Ex vivo efficacy of selective chalcone derivatives on reference strains and field isolates of *Plasmodium falciparum*

Marius Trésor Dable^a, Konan Dominique Tano^a, Mahama Ouattara^b, Kigbafori Dieudonné Silue^c, Eby I. Hervé Menan^d and William Yavo^{a,e}

^aMalaria Research and Control Centre, National Institute of Public Health, Abidjan, Côte d'Ivoire; ^bDepartment of Therapeutic Chemistry and Organic Chemistry, Faculty of Pharmacy and Biological Sciences Félix Houphouët-Boigny University, Abidjan, Côte d'Ivoire; ^cSwiss Scientific Research Centre in Côte d'Ivoire, Abidjan, Côte d'Ivoire; ^dCentre for Diagnosis and Research on AIDS and other infectious diseases, Abidjan, Côte d'Ivoire; ^eDepartment of Parasitology-Mycology, Faculty of Pharmacy and Biological Sciences, Félix Houphouët Boigny University, Abidjan, Côte d'Ivoire

ABSTRACT

The extension of *Plasmodium falciparum* resistance to existing antimalarial drugs is worrying. Faced with this problem, the search for new and effective molecules is necessary. In this context, six chalcone derivatives (B1, B11, B14, B17, SCA02 and SCA03) were tested on field isolates and then reference strains to evaluate their antiparasmodial activity by using the Rieckmann semi-microtest, recommended by WHO, for in vitro and ex vivo activity tests. Compounds B14 and B17 exhibited promising antiparasmodial activities (IC₅₀s: 14.41–16.40 µM) regardless of the type of isolate. Compounds B1, B11, SCA02 and SCA03 showed a moderate inhibition of field isolates (IC₅₀s: 25.63–48.29 µM) and very good activity against reference strains (IC₅₀s: 3.82–10.03 µM). Therefore, more structural modulations should improve their efficiency and make these molecules very good candidates for future effective antimalarial drugs.

KEYWORDS

Malaria; chalcones; ex vivo/invitro; *Plasmodium falciparum*

Introduction

In spite of global control measures, malaria remains a major public health problem. In fact, in 2018, the World Health Organization (WHO) estimated the number of reported malaria cases to be around 228 million worldwide. In the same year, malaria caused nearly 405,000 deaths, the majority (85 %) of which occurred in sub-Saharan Africa [1]. Children under five and pregnant women are the most affected by this condition [2,3]. In Côte d'Ivoire, malaria accounted for 43% of outpatients visits [4]. In addition, the resistance of *Plasmodium falciparum* to most available antimalarial drugs raises serious concerns about the therapeutic management of the disease in endemic countries [5]. Indeed, for the artemisinin-based combination therapies (ACTs) recommended by the WHO for the first-line treatment of uncomplicated malaria in malaria-endemic countries [6], several studies report the emergence and extent of artemisinin resistant strains in the different regions of South-East Asia [7–12]. This threat seems to be present in Africa [13]. These findings imply the need and the urgency to search for new molecules in order to face up to the challenge of falciparum resistance [14]. To address this concern, we are interested in chalcone derivatives, which are considered precursors of flavonoids and isoflavonoids [15]. Chalcones have many interesting pharmacological activities [16], including antibacterial, antiparasmodial,

antileishmanial, antitrypanosomal, antifungal and antiviral activities [15,17,18]. They exist in natural and synthetic forms. The most promising compound of this new class of antimalarials appears to be Licochalcone A. It is a natural substance isolated from a Chinese licorice or *Glycyrrhiza glabra* (Fabaceae). Preliminary study has shown that it inhibits the *in vitro* growth of *P. falciparum* [19]. As a result, research work intensified around this substance and made it possible to identify its structure and to carry out its synthesis in the laboratory [17]. The aim of our study was to measure *ex vivo* and *in vitro* sensitivity of *P. falciparum* to some derivatives of these chalcones.

Materials and methods

Molecules

These are six chalcone synthesis derivatives including derivatives of 1,3-diphenylpropanone variously modulated by groups of chlorine, hydroxyl, methoxyl, nitro and dimethylamine type. These compounds were synthesized by the Department of Therapeutic and Organic Chemistry of the Pharmaceutical and Biological Sciences of the University Felix Houphouët-Boigny of Abidjan, Côte d'Ivoire. The chloroquine phosphate PHR 1258-1G lot # LRAA9061 from the manufacturer SIGMA-ALDRICH was chosen as the reference molecule.

Population and sites of the study

The parasitized blood samples collection was carried out at the El Rapha health center in Abobo and at the Community-Based Urban Health Training Unit (FSU-Com) in Yopougon Toit-Rouge. The municipalities of Abobo and Yopougon are among the ten municipalities of the city of Abidjan, the economic capital of Côte d'Ivoire. They are located to the north of the city and are the most populated with respective populations of 1,030,568 and 1,071,543 inhabitants [20]. Included in the study were patients suffering from uncomplicated malaria with *P. falciparum* mono-infection and a parasite density $\geq 0.08\%$. Written informed consent from the patient, parent or legal representative was obtained prior to participation in this study. Recent administration of antimalarial treatment (within seven days of consultation) resulted in exclusion from this study. The protocol of the study was approved by the National Committee of Ethics and Research of Côte d'Ivoire (reference 043/MSHP/CNER-Kp). After an interview and clinical examination, the blood of the suspected patients was collected on tube containing EDTA. A blood smear and a thick blood-stained GIEMSA were then performed as part of the biological malaria diagnosis [21]. The NF54 (chloroquine-sensitive) and K1 (multi-resistant) strains were used as reference strains for this study.

Ex vivo and in vitro tests

The chemosensitivity tests were carried out in the parasitology-mycology laboratory of the Center for Diagnosis and Research on AIDS and other infectious diseases (CeDReS). The isolates selected for the study were washed three times in RPMI 1640 (Roswell Park Memorial Institute (GIBCO®, Invitrogen™, Ref: 13,018-031, Lot No. 1070029X, Grand Island, N.Y. – USA). This operation consisted in adding a solution of RPMI 1640 to the parasitized blood, homogenize the whole then centrifuge at 1800 rpm for 5 minutes then remove the plasma and the buffy coat. The red blood cells pellet obtained was diluted if necessary, by O Rhesus positive red blood cells and the isolates were kept in culture in complete medium (RPMI 1640 and 10% of Albumax II) [22]. Regarding the reference strains; they were first thawed according to the protocol described by Hodan Ahmed Ismail [23]. They were then transferred into culture flasks which previously contained the optimized medium (medium supplemented with L-glutamine, serum, glucose, hypoxanthine-albumax complex and sodium bicarbonate) prepared for this purpose and then cultured in a CO₂ incubator at 37 ° C. After several days of growth, the strains were synchronized with sorbitol for the selection of ring parasites that were used to carry out the tests [24]. The distribution of drugs was made according to the molecules. The chalcones were diluted in dimethyl sulfoxide (DMSO) and

the chloroquine was diluted in RPMI wash. A range of six concentrations distributed in triplicate was performed for each molecule tested. For the chalcone derivatives, the concentrations were: C6 = 50 µg/ml, C5 = 16.67 µg/ml, C4 = 5.55 µg/ml, C3 = 1.85 µg/ml, C2 = 0.61 µg/ml and C1 = 0.21 µg/ml. For chloroquine, concentrations ranged from C6 = 200 nM to C1 = 6.25 nM after dichotomic dilution. An inoculum prepared from the parasitized blood was dispensed at 200 µL/well into sterile culture plates containing previously 50 µL of molecule solution in each well. The plates were subsequently incubated for 72 hours in an oven at 37 ° C. with an atmosphere enriched with 5% CO₂. The activity of the molecules was measured at the end of the test according to the SYBR green method [25].

Statistical analysis

The data obtained was analyzed by the ICESTIMATOR software for the determination of IC_{50s} by nonlinear regression. The value of the control cups i.e without molecule tested has been considered as 100% parasite growth. The in vitro response was expressed as the geometric mean IC_{50s} values of our different molecules with 95% confidence intervals. The classification of the activity of chalcone derivatives was done according to Liu et al. classification. Thus, the activity was very good when the IC₅₀ was less than or equal to 10 µM (A); good for an IC₅₀ greater than 10 and less than or equal to 20 µM (B); moderate for an IC₅₀ greater than 20 and less than or equal to 50 µM (C); low when the IC₅₀ was greater than 50 and less than or equal to 100 µM (D), and finally very low activity (E) for IC_{50s} greater than 100 µM [26]. The threshold value for chloroquine resistance was 100 nM [22]. The level of significance for statistical tests was set at 0.05.

Results

All the cultures have been validated, ie a success rate of 100%. Derivatives B14 and B17 had a good activity on all the isolates for IC_{50s} between 10.36 and 19.59 µM (Table 1).

The average IC_{50s} according to the *P. falciparum* strains and isolates are presented in Table 2. Chloroquine was used as a control with an IC_{50s} less than 100 nM for strain NF54 (chloroquine sensitive phenotype) and an IC₅₀ greater than 100 nM for strain K1 (chloroquine resistant phenotype). There were 26.31% chloroquine resistant isolates (IC₅₀ from 103.03 to 187.145 nM). Four compounds (B11, B14, B17 and SCA02) showed very good activity on strain K1 (IC_{50s} 3.82–9.87 µM) (Table 2).

Discussion

Chloroquine has been used to prevent and treat malaria for many years. The importance of this drug

Table 1. Antiplasmodial activity according to the type of strain and isolate.

Molécules	Strain						Isolates					
	NF54			K1			CQ-S			CQ-R		
	IC ₅₀ (µg/ml)	IC ₅₀ (µM)	A	IC ₅₀ (µg/ml)	IC ₅₀ (µM)	A	IC ₅₀ (µg/ml)	IC ₅₀ (µM)	A	IC ₅₀ (µg/ml)	IC ₅₀ (µM)	A
Chal_B1	10.03	41.47	C	8.38	34.65	C	5.8	23.99	C	10.73	44.36	C
Chal_B11	6.37	23.42	C	1.04	3.82	A	6.47	23.79	C	12.31	45.26	C
Chal_B14	2.93	10.59	B	2.73	9.87	A	4.53	16.40	B	10.28	37.19	C
Chal_B17	3.29	11.55	B	1.71	6.00	A	2.69	9.45	A	4.1	14.41	B
Chal_SCA02	4.24	12.66	B	3.36	10.03	B	7.33	21.89	C	16.77	50.09	D
Chal_SCA03	2.04	9.09	A	1.08	4.83	A	10.01	44.50	C	15.38	68.35	D

* A = Activity (A = very good; B = good; C = moderate; D = low; E = very low)

* CQ-S: Chloroquinosensitiveisolates; CQ-R: Chloroquioresistantisolates

Table 2. IC_{50s} of the different molecules on all isolates.

MOLECULES	ISOLATES	IC _{50s} GEOMETRIC MEAN (µG/ML)	IC _{50s} GEOMETRIC MEAN (µM)	95% INTERVAL OF TRUST AND CONFIDENCE	IC _{50s} MINIMUM VALUES (µM)	IC _{50s} MAXIMUM VALUES (µM)
Chloroquine	19	-	23.65 10 ⁻³	9.29–38.01 10 ⁻³	6.21 10 ⁻³	157.68 10 ⁻³
Chalcone B1	19	6.67	27.59	24.93–30.25	0.57	34.08
Chalcone B11	19	7.44	27.36	24.29–30.43	0.44	34.23
Chalcone B14	19	5.42	19.59	17.52–21.66	0.21	16.61
Chalcone B17	19	2.95	10.36	7.87–12.85	0.18	32.21
Chalcone SCA02	19	8.58	25.63	22.35–28.91	0.15	32.30
CHALCONESCA03	19	10.86	48.29	46.27–50.31	4.71	30.75

was related to its good tolerance, its reduced cost and its effectiveness [27]. However, given the spread of *P. falciparum* resistance to chloroquine, its withdrawal has been effective in Côte d'Ivoire since 2007 [28]. Nine years later, it was important to evaluate the situation by assessing the prevalence of chloroquine-resistant isolates in the population. Thus, it seemed appropriate to compare the antiplasmodial activity of new antimalarial drug candidates based on the susceptibility pattern of *P. falciparum* isolates to chloroquine. The reported chloroquioresistance prevalence is inferior to the previous ones in Cote d'Ivoire which were from 32 to 56,2% in 2001 to 2005 [29,30]. Therefore, ten years after the withdrawal of chloroquine in Côte d'Ivoire, the prevalence of chloroquine resistance tends to decrease. However, a study in different sentinel sites with more parasites samples is needed to confirm this phenomenon. In Burkina Faso, five years after the change in their national malaria prevention and treatment policy, a study of the prevalence of several antimalarials including chloroquine revealed a 42.1% resistance rate to the molecule [31]. These results show that, despite the withdrawal of chloroquine, chloroquine resistance may persist due to the use of chemically similar (4-aminoquinoline) treatment or the presence of chloroquine on the market for sale informally [32]. The activity of chalconic derivatives tested was generally better on chloroquine-resistant strains. This profile may be related to a mechanism of action different from that of chloroquine, which has a quinoline type profile distinct from the chemical structure of chalcones. The latter could therefore be good drug candidates for the elimination of chloroquine-resistant *Plasmodium*. This inversion of efficiency of chalconic derivatives of similar chemical profile had

previously been described on field isolates. Indeed, some compounds that had moderate activity on CQ-S isolates were highly effective on CQ-R isolates [33]. However, in our study, this trend was not found with isolates collected from patients. This would probably be due to their behavior in culture different from that of the reference strains. A prior adaptation process of *P. falciparum* isolates in culture would have shown similar trends in chloroquine susceptibility patterns. It should be noted that many studies on the search for new antimalarial drug candidates are carried out with reference strains. However, in the field, parasites have since been subjected to many drug pressures and have evolved over several generations. It is therefore essential that the efficacy of the drugs under development be studied both on reference strains and field isolates. Specifically, the compounds B14 (chlorine group) and particularly B17 (dimethylamine group) showed good to very good activity regardless of the type of reference strain. This could be due to the presence of chlorine and dimethylamine groups compared to other derivatives. This trend suggests that these compounds and chloroquine would exert their antimalarial activity according to different modes of action and that the modification of the conformation and the biochemical parameters acquired during the mechanism of action of the chloroquine resistance would be favorable to the activity of B17 and B14 secondarily.

Liu et al. evaluated alkoxyated chalcone derivatives with high efficiency on *P. falciparum* strain K1 [34] with IC_{50s} around 5 µM. In addition, some authors found similar results for benzimidazolyl-chalcone derivatives where IC_{50s} ranged from 0.78 to 8.23 µM. For them, synthetic molecules that had IC_{50s} <10 µM were considered interesting [33]. Moreover, effective inhibition

for IC₅₀s between 4.93 and 8.47 μ M with retinoid derived chalcone compounds has been previously reported on K1 and Thai strains [35].

Conclusion

Discovering new antimalarial drugs to address falciparum drug resistance is critical for malaria elimination. In this study, compounds B14 and B17 respectively carrying the chlorine and dimethylamine group showed a satisfactory efficacy profile. Therefore, they could constitute a new class of promising antimalarial drugs. A determination of the cytotoxicity as well as the anti-gametocyte activity of these molecules could pave the way for the discovery of a new series of antimalarial molecules with improved characteristics.

Acknowledgments

The authors express their gratitude to the Malaria Research and Control Center (MRCC) of the National Institute of Public Health, for the supervision and conduct of this study; the Department of Therapeutic Chemistry and Organic Chemistry, UFR Pharmaceutical and Biological Sciences of Félix Houphouët-Boigny University of Abidjan for the synthesis of compounds; the Center for Diagnosis and Research on AIDS and other infectious diseases (CeDReS) for technical support and the Swiss Center for Scientific Research in Côte d'Ivoire for providing the reference strains.

Conflict of interest

The author's state that there are no conflicts of interest for this article.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

- [1] WHO. World Malaria Report. 2019;1–185.
- [2] Ogouyèmi-Hounto A, Azandossessi C, Lawani S, et al. Therapeutic efficacy of artemether–lumefantrine for the treatment of uncomplicated falciparum malaria in northwest Benin. *Malar J.* 2016;15:1–8.
- [3] De Beaudrap P, Turyakira E, Nabasumba C, et al. Timing of malaria in pregnancy and impact on infant growth and morbidity: a cohort study in Uganda. *Malar J.* 2016;15:1–9.
- [4] Yavo W, Konaté A, Mawili-Mboumba DP, et al. Genetic Polymorphism of msp1 and msp2 in *Plasmodium falciparum* Isolates from Côte d'Ivoire versus Gabon. *J Parasitol Res.* 2016;1–7. DOI:10.1155/2016/3074803
- [5] Chang HH, Childs LM, Buckee CO. Variation in infection length and superinfection enhance selection efficiency in the human malaria parasite. *Sci Rep.* 2016;6:1–9.
- [6] ANONYME. Arrêté N°109/CAB/MSLS/du 14 juillet 2014 modifiant l'arrêté 144/MSHP/CAB/du 23 septembre 2010 portant institution d'un schéma de prise en

charge du paludisme en Côte d'Ivoire. Ministère de la Santé et de Lutte contre le Sida. 2014;1–4.

- [7] Dell'Acqua R, Fabrizio C, DiGennaro F, et al. An intricate case of multidrug resistant *Plasmodium falciparum* isolate imported from Cambodia. *Malar J.* 2017;16:1–4.
- [8] Dwivedi A, Khim N, Reynes C, et al. *Plasmodium falciparum* parasite population structure and gene flow associated to anti-malarial drugs resistance in Cambodia. *Malar J.* 2016;15:1–12.
- [9] Menard D, Arieu F. Towards real-time monitoring of artemisinin resistance. *Lancet.* 2015;15:367–368.
- [10] Tun KM, Imwong M, Lwin KM, et al. Spread of artemisinin-resistant *Plasmodium falciparum* in Myanmar: a cross-sectional survey of the K13 molecular marker. *Lancet.* 2015;15:415–421.
- [11] Thanh NV, Thuy-Nhien N, Tuyen NTK, et al. Rapid decline in the susceptibility of *Plasmodium falciparum* to dihydroartemisinin-piperaquine in the south of Vietnam. *Malar J.* 2017;16:1–10.
- [12] Canavati SE, Lawford HLS, Fatunmbi BS, et al. Establishing research priorities for malaria elimination in the context of the emergency response to artemisinin resistance framework-the Cambodian approach. *Malar J.* 2016;15:1–10.
- [13] Lu F, Culleton R, Zhang M, et al. Emergence of Indigenous Artemisinin-Resistant *Plasmodium falciparum* in Africa. *N Engl J Med.* 2017;1–3.
- [14] Khan S. Recent advances in the biology and drug targeting of malaria parasite aminoacyl-tRNA synthetases. *Malar J.* 2016;15:1–10.
- [15] Carlo G DI, Mascolo N, AA I, et al. Flavonoids: old and new aspects of a class of natural therapeutic drugs. *Life Sci.* 1999;65:337–353.
- [16] Dimmock JR, Elias DW, Beazely M, et al. Bioactives chalcones. *Curr Med Chem.* 1999;6:1125–1149.
- [17] Nath DD. The chemistry of chalcones and related compounds. Vol. 1. New York: John Wiley; 1981. p. 285.
- [18] Asif M. A review on recent advances and potential pharmacological activities of versatile chalcone molecule. *Chem Int.* 2016;2:1–18.
- [19] Chen M, TG T, Christensen SB, et al. Licochalcone A, a new antimalarial agent, inhibit in vitro growth of the human malaria parasite *Plasmodium falciparum* and protect mice from plasmodium yoelii infection 1994. *Antimicrob Agents Chemother.* 1994;38:1–6.
- [20] INS. Recensement général de la Population et de l'habitat de Côte d'Ivoire. Institut National De La Statistique. 2015;14:1–22.
- [21] OMS. *Techniques de base pour le Diagnostic microscopique du paludisme. Partie I guide du stagiaire* Organisation Mondiale de la Santé 2014; 2: 1–90.
- [22] BASCO LK. Field application of in vitro assays sensitivity of human malaria parasites antimalarial drugs. Geneva: World Health Organisation; 2007. p. 1–191.
- [23] Hodan AI. Thawing of glycerolyte frozen parasites with NaCl. *Methode Malaria Res.* 2013;6:1–499.
- [24] WWARN. In vitro Module, WWARN, 2010. Synchronisation of *P. falciparum*. WWARN Procedure INV04 2010; 1: 1–7.
- [25] WWARN. In vitro Module, WWARN 2011. *P. falciparum* drug sensitivity assay using SYBR® Green I Assay Technique. WWARN Procedure INV08 2011; 1–8.
- [26] Liu M, Wilairat P, Croft SL, et al. Structure–activity relationships of antileishmanial and antimalarial chalcones. *Bioorg Med Chem.* 2003;11:2729–2738.

- [27] Parhizgar AR, Tahghighi A. Introducing new anti-malarial analogues of chloroquine and amodiaquine: *a narrative review*. Iran J Med Sci. [2017](#);42:115–128.
- [28] Menan EI, Yavo W, Oga SS, et al. Open randomized study comparing chloroquine and amodiaquine for treatment of uncomplicated *Plasmodium falciparum* malaria in children in Aboisso, Côte d'Ivoire. Med Trop. [2007](#);67:309–310.
- [29] DJaman JA, Yapi A, Dje MK, et al. Sensibilité in vitro à la Chloroquine de *Plasmodium falciparum* à Abidjan. Med Afr Noire. [2001](#);48:371–374.
- [30] Brice BK, William Y, Lacina O, et al. in vitro susceptibility of *Plasmodium falciparum* isolates from Abidjan (Côte d'Ivoire) to artemisinin, chloroquine, dihydroartemisinin and pyromaridine. Tanzania J Health Res. [2010](#);12:73–79.
- [31] Tinto H, Bonkian LN, Nana LA, et al. Ex-vivo antimalarial drugs sensitivity profile of *Plasmodium falciparum* field isolates from Burkina Faso five years after the national policy change. Malar J. [2014](#);13:1–7.
- [32] Asare KK, Boampong JN, Afoakwah R, et al. Use of procried chloroquine is associated with an increased risk of Pfcr1 T76 mutation in some parts of Ghana. Malar J. [2014](#);13:1–8.
- [33] Ouattara M, Sissouma D, Yavo W, et al. Synthèse et criblage antiplasmodial de quelques benzimidazolyl-chalcones. Int J Biol Chem Sci. [2015](#);9:1697.
- [34] Liu M, Wilairat P, Go ML. Antimalarial alkoxylated and hydroxylated chalcones: structure–activity relationship analysis. J Med Chem. [2001](#);44(44):4443–4452.
- [35] Valla A, Valla B, Cartie D, et al. New syntheses and potential antimalarial activities of new 'retinoid-like' chalcones. Eur J Med Chem. [2006](#);41:142–146.