Discovery of 5-Chlorobenzimidazole-based as Promising Inhibitors of Chloroquine-Resistant Plasmodium Strains: Synthesis, Biological Evaluation, Molecular Docking and Computational Studies

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: To overcome drug resistance to current antimalarial drugs, we propose the synthesis and *in vitro* evaluation of the antiplasmodial activity of a series of 5-chlorobenzimidazolyl-chalcones against chloroquino sensitive (CQ-S) and chloroquino resistant (CQ-R) strains of *P. falciparum*.
Objective: This study aimed to establish through structure-activity relationship studies and docking, the structural elements essential for antiplasmodial activities.

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Methods: The antiplasmodial activity of these benzimidazolylchalcones was carried out according to the Rieckmann microtest technique, followed by the determination of the concentrations inhibiting 50% of the production of parasitic HRP2 antigens (IC$_{50}$) by ELISA. Chloroquine was used as a reference molecule with a sensitivity threshold set at 100 µM. Molecular docking was performed using sensitive (PDB ID: 1J3I) and resistant (PDB ID: 4DP3) dihydrofolate reductase-thymidylate synthase proteins (PfDHFR-TS).

Results: All benzimidazolylchalcones tested expressed antiplasmodial activities especially against chloroquine resistant isolates (IC$_{50}$ = 0.32-44.38 µM). The best profile against both isolates was the methoxylated derivative (3e) with an IC$_{50}$ ranging from 0.32 to 1.96 µM. This compound had the best antimalarial activity against CQ-S isolates. On CQ-R isolates, the unsubstituted 5-chlorobenzimidazole derivative (3b) had exalted activity (IC$_{50}$ = 0.78 µM). We selected a weakly active non-chlorinated derivative 3a and chlorinated derivatives 3b, 3d, 3e and 3f) with IC$_{50}$< 3µM against the chloroquine-resistant strain to perform docking studies. These revealed that the pyrrolic nitrogen of benzimidazole and the ketone of propenone are the main chemical entities involved in the interaction at the receptor. Moreover, ADMET studies showed favorable pharmacokinetic properties.

Conclusion: Molecular docking studies confirmed the experimental findings and revealed the possible interactions pattern. Derivatives 3b and 3e, which showed promising binding affinities against PfDHFR-TS, can be proposed as lead compounds for the development of antimalarial drug candidates.

Keywords: Benzimidazole; Chalcone; Chemoresistance; Plasmodium falciparum; Docking; ADME properties.

1. INTRODUCTION

Malaria is a parasitic infection transmitted to people through the bites of infected mosquitoes due to species of the genus *Plasmodium* whose main infectious agent, *Plasmodium falciparum*, is formidable [1]. In fact, *Plasmodium falciparum* and *Plasmodium vivax* are the two species responsible for the most deadly forms of malaria, which mainly affects children and pregnant women [2]. Around 3.4 billion people in the world –more than one third of the world's population today- are at risk of being infected with malaria and developing disease malaria [3, 4]. Estimates point to 229 million malaria episodes in 2019, of which 94% (215 M) were in the WHO African Region. In this region, the number of deaths due to malaria has decreased from 620,000 to 409,000 over the period 2009-2019 representing a reduction in malaria cases and death rates of 18% and 34% since 2010 [5]. These figures are all the more alarming as we are witnessing an increase in morbidity caused by resurgence of *P. falciparum* chemoresistance to almost all available antimalarial drugs, including artemisinin derivatives, the most potent and safe antimalarial drugs [6]. In fact, available classes mainly used for treatment of malaria due to *P. falciparum* can be divided into five classes namely quinolone and arylamino alcohol, artemisinin and its derivatives, antifolates, hydroxynaphthoquinones and antibacterial agents [7].

However, the efficacy of the current first-line agents for curative treatment based on artemisinin-based combination therapy (ACT) in nearly all areas is seriously limited by frequent drug resistance [6,7]. In 2017, the WHO sounded the alarm, declaring that malaria control was at a crossroads of paths. Despite remarkable progress, advances in the fight against malaria worldwide have stabilized in recent years, particularly in high-burden countries [1,2]. Meanwhile, as a result of disruptions to planned routine activities and services due to the current coronavirus pandemic, a modeling study predicts an impact on the malaria burden with an additional loss of life of up to 36% over the next 5 years [8]. Global progress in malaria control is at risk of being undermined by gaps in access, COVID-19, and inadequate funding [3, 4]. In such a context of development of multidrug-resistant malaria and absence of vaccines, a revival of malaria control is needed.

To overcome drug resistant, one of the alternatives proposed is to search for suitable drug inhibitors that are effective against resistant strains of Plasmodium [9]. Recent developments in the understanding of parasite biology as well as current therapeutic approaches, promote action on innovative targets to generate promising new drugs against resistant malaria. Among the metabolic processes of cytoplasmic

enzymes, inhibition of the dihydrofolate reductase (DHFR) pathway, whether or not linked to Thymidylate synthase (TS), has been established as a prime target for antimalarial chemotherapy [10]. In fact, the discovery of the chemoprotective activities against *Plasmodium falciparum* of P218, a potent inhibitor of Dihydrofolate reductase, has given new hope for preventive therapy of the pregnant women and infants may in malaria endemic areas [11]. Indeed, this new candidate for intermittent preventive treatment may become an alternative to the combination of sulfadoxine and pyrimethamine whose efficacy is threatened by resistance resulting from mutations in the *P. falciparum* dihydrofolate reductase [PfDHFR] genes targeted by pyrimethamine [12] (Figure 1). Similarly, antimalarial research has taken a leap forward with the identification of the WR99210, an analog of cycloguanil, which is a selective inhibitor of the bifunctional enzyme DHFR-TS of

the parasite, especially as it has no action on human DHFR, which is not fused with TS (Fig. 1).

Recently, *in silico* antimalarial studies performed for the protein dihydrofolate reductases-thymidylate synthase (PfDHFR-TS) revealed that the chalcone analogues interacted with both the susceptible (1J3I.pdb) and the resistance protein (4DP3.pdb), meaning that they were active against both the chloroquine-sensitive as well as the chloroquine-resistant *Plasmodium* strains [13]. It is in this perspective that we are interested in hybrids of chalcone and benzimidazole as potential new antimalarial agents. In a previous study, we demonstrated that the 5-chlorobenzimidazole group of a series of benzimidazolyl-chalcones would behave as an antiplasmodial pharmacophore similar to the 7-chloroquinoline of amodiaquine and chloroquine [14] (Fig. 2).
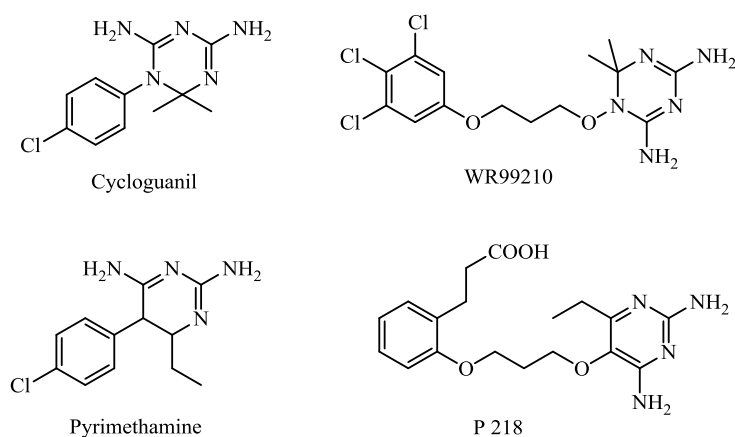

Fig. 1. **Pyrimidines and dihydrotriazines inhibitors of *P. falciparum* DHFR**
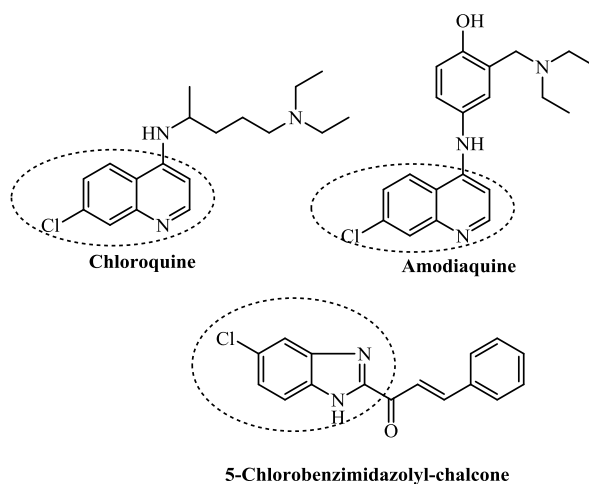

Fig. 2. Pharmacophore of Amodiaquine, Chloroquine and 5-Chlorobenzimidazolyl-chalcone

In the present work, we propose to extend the antiplasmodial evaluations to a wider variety of derivatives possessing this pharmacophore so as to establish the structural elements essential for good serial antiplasmodial activities of benzimidazole-supported chalcone hybrids. Also, *in silico* studies were used to explore the interactions of 4 compounds, selected for their excellent antimalarial activities on both chloroquine-sensitive and chloroquine-resistant strains, with respect to protein binding sites. These computational studies also aimed to address the pharmacokinetics properties of the designed compounds.

Therefore, the objective of this work is to evaluate the antiplasmodial activity of new 5-chlorobenzimidazolyl-chalcones. Specifically, we aimed to determine the concentrations of chalcones capable of inhibiting 50% of the maturation of *P.falciparum* by ELISA assay of the HRP2 antigen. Then, the main observations were pooled with the previously obtained results to establish the structural elements favorable to the induction of antiplasmodial activities as well as understand the interactions between the designed compounds with their biological targets.
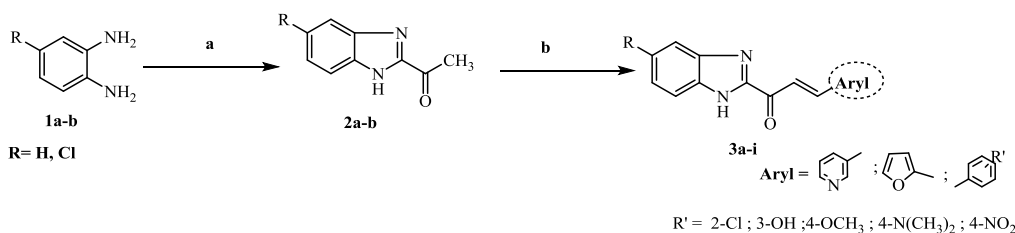
2. MATERIALS AND METHODS

2.1 Chemistry

For all the characterized compounds, the spectra of Nuclear Magnetic Resonance (NMR) spectra of the ^{1}H proton (300MHz) and of the ^{13}C (75 MHz) were recorded on a Brücker Avance 300 instrument. The tetramethylsilane (TMS) is used as a reference reference of the shifts expressed in ppm. The description of the NMR spectra uses symbols : singlet = s; doublet = d; split doublet = dd; triplet = t; quadruplet = q; quintuplet = quint; multiplet = m. The mass spectra were recorded on a JEOL JMS DX300 spectrometer in ESI mode (electrospray/quadrupole ionization). The melting points (FP) were determined using a Kofler bench and are not corrected. The thin layer chromatography (TLC) were performed on silica plates Macherey-Nagel Sil G/UV254 or on Macherey-Nagel ALOX N/UV254 alumina. The eluent system used for the TLC of the synthesized compounds was a DCM / MetOH mixture (95: 5). The products were then revealed with iodine. Solvents and reagents, including benzaldehydes, were obtained from Acros Organics (France) or from Aldrich (France). Chloroquine, an antimalarial drug, supplied as a pure powder comes from Sigma Chemical Co. (USA).

The first step of chemical synthesis is to prepare 2-acetyl benzimidazole (**2a-b**) by heterocyclization from suitably selected orthophenylene diamines (**1a-b**) according to the reported procedures [14, 15]. The ketone intermediates were then reacted with various heterocyclic benzaldehydes or aldehydes to give benzimidazolyl-chalcones (**3a-i**) substituted or not on the benzimidazole ring (Scheme 1).

1- (5-chloro-1H-benzimidazol-2-yl) -3-phenylprop-2-en-1-one (**3b**)
Pale yellow solid, m.p.: 237-239°C, yield 78%. ^{1}H NMR (300MHz, DMSO-d6) δ: 13.50 (s, 1H, NH); 8.10 (s, 1H, H4); 7.97 (d, 1H, *J* = 16 Hz, CH=CH); 7.85 (m, 2H, Ph-2,6); 7.76 (m, 1H, H7) ; 7.46-7.51 (m, 3H, Ph-3,4,5); 7.25 (m, 1H, H6); 6.77 (d, 1H, *J* = 16 Hz, CH=CH). ^{13}C NMR (75MHz, DMSO-d6) δ: 181.2 (C=O), 153.7 (CH=CH), 144.0 (C2), 143.8 (C3a), 142.6 (C7a), 141.3 (Ph-1), 139.9 (C5), 128.8 (Ph-3,5), 124.6 (Ph-2,6), 122.7 (Ph-4), 122.5 (C6), 121.7 (CH=CH), 117.0 (C7). MS: for $C_{16}H_{11}ClN_2O$, calcd. 283.0567[M+H]$^+$, found 282.9731.

Reagents and conditions : **a. 1)** lactic acid, .HCl diluted, NH$_4$OH diluted ; **2)** K$_2$CrO$_7$ / H$_2$SO$_4$ diluted, NH$_3$ solution

b. Het-CHO, NaOH / EtOH, AcOH 20%

Scheme 1. General synthesis method of 5-chlorobenzimidazolylchalcones

1-(5-chloro-1H-benzo[d]imidazol-2-yl)-3-(2-chlorophenyl)prop-2-en-1-one (**3c**)

Yellow solid, m.p.: 118 -120°C, yield 78%. ^{1}H NMR (300MHz, DMSO-d6) δ: 13.50 (s, 1H, N-H); 8.12 (s, 1H, H4); 8.08 (d, 1H, J = 16 Hz, CH=CH); 7.97 - 7.92 (m, 1H, Ph-3); 7.79 – 7.81 (m, 1H, H7); 7.75 - 7.72 (m, 2H, Ph-5,6); 7.69 - 7.66 (m, 1H, H6); 7.55 - 7.51 (m, 1H, Ph-4) ; 7.50 - 7.42 (d, 1H, J = 16 Hz, CH=CH). ^{13}C NMR (75MHz, DMSO-d6) δ: 180.1 (C=O), 153.7 (Ph-3), 145.3 (CH=CH), 143.8 (C2), 141.7 (C3a), 141.1 (C7a), 139.3 (Ph-1), 135.2 (Ph-5), 130.7 (C5), 130.1 (C6), 129.2 (CH=CH), 122.7 (Cl-Ph-2), 122.5 (Ph-6), 121.6 (C7), 120.8 (C4), 117.0 (Ph-4). MS: for $C_{16}H_{10}Cl_2N_2O$ calcd. 317.7945[M+H]+, found 317.7902.

1-(5-chloro-1H-benzo[d]imidazol-2-yl)-3-(3-hydroxyphenyl)prop-2-en-1-one (**3d**)

Yellow solid, m.p.: 212 -214°C, yield 66%. ^{1}H NMR (300MHz, DMSO-d6) δ: 13.50 (s, 1H, N-H); 8,03 (d, 1H, J = 8.4 Hz, OH-Ph-3); 8.15 (s, 1H, H4); 7.75 (d, 1H, J = 16 Hz, CH=CH); 7.51 - 7.45 (m, 1H, H7); 7.32 -7.25 (m, 2H, Ph-5,6); 7.24 - 7.18 (m, 2H, H6, Ph-2); 6.86 - 6.81 (m, 1H, Ph-4); 6.80 (d, 1H, J = 16 Hz, CH=CH). ^{13}C NMR (75MHz, DMSO-d6) δ: 180.6 (C=O), 161.2 (OH-Ph-3), 158.0 (CH=CH), 152.6 (C2), 143.8 (C3a), 141.7 (C7a), 139.3 (Ph-1), 135.2 (Ph-5), 130.1 (C5), 124.2 (C6), 122.7 (Ph-6), 122.5 (CH=CH), 121.6 (Ph-2), 118.2 (C7), 117.0 (C4), 116.7 (Ph-4). MS: for $C_{16}H_{11}ClN_2O_2$ calcd. 299.8560[M+H]+, found 299.8545.

1-(5-chloro-1H-benzo[d]imidazol-2-yl)-3-(4-methoxyphenyl)prop-2-en-1-one (**3e**)

Yellow solid, m.p.: 194 – 196 °C , yield: 69%. ^{1}H NMR (300MHz, DMSO-d6) δ: 13.04 (s, 1H, N-H), 8.58 (s, 1H, H4), 8.08 (d, 1H, J = 16 Hz, CH=CH),7.81 - 7.75 (m, 2H, Ph-2,6), 7.67 - 7.63 (m, 1H, H7),7.24 (m, 1H, H6), 7.16- 7.10 (m, 2H, Ph-3,5),6.91 (d, 1H, J = 16 Hz, CH=CH), 3.87 (s, 3H, Ph-OCH$_3$).^{13}C NMR (75MHz, DMSO-d6) δ: 182.3 (C=O), 158.4 (OCH$_3$-Ph-4), 143.4 (CH=CH), 139.6 (C2), 138.5 (C3a and C7a), 128.2 (Ph-2,6), 127.8 (C5), 125.9 (Ph-1), 122.3 (C6), 119.7 (CH=CH), 114.7 (C7), 113.5 (C4), 112.4 (Ph-3,5),53.1 (OCH$_3$). MS: for $C_{17}H_{13}ClN_2O_2$ calcd. 312.1705[M+H]+, found 312.1687.

1-(5-chloro-1H-Benzo[d]imidazol-2-yl)-3-(4-(dimethylamino)phenyl)prop-2-en-1-one (**3f**)

Red solid, m.p.: 196 – 198°C ,yield: 55%; ^{1}H NMR (300MHz, DMSO-d6) δ: 13.11 (s, 1H, N-H), 8.16 (s, 1H, H4), 7.83 (d, 1H, J = 16 Hz, CH=CH), 7.69-7.61 (m, 2H, Ph-2,6), 7.56(d, 1H, J = 8.5 Hz, H7), 7.27 (d, J = 8.5 Hz, 1H, H6), 6.91–6.83 (m, 2H, Ph-3,5), 6.72 (d, 1H, J = 16 Hz, CH=CH), 3.46 (s, 6H,N(CH$_3$)2).^{13}C NMR (75MHz, DMSO-d6) δ: 185.2 (C=O), 149.7 (N(CH$_3$)2-Ph-4), 143.3 (CH=CH), 140.1(C2), 139.1 (C3a), 136.1(C7a), 129.1 (Ph-2,6), 128.9 (C5), 123.5 (Ph-1), 123.1 (C6), 119.2 (CH=CH), 115.2 (C7), 114.2 (C4), 111.8 (Ph-3,5), 40.4 (N(CH$_3$)2). MS: for $C_{18}H_{16}ClN_3O$ calcd. 325.1106 [M+H]+, found 325.1099.

1-(5-chloro-1H-benzo[d]imidazol-2-yl)-3-(4-nitrophenyl)prop-2-en-1-one (**3g**)

Yellow solid, m.p.: 212 -214°C, yield 66%. ^{1}H NMR (300MHz, DMSO-d6) δ: 13.50 (s, 1H, N-H); 8.15 (s, 1H, H4); 8.11 (s, 1H, Ph-2); 7.98 - 7.95 (m, 1H, Ph-4); 7.76 - 7.72 (m, 1H, Ph-6); 7.57 (d, 1H, J = 16 Hz, CH=CH); 7.49 - 7.45 (m, 2H, Ph-5 and H7); 7.32 - 7.25 (m, 1H, H6); 6.83 (d, 1H, J = 16 Hz, CH=CH). ^{13}C NMR (75MHz, DMSO-d6) δ: 185,1 (C=O), 151.8 (NO$_2$-Ph-3), 148.7 (CH=CH), 142.6 (C2), 138.8 (C3a), 135.3 (Ph-1), 133.7 (C7a), 132.7 (Ph-6), 132.2 (Ph-5), 130.2 (C5), 126.6 (C6), 125.7 (Ph-4), 123.9 (Ph-2), 122.5 (CH=CH), 118.2 (C7), 117.3 (C4). MS: for $C_{16}H_{10}ClN_3O_3$ calcd. 328.1045[M+H]+, found 328.1034.

1- (5-chloro-1H-benzimidazol-2-yl)-3-pyridin-3-ylprop-2-en-1-one (**3h**)

Yellow solid, m.p.: 187 -189°C, yield 61%. ^{1}H NMR (300MHz, DMSO-d6) δ: 13.50 (s, 1H, N-H); 9.07 (s, 1H, Pyr-2); 8.96 (m, 1H, Pyr-4) ; 8.10 (s, 1H, H4); 8.08 - 8.05 (m, 2H, Pyr-6 and CH=CH); 7.51 - 7.48 (m, 1H, H7); 7.32 - 7.27 (m, 1H, Pyr-5); 7.14 - 7.10 (m, 1H, H7); 7.85 - 7.82 (m, 1H, H6) ; 7.70 (d, 1H, J = 16 Hz, CH=CH). ^{13}C NMR (75MHz, DMSO-d6) δ: 181.0 (C=O), 154.5 (Pyr-4), 151.1 (Pyr-2), 150.2 (C2), 145.0 (C3a), 143.8 (CH=CH), 141.7 (C7a), 139.2 (Ph-6), 133.0 (Pyr-1), 127.6 (C5), 124.2 (CH=CH), 122.6 (C6), 121.7 (Pyr-5), 120.8 (C7), 117.5 (C4). MS: for $C_{15}H_{10}ClN_3O$ calcd. 284.0498[M+H]+, found 284.0517.

1- (5-chloro-1H-benzo[d]imidazol-2-yl)-3- (furan-2-yl) prop-2-én-1-one (**3i**)

Brown solid, m.p.: 237 -239°C, yield 61%. ^{1}H NMR (300MHz, DMSO-d6) δ: 13.50 (s, 1H, N-H); 8.32 (s, 1H, H4); 8.17- 8.14 (m, 1H, Fur-5); 7.80 - 7.78 (m, 1H, Fur-3); 7.65 (d, 1H, J = 16 Hz, CH=CH); 7.48 – 7.43 (1H, m, H7); 7.10 – 7.07 (m, 1H, H6); 6.82 – 6.79 (m, 1H, Fur-4) ; 6.72 (d, 1H, J = 16 Hz, CH=CH). ^{13}C NMR (75MHz, DMSO-d6) δ: 183.0 (C=O), 151.2 (Fur-1); 144.5 (Fur-5), 141.2 (C2), 140.8 (C3a), 138.7 (C7a); 128.6 (C5), 126.8 (CH=CH), 123.6 (C6), 122.2

(CH=CH), 117.5 (C7), 116.8 (C4); 113.7 (Fur-3), 112.6 (Fur-4). MS: for $C_{14}H_9ClN_2O_2$ calcd. 273.1276[M+H]+, found 273.1204.

2.2 Antiplasmodial Assay

Clinical isolates of *Plasmosium falciparum* were obtained from patients brought to the laboratory of the general hospital of Abobo, Abidjan Ivory Coast, for suspected malaria. Patients were included if they had a monospecific *P. falciparum* infection and had not used any antimalarial treatment for at least 30 days. Once informed consent was obtained, samples with a positive thick drop and thin blood smear with a parasite density greater than 100 parasites/µl were stored in a cooler containing a cold accumulator and then transported to the cell culture room of the Immunology Unit of the Center for Diagnosis and Research on AIDS and Opportunistic Diseases (CeDReS). The protocol of the study was approved by the ethical research committee of CeDReS of the CHU of Treichville, Ivory Coast. The samples were analyzed at the Immunology Unit of CeDres, Treichville University Hospital.

The assays to evaluate the antiplasmodial activities of the benzimidazolyl-chalcones (**3a-3i**) were performed following the colorimetric assay of histidine rich protein 2 (HRP2) production by ELISA [16, 17]. This HRP2 assay, which reflects the specific growth of *Plasmodium falciparum*, was performed against two clinical isolates of *P. falciparum*: one was sensitive to chloroquine and the other was resistant to chloroquine. Thus, we used the malaria Ag CELISATM kit. From the parasitized blood sample, we prepared an inoculum (washed parasitized blood + RPMI 1640 with HEPES and Na bicarbonate + BSA +/- washed GRS O) [11,12]. A duplicate inoculum sample (200 µL) was cultured on plates containing 50 µL of test chalcones at different concentrations (50 µg/mL, 10 µg/mL, 2 µg/mL, 0.4 µg/mL) and chloroquine (12.5 µg/mL, 25 µg/mL, 50 µg/mL, 100 µg/mL, 200 µg/mL, 400 µg/mL, 800 µg/mL). After 72 h of incubation at 37°C, the anti-plasmodial activity of the chalcones was determined by the HRP2 colorimetric method.

2.2.1 Malaria parasites and cultivation

Briefly, the parasites were cultured in human erythrocytes (obtained from an o positive blood group donor) and RPMI-1640 culture medium (Gibco by Life Technologies, Grand Island, NY, USA) supplemented with HEPES. This medium was enriched with 10% albumax II (Gibco by life technologies, Grand Island, NY, USA) used as a substitute for human serum and bicarbonate maintained at the fixed temperature of 37° C in a water bath. Each parasitized blood sample (GRP) was washed three times with RPMI 1640 before use in culture. Before the incorporation of the parasitized red blood cells (GRP) in the culture inoculum, a step of dilution of the GRP pellet was carried out using group O red blood cells in order to reduce the parasitaemia to between 0.1 and 0.2% when the parasite density was greater than this interval. For incubation, the culture was kept in a humid atmosphere in an oven thermostatically controlled at 37° C and, containing a gas mixture of 5% CO_2, 5% O2 and 90% N_2, for 72 hours. At the end of this incubation time, the plates were transferred from the oven to the laboratory bench. A rapid visual assessment of the maintenance of the blood-red coloration of the wells, indicating an absence of bacterial contamination, was necessary for the validation of the culture. After validation of the culture, the culture plate was left in the still sealed state, then stored by freezing at -20° C before the ELISA test step. This final parasite culture was immediately used for antimalarial assay.

2.2.2 Antiplasmodial screening method

An *in vitro* histidine-rich protein 2 (HRP2) assay was performed to assess anti-*P. falciparum* activity. HRP2 assays were performed according to standard procedures. Briefly, *P. falciparum* isolates were grown in the presence of increasing dilutions of synthetic chalcone derivative and chloroquine, the reference antimalarial drug. In practice, one culture plate was used to culture 2 isolates. Each drug concentration was tested in duplicate in the culture wells. Thus, 4 concentration ranges (50 µg/ml, 10 µg/ml, 2 µg/ml and 0.04 µg/ml) of the tested chalcones were present in duplicate in the culture wells. For chloroquine, a range of 7 daughter solutions from 800 nM to 12.5 nM was obtained by a series of dichotomous dilutions in RPMI of a 1600 nM stock solution. In each well of a microplate, 100 µl of each dilution tested were added to 100 µl of final parasite culture. After incubation at 37°C for 72 h, the plates were then freeze-thawed twice to achieve complete hemolysis and 100 µl of each hemolysis culture sample was transferred to the ELISA plates. The microwells were previously coated with *P. falciparum* monoclonal anti-HRP II antibody and incubated at laboratory room temperature for 1

hour in a humidified chamber. Plates were washed five times with the PBS/Tween mixture called wash buffer and 100 µl of the diluted antibody conjugate was added to each well. After incubation for an additional 1 hr in a humidified chamber, the plates were washed with the wash buffer mixture (200 µl/well) and 100 µl of the substrate enzyme was added to each well. The plates were then incubated for an additional 15 minutes at room temperature and protected from light; and 50 µl of the stop solution was added. Absorbance values were read using an ELISA plate reader (iMarkTM, Bio-Rad, USA) at a maximum absorbance of 450 nm. The measurement of antimalarial activity of the drugs was expressed as 50% inhibitory concentration (IC_{50}) which is the concentration of antimalarial drug inhibiting 50% of the parasite HRP2 released compared to the control without antimalarial drug. IC_{50} values were calculated using a nonlinear dose-response curve fitting analysis via ICEstimator 1.2 software. The threshold value for the chloroquine IC_{50} was 0.1 µM. This is a conventional value used by several authors. Thus, if the IC_{50} was lower than 0.1 µM, the isolate was said to be chloroquinosensitive. It was qualified as chlororesistant for an IC_{50} greater than or equal to 0.1 µM.

2.3 Computational Studies

2.3.1 Molecular Docking and MM-GBSA studies

Molecular docking studies were conducted to explore protein ligands interactions. Crystal structures of sensitive (PDB ID: 1J3I) and resistant (PDB ID: 4DP3) proteins of dihydrofolate reductases–thymidylate synthase (PfDHFR) were obtained from protein data bank (RCSB). The proteins were prepared using protein preparation wizard of maestro v12.7 of Schrodinger suite. The compounds were prepared using ligprep. The binding cavities of the two proteins were identified using the receptor grid generation tool using the cocrystalized ligands. Docking was carried out using XP mode of Glide. MM-GBSA binding free energies were calculated using Prime module of Schrodinger.

2.3.2 ADME prediction

Pharmacokinetics properties of the compound were predicted using qikprop tool of Schrodinger.

3. RESULTS AND DISCUSSION

3.1 Biological Evaluation

The threshold value of the chloroquine IC_{50} was 0.1 µM. This is a conventional value used by several authors. Thus, if the IC_{50} was less than 100nM, the isolate was said to be chloroquino sensitive. It was qualified as chlororesistant for an IC_{50} greater than or equal to 0.1 µM. The results of the antiplasmodial screening of the height 5-chlorobenzimidazolylchalcone derivatives and IC_{50} of chloroquine and Chalcone (1,3-diphenylpropenone) used as reference for comparison is presented in table 1.

The results showed a higher efficacy of our synthetic chalcones on chloroquine-resistant isolates with IC_{50}s ranging from 0.78 to 31.28µM compared to chloroquine-sensitive isolates with IC_{50}s ranging from 0.32 to 44.38µM. Indeed, in a previous study it had been shown that 1,3-diphenylpropenone or chalcone had a moderate antiplasmodial activity on both chloroquine-sensitive and chloroquine-resistant isolates, with IC_{50} of 38.56 and 1.44 µM respectively. Benzimidazolyl-chalcones (3a-3i) were designed as a result of the juxtaposition of the benzimidazole ring and the arylpropenone linkage of chalcones. These two chemical entities have proven their strong antiplasmodial potentialities [13, 18]. Moreover 3a, first compound directly derived from this concept of molecular hybridization by replacing the phenyl in position 1 of the 1,3-diphenylpropenone by benzimidazole, has also shown good antiplasmodial properties. Indeed, this compound not substituted on benzimidazole, showed significant activity against both chloroquine-resistant *P. falciparum* isolate (IC_{50} = 6.81 µM) and chloroquine-sensitive *P. falciparum* isolate (IC_{50} = 44.38 µM). However, this activity remainsunsatisfactory compared to the chalcone without the benzimidazole ring. Tests to improve the antiplasmodial activities of compound **3a** consisted in introducing a chlorine atom on the benzimidazole ring in positon 5 (compound **3b**). This modulation contributes to the exaltation of antiplasmodial activities on both chloroquine-sensitive (IC_{50} = 10.65 µM) and chloroquine-resistant (IC_{50} = 0.78 µM) *P. falciparum* isolates by 4 to 9 times compared to its non-chlorinated analogue **3a**.

Therefore, in order to optimize the antiplasmodial activities of compound **3b**, two types of chemical modulations were undertaken on the benzene

homocycle. The first modulation consisted in the introduction on the benzene ring of various substituents known as anti-infectious activity performers. In general, the presence of an electron-donor group (OH, OCH$_3$, N(CH$_3$)$_2$ on the benzene homocycle of 5-chlorobenzimidazolylchalcone **3b** led to an improvement in the antiplasmodial activity. Indeed, compounds **3d**, **3e** and **3f** had very good antimalarial activity on chloroquinosensitive isolates, with IC$_{50s}$ ranging from 9.40 µM to 0.32 respectively. These derivatives were at least 4-fold more potent than the reference chalcone with an IC$_{50}$ of 38.56 µM. Remarkably, among the screened hybrid of chalcones, the para-methylated derivative **3e** was the most active against the chloroquine-sensitive isolate. Although these activities are below that of chloroquine (IC$_{50}$ = 0.076 µM), these compounds remain remarkably effective against the chloroquino-sensitive isolate of *P. falciparum*. On the other hand, the presence of an electron-withdrawing group of the chloro or nitro type reduced the antiplasmodial activity of the corresponding derivatives towards the susceptible strain.

Even if, on the chloroquinoresistant isolate, the tendency of electron-withdrawing groups to induce less good antimalarial activities remains. Among the electron-donor derivatives, the best activity on the chloroquinoresistant isolate was obtained by the dimethyl amine derivative **3f**. Similarly, with an IC$_{50}$ of 1.8 µM, the presence of a dialkylamine group like the one present in Chloroquine, but this time of dimethylamine type, didn't allow **3f** to improve antiplasmodial activity on chloroquine-resistant isolates. In the end, no substitution of the benzene homocycle allowed to obtain a derivative with a better activity than the unsubstituted compound **3b**.
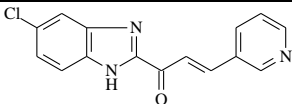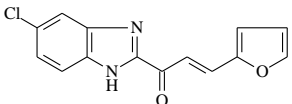
The second modulation consisted in replacing the benzene homocycle by a pyridine or furanic heterocycle. On the one hand, the pyridine derivative **3h** with an activity around 25 µM, did not improve the activities obtained with compound **3b**. On the other hand, the presence of the furanic heterocycle induced a loss of activities against the susceptible *P. falciparum* isolate while maintaining a minimal activity on the resistant strain (IC$_{50}$ = 9.34 µM).

Overall, as summarize in figure 3, general trends of SAR in benzimidazole chalcone series established that

- the replacement of the benzene homocycle by a heterocycle is not appropriate to have excellent antiplasmodial activities.
- the electro withdrawing groups seems to induce a reduction of activity on chloroquinosensitive isolates as well as on chloroquine-resistant isolates
- the presence of methoxygroup, a modulator of the antiplasmodial activity of quinine, increased the antiplasmodial efficacy only on chloroquinosensitive isolates.

Table 1. **Median IC$_{50}$ of all tested compounds by type of isolates**

Compounds	Structures			Chloroquine-sensitive *P. falciparum* isolate IC$_{50}$ (µM) ±SD	Chloroquine-resistant *P. falciparum* isolate IC$_{50}$ (µM) ± SD
Chalcone or 1,3-diphenyl prop-2-en-1-one				38.56 ± 3.86	1.44 ± 0.14
3a[14]				44.38	6.81
3b		R	H	10.65 ± 1.07	0.78 ± 0.08
3c			2-Cl	32.10 ± 3.21	31.28 ± 3.13
3d			3-OH	6.23 ± 0.62	2.34 ± 0.23
3e			4-OCH$_3$	0.32 ± 0.03	1.96 ± 0.20
3f			4-N(CH$_3$)$_2$	9.40 ± 0.94	1.82 ± 0.18
3g			4-NO$_2$	29.39 ± 2.94	10.61 ± 0.11

Compounds	Structures	Chloroquine-sensitive *P. falciparum* isolate IC$_{50}$ (µM) ±SD	Chloroquine-resistant *P. falciparum* isolate IC$_{50}$ (µM) ± SD
3h		29.61 ± 2.96	22.42 ± 2.24
3i		-	9.34 ± 0.93
Chloroquine		0.076 ± 0.008	0.13 ± 0.013

*Mean ± SD = Mean values ± Standard deviation of means of three experiments
* - = inactive at the threshold concentration of 100 µM

Halogen atom dramatically improved the antiplasmodial activity

Para methoxy substituent considerably enhanced antiplasmodial activity against chloroquine-sensitive isolate

Presence of heterocycle is not favorable to antimalarial activities
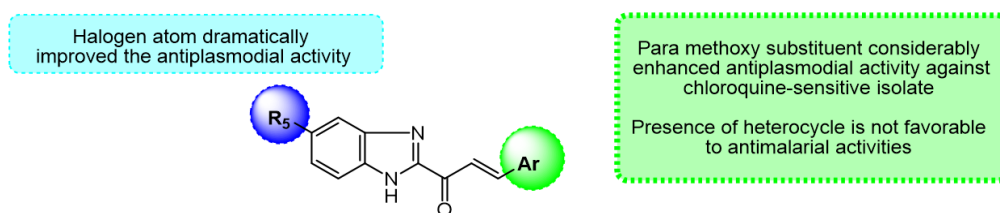
Fig. 3. SAR of benzimidazolyl-chalcones with antiplasmodial properties

This activity would be related to the basic character of the benzimidazole heterocycle, like the quinoline nucleus of antimalarial drugs such as chloroquine and amodiaquine. This better performance on chloroquinoresistant isolate can be explained by either a different mechanism of action or a conformational modification of the target receptors of resistant strains in favor of chalcones with Benzimidazole vector.

3.2 Computational Studies

3.2.1 Molecular docking and MM-GBSA binding free energy studies

To understand the mechanism of the antiplasmodial activity of the designed compounds, molecular docking studies were conducted between the non-chlorinated derivative **3a** less active and chlorinated derivatives **3b**, **3d**, **3e**, and **3f**, which exhibited the best antimalarial activities towards the chloroquine-resistant strain with IC$_{50}$< 3µM. These studies were performed against the dihydrofolate reductases–thymidylate synthase (PfDHFR-TS) enzyme using both sensitive (PDB ID: 1J3I) and resistant proteins (PDB ID: 4DP3). As depicted in **table 2**, the docking scores of these hybrids of chalcone **3a**, **3b**, **3d**, **3e**, and **3f** were-6.62, -7.68, -8.31, -5.55 and -7.73 kcal,

respectively, in the case of the sensitive protein, and -8.22,-7.98, -6.43, -8.19 and -8.74 kcal/mol in the case of the resistant protein for the respective five compounds, in succession. All the compounds exhibited strong binding towards the two proteins in agreement with experimental results. MM-GBSA studies showed compounds **3a**, **3b**, **3d**, **3e**, and **3f** possess some higher negative binding free energy values, viz., -44.4, -57.89 kcal/mol, -63.2 kcal/mol, -53.45 kcal/mol and -52.01 kcal/mol, respectively in case of sensitive protein; while, in case of resistant protein, binding free energies were better, -46.54, -63.09 kcal/mol, -53.59 kcal/mol, -60.13 kcal/mol and -68.41kcal/mol respectively for the four compounds.

In silico antimalarial studies performed for the PfDHFR-TS resistance protein (PDB ID: 4DP3) confirm that the ketone of propenone and the pyrrolic nitrogen of benzimidazole establish key hydrogen bonds with the target amino acids (Leu 164, Leu 40 and ALA 16) (Figure **4a-e**). These key interactions as well as the chlorine on the benzimidazole, which allows for better hydrophobic pole occupancy, would account for the better antiplasmodial activities of the 5-chlorobenzimidazolyl-chalcones (**3b-3i**) compared to benzimidazolyl-chalcone **3a**.

Table 2. Docking score and MM-GBSA free binding energies for molecules 3a, 3b, 3d, 3e, and 3f against both sensitive (PDB ID: 1J3I) and resistant (PDB ID: 4DP3) proteins of PfDHFR-TS

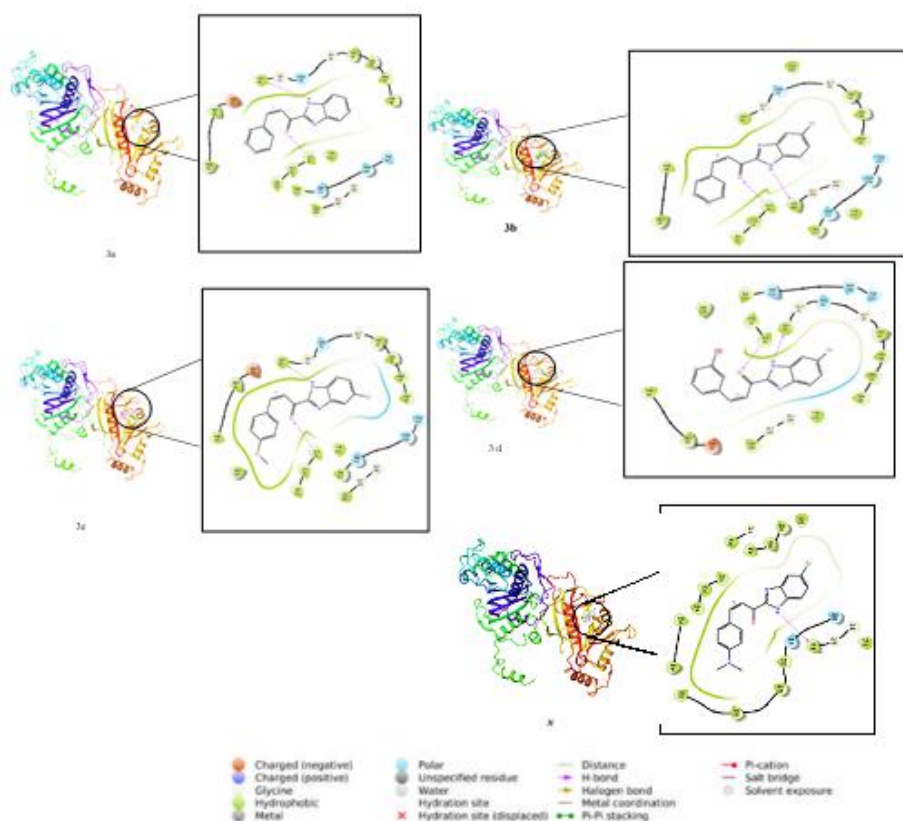| Compounds	1J3I		4DP3	
	docking score (kcal/mol)	MMGBSA dG Bind (kcal/mol)	docking score (kcal/mol)	MMGBSA dG Bind (kcal/mol)
3a	-6.62	-44.34	-8.22	-46.54
3b	-7.68	-57.89	-7.98	-63.09
3d	-8.31	-63.2	-6.43	-53.59
3e	-5.55	-53.45	-8.19	-60.13
3f	-7.73	-52.01	-8.74	-68.41

Fig. 4. 2D and 3D interactions of five synthesized compounds 3a, 3b, 3d, 3e and 3f with the amino acid residues of resistant PfDHFR-TS (PDB ID: 4DP3)

Table 3. Predicted ADME properties of compounds 3a, 3b, 3d, 3e, and 3f

Compounds	QPlogPo/w[1]	QPlogS[2]	QPPCaco[3]	QPlogBB[4]	QPPMDCK[5]	Percent Human Oral Absorptio[6]	Rule of Five[7]
3a	3.14	-3.65	2599.99	-0.24	1389.58	100.00	0
3b	3.51	-4.22	1760.71	-0.22	2247.57	100.00	0
3d	2.81	-3.98	589.88	-0.76	688.75	93.00	0
3e	3.69	-4.53	2607.45	-0.153	3424.83	100.00	0
3f	4.18	-5.44	2605.61	-0.189	3422.22	100.00	0

[1]*Predicted octanol/water partition coefficient log P (acceptable range -2.0–6.5).,* [2] *Predicted aqueous solubility in mol/L (acceptable range -6.5–0.5),* [3] *Predicted caco cell permeability in nm/s (acceptable range: <25 is poor and >500 is great),* [4] *Predicted blood brain barrier permeability (acceptable range -3–1.2).*
[5] *Predicted apparent MDCK cell permeability in nm/s (acceptable range in nm/s (acceptable range: <25 is poor and >500 is great),* [6] *Percentage of human oral absorption (acceptable range: <25 is poor and >80% is high.* [7]*Lipinski rule of five.*

3.2.2 *In silico* ADME analysis

The interesting *in vitro* and *in silico* docking results encouraged us to conduct ADME prediction studies for the synthesized molecules to study the pharmacokinetic properties. ADME prediction was performed using qikprop of Schrodinger suite. The predicted properties are presented in table 3. All five synthesized molecules showed acceptable lipophilicity (QPlogPo/w), high aqueous solubility (QPlogS), excellent cell permeability (QPPMDCK and QPPCaco), good CNS penetration (QPlogBB) and excellent oral absorption. None of the compounds violated Lipinski rule of five.

4. CONCLUSION

The present study was carried out with the aim of extending the evaluation of the antiplasmodial activities of benzimidazolyl-chalcones initiated in a previous study and which highlighted the excellent potentiality of the 5-chlorinated derivative. Ultimately, the 5-chlorobenzimidazolyl-chalcones were found to be chalcone hybrids with strong antiplasmodium activity. In summary, based on the MICs, it should be noted that all 5-chlorobenzimidazolyl-chalcones have better antiplasmodium activity on chloroquine-resistant isolates (CQ-R) than on chloroquinosensitive isolates (CQ-S). In addition to the important role played by the 5-chlorobenzimidazole core in the appearance of the antiplasmodial activities of the new chalcones, it appears from the pharmacomodulations that only the introduction of electrodonor substituents such as hydroxyl, méthoxy or a dimethylamine group on the phenyl ring, allowed to improve the activities on chloroquinosensitive isolates but not on chloroquinoresistant isolates. On chloroquinoresistant isolates, the unsubstituted derivative remains the one with the most remarkable antimalarial performance in this series of 5-chlorobenzimidazoles. However, for development as a drug candidate in the treatment of drug-resistant malaria, we can propose the methoxylated derivative. We selected 5 compounds namely **3a**, **3b**, **3d**, **3e** and **3f**, to perform docking studies, as these compounds presented the best antimalarial activities. Molecular docking studies showed that all potent compounds presented significantly high binding affinity against resistant and sensitive dihydrofolate reductase plasmid-thymidylate synthase proteins. In addition, the compounds exhibited favorable *in silico* ADME properties.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The protocol of the study was approved by the ethical research committee of CeDReS of the CHU of Treichville, Ivory Coast.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Zekar L, Sharman T. *Plasmodium Falciparum* Malaria. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021. Accessed 21 September 2021.
Available:https://www.ncbi.nlm.nih.gov/books/NBK555962/
2. Global malaria gains threatened by access gaps, COVID-19 and funding shortfalls. World Health Organization. 2020. Accessed 01 august 2021.
Available:https://www.who.int/news/item/30-11-2020-who-calls-for-reinvigorated-action-to-fight-malaria
3. Howes RE, Battle KE, Mendis KN, Smith DL, Cibulskis RE, Baird JK, Hay SI. Global Epidemiology of *Plasmodium vivax*. Am J Trop Med Hyg. 2016;28(95)(6 Suppl):15-34.
4. World malaria report 2019. Global Malaria Programme. WHO. 20; 232p. Accessed 01 September 2021.
Available:https://www.who.int/publications/i/item/9789241565721
5. World malaria report 2020. 20 years of global progress & challenges. Global Malaria Programme. World Health Organization. 2020; 299 p. Accessed 11 September 2021.
Available:https://www.who.int/publications/i/item/9789240015791

6. 6- Ariey F, Witkowski B, Amaratunga C. A molecular marker of artemisinin-resistant *Plasmodium falciparum* malaria. Nature. 2014;505:50–5.

7. Turkson BK, Agyemang AO, Nkrumah D, Nketia RI, Baidoo MF, Mensah MLK. Treatment of Malaria Infection and Drug Resistance, *Plasmodium* Species and Drug Resistance. IntechOpen DOI:10.5772/intechopen.98373. Accessed 17 September 2021. Available:https://www.intechopen.com/chapters/77119

8. Hogan AB, Jewell BL, Sherrard-Smith E, Vesga JF, Watson OJ, Whittaker C, et al. Potential impact of the COVID-19 pandemic on HIV, tuberculosis, and malaria in low-income and middle-income countries: a modelling study.The Lancet Global Health. 2020;8(9):e1132–e41.

9. Cui L, Mharakurwa S, Ndiaye D, Rathod PK, Rosenthal PJ. Antimalarial Drug Resistance: Literature Review and Activities and Findings of the ICEMR Network.The American Society of Tropical Medicine and Hygiene. 2015;93(3_Suppl):57-68.

10. Kumara S, Bhardwaja TR, Prasadb DN, Singhb RK. Drug targets for resistant malaria: Historic to future perspectives. Biomed Pharmacother.2018;104:8–27.

11. Chughlay MF, Rossignol E, Donini C, El Gaaloul M, Lorch U, Coates S. Grant Langdon, Tim Hammond, Jörg Möhrle, Stephan Chalon. First-in-human clinical trial to assess the safety, tolerability andpharmacokinetics of P218, a novel candidate for malaria chemoprotection. Br J Clin Pharmacol. 2020;86:1113-24.

12. Chughlay MF, El Gaaloul M, Donini C, Campo B, Berghmans P-J, Lucardie A, Marx MW, Cherkaoui-Rbati MH, Langdon G, Angulo-Barturen I, Viera S, Rosanas-Urgell A, Van Geertruyden J-P, and Chalon S. Chemoprotective Antimalarial Activity of P218 against *Plasmodium falciparum*: A Randomized, Placebo-Controlled Volunteer Infection Study. Am J Trop Med Hyg. 2021;104(4): 1348–58.

13. Syahri J, Yuanita E, Achromi NB, Armunanto R, Purwono B. Chalcone analogue as potent anti-malarial compounds against *Plasmodium falciparum*: Synthesis, biological evaluation, and docking simulation study. Asian Pac J Trop Biomed. 2017;7(8): 675–9.

14. Ouattara M, Sissouma D, Yavo, W, Kone MW. Synthèse et criblageantiplasmodial de quelquesbenzimidazolyl-chalcones. Int j biol chem sci. 2015;9:1697-710.

15. Coulibaly S, N'guessan DUJP, Ouattara M, Koné WM, Sissouma D. Synthesis and Antifungal Activities of Benzimidazolyl-Arylpropenone Scaffolds as Promising Inhibitors of Azole-Resistant *Candida* Strains. European Journal of Biomedical and Pharmaceutical Sciences. 2019;6: 19-25.

16. Noedl H, Attlmayr B, Wensdorfer WH, Killarritsh H, Miller RS. A histidine-rich protein 2-based malaria drug sensitivity assay for field use. Am J Trop Med Hyg.2004;71(6):711-4.

17. Estimation of *Plasmodium falciparum* drug susceptibility ex vivo by HRP2 Procedure.Antimalarial Resistance Network (WWARN) 2011. Accessed 11 September 2021. Available:https://www.wwarn.org/tools-resources/procedures/estimation-plasmodium-falciparum-drug-susceptibility-ex-vivo-hrp2-elisa

18. Singh K, Okombo J, Brunschwig C, Ndubi F, Barnard L, Wilkinson C et al. Antimalarial pyrido[1,2-*a*]benzimidazoles: Lead optimization, parasite life cycle stage profile, mechanistic evaluation, killing kinetics, and *in vivo* oral efficacy in a mouse model. J Med Chem. 60: 1432-48.

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