenone (**I**) and 5-diprenyl acetophenone (**II**) in *in vivo*. In this study, compounds **I** and **II** were synthesized using an aromatic substitution reaction. The *in-vivo* antimalarial potential of compounds **I** and **II** was analyzed in *Plasmodium berghei*-infected mice. Our data showed that compound **I** (25, 50, and 100 mg/kg) had promising antimalarial activity, with parasitemia inhibited rate being 68.03, 65.16, and 69.75%, respectively. Compound **II** dose-dependently inhibited parasitemia levels, it demonstrated an infinitesimally higher activity (72.12%) when compared with compound **I** (69.75%) at 100 mg/kg dose. The two compounds passed the rule of three, Lipinski's rule of five, predicted plausible pharmacokinetic profile (ADME), and apparent safety profile, and demonstrated drug-like fragments. The study provided guidance in exploring novel antimalarial compounds based on the scaffolds of prenylated acetophenones.

Keywords
- malaria
- prenylated acetophenone
- 3,5-diprenyl acetophenone
- 5-diprenyl acetophenone
- fragment-based drug design

Introduction

Malaria is an infectious and life-threatening disease that affects nearly half the population of the world, it is typically found in tropical and subtropical climates where the parasite can grow. Africa is responsible for 90% of worldwide malaria morbidity and death, particularly pediatric mortality.[1] *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae*, and *Plasmodium knowlesi* are the most common plasmodium parasites that cause malaria.[2,3] *P. falciparum* is the most dangerous human malaria parasite, and it is responsible for the majority of malaria infections in Sub-Saharan Africa, such as Nigeria. It is also the parasite most likely to develop drug resistance.[3]

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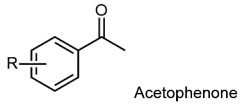

Fig. 1 General structure of acetophenone.

Several antimalarial drugs have been used to reduce sensitivity of these parasites to these antimalarial drugs. The most potent antimalarial agents are the artemisinin-based combination therapies (ACTs); however, with reports of potential resistance to ACTs, there are great concerns among researchers in the field of drug design for potent antimalarial agents against the drug-resistant strains of *Plasmodium*. Although ACTs remain the first line of defense against malaria, backup or alternative solutions are needed to deal with ACT-resistant strains produced by these parasites.[2]

One of the more recent areas of exploration in drug design is the fragment-based drug design, where most active scaffolds or moieties of active drug compounds can be used in designing drug compounds with synergistic-like effects in terms of activity and physicochemical properties such as reduced or less toxic effects caused by a process called molecular hybridization.[1,4] To combat this disease, new antimalarial agents with novel biological targets are required.

Acetophenone is an organic compound that is used in the synthesis of many organic pharmaceuticals, and it is also a precursor for many resins and fragrances. General structure of acetophenone is shown in ►**Fig. 1**. Proteases form one of the most explored or studied antimalarial mediating targets.[5–8] And acetophenone is one of the important fragments with protease-inhibiting activity. A condensation of acetophenone and aldehyde gives a very important class of organic compounds known as chalcones. Chalcones offer a wide range of medicinal properties, including antiplasmodial activity.[2] Acetophenones form part of the most active fragment of the chalcones and other classes of organic compounds. Modifications on the acetophenone ring improve its activity. Some acetophenones used in the synthesis of chalcones have been modified through the addition of electron-withdrawing or -donating substituents such as methoxy, hydroxy, chlorine, and alkyl to give different pharmacological activities. The presence of electron-donating groups on the acetophenone ring, according to Li et al,[9] is advantageous to antimalarial activity. Structure–activity relationship (SAR) evaluation shows that the size and hydrophobicity of the substituents attached on the acetophenone are critical parameters for regulating the activity of the ring.[10]

In this work, two prenylated acetophenones, 3,5-diprenyl acetophenone (**I**) and 5-diprenyl acetophenone (**II**), were chosen to assess their antimalarial activity to correlate the theoretical studies, e.g., rule of three (Ro3) for active fragment and rule of five (Ro5) for orally bioavailable agents, and *in silico* pharmacokinetic (PK) studies with their experimental activity, and deduce further insights into their SAR. This is the first report of the *in vivo* antimalarial activity of the two prenylated acetophenones.

Materials and Methods

Chemical Reagents and Instruments

All reagents and solvents utilized in this study were of analytical grade. The reagents were 2,4-dihydroxyacetophenone, anhydrous 1,4-dioxane, boron trifluoride diethyl etherate, 3-methyl-2-buten-1-ol, 2-chloroquinolinyl-3-carbaldehyde, ethanol, sodium hydroxide, hexane, and ethyl acetate. All reagents were procured from Sigma Aldrich, Germany.

The melting points of the compounds were measured without correction using the Gallenkamp melting point instrument. Experiments of proton nuclear magnetic resonance (^{1}H-NMR) and carbon-13 nuclear magnetic resonance (^{13}C-NMR) were conducted in the Department of Chemistry, University of Kwazulu-Natal, South Africa, and Multi-user Laboratory, Ahmadu Bello University, Zaria, using 400 MHz Bruker and 400 MHz Agilent, respectively. Chemical shift (d) in ppm downfield from tetra-methyl silane as the internal standard was used to capture the nuclear magnetic resonance data. Wavenumbers were captured as Fourier-transform infrared spectroscopy (FTIR) using an Agilent spectrophotometer (cm^{-1}).

Synthesis of Prenylated 2,4-Dihydroxyacetophenone

The synthesis of compound **I**, **II**, and **III** was described in ►**Fig. 2**. The two prenylated acetophenones (**I** and **II**) were synthesized through an aromatic electrophilic substitution reaction. Equimolar quantities of 3-methyl-2-buten-1-ol and boron trifluoride diethyl etherate catalyst (20 mmol) were dissolved in anhydrous 1,4-dioxane to acquire a pink-red solution, to which 20 mmol of 2,4-dihydroxyacetophenone was subsequently added. The solution was continuously stirred for 2 hours. This reaction was performed at room temperature under inert conditions (nitrogen atmosphere). Thin-layer chromatography profile was used to monitor the progress of the reaction. On completion of the reaction, the mixture was diluted with diethyl ether (100 mL) and washed with water (50 mL × 3). The organic phase was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to obtain a residue, which was purified by a column chromatography using *n*-hexane and *n*-hexane/ethylacetate (7:3) to afford compounds **I**, **II**, and trace amount of compound **III**.

Animals

Forty locally bred adult Swiss Albino mice, weighing 18 to 22 g, were housed in conventional laboratory settings with unlimited access to pellet feed and water *ad libitum*. The animals were housed in clean polypropylene cages at the Department of Pharmacology and Therapeutics' Animal House. All animal experiments followed Ahmadu Bello University's research policy and guidelines for the use and care of laboratory animals, which are widely acknowledged globally. Ethical approval was sought and obtained from ABU Committee on Animal Use and Care.

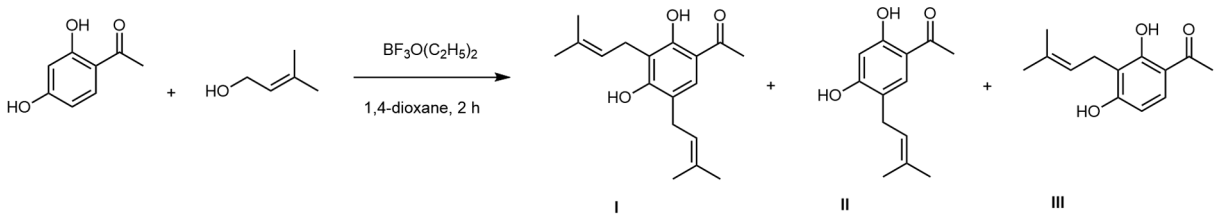

Fig. 2 Reaction scheme.

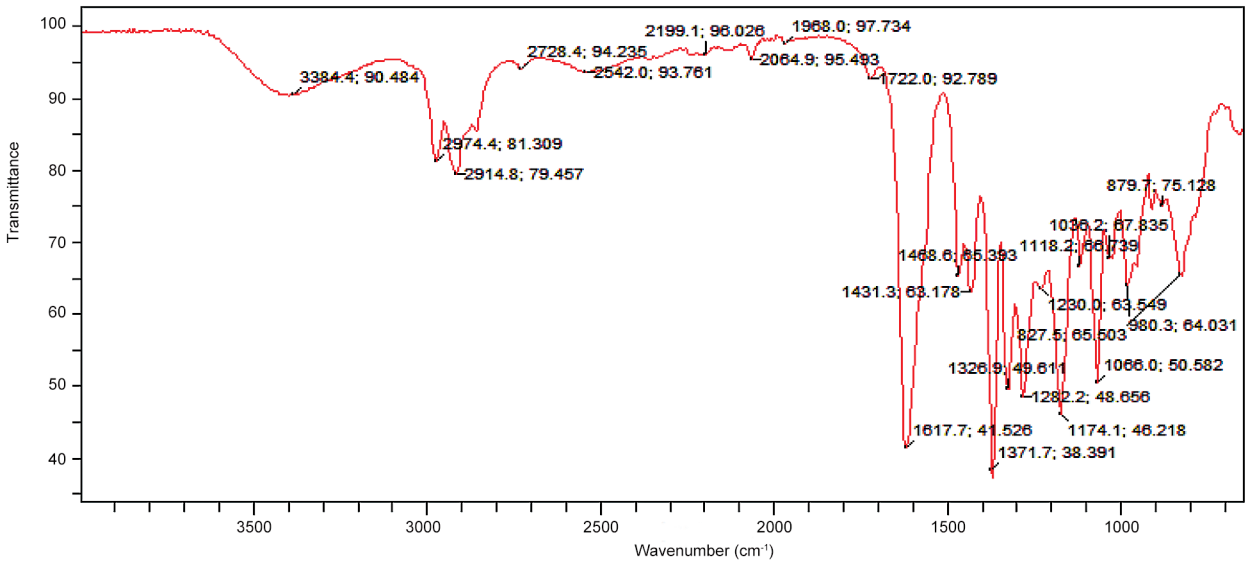

Fig. 3 FTIR spectrum of compound **I**. FTIR, Fourier-transform infrared spectroscopy.

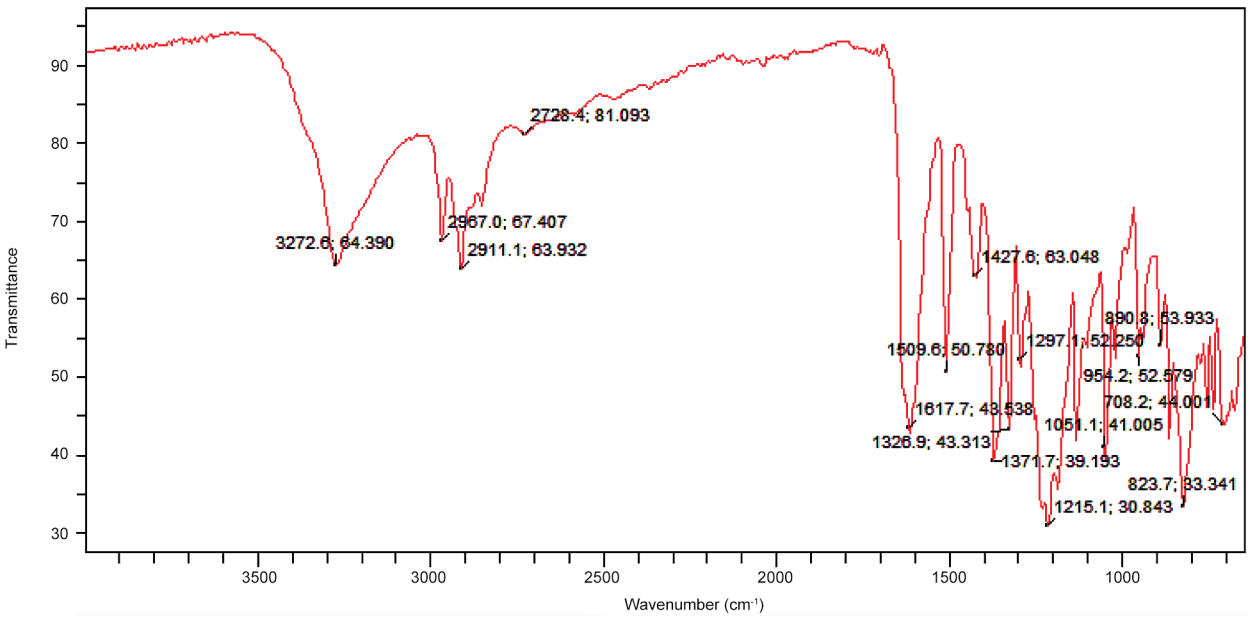

Fig. 4 FTIR spectrum of compound **II**. FTIR, Fourier-transform infrared spectroscopy.

Malarial Parasite

Malaria parasites (Plasmodium berghei NK 65) were provided by the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. The mice were inoculated intraperitoneally with 0.2 mL standard inoculum containing approximately 10^7 parasitized red blood cells.

Evaluation of Theoretical Oral Bioavailability, Pharmacokinetics, and Toxicity

Prior to synthesis, the oral bioavailability of the two prenylated acetophenones was theoretically predicted using Lipinski's Ro5 on SWISS ADME web tools. (http://www.swissadme.ch). Ro3 was used to predict the propensity of

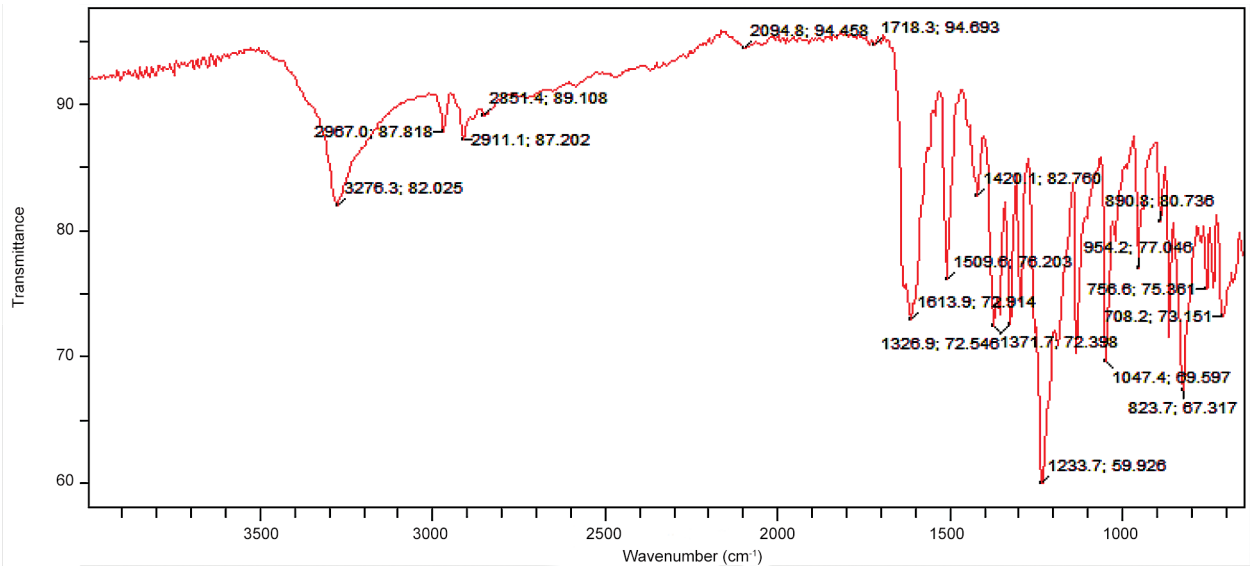

Fig. 5 FTIR spectrum of compound **III**. FTIR, Fourier-transform infrared spectroscopy.

their activity. *In silico* PK study and toxicity evaluation was performed on Admetlab2.0 and Protox-II.

Antimalarial Activity Evaluation *In Vivo*

Inoculation of *Plasmodium berghei* Parasite

Infected blood was taken from the tail vein of a donor mouse with a parasitemia level of 20 to 25% using heparinized capillary tubes and then transferred to a sterile plain beaker. Two milliliters of blood were diluted with 10 mL of normal saline, yielding 0.2 mL of infected red blood cells. The mice were then given 0.2 mL of blood suspension intraperitoneally.

Treatment

To test the curative efficacy of the synthesized compounds against an established plasmodium infection, Ryley and Peters' approach was used.[11] The mice were infected with the parasite as described by Ryley and Peters' method and were left untreated for 72 hours, following which parasitemia levels were estimated to be between 20 and 25%.[11] The mice with the required parasitemia levels were split into eight groups ($n = 5$ in each group). Group 1 was given the negative control intraperitoneally [1% (w/v), acacia (Sigma Aldrich)]. Groups 2 to 7 were given 25, 50, and 100 mg/kg of test compounds, while group 8 was given 5 mg/kg of the positive control [chloroquine (Jiangsu Ruinian Quianjin Pharmaceutical Co. Ltd.)]. All treatments were given intraperitoneally for 4 days consecutively. On the fifth day, blood was obtained from the tail vein of the treated mice from all groups. A thin film was made by smearing the blood samples on microscopic slides. The slides were fixed in absolute methanol and stained with 3% Giemsa solution at pH 7.2. Average levels of parasitemia were calculated from six different fields. The average suppression percentage of the parasite was calculated for each group using Equation (1):

$$\text{Percentage chemosuppression} = \frac{A-B}{A} \times 100\% \quad (1)$$

where A is average levels of parasitemia of negative control; B is average levels of parasitemia in each treated group.

Statistical Analysis

The data were analyzed with SPSS 20.0 software and displayed as mean ± standard error of the mean. The mean difference between the results obtained was compared using a one-way ANOVA (analysis of variance) followed by Dunnett's post-hoc test. Statistical significance was defined as a p-value of less than 0.05.

Results

Synthesis and Characterization

Following the synthetic procedure, compounds **I**, **II**, and **III** were obtained and characterized by the following spectral data:

Compound **I**: chemical name "1-[2,4-dihydroxy-3,5-bis (3-methylbut-2-en-1-yl)-phenyl]-ethan-1-one," white crystals, yield: 6.25%, mp 110–112°C, FT-IR (v cm^{-1}): 3,384 (Ar-OH), 2,974 and 2,914 (sp^2 C-H), 2,728 (sp^3 C-H), 1,617 (C = O). ^{1}H-NMR (MeOD, 400 MHz) δ 7.49 (d, 1H), 6.27 (d, 1H), 5.31 (t, 1H), 3.33 (d, $J = 7.2$ Hz, 2H), 3.23 (d, $J = 8.0$ Hz, 2H), 2.50 (s, 3H), 1.76 (s, 12H). ^{13}C NMR (MeOD, 400 MHz) δ 203.91, 160.67, 159.84, 132.74, 131.01, 128.78, 121.89, 119.63, 115.05, 113.86, 27.62, 24.53, 21.19, 16.54.

Compound **II**: chemical name "1-[2,4-dihydroxy-5-(3-methylbut-2-en-1-yl)-phenyl] ethan-1-one," white crystals, yield: 10%, mp 145–146°C, FT-IR (v cm^{-1}): 3,272 (Ar-OH), 2,967 and 2,911 (sp^2 C-H), 2,728 (sp^3 C-H), 1,617 (C = O). ^{1}H-NMR (CD_3OD 400 MHz) δ 7.58 (s, 1H), 5.51 (s, 0.5H), 5.37 (s, 0.5H), 5.11 (s, 1H), 3.54 (s, 1H), 3.45 (s, 1H), 2.69 (s, 3H), 1.86 (s, 6H). ^{13}C NMR (CD_3OD, 400MHz) δ

Table 1 Rule of three evaluation of the fragments

Compound	MW $\leq$ 300 (g/mol)	MlogP $\leq$ 3	HBA $\leq$ 3	HBD $\leq$ 3	nRB $\leq$ 3	PSA $\leq$ 60 Å^2
I	288.38	3.05	3	2	5	57.53
II	220.26	1.89	3	2	3	57.53

Abbreviations: MW, molecular weight; HBA, number of hydrogen bond acceptor; HBD, number of hydrogen bond donor; MlogP, lipophilicity; nRB, number of rotatable bonds; PSA, polar surface area.

202.65, 163.11, 132.03, 131.62, 122.25, 120.57, 112.64, 101.63, 27.18, 24.73, 24.53, 16.43.

Compound **III**: chemical name "1-[2,4-dihydroxy-3-(3-methylbut-2-en-1-yl)-phenyl] ethan-1-one," white crystals, yield: 0.07%, mp 154–156°C, FT-IR (ν cm^{-1}): 3,276 (Ar-OH), 2,967 and 2,911 (sp^2 C-H), 2,851 (sp^3 C-H), 1,613 (C = O).

Drug-Likeness

Compound **II** efficiently passed Ro3 for active fragments having molecular weight $\leq$300 g/mol, MlogP $\leq$ 3, number of hydrogen bond acceptor $\leq$3, number of hydrogen bond donor $\leq$3, number of rotatable bonds $\leq$3, polar surface area $\leq$60 Å, while compound **I** passed Ro3 on the average according to results in ►**Table 1**. Ro3 has been useful in ensuring that fragment libraries really do consist of compounds with active fragment-like properties. Any compound that passes the Ro3 on average could be useful when constructing fragment libraries for efficient lead discovery. As shown in ►**Table 2**, both compounds passed the Lipinski's Ro5 as indicated in the reported studies,[12,13] suggesting that they have high probability of being orally bioavailable.

In Silico PK (ADME)

Absorption

As shown in ►**Table 3**, compounds **I** and **II** had optimal and medium permeability in both *in-vivo* and *in-vitro* predictive models, respectively. The *in-vivo* permeability of compound **II** in the predictive human colon adenocarcinoma cell lines (Caco-2) was slightly higher when compared with compound **I,** with values of being −4.64 and −4.70 cm/s, respectively. However, permeability co-efficient (*Papp*) values of both compounds were 15×10^{-6} cm/s, which is greater than 2×10^{-6} cm/s but less than 20×10^{-6} cm/s in Madin – Darby canine kidney cells (MDCKs), suggesting that they had medium permeability in MDCK model.

Compound **I** had strong substrate affinity, while poor inhibitory affinity for P-gp enzyme with scores of 0.05 and 0.82, respectively. On the other hand, compound **II** had comparable substrate affinity for P-gp with scores of 0.02 and stronger inhibitory affinity for P-gp with scores of 0.07, respectively. The observed disparities between Caco-2 permeability and MDCK permeability for both compounds can be explained by P-gp inhibitory and substrate affinities. Drugs that are P-gp substrates usually have disparities in their Caco-2 and MDCK permeability. An instance is quinidine, a P-gp substrate that had high permeability in the Caco-2 model but medium permeability in the MDCK model. This explained why both compounds being P-gp substrates with comparable affinities indicated high permeability in the Caco-2 model and medium permeability in the MDCK model.[14]

Compounds **I** and **II** had high human intestinal absorption with scores of 0.02 and 0.01, suggesting that more than 30% of the compounds is absorbed in human intestine. Also, the result showed that compound **II** is more readily absorbed in the human intestine in comparison to compound **I**.

Oral bioavailability prediction suggested that the two compounds are orally bioavailable complementing the results of Lipinski's rule's results. Compounds **I** and **II** had

Table 2 Theoretical oral bioavailability of compounds I and II based on Lipinski's rule of five

Lipinski's rule of five[a]						
Compound	Mol. Wt[b]	HBA	HBD	nRB	MlogP	Remarks
I	288.38	3	2	5	3.05	Pass
II	220.26	3	2	3	1.89	Pass

Abbreviations: HBA, hydrogen bond acceptor; HBD, hydrogen bond donor; nRB, number of rotatable bonds; MlogP, lipophilicity.
[a]Lipinski rule (Mol. Wt $\leq$ 500, MlogP $\leq$ 4.15, N or O $\leq$ 10, NH or OH $\leq$ 5 and nRB $\leq$ 10).[4,5]
[b]Molecular weight in g/mol.

Table 3 Absorption

Compound	Caco-2 permeability	MDCK permeability	Pgp-inhib.	Pgp-subs.	HIA	F30%	F20%	F10%
I	−4.70	15×10^{-6}	0.82	0.05	0.02	0.36	0.96	0.55
II	−4.64	15×10^{-6}	0.07	0.02	0.01	0.29	0.92	0.55

Note: Empirical decision for P-gp, HIA, and F: 0–0.3, excellent; 0.3–0.7, good; 0.7–1.0, poor.

Table 4 Distribution

Compound	PPB (%)	Volume distribution (L/kg)	BBB (%)	F_u (%)
I	89.96	3.452	0.049	7.638
II	97.49	0.800	0.082	3.974

Abbreviations: PPB, plasma protein binding; BBB, blood–brain barrier; F_u, fraction unbound.
Note: Empirical decision for BBB: 0–0.3, excellent; 0.3–0.7, good; 0.7–1.0, poor.

excellent 30% oral bioavailability with F30% scores of 0.36 and 0.29, good 10% oral bioavailability with F10% scores of 0.55 and 0.55 (SwissADME), yet, poor 20% oral bioavailability with F20% scores of 0.96 and 0.92, respectively.

Distribution

As shown in ►**Table 4**, compound **I** was predicted to have protein plasma binding (PPB) of 89.96%, a value considered as optimal PPB, and compound **II** predicted a higher PPB value of 97.49%. However, statistics have shown that lump-sums of United States Food and Drug Administration (FDA)-approved drugs have PPB greater than 99%.[14] The volume distribution (VD) values of both compounds fall between 0.04 and 20 L/kg for optimal distribution as indicated by the results in ►**Table 4**. As a consequence of their PPB values, compound **I** demonstrated medium fraction unbound (F_u) of 7.638% while compound **II** indicated a low F_u of 3.974%. In spite of their low F_u, both compounds had excellent blood–brain barrier penetration with scores less than 0.1.

Metabolism

Compound **I** is a strong inhibitor and substrate of CYP2D6 and CYP3A4, a weak inhibitor of CYP2C9, and a noninhibitor of CYP1A2 and CYP2C19. Also, the compound has high substrate affinity for CYP1A2 and CYP2C19, and was nonsubstrate of CYP2C9 enzyme (►**Table 5**). On the other hand, compound **II** was a strong inhibitor and substrate of CYP3A4, a weak inhibitor of CYP2C9, and a noninhibitor of CYP2D6, CYP1A2, and CYPC19 enzymes. Compound **II** is also a strong substrate of CYP2C19, a weak substrate of CYP1A2 and CYP2D6, and a nonsubstrate of CYP2C19 (►**Table 5**).

Excretion

Clearance of compound **I** (15.726 mL/min/kg) is slightly higher than that of compound **II** (15.045 mL/min/kg), suggesting a high clearance rate of both of the compounds. The half-life ($T_{1/2}$) score of compound **II** was 0.85 (►**Table 6**), suggesting a short half-life of compound **II** within 3 hours. However, $T_{1/2}$ of compound **I** was 0.57, suggesting a moderate half-life of compound **I** of $\leq$3 hours.

Table 6 Excretion

Compound	Clearance (mL/min/kg)	Half-life ($T_{1/2}$)
I	15.726	0.568
II	15.045	0.850

Note: Empirical decision for $T_{1/2}$: 0–0.3, excellent; 0.3–0.7, good; 0.7–1.0, poor.

Toxicity

►**Tables 7** to **12** illustrate the different toxicity endpoints of compounds **I** and **II**. The percentage of predicted accuracy and percentage of average similarity of each compound, compared with the datasets of the models used on Protox-II, were 68.07 and 69.12% for compounds **I** and **II** (►**Table 9**).

Organ Toxicity

Lethality dose (LD_{50}) of compounds **I** and **II** were 2,830 mg/kg (►**Table 7**), which were categorized as Class V: (2,000 mg/kg $< LD_{50} \leq$ 5,000 kg/kg), suggesting that they may be harmful if swallowed according to the toxic class of the globally harmonized system of classification of labeling of chemicals. Predicted rat or mice oral acute toxicity (OAT) scores of compounds **I** and **II** were 0.21 and 0.07, which fall in the category of OAT >500 mg/kg for low toxicity translating that the compounds are safe. Our data also showed that compounds **I** and **II** are predicted as noncauser of human hepatotoxicity or drug-induced liver injury with inactivity probabilities of 0.68 and 0.57, noncarcinogenic with probabilities of 0.68 and 0.70, nonimmunotoxic with probabilities of 0.99 and 0.62, nonmutagenic with probabilities of 0.75 and 0.75, and noncytotoxic with probabilities of 0.76 and 0.71, respectively (►**Table 7**).

Furthermore, compounds **I** and **II** are also nonblocker of the human ether-a-go-go related gene (hERG) as indicated by their scores of 0.01 (►**Table 8**), which translates to an excellent safety profile. Therefore, they may not cause hERG toxicities which include long QT syndrome, arrhythmia, and Torsades de Pointes that were associated with palpitations, fainting, or even sudden death.[14] Compound **I** could not cause respiratory toxicity with excellent safety score of 0.16, while compound **II** has a score of 0.40 (►**Table 8**), suggesting a lower safety profile when compared

Table 5 Metabolism

Compound	CYP1A2		CYP2C19		CYP2C9		CYP2D6		CYP3A4	
	Inhib.	Subs.	Inhib.	Subs.	Inhib.	Subs.	Inhib.	Subs.	Inhib.	Subs.
I	0.85	0.33	0.74	0.16	0.69	0.82	0.50	0.32	0.13	0.14
II	0.97	0.64	0.81	0.08	0.59	0.81	0.88	0.53	0.28	0.20

Abbreviations: Inhib., inhibitors; Subs., substrate.
Note: Empirical decision: 0–0.3, excellent; 0.3–0.7, good; 0.7–1.0, poor.

Table 7 Organ toxicity

Compound	LD_{50} (mg/kg)	Toxic class	H-HT/DILI	Carcinogenic	Immunotoxic	Mutagenic	Cytotoxic
I	2,830	5	0.68 (I)	0.68 (I)	0.99 (I)	0.75 (I)	0.76 (I)
II	2,830	5	0.57 (I)	0.70 (I)	0.62 (I)	0.75 (I)	0.71 (I)

Abbreviation: I, inactive.
Note: Empirical decision for H-HT/DILI: 0–0.3, excellent; 0.3–0.7, good; 0.7–1.0, poor.

Table 8 Organ toxicity continued

Compound	hERG blocker	OAT	FDAMDD	Skin sensitivity	Respiratory toxicity
I	0.01	0.21	0.04	0.84	0.16
II	0.01	0.07	0.19	0.53	0.40

Abbreviations: FDAMDD, FDA maximum recommended daily dose; hERG, human ether-a-go-go-related gene; OAT, oral acute toxicity.
Note: Empirical decision: 0–0.3, excellent; 0.3–0.7, good; 0.7–1.0, poor.

Table 9 Organ toxicity continued

Compound	Eye corrosion	Eye irritation	Prediction-accuracy (%)	Average similarity (%)
I	0.004	0.468	68.07	69.12
II	0.055	0.935	68.07	69.12

Note: Empirical decision for eye corrosion/irritation: 0–0.3, excellent; 0.3–0.7, good; 0.7–1.0, poor.

Table 10 Nuclear receptor pathway toxicity

Compound	NR-AR	NR-AR-LBD	NR-AhR	NR-Ar	NR-ER	NR-ER-LBD	NR-PPAR-γ
I	0.99 (I)	0.99 (I)	0.91 (I)	0.98 (I)	0.85 (I)	0.93 (I)	0.96 (I)
II	0.98 (I)	0.99 (I)	0.76 (I)	0.94 (I)	0.77 (I)	0.86 (I)	0.96 (I)

Abbreviations: I, inactive.

Table 11 Stress response pathway toxicity

Compound	SR-ARE	SR-ATAD5	SR-HSE	SR-MMP	SR-p53
I	0.87 (I)	0.97 (I)	0.87 (I)	0.76 (I)	0.84 (I)
II	0.80 (I)	0.97 (I)	0.80 (I)	0.61 (A)	0.77 (I)

Abbreviations: A, active; I, inactive.

Table 12 Environmental toxicity

Compound	BCF [log10(L/kg)]	IGC_{50}	LC_{50}FM	LC_{50}DM
I	1.09	3.77	5.12	6.13
II	0.85	3.77	4.44	5.31

Abbreviations: BCF, bioconcentration factor; IGC_{50}, concentration of a substance in water in mg/L that could cause 50% growth inhibition to *Tetrahymena pyriformis* after 48 hours; LC_{50}FM, concentration of a substance in water in mg/L that could cause 50% of fathead minnow to die after 96 hours; LC_{50}DM, the concentration of the designed hydrazones in water in mg/L that could cause 50% of *Daphnia magna* to die after 48 hours.
Note: Unit for IGC_{50}, LC_{50}FM, and LC_{50}DM is $-\log10[(mg/L)/(1{,}000 \times MW)]$

with compound **I**. Also, both compounds are nontoxic, with FDA maximum recommended daily dose below 0.2 mmol/kg-bw/d (►**Table 8**). However, compound **I** was predicted to be skin sensitive with a score of 0.84, therefore may not be formulated for topical application. While compound **II** was relatively non-skin sensitive with a score of 0.53, it may be formulated for topical application.

As shown in ►**Table 9**, compounds **I** and **II** are non-eye corrosive with excellent safety scores of 0.004 and 0.06, respectively. However, compound **II** is an eye irritant with

Table 13 Effect of compounds I and II on curative activity in *Plasmodium berghei*-infected mice

Treatment	Dosage (mg/kg)	Six fields (% inhibition of parasite growth)	Parasitemia levels
DW	10	–	27.90 ± 2.75
Compound I	25	68.03	8.92 ± 0.41
	50	65.16	9.72 ± 0.30
	100	69.75	8.44 ± 0.91
Compound II	25	33.33	18.60 ± 2.13
	50	39.43	16.90 ± 1.30
	100	72.16	7.77 ± 0.83
CQ	5	95.69	1.20 ± 0.10

Abbreviations: CQ, chloroquine; DW, distilled water.
Note: Values are presented as mean ± standard error of the mean; data were analyzed by one-way ANOVA followed by Dunnett's post-hoc test, $n = 5$.

a score of 0.94, while compound **I** is relatively non-eye irritant with a score of 0.47.

Tox21 Pathway

Nuclear receptor pathway toxicity. Compounds **I** and **II** was predicted not to interact with any of the nuclear receptors with probabilities between 0.85 for estrogen receptor (NR-ER) and 0.99 for androgen receptor (NR-AR) according to results in ►**Table 10**. This suggests that the compounds may not cause nuclear receptor pathway toxicity.

Stress response pathway toxicity. Compound **I** was predicted as nontoxic to stress response pathways indicating noninteraction with any of the stress response receptors. The noninteraction probabilities of the compound ranged between 0.76 for mitochondrial membrane potential (SR-MMP) to 0.97 for ATPase family AAA domain-containing proteins 5 (SR-ATAD5) (►**Table 11**). While compound **II** was also inactive against nearly all of the stress response receptors with noninteraction probabilities between 0.77 for phosphoprotein (tumor suppressor) p53 SR-p53 and 0.97 for SR-ATAD5 (►**Table 11**). It is found to interact with SR-MMP with 0.61 activity probability.

Environmental toxicity. Compounds **I** and **II** had bioconcentration factors (BCFs) <3.000 log10(L/kg) according to the results in ►**Table 12**, which corresponds to BCF <1,000 L/kg categorized as nonbioaccumulative by the United States Environmental Protection Agency under the Toxic Substances Control Ac. These values are also below the 3.700 earmarked by Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) threshold for very bioaccumulative chemicals. While compounds **I** and **II** had equal tetrahymena pyriformis (IGC_{50}) values (3.77). Compound **I** demonstrated safer fathead minnow LC_{50} (LC_{50}FM, 5.12) and daphnia magna LC_{50} (LC_{50}DM, 6.13) when compared with compound **II** (4.44 and 5.31, respectively).

Antimalarial Activity

Mice with plasmodium infection were treated with compound **I** (25, 50, 100 mg/kg) and compound **II** (25, 50, 100 mg/kg). Chloroquine was used as a control drug. Then, parasitemia levels were evaluated, and the results are shown in ►**Table 13**. Our data showed that parasitemia levels in mice treated with compounds **I** and **II** were significantly lowered than those treated with distilled water, suggesting the antimalarial activity of the two compounds; however, parasitemia levels of compound **I** and **II**-treated group were much higher than the control drug (chloroquine), suggesting a much weaker activity of the two compounds when compared with the reference drug.

Discussion

Synthesis and Characterization

Two prenylated acetophenones were synthesized using 3-methyl-2-buten-1-ol under nitrogen conditions to minimize oxidative side-product(s) and efficient prenylation on positions 3 and 5 of the dihydroxy acetophenone. The synthesis was achieved in low yields of 6.25, 10.0, and 0.07% for compounds **I**, **II**, and **III**, respectively. Oral bioavailability is an important parameter in drug design as it reduces drug failure resulting from poor PK profile. The two compounds passed Lipinski's rule as revealed in ►**Table 2**. This showed that the compounds would be orally bioavailable.

Compounds **I**, **II**, and **III** were found to be white crystalline solids according to a reported study.[15] Melting points were within the range of 110 to 112°C for compound **I**, 145 to 146°C for compound **II**, and 154 to 156°C for compound **III**. The FTIR spectra showed the presence of prominent bands at 3,384–3,272 cm^{-1} (OH stretching vibration), 1,613–1,617 cm^{-1} (C = O stretching vibration), 2,851–2,728 cm^{-1} (sp^3 C-H stretching vibration), 2,914–2,911 cm^{-1} and 2,974–2,967 cm^{-1} (sp^2 C-H stretching vibration) (►**Figs. 3–5**). The sp^2 and sp^3 stretches indicated the presence of the prenylated groups on compounds **I**, **II**, and **III**. The ^{1}H NMR and ^{13}C NMR spectra were used to elucidate the structures of compounds **I** and **II**. Also as shown by ^{1}H and ^{13}C NMR spectra, there were appearances of distinct peaks indicating prenylation had occurred on the substituted acetophenone ring. The ^{1}H-NMR of compound **I** showed distinct peaks at 5.31 and 6.27 ppm, indicating the presence of phenolic protons on the aromatic group, peaks at 3.33 and 3.23 ppm corresponded to allylic protons in the prenylated groups. Peaks at 7.49 ppm indicated the aromatic proton. Peaks at 1.76 ppm corresponded to signals for terminal methyl groups of the

prenyl groups, while the peak at 2.50 ppm corresponded to α-methyl of the ketone. For compound **II**, the proton peaks at 7.58 and 5.51 ppm corresponded to aromatic protons, the peaks at 5.37 and 5.11 ppm indicated for phenolic protons, the peak at 3.54 ppm indicated for allylic proton, the peak at 3.45 ppm corresponded to methylene protons of the prenyl group, the peak at 2.69 ppm corresponded to α-methyl of the ketone, and the peak at 1.751 ppm indicated for the terminal methyl groups on compound **II** accordingly.

Antimalarial Activity

This study is the first report of antimalarial activity of prenylated acetophenone. As shown in ►**Table 13**, at doses of 25 and 50 mg/kg, compound **I** showed promising activity with percentage inhibition of 68.03 and 65.16% respectively, demonstrating superior activities when compared with compound **II** (33.33 and 39.43%). This suggests that prenylation at position 3 of the acetophenone is important for antimalarial activity. Furthermore, the superior activities of compound **I** may be due to its better PK profiles in respect to their PPB, VD, and F_u as displayed in ►**Table 4**.

However, at a higher dose of 100 mg/kg, compound **II** demonstrates supper-activity with the inhibition rate of parasitemia level being 72.16% when compared with compound **I** (69.75%). Furthermore, the antimalarial activity of compound **II** was dose-dependent. A closer analysis also indicated 100% increase in activity of compound **II** when the dose was increased from 50 to 100 mg/kg.

To evaluate the possible prodrug effect of the compounds, the curative model was chosen for the study. Krettli et al suggested that a compound should be considered active when its parasitemia reduction is $\geq$30%.[16] In another study, antimalarial agents are classified into three categories as moderate, good, and very good if the compound showed parasitemia suppression percentage equal or greater than 50%.[17,18] Consequently, based on these criteria, compounds **I** and **II** were presumed to have very good antimalarial activity. Furthermore, compounds **I** and **II** demonstrated a superior antimalarial activity compared with nerolidylcatechol and its derivatives in a similar study,[19] suggesting that the resorcinol moiety of compounds **I** and **II** is important for antimalarial activity and therefore confers more antimalarial activity compared with the catechol moiety. Early communication of these findings has been reported in a preprint.[20]

Conclusion

The two prenylated compounds showed significant antimalarial activity as displayed by their ability to suppress *Plasmodium berghei* infection in mice. The two prenylated acetophenones demonstrated drug-like physicochemical parameters with good PK profile and excellent safety profile. Thus, these scaffolds should play significant roles in designing compounds with better antimalarial activity.

Supporting Information

Proton and carbon NMR of compound **I** and **II** are included in the ►**Supporting Information (►Figs. S1–S4** [online only]).

Conflict of Interest

The authors declare no conflict of interest.

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