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Original article

# Design, economical synthesis and antiplasmodial evaluation of vanillin derived allylated chalcones and their marked synergism with artemisinin against chloroquine resistant strains of *Plasmodium falciparum*☆

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ABSTRACT

The *in vitro* blood stage antiplasmodial activity of a series of allylated chalcones based on the licochalcone A as lead molecule was investigated against chloroquine (**CQ**) sensitive *Pf*3D7 and **CQ** resistant *Pf*INDO strains of *Plasmodium falciparum* using SYBR Green I assay. Of the forty two chalcones tested, eight showed $IC_{50} \leq 5$ μM. Structure–activity relationship (SAR) studies revealed **9** {1-(4-Chlorophenyl)-3-[3-methoxy-4-(prop-2-en-1-yloxy)phenyl]-prop-2-en-1-one} as the most potent ($IC_{50}$: 2.5 μM) against *Pf*3D7 with resistance indices of 1.2 and 6.6 against *Pf*Dd2 and *Pf*INDO strains, respectively. Later on, the synergistic effects **9** with standard antimalarials {artemisinin (**ART**) and chloroquine (**CQ**)} were studied in order to provide the basis for the selection of the best partner drug. *In vitro* combinations of **9** with **ART** showed strong synergy against *Pf*INDO ($\Sigma FIC_{50}$: 0.31–0.72) but additive to slight antagonistic effects ($\Sigma FIC_{50}$: 1.97–2.64) against *Pf*3D7. $\Sigma FIC_{50}$ 0.31 of **ART**+**9** combination corresponded to a 320 fold and 3 fold reduction in $IC_{50}$ of **9** and **ART**, respectively. Similar combinations of **9** with **CQ** showed synergy to additivity to mild antagonism against the two strains {$\Sigma FIC_{50}$: 0.668–2.269 (*Pf*INDO); 1.45–2.83 (*Pf*3D7)}. Drug exposure followed by drug withdrawal indicated that **9** taken alone at $IC_{100}$ killed rings, trophozoites and schizonts of *P. falciparum*. The combination of **ART** and **9** (1X $\Sigma FIC_{100}$) selectively inhibited the growth of rings while the 2X $\Sigma FIC_{100}$ combination of the same caused killing of rings without affecting trophozoites and schizonts. In contrast, the 1X combination of **CQ** and **9** ($\Sigma FIC_{100}$: 0.5) killed rings and trophozoites. DNA fragmentation and loss of mitochondrial membrane potential ($\Delta\Psi m$) in the **9** treated *P. falciparum* culture indicated apoptotic death in malaria parasites. Prediction of ADME properties revealed that most of the molecules did not violate Lipinski's parameters and have low TPSA value suggesting good absorption. The results suggest the promising drug-like properties of **9** against **CQ** resistant *Pf* and propensity for synergy with classical antimalarial drugs together with easy and economical synthesis.



## 1. Introduction

Despite years of continual efforts for its eradication, malaria that kills approximately three million people per annum [1,2] still remains a globally prevalent parasitic disease. Almost all these deaths are caused by *Plasmodium falciparum*, one of the four species of malaria parasites in humans [2]. The principal reason hampering malaria control is the emergence of resistance by *P. falciparum* strains to the first-line antimalarial drugs like chloroquine [3,4].

*Abbreviations:* SAR, Structure–activity relationship; CQ, Chloroquine; ART, Artemisinin; Pf, *Plasmodium falciparum*.

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Natural products have always remained in focus for the discovery of new drug leads intended for the treatment of human diseases [5]. The major breakthroughs in the use of natural products as antimalarials are the discoveries of quinine [6] and artemisinin [7]. At present, artemisinin is the most effective treatment for curing chloroquine-resistant *P. falciparum* infections [6–9]; however its indiscriminate use as monotherapy has raised the concern of emergence of drug resistance [10–14]. To slow down the resistance to this vital class of drugs; artemisinin based combination therapies (ACTs) are being advocated by WHO [15]. This should also lessen pressure on the rising demand for artemisinin-a natural product in short supply and with commercially unviable synthesis [16]. Further, this urgent need to develop new antimalarial agents or drug combinations that are effective and support treatment at affordable cost is supplemented by the recent emergence of resistance against ACTs manifested in the form of delayed parasite clearance [17].

In the light of this perspective, natural products or their synthetic derivatives as a partner compound are an attractive search option for novel ACTs. One such important class of natural compounds is 1,3-diarylprop-2-en-1-ones, commonly termed as chalcones. The appeal of working with chalcones stems from their synthetic accessibility, the various ways the core structure can be diversified depending on the substitution patterns on the two aromatic rings and their ability to confer drug-like properties to compound libraries modeled on them [18]. Chalcones drew the attention of chemists when licochalcone A (a 1,1-dimethyl allylated natural product) isolated from Chinese liquorice roots, was reported to exhibit potent *in vivo* and *in vitro* antimalarial activity against both chloroquine-susceptible and chloroquine-resistant *P. falciparum* strains [19]. Since then, several natural chalcones such as xanthohumol, 5-prenylbutein, licoagrochalcone A, homobutein and crotaorixin have been reported to exhibit *in vitro* antiplasmodial activity against *P. falciparum* strains with $IC_{50}$ values in the range 10.3–16.1 μM [20–22]. However, limitations such as low percentage in natural resources, toxicity [23], low bioavailability, poor solubility and tedious total synthesis have generally restrained their use in humans [22,23]. Nevertheless, the compounds described above provide useful synthons for semi-synthetic transformations of easily available precursors into newer and modified antimalarials [24–29] against not only drug-sensitive, but also drug-resistant strains of *Plasmodium*. In this context, an analogue of licochalcone A, 2,4-dimethoxy-4′-butoxychalcone was found to exhibit potent activity against *P. falciparum in vitro* and the rodent parasites *Plasmodium berghei* and *Plasmodium yoelii in vivo* [23].

A close scrutiny of natural antimalarial chalcones has revealed that a vast majority of these possess substituted allylated aromatic rings (prenyl or geranyl group) as important elements of their pharmacophores. The importance of prenyl or allyl group for enhancing the bioactivities of flavonoids and chalcones is well documented in literature [22,30–33]. Furthermore, the prenyl moiety contributes to the lipophilicity of the molecule, an important requirement for antimalarial activity [34]. However, its incorporation needs tedious multi-step synthesis and hence could be replaced by groups with comparable lipophilic characters involving economical synthons without any decrease in antimalarial activity.

Here we report the design, synthesis and *in vitro* antiplasmodial evaluation of forty-two allylated chalcones which are structurally similar to natural licochalcone A. To address the demands of green chemistry [35], vanillin [36] – an easily available natural precursor [37], has been utilized for the synthesis of these chalcones. The chalcones under study are divided into four main types according to the substitution of ring A: (i) *C*-allylated, (ii) *O*-allylated, (iii) both *C*- and *O*-allylated, and (iv) *O*-diallylated. First, we have examined the antiplasmodial structure activity relationship (SAR) of all allylated chalcones. Next, we have studied the antiplasmodial effect of the combinations of one of the most potent allylated chalcones i.e. **9** with chloroquine and artemisinin against **CQ** sensitive (*Pf*3D7) and resistant (*Pf*INDO) strains of *P. falciparum*. Finally, we studied the mechanistic features of the antiplasmodial action of **9** by microscopic evaluation of stage specific activity, kill kinetics and mode of cell death by using fluorescent and non-fluorescent staining methods against erythrocytic stages of *P. falciparum* which indicated that **9** induce apoptotic cell death. Also, combination of **9** with **ART** exhibits strong synergy against a highly **CQ** resistant strain of *P. falciparum*.

## 2. Results and discussion

### 2.1. Chemistry

The synthesis of allylated chalcones is summarized in Schemes 1–5. 4-allyloxy-3-methoxybenzaldehyde (**1b**) was obtained by refluxing vanillin (**1a**) with allyl bromide in the presence of $K_2CO_3$ [38,39]. Compound **1b** upon microwave irradiation at 200 °C underwent Claisen reaction to yield **2b** which was transformed into **3b** using dimethyl sulfate as methylating agent [40,41] and into **4b** by reacting with allyl bromide [40]. Claisen–Schmidt condensation of **1a** and **1b**–**4b** with the corresponding acetophenones [42,43] gave chalcones (**1**–**19**) which were purified by chromatography and crystallization (Scheme 1).

Similarly, 4-allyloxyacetophenone (**5b**), obtained by reacting 4-hydroxyacetophenone (**2a**) with allyl bromide [38] was condensed with **1b** to give corresponding chalcone **20**. Likewise, corresponding allyloxy benzaldehydes (**6b**, **7b**) were obtained by refluxing 2-hydroxy-3-methoxybenzaldehyde (**3a**) and 3-hydroxy-4-methoxybenzaldehyde (**4a**), respectively with allyl bromide in the presence of $K_2CO_3$ in dry acetone [38,39]. Compounds **6b** and **7b** upon Claisen–Schmidt condensation with 4-chloroacetophenone in the presence of aqueous NaOH [42,43] gave chalcones (**21** and **22**) which were purified by chromatography and crystallization (Scheme 2). Similarly, **23** obtained by condensation of **1a** with chloroacetophenone [42,43]. **23** upon reaction with prenyl bromide, 1-butyl bromide and 4-bromobenzyl bromide yielded **24**–**26**, respectively [39] (Scheme 3).

In a similar vein, analogues of vanillin, such as syringaldehyde (**5a**), 4-hydroxybenzaldehyde (**6a**), 2-hydroxybenzaldehyde (**7a**), 3-ethoxy-4-hydroxybenzaldehyde (**8a**) and 3,4-dihydroxybenzaldehyde (**9a**) were reacted with allyl bromide [38,39] to provide *O*-allylated benzaldehydes (**8b**–**12b**). Compounds **8b**–**12b** upon Claisen–Schmidt condensation with 4-chloroacetophenone gave chalcone products (**27**–**31**). Similarly, 4-allyloxy-3-methoxyacetophenone (**13b**) was obtained by reaction of 4-hydroxy-3-methoxyacetophenone (**10a**) with allyl bromide which on condensation with 4-chlorobenzaldehyde provided chalcone **32**. **14b** was obtained by reaction of 2,4-dihydroxyacetophenone (**11a**) with allyl bromide in the presence of $K_2CO_3$ [42,43] (Scheme 4).

Chalcones **33**–**38** were prepared by reacting **1b** with various heteroaromatic acetophenones (Scheme 5) as incorporation of heteroatom generally increases the biological activities. Hence, compound **39** was synthesized by reaction of **18** with 4,7-dichloroquinoline in THF in the presence of $K_2CO_3$ [44] which upon further reaction with allyl bromide in the presence of KOH and THF yielded **40**. Compounds **41** and **42** were prepared by refluxing **9** with phenylhydrazine [45] and guanidine hydrochloride [46], respectively (Scheme 5).