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New quinolinyl chalcones with potential antiplasmodial activity: synthesis and *in-vivo* studies on *Plasmodium berghii*

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Abstract: Malaria remains a devastating parasitic disease of the tropics, with reported decreased sensitivity to all the available drugs. Spurred by the previous literature and computational studies against four *plasmodium* proteases, three quinolinyl chalcone derivatives; 3-(2-chloro-6-methoxyquinolin-3-yl)-1-(2, 4-dimethoxy phenyl) prop-2-en-1-one (P3), 3-(2-chloroquinolin-3-yl)-1-(2, 4-dimethoxy phenyl) prop-2-en-1-one (P4) and 3-(2-chloro-6-methoxyquinolin-3-yl)-1-(2, 3, 4-trimethoxyphenyl) prop-2-en-1-one (P5) were synthesized and evaluated for their *in vivo* antiplasmodial activity. The synthesis was achieved through the Claisen–Schmidt condensation reaction of polymethoxylated acetophenones and substituted 3-quinolinyl carbaldehydes. The structures of these compounds were resolved using Fourier transform infrared (FT-IR), Proton, Carbon-13 and, two-dimensional nuclear magnetic resonance (NMR) spectroscopy in addition to, mass spectrometry (MS). *In vivo* antiplasmodial activity in mice infected with *Plasmodium berghei* parasite, was evaluated using a curative model. One-way analysis of variance (ANOVA) and Bonferroni post-hoc test was used to determine the statistical significance compared to the control.

Findings revealed that P5 showed significant ($p < 0.001$) dose-dependent chemo suppressive activity of 59.09, 70.36 and 77.6% at doses of 25, 50 and 100 mg kg-1 respectively, in comparison to 10 mg kg-1 quinine (88.5%) and 25 mg kg-1 chloroquine (85.5%). Thus, P5 displayed the highest chemo suppressive activity, followed by P3 when compared to P4, indicating the importance of the 6"-OCH3 substitution on the quinolinyl ring for antiplasmodial activity. The activity of P5 at 100 mg kg-1 is close to that of 25 mg kg-1 chloroquine, therefore P5 is a potential antimalarial compound with a novel target. P3 and P5 are new compounds not reported in any chemical literature.

Keywords: Malaria; Antiplasmodium; Quinolinyl-Chalcones; Proteases; Schmidt-Claisen condensation.

1. INTRODUCTION

Malaria is a devastating infectious disease of the tropics caused by a parasitic protozoan. Among the four important *Plasmodium* (P) species responsible for causing malaria in humans, *P.falciparum* is the most virulent and predominant in Africa[1]. In 2019 alone, there were an estimated 229 million cases of malaria worldwide and malaria deaths stood around 409 000[2].

Despite the global efforts to defeat malaria, it is still a major threat to public health. One of the major hurdles to control malaria is the resistance of malaria parasites to most of the commonly used antimalarials. Presently, Artemisinin Combination Therapy (ACT) is the first-line intervention in *P. falciparum* malaria[2]. However, there is a reported decrease in sensitivity of the ACTs in some parts of Asia[3,4,1], and development of resistance in *P falciparum* culture as well as clinical isolates. ACT's resistance to *Plasmodium falciparum* is also reported in Africa[5]. This, coupled with the unavailability of a clinically effective vaccine has motivated scientists to develop new drugs with novel targets. With the unveiling of full genome sequences of *P. falciparum*, numerous new drug targets have been proposed[6,7].

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These are quite diverse and of our interest are the *P. falciparum*, proteases[8-10]. Proteases have been identified as promising drug targets through several experiments [11,12]. They are said to be responsible for degrading host hemoglobin to provide essential amino acids for the parasite survival[13-16]. Proteases having key roles in hemoglobin degradation include aspartic (plasmepsins) and cysteine (falcipains) proteases [7]. Another role of plasmodium proteases in the pathogenesis of malaria disease includes activation of inflammation, cell/tissue penetration, invasion of erythrocyte, development of the parasite, immune evasion, autophagy, and other proteins breakdown [17,18].

Interestingly, chalcone (Figure 1) was identified through computational studies as *Plasmodium* cysteine protease inhibitor[19]. At the same time, Licochalcone A isolated from Chinese licorice roots was identified as a potent antimalarial compound[20]. Since then, there was substantial interest in the scientific community to develop chalcone as an effective antimalarial drug. Various derivatives have been synthesized and were found to possess promising antimalarial activity [19,21-24]. Several efforts were made to determine the features necessary for the antimalarial activity for optimization through QSAR and molecular modeling studies[16,25.] From these studies, the size and hydrophobicity of substituents on both rings of chalcones were identified as critical parameters. In addition, most of the hydroxylated chalcones were found to be less active than the corresponding alkoxylated derivatives[16].

Quinolines represent a significant class of heterocycles and are established chemical scaffolds in many important antimalarials such as; quinine and chloroquine[26]. For more than a decade, several quinolinated chalcones have been synthesized and assessed for antiplasmodial activity with promising outcomes[18,27-30].

The present study describes the synthesis and *in-vivo* antiplasmodial activity of three new quinolinyl chalcones (Table 1) in mice infected with the *Plasmodium berghei* parasite. The compounds designed in this study are based on features of some active compounds from previous studies, constituting various active chemical scaffolds. Hybrid design was applied, where 2-chloro quinolinyl ring A scaffold and, 2,3,4 trimethoxy / 2,4 dimethoxy ring B scaffold associated from previous studies[18,27], with potent antimalarial activity were merged to form a single chemical entity (Figure 2). Additionally, the selection of these compounds was guided by computational studies against four plasmodium proteases (manuscript in preparation).

Figure 1. Chalcone.

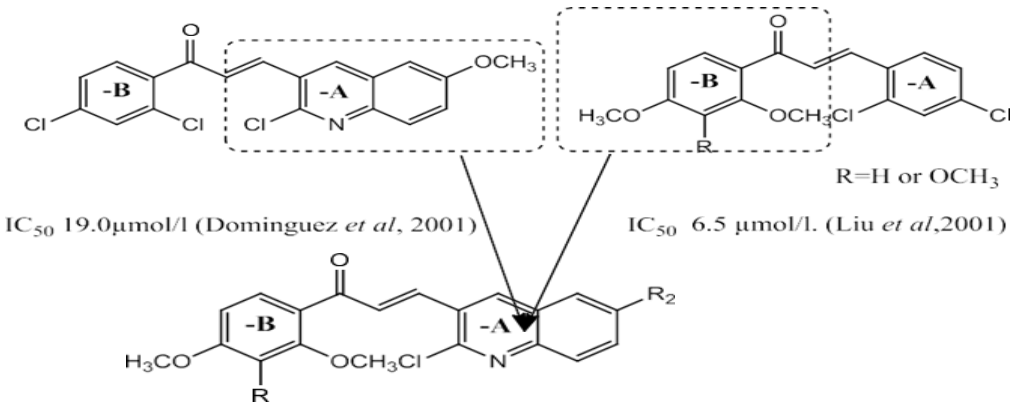
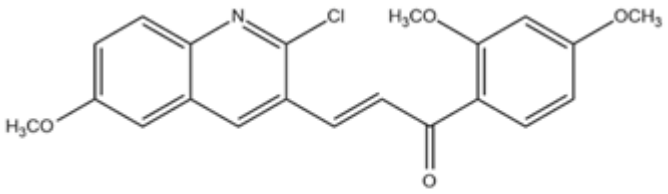
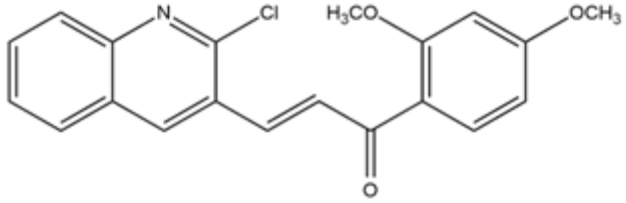
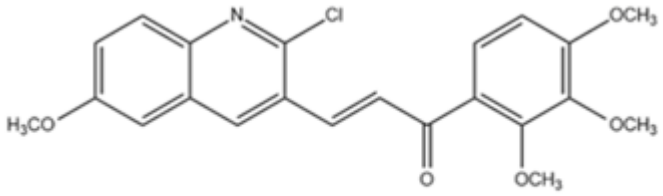


Figure 2. Hybrid design of quinolinyl chalcone (R, R_2 = H or OCH_3).

Table 1. Chemical description of P3, P4, and, P5**.**

Compound ID	Molecular formula	2D- Representation/IUPAC Name
P3	$C_{21}H_{18}ClNO_4$	 3-(2-chloro-6-methoxyquinolin-3-yl)-1-(2,4-dimethoxyphenyl)prop-2-en-1-one
P4	$C_{20}H_{16}ClNO_4$	 3-(2-chloroquinolin-3-yl)-1-(2,4-dimethoxyphenyl)prop-2-en-1-one
P5	$C_{22}H_{20}ClNO_5$	 3-(2-chloro-6-methoxyquinolin-3-yl)-1-(2,3,4-trimethoxyphenyl)prop-2-en-1-one

2. METHODS

2.1. Equipment

All glassware used has been made from Pyrex and were properly cleaned and dried before use. Melting points are uncorrected and, were determined in a Gallenklamp melting point apparatus. FT-IR spectra were determined on an Agilent model 470 spectrophotometer. ^{1}H-NMR and ^{13}C-NMR spectra were recorded using a 400 MHz Agilent and 500 MHz Bruker spectrometer and are reported in ppm downfield from TMS as an internal standard. Mass spectra were performed in an Agilent model 5995/gas chromatography ionization energy 70 eV.

2.2. Reagents, solvents, and standard Drugs

All starting reagents and solvents used for the experiments were of analytical grade and were used without further purification, including; 2,3,4-trimethoxyacetophenone, 2, 4-dimethoxyacetophenone, 2-chloro-6-methoxy-3-quinoline carbaldehyde, 2-chloro-3-quinoline carbaldehyde, Sodium hydroxide (50%), ethanol, hydrochloric acid, 10% Giemsa stain, Acacia, Chloroquine phosphate, quinine, artemether. Most of the reagents were obtained from Sigma Aldrich Germany. The purity of the starting materials was ascertained using thin-layer chromatography (TLC).

2.3. Experimental animals

The animals used in this study were Swiss albino mice of both sexes, weighing from 20 to 24 g and obtained from the Animal House of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. They were housed in clean polypropylene cages under standard laboratory conditions and allowed free access to a standard rodent pellet diet and water *ad libitum*. All animal experimental protocols complied with the Ahmadu Bello University research guidelines and internationally recognized guidelines for the use and care of laboratory animals.

2.4. Malarial parasite

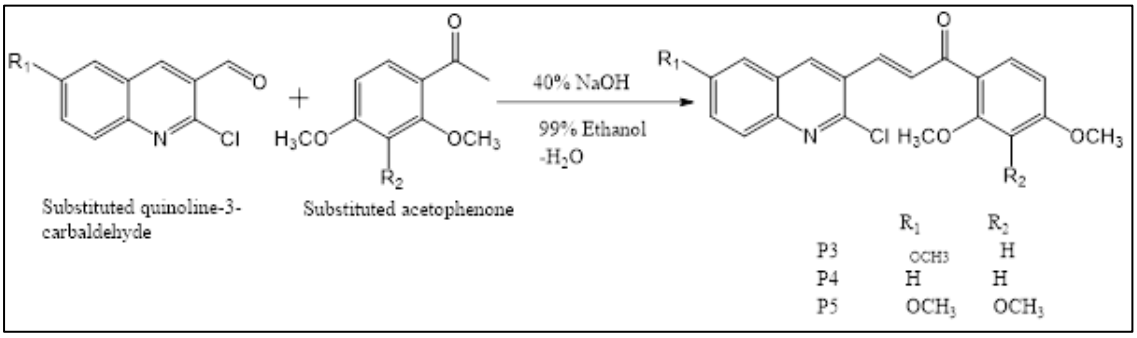
The parasite used for the study is a chloroquine-sensitive malaria parasite (*Plasmodium berghei* NK 65), obtained from the Department of Microbiology- National Medical Research Institute (NIMR), Lagos. The parasites were kept viable in new groups of mice by continuous intraperitoneal injection of 0.2ml of infected red blood cells every four days[31].

2.5. Evaluation of theoretical oral bio-availability

The in silico oral bio-availability was predicted according to Lipinski 's rule of five[32] before the synthesis on the SwissADME server (http://www.swissadme.ch).

2.6. Synthesis and characterization of quinolinyl chalcones (Claisen –Schmidt condensation)

The synthesis of the three quinolinyl chalcones was achieved by base-catalyzed condensation of corresponding equimolar amounts of substituted acetophenone and substituted benzaldehyde using NaOH as a catalyst. The chemical equations for the synthesis are as shown in Scheme 1. The progress of the reactions was monitored by thin-layer chromatography (TLC) on silica gel plates. Ethyl acetate: n-hexane (7:3) was used as the developing solvent, the appearance of a single new spot and the disappearance of the reactant's spot indicate the formation of the product, which were visualized under 254 nm ultraviolet light and iodine vapor.



Scheme 1. Synthesis of substituted 3-quinolinyl chalcones.

2.6.1. General procedure for the synthesis of the three quinolinyl chalcones (P3, P4, and P5)

Equimolar amounts of the substituted acetophenone (0.01 moles) and substituted quinoline-3-carbaldehyde (0.01 moles) were mixed with 20 ml ethanol in a round bottom flask. To this 10 ml of 40%, sodium hydroxide solution was added drop-wise with continuous stirring for 30 minutes while keeping the mixture cold. The mixing was then continued for another 6 - 8 hours at room temperature, using a magnetic stirrer, and kept in the refrigerator overnight until it formed a solid mass. Drops of 10% HCl were added to neutralize the reaction. The mixture was then diluted using 40 ml ice-cold distilled water and filtered. The residue was washed well with more ice-cold distilled water and air-dried. The product was crystallized from 99% ethanol. P3 and P5 were however purified using anti-solvent recrystallization. The purity of the final products was checked using TLC and melting point.

2.6.2. Spectroscopic analysis

Detailed structural assignment of the synthesized compounds was carried out using FT-IR, mass spectrometry (MS), and various types of nuclear magnetic resonance (NMR). FT-IR data are reported in terms of frequency of absorption ν cm^{-1}. Data for ^{1}H-NMR are reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, dd = doublet of a doublet, m = multiplet), integration (J in Hz). Data for ^{13}CNMR expressed as chemical shift δ (ppm).

2.7. Evaluation of *in vivo* antiplasmodial activity

2.7.1. Inoculation of plasmodium berghei parasites

Blood was collected into a heparinized capillary tube via the tail vein of a donor mouse with a *P berghii* Parasitemia level of approximately 20-25% and then transferred to a sterile plain bottle. Physiological saline (0.9%) was used to dilute the blood based on the level of parasitemia of the donor mice to obtain a blood suspension in which there were approximately 5×10^7 infected erythrocytes per milliliter. Each mouse was then inoculated intraperitoneally with 0.2ml of blood suspension containing approximately 1×10^7 infected erythrocytes per milliliter [33].

2.7.2. Curative test in mice

Evaluation of the curative potential of the compounds against an established plasmodia infection was done as described by Ryley and Peters[34]. Sixty (60) mice were infected intraperitoneally with parasites as described above and left for 72 hours before treatment was initiated. After Parasitemia was established, blood smears were collected and examined microscopically to determine the level of parasitemia. The mice were randomly divided into twelve groups of five mice each. Groups 1-9 received three grade doses (25, 50 and, 100 mg kg^{-1} body weight) of the three quinolinyl chalcones. Groups 10, 11, and 12 were administered 25 mg kg^{-1} of chloroquine, 10 mg kg-1 of quinine, and 0.2 ml of 1%w/v acacia suspension intraperitoneally respectively. The treatment was given for four consecutive days. On the fifth day, blood was collected from the tail vein of each mouse and smeared on a slide to produce a thin film for parasitemia determination as described by[35].

Average percent parasite suppression relative to the control was calculated for each treatment group using the formula below [36];

$$\% \text{ Parasitemia} = \frac{mean\ parasitemia\ (-ve\ control)\ -\ mean\ parasitemia\ (+ve\ control)}{mean\ parasitemia\ (-ve\ control)} \times 10$$

2.8. Data analysis

SPSS statistical software was used to analyze the data obtained from all tests using one-way ANOVA followed by Dunnett's post hoc test. The results were considered statistically significant with p-values < 0.05.

3. RESULTS

3.1. Evaluation of theoretical oral bio-availability

All the quinolinyl chalcones designed have passed the criteria put forward by Lipinski *et al.* (2009) for a chemical compound to be orally bioavailable[32] (Table 2).

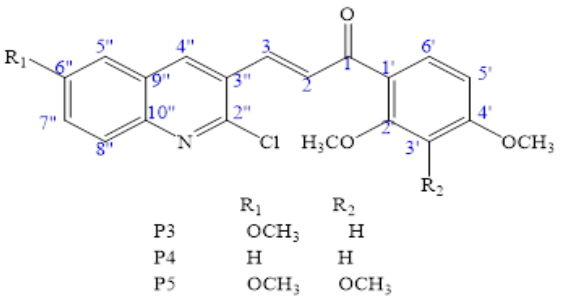
3.2. Synthesis and characterization

The procedure using Claisen-Schmidt condensation furnishes good yields, and the physical appearance of the quinolinyl chalcones is in agreement with their reported form[16]. Figure 3, was used to assign the spectral data obtained.

Table 2. Analysis of theoretical oral bioavailability based on Lipinski's rule of five.

Compound ID	Lipinski's rule of five[b]					
P3	383.82	5	0	6	2.35	Pass
P5	413.85	6	0	7	2.01	Pass

(a)Molecular weight in g/mol, (b) Lipinski et al, 2001 (Mwt≤500, MLogP≤4.15, N or O≤10, NH or OH≤5 and number of rotatable bonds≤ 10)[32].



	R_1	R_2
P3	OCH_3	H
P4	H	H
P5	OCH_3	OCH_3

Figure 3. Atom numbering of 3-quinolinyl-substituted chalcones (P3, P4 and, P5), for structural assignments.

3.2.1. Compound P3: 3-(2-chloro-6-methoxyquinolin-3-yl)-1-(2,4-dimethoxyphenyl) prop-2-en-1-one

Light yellow crystal; % Yield 50.64; R_f value 0.40 (n-Hexane: Ethyl acetate (7:3); mp 109 – 111^{0C}; FT-IR (ν cm^{-1}) 3011 (Ar CH), 2983 (=CH), 2848 (-CH), 1664 (C=O), 1579 (C=N) 1550 (C=C), 1238 and 1017 (C-O-C); ^{1}HNMR $CDCl_3$ – δ 7.84(d, 1H, J=21Hz, H3), 7.77(d, 1H, J=8.68Hz, H6'), 7.19(d, 1H, J=9.2Hz, H8''), 7.16(dd, 1H, J=2.24Hz, 8.88Hz, H7''), 6.81(d, 1H, J=20Hz, H2), 6.45(dd, 1H, J=2.68Hz, 9.16Hz, H5'), 6.24(d, 1H, J=2.12Hz,

H3'), 4.93(d, 1H, J=2.40Hz, H4''), 4.79(d, 1H, J=6.36Hz, H5''), 3.76 (s, 3H, OMe), 3.74 (s, 3H, OMe), 3.38 (s, 3H, OMe); ^{13}CNMR 196.42 (C1), 164.69 (C4'), 160.39 (C2'), 158.04 (C6''), 148.88 (C2''), 142.16 (C10''), 135.09 (C3), 133.24 (C6'), 132.91 (C3''), 129.44 (C8''), 127.92 (C9''), 122.34 (C7''), 119.75 (C1'), 105.84 (C5'), 104.86 (C2), 97.89 (C3'), 55.55 (OCH_3 2'), 55.51 (OCH_3 4'), 55.09 (OCH_3 6''), 53.09 (C4''), 41.44 (C5'');

3.2.2. Compound P4: 3-(2-chloroquinolin-3-yl)-1-(2,4-dimethoxyphenyl) prop-2-en-1-one

Yellow cotton-like; % Yield 28.27; R_f value 0.55 (n-Hexane: Ethyl acetate (7:3)); mp 80 – 81^{0C} FT-IR (ν cm^{-1}) 2999 (Ar CH), 2944 (=CH), 2838 (-CH), 1642 (C=O), 1585 (C=N) 1552 (C=C), 1257 and 1093 (C-O-C); ^{1}HNMR $CDCl_3$ – δ 8.39(d, 1H, J=2.40Hz, H4''), 8.03(d, 1H, J=15.9Hz, H3), 7.82(d, 1H, J=10.0Hz, H5''), 7.80(d, 1H, J=9.00Hz, H6'), 7.72(d, 1H, J=16Hz, H2), 7.72(d, 1H, J=9.2Hz, H8''), 7.61(d, 1H, J=9.0Hz, H6''), 7.46(dd, 1H, J=4.04Hz, 9.0Hz, H7''), 6.57(dd, 1H, J=2.1Hz, 8.6Hz, H5'), 6.49(d, 1H, J=2.00Hz, H3'), 3.76 (s, 3H, OMe), 3.74 (s, 3H, OMe); ^{13}CNMR 189.55 (C1), 164.64 (C4'), 160.75 (C2'), 150.51 (C2''), 147.47 (C10''), 136.24 (C3), 133.24 (C4''), 133.07 (C8'''), 131.50 (C2), 131.38 (C3''), 128.65 (C5''), 128.27 (C6'), 127.80 (C6''), 127.12 (C9''), 123.84 (C1'), 121.61 (C7''), 105.4 (C5'), 98.67 (C3'), 55.84 (OCH_3 2'), 55.61 (OCH_3 4'); Mass spectrum (*m/z*) M^+ 354.

3.2.3. Compound P5: 3-(2-chloro-6-methoxyquinolin-3-yl)-1-(2,3,4-trimet hoxyphenyl) prop-2-en-1-one

Yellow cotton-like; % Yield 50.29; Rf value 0.43 (n-Hexane: Ethyl acetate (7:3)); mp 88-89 – 94^{0C} FT-IR (ν cm-1) 3093 (Ar CH), 3000 (=CH), 2847 (-CH), 1664 (C=O), 1613 (C=N) 1579, (C=C), 1274 and 1095 (C-O-C); ^{1}HNMR $CDCl_3$ – δ 7.80(d, 1H, J=16.0Hz, H3), 7.72(d, 1H, J=9.20Hz, H6'), 7.42(d, 1H, J=8.84Hz, H8''), 7.21(d, 1H, J=8.84Hz, H5'), 6.82(d, 1H, J=16Hz, H2), 6.62(dd, 1H, J=4.04Hz, 8.84Hz, H7''), 4.97(d, 1H, J=5.10Hz, H4''), 4.86(d, 1H, J=6.36Hz, H5''), 4.09 (s, 3H, OMe 2'), 3.83 (s, 3H, OMe 4'), 3.73 (s, 3H, OMe 6''), 3.65 (s, 3H, OMe 3') ; ^{13}CNMR 197.91 (C1), 158.10 (C4'), 157.57 (C6''), 153.35 (C2'), 148.84 (C2''), 142.32 (C3'), 141.29 (C10''), 135.08 (C3), 132.12 (C3''), 129.59 (C6'), 127.82 (C9''), 126.19 (C8''), 124.53 (C1'), 122.40 (C5'), 107.20 (C7''), 104.94 (C2), 61.38 (OCH_3 2'), 60.77 (OCH_3 4'), 56.12 (OCH_3 6''), 55.60 (OCH_3 3'), 51.85 (C5''), 41.73 (C4'').

3.3. *In vivo* antiplasmodial activity against *P. berghei* infected mice.

Table 3 showed the effect of compounds P3, P4, and P5 on the parasitemia level of *P. berghei* infected mice.

Table 3. Effect of compound P3, P4 and P5 on the parasitemia level of *P. berghei* infected mice.

Treatment groups	Dose (mg/kg)	Average parasitemia	Percentage suppression
Acacia	5ml/kg	22.00±1.50	0
CQ	25	3.20±1.01^c	85.45
Quinine	10	2.52±0.41^c	88.54
P3	25	5.74±1.37^c	73.90
P3	50	7.80±1.31^c	64.54
P3	100	6.57±3.52^c	70.15
P4	25	8.34±2.26^a	62.09
P4	50	7.22±1.24^c	67.18
P4	100	10.28±1.00	53.27
P5	25	10.76±3.12	51.09
P5	50	6.52±2.25^b	70.36
P5	100	4.93±0.83^c	77.61

Values are presented as Mean ± SEM; Data analyzed by one way ANOVA followed by Bonferroni 's post-hoc test; n=5 a=p<0.05, c=p<0.001 versus control; CLQ=chloroquine Route of administration=ip.

4. DISCUSSION

Three chalcone derivatives; P3, P4, and P5 which differed from each other in the number of methoxy substitutions were synthesized in a good yield. The choice of the methoxy group is based on previous studies that indicated its importance for good antimalarial activity[16,18]. In addition, the importance of quinoline nuclei in the development of antimalarial drugs cannot be overstated. P3 and P5 are new chalcone derivatives that have not been reported in any literature or chemical database, as determined by a search in the Sci-Finder ^{n} (www.scifinder.com). The three compounds met the criteria proposed by Lipinski[32], for a chemical compound to be orally bio-available (Table 2). Predicting theoretical oral bioavailability is an important step in the drug development process. This

is intended to reduce the likelihood of drug failure later in the process due to poor pharmacokinetics.

The percentage yields for P3, P4, and P5 are 57%, 78%, and 65%, respectively. Both compounds precipitate as mixtures and were separated and purified using recrystallization. P3 appeared as a light-yellow crystal, while P4 and P5 are yellow cotton-like.

The FT-IR data of all the three quinolinyl chalcones (Supplementary Information) showed prominent carbonyl peaks (C=O) that emerged at 1613 cm^{-1} and olefinic (=C-H) vibration at 2983 cm^{-1}. A sharp strongly coupled absorption of C-O-C stretching also occurred at 1236 and 1017 cm^{-1}.

The ^{1}H-NMR, ^{13}C-NMR a, and 2D-NMR spectra confirmed the structures of P3, P4, and P5 (Supplementary Information). In addition to the characteristics α, β-unsaturated ketone linker protons observed as doublets in the region of 6.81 – 7.72 ppm (H- α) and 7.84 - 8.03 ppm (H- β) with coupling constants of 15.9-20 Hz (*trans*-isomers) and the methoxy protons as a singlet at the region of 3.38 – 4.09 ppm, the rest of the protons appear in their expected regions with their usual coupling constants. It is evident from these coupling constants that the products formed are predominantly the *trans*-isomers (E-form). The configuration of the Z isomer is unstable due to the strong steric effects between the carbonyl group and the A-ring[37]. Another noticeable observation is the appearance of C4" and C5" protons up-field for P3 and P5. The two protons of P3 are at 4.93 and 4.79 ppm for C4" and C5" respectively. For P5 the C4" and C5" protons are at 4.97 and 4.86 ppm respectively. The shift of delta value could be explained by the strong influence of electron-donating methoxy group at C6" position causing a shielding effect.

Additional support for the structures of P3, P4, and P5 is also provided by the ^{13}C-NMR spectra (Supplementary Information), in which the carbonyl carbon (C=O) of the α, β-unsaturated ketone linker is observed in the region 196.42, 189.55, 197.91 ppm for P3, P4 and P5, respectively. Also, the α and β-carbon atoms with respect to the carbonyl group give rise to characteristic signals in between δ 104.86 to 131.50 ppm and δ 135.08 to136.24 ppm respectively.

The positions of all carbon atoms in P3, P4, and P5 are assigned (Supplementary Information). The spectroscopic differences between P4 without a methoxy group at C6" and P3, P5 with a methoxy group at the C6" positions were remarkable. Indeed, the presence of the methoxy group had strongly influenced both the ^{1}H-NMR and ^{13}C-NMR. The nature of carbon atoms (CH_3- or –CH) was established using Distortionless Enhancement by Polarization Transfer (DEPT) spectroscopy (Supplementary Information). The two-dimensional (COSY, HSQC, and HMBC) NMR spectroscopic data confirmed the tentative structural assignment that was made using ^{1}H and ^{13}C-NMR for both compounds. The connection between each carbon atom and the attached hydrogen atom (atoms) was established using HSQC and a long-range correlation between protons and carbon was established with HMBC, which led to the linking of substructural fragments (Supplementary Information). The COSY correlation was observed on protons at C3'–C5', C5'- C6', C4"- C5" and C7"- C8" for P3, C5'- C6', C4"- C5" and C7"- C8" for P5 and, C2'–C3', C5'- C6', C5"- C6", C6"- C7" and C7"- C8" for P4 (Supplementary Information).

Mass spectral (MS) data further confirmed the structural assignment using NMR of P4, which indicated its molecular ion peak (m/z) corresponding to the molecular weight. The molecular ion peak $(M + 1)^{+}$ is 354 (Supplementary Information).

In the curative study (Table 3), the quinolinyl chalcones did not completely eliminate the *P. falciparum* parasite after four days of treatment but showed significant parasite suppressive effects. P3 and P4 showed a significant ($p < 0.001$) non-dose dependent parasitemia suppression with the highest percentage inhibition of 73.9 % at 25 mg kg^{-1} and 67.1 % at 50 mg kg^{-1}, respectively. However, P5 showed significant ($p < 0.001$) dose-dependent chemosuppressive activity of 59.09, 70.36 and 77.6% at doses of 25, 50 and 100 mg kg^{-1} respectively, compared to 10 mg kg^{-1} quinine (88.5%) and 25 mg kg^{-1} chloroquine (85.5%). The highest chemosuppressive activity displayed by P5 could indicate the importance of the 6"-OCH_3 substitution on the quinolinyl ring for antiplasmodial activity. Additionally, P3 and P4 have dimethoxy substituted ring B and P5 a trimethoxy substituted ring B, but P3 and P5 were more active than P4, further indicating the importance of the 6"-OCH_3 substitution. A chalcone with 6,7-dimethoxy quinolinyl ring A and 2,4- dichloro ring B have been reported as the most active compound among the derivatives studied by Dominguez *et al.*, (2001).

Similarly, Shikha *et al.* (2009) reported 6-methoxyquinolinyl ring A and 2-bromo, 4-chloro ring B chalcones as the most active in the series investigated.

5. CONCLUSIONS

P3, P4, and P5 have shown promising antimalarial activity *in vivo* in mice infected with *P berghii.* The activity of P5 at 100 mg kg^{-1} is close to that of 25 mg kg^{-1} chloroquine, therefore P5 is a potential antimalarial compound for further development.

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Ethical Statement: All animal experimental protocols complied with the Ahmadu Bello University research guidelines and internationally recognized guidelines for the use and care of laboratory animals (ABUCAUC/2016/004).

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List of Abbreviations: P.: Plasmodium, ACT: Artemisinin Combination Therapy, QSAR: Quantitative Structure Activity Relationship, ID: Identity, 2D: Two Dimensional, TMS: TetraMethylSilane, TLC: Thin-Layer: Chromatography, NIMR: National Medical Research Institute, HCL: Hydrochloric acid, -VE: Negative, +VE: Positive, Mol. Wt: Molecular Weight, HbA: Hydrogen bond Acceptor, HbD: Hydrogen bond Donor, nRB: Number of rotatable bonds, MLogP: Moriguchi octanol-water Partition coefficient, Rf: Retention Factor, mp: Melting point, CLQ: Chloroquine, ip: Intraperitoneal, COSY: Correlation Spectroscopy, HSQC: Heteronuclear Single Quantum Coherence, HMBC: Heteronuclear Multiple Bond Correlation

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