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Synthesis, *In-vitro* Antimalarial Activity and *In silico* Molecular Docking Study of Amino Chalcone Derivatives from 1-(2-aminophenyl)-3-(4- substituted-phenyl) prop-2-en-1-one and Dihydroquinolone Derivatives

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ABSTRACT

Background: Malaria is one of the major global health problems in developing countries and faced to the increased resistance of *Plasmodium falciparum* against existing malarial agents, it is important to look for new antimalarial compounds that will be active in multiple stage of *Plasmodium falciparum*'s life cycle. **Objective:** The goal of this work was to synthesize Amino Chalcone derivatives and Dihydroquinolone derivatives, then evaluate their antimalarial activity by standard computational and biological methods. **Methods:** These amino chalcones were synthesized by the Claisen-Schmidt condensation and by intramolecular cyclization of substituted amino chalcones for the Dihydroquinolones derivatives. Their structures have been determined by NMR (^{1}H and ^{13}C). The *in-vitro* antimalarial assays were carried out by using the maturation test of trophozoites into schizonts. The molecular docking of these compounds was performed by AutoDock vina program using Plasmodium falciparum dihydrofolate reductase-thymidylate synthase (PfDHFR-TS) (PDB ID 1J3I) as target protein. **Results:** All synthesized amino chalcones and dihydroquinolone derivatives were active against Fresh clinical isolates of *Plasmodium falciparum* with a range of EC_{50} ranging from 1.56 to 25µg/mL. However, the 2-phenyl-2, 3-dihydroquinolin-4-(1H)-one (DHQ 2) and 2- (4-methoxyphenyl)-2, 3-dihydroquinolin-4-(1H)-one (DHQ 4) showed excellent antimalarial activity with IC_{50} of 3.125 and 1.56 µg/mL, respectively. Whereas the IC_{50} of Chloroquine use as reference was 1.56µg/mL. Based on absorption, distribution, metabolism and excretion (ADME) properties, all synthetized compounds satisfied the Lipinski rule. **Conclusion:** The results suggest that these synthesized compounds (DHQ 2 and DHQ 4), could be used, after in vivo and clinical tests, like antimalarial supplement or even replace current drug therapies.

Keywords: *Amino chalcones, Dihydroquinolones, Antimalarial, NMR, AutoDock vina; Swiss ADME.*

INTRODUCTION

For decades, Malarial caused by the *Plasmodium* parasite, which is transmitted by the Anopheles mosquitoes' bite, has been one of the major global health problems in the Tropical and subtropical regions. According to recent reports from the World Health Organization (WHO), the number of malaria cases worldwide had reached 229 million in 2019 in 87 malaria endemic countries[1,2]. In general, about fifty percent of the world's community lives under the continuing threat of malaria, which is currently treated by Artemisinin, its derivatives and chloroquine in endemic countries[1,2]. This disease caused approximately 619, 000 deaths (in 2021), 90% of which were in Sub - Saharan Africa, and 78% of these deaths for children under 5 years of age[3,4]. It is the most prevalent parasitic disease and the most common cause of hospital visitation in Democratic Republic of the Congo[3]. This is explained by the increased resistant of parasite to existing drugs such as Chloroquine and Artemisinin, which largely target the asexual blood stage of *Plasmodium falciparum* life cycle[3-6]. Faced to this alarming situation, much scientific research is directed towards the discovery of new antimalarial compounds with other mechanisms of action against *Plasmodium falciparum* and that will be active in multiple stage of life cycle of the latter.

Natural medical compounds have always been considered as an inspiration for new antimalarial, antibacterial, antifungal, antiviral, antioxidants, anti-inflammatory drugs development[7-13]. Among these natural medical compounds with many biological activities, there is also the class of Chalcones and Quinolones.

The Chalcones, known as 1,3-diphenylprop-2-en-1-one, are the aromatic ketones and the enones that form a variety of biological agents and they considered the main precursors for flavonoids, and isoflavonoids biosynthesis in plants[14-21]. They are widely located in nature (in plants, bacteries, fungi, etc.) and are generally synthesized in the Laboratory from aromatic aldehydes and aliphatic aldehydes or ketones via the condensation reaction Claisen-Schmidt in the presence of base or acid catalysts [17–22]. Chalcones possess several physiological activities (antioxidant, antimalarial, antibacterial, anti-ulceral, anti-inflammatory, anti-HIV, antiviral, antifungal, antileishmanial, anticancer, antitubercular, antihyperglycemic, carboxygenase inhibitor, and insecticidal) which are generated by the presence of the reactive function α,β-unsaturated keto present in the molecule[23-29]. Quinolone, being found for the first time accidentally in the Chloroquine's synthesis in 1962, belongs to the large classes of synthetic molecules with various pharmacological and biological properties such as antimicrobial, antimalarial, antitubercular, antifungal, antileishmanial, anti-inflammatory, anticancer, antiviral, antiprotozoal, etc[4, 30,31,32]. Hence, the quinolone scafold is more important for researchers. The goal of this work is to synthesize Amino Chalcone derivatives from 1-(2-aminophenyl)-3-(4- substituted-phenyl) prop-2-en-1-one and Dihydroquinolone derivatives, then evaluate their antimalarial activity by standard computational and biological methods.

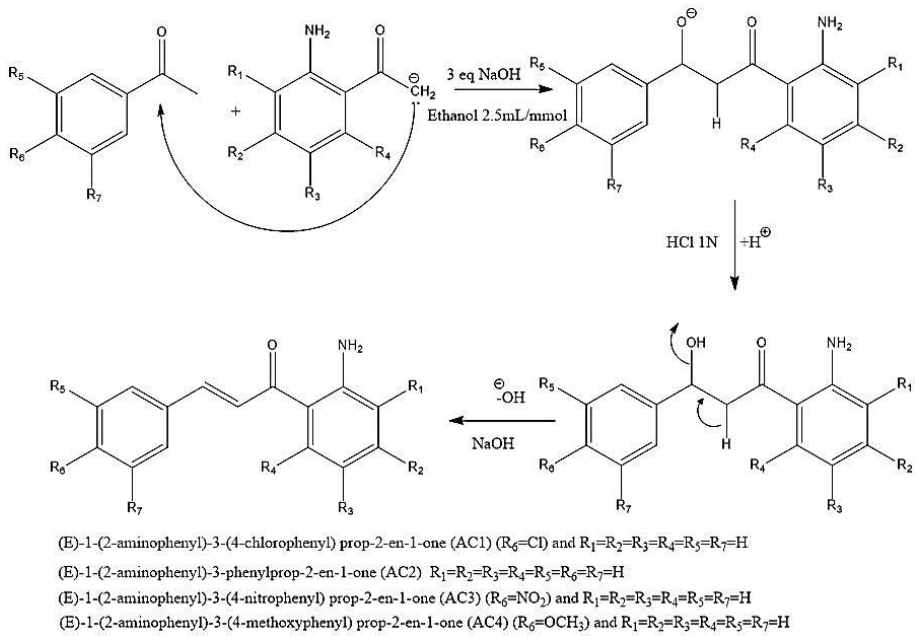
MATERIAL AND METHODS

Part 1- Chemistry

All the starting materials were commercially obtained (Merck). Thin-layer chromatography was carried out on silica gel plates (Merck Kieselgel 60 F254) and visualized by UV light (254 nm). The melting points are determined using a Büchi M-565 melting point apparatus (Büchi Labortechnick AG). NMR spectra were obtained using a Jeol ECA 400 (400 MHz) and Lambda 400 NMR spectrometers. All chemical shifts are expressed in ppm. FT-IR spectra were taken in KBr pellets (100 mg) using Shimadzu FT-IR spectrophotometer and the values were represented as wavenumber in cm^{-1}.

The 4 substituted amino chalcones from 1-(2-aminophenyl)-3-(4- substituted-phenyl) prop-2-en-1-one have been synthesized by Claisen-Schmidt reaction using Sodium hydroxide (NaOH) as catalyst in anhydrous ethanol according to the method described by Mulula and al[17,18,19]. Whereas, the four substituted Dihydroquinolones were synthesized by intramolecular cyclization of substituted amino chalcones by using 1 mol % phosphomolybdic acid ($H_3PMo_{12}O_{40}$) PMA-SiO_2 a reusable catalyst in ethanol according to the literature[33, 34].

a) Synthesis of four (4) Amino Chalcone Derivatives from 1-(2-aminophenyl)-3-(4-substituted-phenyl) prop-2-en-1-one. To a solution of 2-aminoacetophenone (1 eq) in Ethanol (2.5mL/mmol), Sodium hydroxide (3 eq) was added. After10 min, 1.2 eq of substituted benzaldehydes (4-clhorobenzaldehyde, benzaldehyde, 4-nitrobenzaldehyde and 4-methoxybenzaldehyde for (E)-1-(2-aminophenyl)-3-(4-chlorophenyl) prop-2-en-1-one, (E)-1-(2-aminophenyl)-3-phenylprop-2-en-1-one, (E)-1-(2-aminophenyl)-3-(4-nitrophenyl) prop-2-en-1-one and (E)-1-(2-aminophenyl)-3-(4-methoxyphenyl) prop-2-en-1-one, respectively) were added and the mixture was stirred for 30 min at room temperature, then left to stand for 24 h. After cooling the reaction mixtures with ice, the mixture was neutralized carefully using 1N hydrochloric acid. The crude mixture was extracted by ethyl acetate solvent, washed with water and brine afforded chalcones, which were purified by column chromatography using hexane: ethyl acetate as eluent to give four pure aminochalcones (E)-1-(2-aminophenyl)-3-(4-chlorophenyl) prop-2-en-1-one, (E)-1-(2-aminophenyl)-3-phenylprop-2-en-1-one,



Scheme I. General mechanism of amino chalcone by the Claisen-Schmidt reaction using Sodium hydroxide (NaOH) as catalyst.

(E)-1-(2-aminophenyl)-3-(4-nitrophenyl) prop-2-en-1-one and (E)-1-(2-aminophenyl)-3-(4-methoxyphenyl) prop-2-en-1-one. The purity of these 4 compounds was evaluated by using HPLC and thin layer chromatography (TLC) methods and these compounds have been characterized by nuclear magnetic resonance (^{1}H-NMR and ^{13}C-NMR).

(E)-1-(2-aminophenyl)-3-(4-chlorophenyl) prop-2-en-1-one (AC_1). Pale yellow solid, 60% yield, m.p; 168-171ºC, **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.84 (d, J=8.09Hz, 1H), 7.67 (d, J=15.21Hz, 1H), 7.54 (d, J=8.79Hz, 2H), 7.37 (d, J=8.79Hz, 2H), 7.24- 7.33 (m, 2H), 6.63-6.67 (m, 2H), 6.37 (bs, 2H, NH_2); **^{13}C NMR** (400 MHz, $CDCl_3$): δ 191.40, 171.27, 151.25, 141.55, 136.06, 134.53, 133.90, 131.09, 129.56, 129.04, 123.69, 117.44, 115.91.

(E)-1-(2-aminophenyl)-3-phenylprop-2-en-1-one (AC_2). Golden yellow solid, 65% yield, m.p; 145-147ºC, **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.85 (d, J=8.51Hz, 1H), 7.73 (d, J=15.14Hz, 1H), 7.58-7.64(m, 3H), 7.36-7.42 (m, 3H), 7.24-7.29 (m, 1H), 6.97-6.71(m, 2H), 6.33 (bs, 2H, NH_2); **^{13}C NMR** (400 MHz, $CDCl_3$): δ 191.84, 151.21, 143.08, 135.35, 134.57, 131.34, 130.18, 129.02, 128.51, 123.35, 119.09, 117.55, 115.87.

(E)-1-(2-aminophenyl)-3-(4-nitrophenyl) prop-2-en-1-one (AC_3). Orange solid, 64% yield, m.p; 194-196ºC, **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.85 (d, J=8.41Hz, 3H), 7.67-7.90 (m, 6H), 7.32(t, J=7.65Hz, 1H), 6.69 (bs, 2H, NH_2); **^{13}C NMR** (400 MHz, $CDCl_3$): δ 190.70, 169.90, 151.49, 148.26, 141.45, 139.71, 135.18, 131.15, 128.76, 127.05, 124.53, 117.59, 115.98.

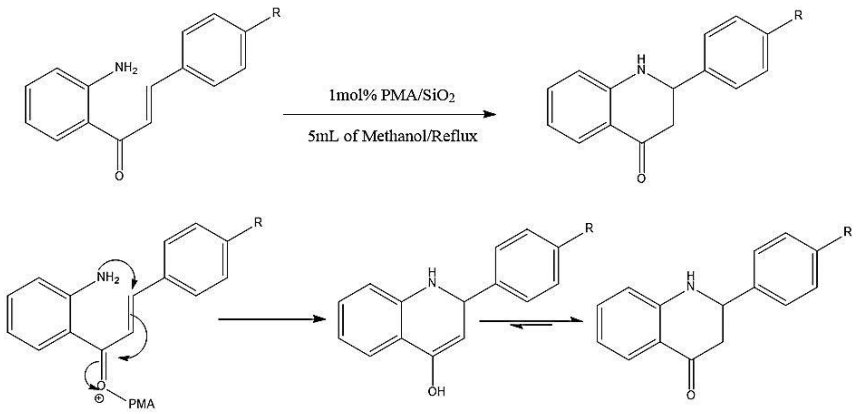
(E)-1-(2-aminophenyl)-3-(4-methoxyphenyl) prop-2-en-1-one (AC_4). Yellow solid, 73% yield, m.p; 150-152ºC, **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.79 (d, J=8.46Hz, 1H), 7.66 (d, J=15.23Hz, 1H), 7.52 (d, J=8.80Hz, 2H), 7.44 (d, J=15.23Hz, 1H), 7.21-7.24 (m, 1H), 6.87 (d, J=8.12Hz, 2H), 6.60-6.65 (m, 2H), 6.22 (bs, 2H, NH_2), 3.78 (s, 3H, OCH_3); **^{13}C NMR** (400 MHz, $CDCl_3$): δ 191.83, 161.40, 151.08, 142.90, 134.21, 131.04, 130.11, 128.06, 120.82, 119.35, 117.43, 115.89, 114.41, 55.47.

b) Synthesis of four (4) Dihydroquinolones from amino chalcones.

Preparation of catalyst PMA/SiO_2 (1 mol %)

1 eq of PhosphoMolibdic acid (PMA: $H_3PMo_{12}O_{40}$) and 0.9 eq of Silica gel were added methanol by stirring at the room temperature for 6 hours. After, the methanol solvent was removed using rotary evaporator and the solid catalyst PMA/SiO_2 was collected.

The four (4) Dihydroquinolone derivatives were obtained by adding the mixture of 1 mol of previous obtained aminochalcone derivatives (AC_1, AC_2, AC_3 and AC_4, respectively) and the catalyst PMA/SiO_2 (1 mol %) in 5 mL of methanol under N_2 atmospheric. The mixture was stirred under reflux for 8 hours. After this,



Scheme II. Synthesis of Dihydroquinolones from amino chalcones.

the methanol solvent was removed using rotary evaporator and the formed residue was dissolved in 10 mL of diethylether and filtered. The filtrate was concentrated using rotary vapor and purified by column chromatography using cyclohexane/ethyl acetate as eluent to afford four pure Dihydroquinolone derivatives : 2- (4-clorophenyl)-2, 3-dihydroquinolin-4-(1H)-one (DHQ_1), 2- phenyl-2, 3-dihydroquinolin-4-(1H)-one (DHQ_2), 2- (4-nitrophenyl)-2, 3-dihydroquinolin-4-(1H)-one (DHQ_3) and 2- (4-methoxyphenyl)-2, 3-dihydroquinolin-4-(1H)-one (DHQ_4), respectively.

2- (4-clorophenyl)-2, 3-dihydroquinolin-4-(1H)-one (DHQ_1). Yellow solid, 73% yield, m.p; 169-171ºC, **^{1}H NMR** (400MHz, $CDCl_3$): δ 7.87 (d, J= 8.12 Hz, 1H), 7.33-7.41 (m, 5H), 6.81 (t, J=7.71 Hz 1H), 6.72 (d, J=7.57 Hz, 1H), 4.74 (dd, J=3.45Hz, J=13.41Hz , 1H), 4.41 (bs, NH), 2.83 (dd, J=13.41Hz, J=16.60Hz, 1H), 2.74 (dd, J=3.41Hz, J=15.95Hz, 1H);**^{13}C NMR** (100MHz, $CDCl_3$): δ 193.00, 151.44, 139.61, 135.61, 134.25, 129.49, 128.09, 127.70, 119.14, 118.80, 116.06, 57.99, 46.51.

2-phenyl-2, 3-dihydroquinolin-4-(1H)-one (DHQ_2) Yellow solid, 54% yield, m.p; 149ºC, **^{1}H NMR** (400MHz, $CDCl_3$): δ 7.89 (d, J= 8.15Hz, 1H), 7.32-7.50 (m, 6H), 6.80 (t, J=7.95 Hz, 1H), 6.72 (d, J=8.16 Hz, 1H), 4.76 (dd, J=3.21Hz, J=13.67Hz , 1H), 4.50 (bs, NH), 2.90 (dd, J=13.67Hz, J=17.70Hz, 1H), 2.80 (dd, J=3.21Hz, J=17.23Hz, 1H);**^{13}C NMR** (100MHz, $CDCl_3$): δ 193.41, 151.64, 141.09, 135.51, 129.09, 128.57, 127.71, 126.72, 119.11, 118.55, 116.00, 58.61, 46.55.

2- (4-nitrophenyl)-2, 3-dihydroquinolin-4-(1H)-one (DHQ_3). Dark orange solid, 69% yield, m.p; 198-201ºC, **^{1}H NMR** (400MHz, $CDCl_3$): δ 8.20 (d, J= 8.93Hz, 2H), 7.81 (dd, J= 8.03Hz, J= 1.70 Hz, 1H), 7.59 (d, J= 8.95 Hz, 2H), 7.47(d, J= 8.93 Hz, 1H), 6.78 (dt, J=7.82 Hz, J= 1.68 Hz, 1H),6.70(d, J= 8.19 Hz, 1H), 4.83 (dd, J=6.71Hz, J=10.06Hz , 1H), 4.46 (bs, NH), 2.76- 2.79 (m, 2H);**^{13}C NMR** (100MHz, $CDCl_3$): δ 192.52, 151.09, 148.49, 135.71,127.69, 127.51, 126.99, 124.33, 123.75, 119.22, 116.04, 57.91, 46.11.

2- (4-methoxyphenyl)-2, 3-dihydroquinolin-4-(1H)-one (DHQ_4). Yellow solid, 58% yield, m.p; 148-149ºC, **^{1}H NMR** (400MHz, $CDCl_3$): δ 7.80 (d, J= 8.62 Hz, 1H), 7.23-7.32 (m, 3H), 6.85 (d, J= 8.56 Hz, 2H), 6.71 (t, J=8.01 Hz, 1H),6.61 (d, J= 8.05 Hz, 1H), 4.62 (dd, J=3.53Hz, J=13.21Hz , 1H), 4.39 (bs, NH), 3.74 (s, 3H), 2.79 (dd, J=13.58Hz, J=15.73Hz, 1H), 2.66 (dd, J=3.55Hz, J=16.05Hz, 1H);**^{13}C NMR** (100MHz, $CDCl_3$): δ 193.81, 160.91, 151.15, 135.45, 132.55, 127.92, 127.71, 118.48, 115.96, 114.38, 58.02, 55.45, 46.63.

Part 2- Biology

Antimalarial Activity

Parasites

Fresh clinical isolates of *Plasmodium falciparum* were obtained from Hospital center of Kindele (Mont - ngafula township), in Kinshasa, Democratic Republic of the Congo (DRC). Rapid diagnostic test was realized for Plasmodium species identification. The parasite density was determined by counting the number of infected erythrocytes. From donor, 4 mL of venous blood was collected in a tube

coated with EDTA (Greiner Labortechnik). Samples with monoinfection due to *Plasmodium falciparum* and a parasite density between 1% and 2% were used for the in vitro antimalarial tests. The parasites were cultivated and maintained continuously in human erythrocytes according to previously described methods[35].

***In-vitro* Antimalarial Assay Procedure.**

The antiplasmodial activity of each synthesized compound was evaluated against the strain of *Plasmodium falciparum* using maturation test of trophozoites into schizonts described by Mulula and al[3]. A stock solution of 100µg/mL of compound was prepared in methanol (MeOH). These were further diluted in complete medium to attain the final concentrations of 50µg/mL. The stock solutions were prepared on the assay day. Chloroquine was used as the standard reference drug (positive control). Compounds were serially diluted two fold in complete medium (RPMI) up to 0.195µg/mL using a flat bottomed, 96 - well microtitre plate. Erythrocyte non parasitised was added to column 1 (blank) which had no drugs, while parasitized red blood cells were added to columns 2–12. The plate was incubated at 37°C for 48 hours. After incubation, 50µL of each well were placed on the glass slide and air - dried for 24 hours. GIEMSA was added and microscope lecture was realized. The concentration of any substance that inhibited 50% of the parasite growth (IC_{50}) was determined in triplicata.

Molecular docking studies

The molecular docking of the synthesized compounds (4 Amino Chalcone and 4 dihydroquinolone derivatives) on the target proteins of microorganisms was carried out by using Autodock vina program with standard protocol according the literature for understanding the possible interactions between the compounds and parasites[18]. *Plasmodium falciparum* target was Plasmodium falciparum dihydrofolate reductase-thymidylate synthase (PfDHFR-TS) (PDB ID 1J3I), which was obtained from the protein data bank (http://www.rcsb.org).

The ligands were downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/) in SDF format and then converted to pdbqt format using pymol logiciel. Whereas, the protein target Plasmodium falciparum dihydrofolate reductase-thymidylate synthase (PfDHFR-TS) (PDB ID 1J3I) was downloaded from the protein data bank (http://www.rcsb.org) and prepared for pre-docking process. All water molecules were deleted while hydrogen atoms and charges were added to the target protein. The molecular docking of these ligands to the protein target Plasmodium falciparum dihydrofolate reductase-thymidylate synthase (PfDHFR-TS) was carried out by using Autodock vina program with these parameters: center_x = 18.567, center_y = 15.817, center_z = 11.27, size_x = 40, size_y = 40, size_z = 40, energy_range = 4, exhaustiveness = 8. The outputs were analyzed by using Discovery Studio Visualizer.

In silico drug-likeness predictions

In *silico* Drug-likeness helps to know whether a particular pharmacological agent has properties consistent with being an orally active drug. The properties of these 4 Amino Chalcone and 4 dihydroquinolone derivatives were evaluated for their *in silico* parameters using SwissADME web [http://www.swissadme.ch/ (accessed on 8th February 2023).

RESULTS AND DISCUSSION

Part-1 Chemistry

Amino Chalcones (AC) and Dihydroquinolone (DHQ) derivatives synthesis

Chalcones possess several physiological activities (antioxidant, antimalarial, antibacterial, anti-ulceral, anti-inflammatory, anti-HIV, antiviral, antifungal, antileishmanial, anticancer, antitubercular, antihyperglycemic, carboxygenase inhibitor, and insecticidal) which are generated by the presence of the reactive function α,β-unsaturated keto present in the molecule[23-29]. The same applies to quinolone-based compounds which have already shown several biological activities[4, 30,31,32]. These two compounds are therefore very promising for the discovery of new antimalarial agents.

The melting temperature and yield of these 4 synthesized amino chalcones (AC) were (168-171 ºC; 60%), (145-147ºC; 65%), (194-196ºC; 64%) and (150-152ºC; 73%) for AC_1, AC_2, AC_3 and AC_4, respectively. Whereas those of dihydroquinolones (DHQ) were (169-171 ºC; 73%), (149ºC; 54%), (198-201ºC; 69%) and (148-149ºC; 58%) for DHQ_1, DHQ_2, DHQ_3 and DHQ_4, respectively.

^{1}H-NMR spectrum of these four synthesized amino chalcones (AC_1, AC_2, AC_3 and AC_4) each revealed that the characteristic two protons of the amino group have the chemical shifts at δ 6.37; 6.33; 6.69 and 6.22 ppm, respectively. Whereas, the single characteristic proton of amino group from dihydroquinolones derivatives (DHQ_1, DHQ_2, DHQ_3 and DHQ_4) have the chemical shifts at δ 4.41; 4.50; 4.46 and 4.39 ppm, respectively. The single proton of the amino group from dihydroquinolone is more shielded than the two from amino chalcone because the amino group of dihydroquinolone is on the same ring as the carbonyl group while the amino group of aminochalcone is on outside of the benzene ring. In addition, the 3 protons of the methoxy group of the compounds AC_4 and DHQ_4 have the chemical shift (δ) of 3.78 and 3.74 ppm, respectively.

Part-1 Biology

Antimalarial Activity

Antimalarial activity of four synthesized amino chalcones and four dihydroquinolone derivatives against Fresh clinical isolates of *Plasmodium falciparum* were determined using the method described by Mulula and al[3]. Chloroquine was used as reference compounds for antimalarial activity. The IC_{50} (µg/mL) values of these synthesized compounds and Chloroquine are reported in **Table 1**. IC_{50} is the concentration of each test compound needed to hinder multiplication of parasites by 50%.

Table 1. Antimalarial activity against Fresh clinical isolates of *Plasmodium falciparum* (µg/mL).

Compounds	IC_{50} (µg/mL)	Antimalarial activity
AC_1	12.5	Active
AC_2	6.25	Active
AC_3	25	Less active
AC_4	6.25	Active
DHQ_1	6.25	Active
DHQ_2	3.125	Very active
DHQ_3	12.5	Active
DHQ_4	1.56	Very active
Chloroquine	1.56	Very active

All synthesized amino chalcones and dihydroquinolone derivatives were active against Fresh clinical isolates of *Plasmodium falciparum* compared to the reference (Chloroquine). According to the literature, drug that have very active antimalarial activity with IC_{50} values ≤ 5 µg/mL, were classified into active, less active and inactive when IC_{50} values were 5-15, 15-30 and > 30 µg/mL, respectively[3].

Among all the tested compounds, DHQ_2 and DHQ_4 were found to be the most active with IC_{50} values of 3.125µg/mL and 1.56µg/mL, respectively. In addition, the compound DHQ_4 showed also best antimalarial activity than Chloroquine (IC_{50}= 1.56 µg/mL) which was use as reference. This could be explained by the structure of this synthesized compound that has electron donating groups $-OCH_3$ in para position of Aryl ring B. According the literature, the presence of methoxy group ($-OCH_3$) in para position of Aryl ring B of Chalcones and quinolones compounds, enhances the antimalarial activity because this substituent has the potential to increase the lipophilicity of a compound which is an important property in antimalarial activity[2, 36]. In this work, the presence of the substituent (methoxy, Benzyl, nitro, Chloro) in para position of Aryl ring B of Chalcones and quinolones compounds plays a very important role in the antimalarial activity. This increases the antimalarial activity following the order $-OCH_3$> -Phenyl> -Cl> $-NO_2$. In addition, amino chalcones AC_1, AC_2, AC_3 and AC_4 have shown less antimalarial activity against fresh clinical isolates of *Plasmodium falciparum* compared to the dihydroquinolones compounds (DHQ_1, DHQ_2, DHQ_3 and DHQ_4) derived from these amino chalcones. This could be explained by the presence of heterocyclic nitrogen associated with the carbonyl group already present in this cycle.

Molecular docking studies

The *in-silico* antimalarial activity results of synthesized amino chalcones and dihydroquinolone derivatives (Ligands) against Plasmodium falciparum dihydrofolate reductase-thymidylate synthase (PfDHFR-TS) (PDB ID 1J3I) was reported in terms of binding energy and ligand interactions in order to predict the binding energy of ligands within the binding site of target proteins. These results are reported in **Table 2**, and **Figures 1, 2** and **3**.

Plasmodium falciparum dihydrofolate reductase-thymidylate synthase (PfDHFR-TS) (PDB ID 1J3I) is one of the important enzymes responsible for the production of folates and thymidylates, which are required in DNA synthesis of *Plasmodium falciparum*[2]. Thus, the molecules that would block this enzyme will play a key role on the antimalarial activity. The in *silico* antimalarial results indicated that all synthesized amino chalcones and dihydroquinolone derivatives showed strong binding affinity (ranges from −7.5 to −8.2 Kcal/mol, given in **Table 2**) towards the amino acid residues in active pocket Plasmodium falciparum dihydrofolate reductase-thymidylate synthase (PfDHFR-TS) (PDB ID 1J3I) protein through H-bond, Van der Wals and residual interactions, compared to standard drug Chloroquine (-5.9 Kcal/mol).

Dihydroquinolone 4 (DHQ4), which exhibited best antimalarial activity like the drug standard, showed good affinity by interacting with 12 target protein amino acids including PHE-D520; GLY-D517; ARG-D345; THR-D346; ASN-D555; ASP-D605, HIS-D556; LEU-D516; VAL-D348; UMP-D711; TPR-D553 and ASP-D513 (**Figure 2**). Whereas the antimalarial drug reference (Choloroquine) showed less affinity against Plasmodium falciparum dihydrofolate reductase-thymidylate synthase (-5.9 Kcal/mol) and interacted with 10 target protein amino acids including ASN-D407; ASP-D513; GLY-D517; ASN-D521; GLU-D382; SER-D524; ILE-D403; ILE-D379; LEU-D516; UMP-D711. From the above, the molecule 2- (4-methoxyphenyl)-2, 3-dihydroquinolin-4-(1H)-one (DHQ4) with the best antimalarial activity such as Chloroquine, would intervene better than the latter during the biosynthesis of the DNA of *Plasmodium falciparum*.

Table 2. Molecular docking simulation of synthetized chalcones (Ligands) against Plasmodium falciparum dihydrofolate reductase-thymidylate synthase (PfDHFR-TS) (PDB ID 1J3I).

Compounds	Binding affinity (Kcal/mol)	H-bond	Residual interactions			
			VDW	π-alkyl	π- π	π-anion
AC 1	-7.9	GLU-D382, ASN-D521	PHE-D386; HIS-D491; UMP-D711; ASN-D407; SER-D524; GLY-D517; PHE-375; GLY-D378; ILE-D379	ILE-D403	PHE-D520; TPR-D404	-
AC 2	-7.8	GLU-D382; ASN-D521	PHE-D386; HIS-D491; UMP-D711; ASN-D407; SER-D524; GLY-D517; PHE-375; GLY-D378; ILE-D379	ILE-D403	PHE-D520; TPR-D404	-
AC 3	-7.9	GLU-D382; ASN-D521; UMP-D711	PHE-D375; HIS-D491; GLY-D378; ASN-D407; SER-D524; GLY-D517; PHE-375; GLY-D378; ILE-D379.	ILE-D403	PHE-D520; TPR-D404	-
AC 4	-7.7	GLU-D382; ASN-D521; UMP-D711	PHE-D375; HIS-D491; GLY-D378; ASN-D407; GLY-D517; PHE-375; GLY-D378; ILE-D379.	-	PHE-D520; TPR-D404	-
DHQ 1	-7.7	ASP-D513; ARG-D345	THR-D346; VAL-D348; HIS-D556; GLY-D517; ASN-D555; ASP-D605; LEU-D516	ILE-D403	UMP-D711; TPR-D553	-
DHQ 2	-7.5	-	PHE-D375; GLY-D378; ASN-D521; ILE-D379, UMP-D711	-	PHE-D520	
DHQ 3	-8.2	GLY-D378; ASN-D521.	PHE-D375; GLY-D517; ARG-D377; ILE-D379 ASN-D400; LEU-D376, UMP-D711	ILE-D403; ARG-D402	PHE-D520	-
DHQ 4	-7.9	-	PHE-D520; GLY-D517; ARG-D345; THR-D346; ASN-D555; ASP-D605, HIS-D556	LEU-D516; VAL-D348	UMP-D711; TPR-D553	ASP-D513
Chloroquine	-5.9	-	ASN-D407; ASP-D513; GLY-D517; ASN-D521; GLU-D382; SER-D524	ILE-D403; ILE-D379; LEU-D516; UMP-D711	PHE-D520	-

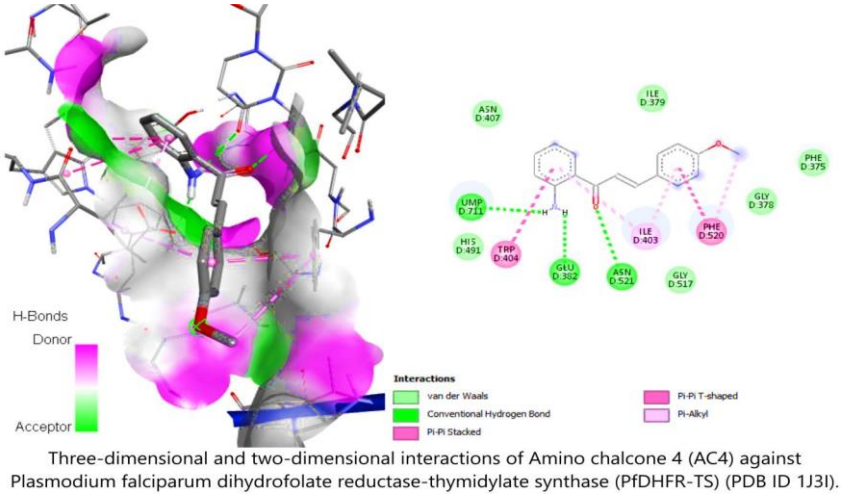


Figure 1. Three-dimensional and two-dimensional interactions of Amino chalcone 4 (**AC4**) against *Plasmodium falciparum dihydrofolate reductase-thymidylate synthase* protein amino acid residues.

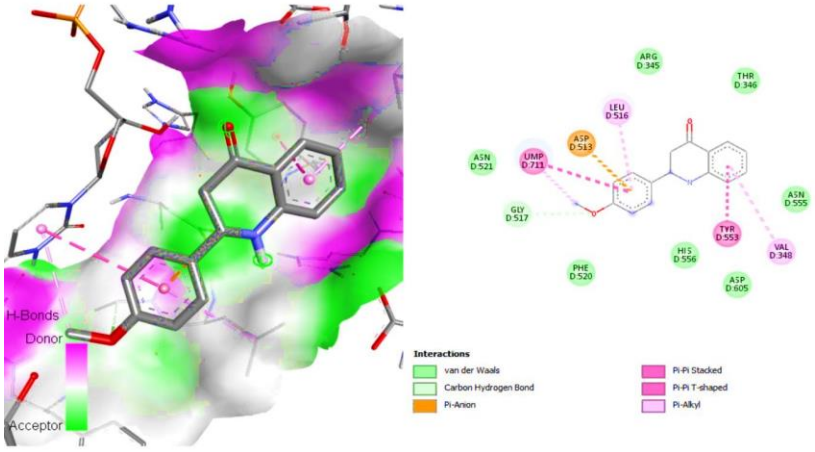


Figure 2. Three-dimensional and two-dimensional interactions of Dihydroquinolone 4 (DHQ4) against *Plasmodium falciparum dihydrofolate reductase-thymidylate synthase* protein amino acid residues.

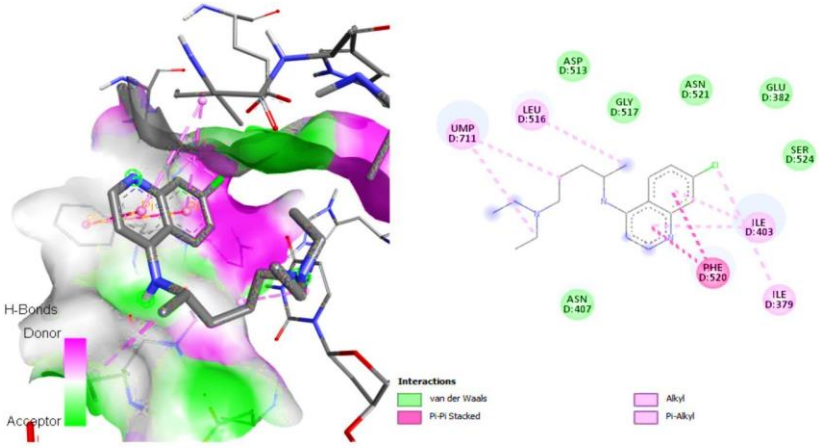


Figure 3. Three-dimensional and two-dimensional interactions of Chloroquine against *Plasmodium falciparum dihydrofolate reductase-thymidylate synthase* protein amino acid residues.

For synthesized aminochalcones, (E)-1-(2-aminophenyl)-3-phenylprop-2-en-1-one (AC_2) and (E)-1-(2-aminophenyl)-3-(4-methoxyphenyl) prop-2-en-1-one (AC_4) showed good antimalarial activity compared to the remaining two with IC_{50} of 6.25µg/mL. This could be explained by the structure of these both synthesized compound that have electron donating groups -OCH_3 and phenyl which increase the lipophilicity of these compounds. In addition, they showed also good affinity by interacting with GLU-D382; ASN-D521, UMP-D711, PHE-D520; GLY-D517; ARG-D345; THR-D346; ASN-D555; ASP-D605, HIS-D556, LEU-D516; VAL-D348, PHE-D520; TPR-D404, PHE-D386; HIS-D491; UMP-D711; ASN-D407; SER-D524; GLY-D517; PHE-375; GLY-D378; ILE-D379.

In-silico drug-likeness predictions

In silico Drug-likeness helps to know whether a particular pharmacological agent has properties consistent with being an orally active drug. To be effective, a compound must have optimal hydrophilic and hydrophobic properties to carry in the blood before penetrating the cell membrane. A simple method for evaluating drug properties is to verify compliance with the Lipinski rule (rule of 5), which specifies the number of hydrophilic; molecular groups weight and

hydrophobicity[18]. Lipinski rule proposed that drug target must have; the molecular weight (MW) ≤500, hydrogen bond acceptor (HBA)≤10, hydrogen bond donor (HBD)≤5, lipophilicity (logP)≤5. The absorption, distribution, metabolism and excretion (ADME) properties of synthetized amino chalcones and dihydroquinolone derivatives are represented in **Table 3**.

Table 3. ADME properties of synthetized chalcones

Compound	Log P	MW (g/mol)	HBA	HBD
AC 1	2.60	257.71	1	1
AC 2	3.65	462.54	3	2
AC 3	2.20	268.27	3	1
AC 4	2.97	253.30	2	1
DHQ 1	2.49	258.70	1	1
DHQ 2	2.67	223.27	1	1
DHQ 3	2.51	347.16	3	1
DHQ 4	2.65	253.30	2	1

With regard to **Table 3**, all synthetized amino chalcones and dihydroquinolone derivatives satisfied the Lipinski rule, Ghose, Veber, Egan and muegge rule of five and also showed very good solubility because the logP is between 2 and 6 (2< log P<6). Thus, these molecules could be used as orally active drug.

CONCLUSION

In this study, we synthetized four amino chalcones (AC_1, AC_2, AC_3 and AC_4) and four dihydroquinolones (DHQ_1, DHQ_2, DHQ_3 and DHQ_4) derivatives, which were identified by NMR (^1H and ^{13}C) spectroscopy. These synthetized compounds are screened for antimalarial activity. All synthesized amino chalcones and dihydroquinolone derivatives were active against Fresh clinical isolates of *Plasmodium falciparum* compared to the reference (Chloroquine) with a range of EC_{50} ranging from 1.56 to 25µg/mL. However, the 2-phenyl-2, 3-dihydroquinolin-4-(1H)-one (DHQ_2) and 2-(4-methoxyphenyl)-2, 3-dihydroquinolin-4-(1H)-one (DHQ_4) showed excellent antimalarial activity with IC_{50} of 3.125 and 1.56 µg/mL, respectively. Whereas, the IC_{50} of Chloroquine use as reference was 1.56µg/mL. The in *silico* antimalarial of these synthesized compounds showed strong binding affinity ranges from ranges from −7.5 to −8.2 Kcal/mol against Plasmodium falciparum dihydrofolate reductase-thymidylate synthase (PfDHFR-TS) (PDB ID 1J3I) protein. Based on absorption, distribution, metabolism and excretion (ADME) properties, all synthetized compounds satisfied the Lipinski rule, Ghose, Veber, Egan and muegge rule and also showed very good solubility because the logP is between 2 and 6 (2< log P<6). The results suggest that the synthesized compounds, especially the 2- phenyl-2, 3-dihydroquinolin-4-(1H)-one (DHQ_2) and 2- (4-methoxyphenyl)-2, 3-dihydroquinolin-4-(1H)-one (DHQ_4) could be used, after in vivo and clinical tests, like antimalarial supplement or even replace current drug therapies.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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