Design, Synthesis and Biological Evaluation of Some Novel 3-Cinnamoyl-4-Hydroxy-2H-Chromen- 2- ones as Antimalarial Agents

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Design, synthesis and biological evaluation of some novel 3-cinnamoyl-4-hydroxy-2H-chromen-2-ones as antimalarial agents

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Abstract A novel series of 3-cinnamoyl-4-hydroxy-2H-chromen-2-ones were designed, synthesized and screened for antiplasmodial activity. Eleven compounds of the series exhibited micromolar potency against chloroquine sensitive and chloroquine resistant strains. The most potent compound 4-hydroxy-3-(3-(4-nitrophenyl)acryloyl)-2H-chromen-2-one showed inhibitory potency (IC_{50}) of 3.1 and 4 μg/ml against chloroquine sensitive and chloroquine resistant strains, respectively. A structure activity relationship study was performed by correlating the effect of substituents with the antimalarial activity of the title compounds. The novel 3-cinnamoyl-4-hydroxy-2H-chromen-2-ones reported here should be good lead for further development of antimalarial agents that can overcome resistance.

Kuldeep Patel and Chandrabose Karthikeyan contributed equally to this study.

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Introduction

Malaria constitutes the most important infectious disease problems of humans, affecting third-world countries, with over 275 million new cases annually and mortality reaching 2 million (Dominguez, 2002; Guerin *et al*., 2002). The majority of these cases are pediatric deaths in developing countries which exemplify the tragedy of the disease caused by this parasite. There are four species of malaria or the genus *Plasmodium*, which include *Plasmodium falciparum*, *P. vivax*, *P. ovale* and *P. malariae*. *P. falciparum* is the foremost killer and is found primarily on the African continent. There is increasingly serious problem of malaria parasite resistance to currently used antimalarials (Macreadie *et al*., 2000; Neekhara *et al*., 2006a, b) and the emergence of drug-resistant parasites especially *P. falciparum* poses significant challenges to the future of drug discovery efforts (Sahu *et al*., 2008). Hence, there is an urgent need to discover new and effective chemical entities that can combat *P. falciparum*.

Chalcones are structurally simple compounds of the flavonoid family and are present in variety of plant species (Boeck *et al*., 2006; Begum *et al*., 2011). Chemically, these are 1,3-diphenyl-2-propen-1-one and have broad spectrum activities (Go *et al*., 2005; Chimenti *et al*., 2009) which include antileishmanial, antiinflammatory, antimitotic, modulation of P-glycoprotein-mediated multidrug resistance, antimalarial, etc. The antimalarial activity of chalcones was apparent (2,4-dimethoxy-4′-allylchalcone and 2,4-dimethoxy-4′-butoxychalcone) after the first report on licochalcone A with potent in vivo and in vitro antimalarial activity at micromolar concentrations (Kharazmi *et al*.,

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1997; Narender and Reddy, 2007). Since then, numerous reports have emerged on the antimalarial activity of chalcones, e.g. quinolinyl chalcone analogues (Dominguez *et al*., 2001), prenylated chalcones (Frolich *et al*., 2005), sulphonamide chalcones (Domiinguez *et al*., 2005a), phenylsulphonyl urenyl chalcones (Araico *et al*., 2006), ferrocenyl chalcones (Wu *et al*., 2002; 2006) in the literature (Tomar *et al*., 2010).

There are multiple mechanisms of action reported for the antimalarial activity of chalcones. Reported mechanisms include inhibition of malaria proteases responsible for hemoglobin digestion, interference with the detoxification process of hemozoin formation, inhibition of the new permeation pathways that uptake essential nutrients, and inhibition of the Pfmrk cell cycle kinase (Dominguez *et al*., 2005b; Go *et al*., 2004; Ziegler *et al*., 2004; Geyer *et al*., 2009).

Coumarin, an organic heterocyclic scaffold constitutes an important class of compound with versatile biological activities and can be found in many natural or synthetic drug molecules (Sun and Cui, 2008). Precedence exists in literature on antimalarial activity of coumarins for example Daphnetin (7,8-dihydroxycoumarin), a Chinese herbal product used for the treatment of coagulation disorders showed potency against malarial parasite both in vitro and in vivo (Yang *et al*., 1992; Huang *et al*., 2006). In vitro, Daphnetin causes a 50% inhibition (IC_{50}) of ^{3}H-hypoxanthine incorporation by *P. falciparum* at concentrations between 25 and 40 μM (Yang *et al*., 1992). In the light of aforementioned findings and the growing interest in chalcones as potential antimalarials, we hypothesized that a novel chalcone incorporating coumarinyl moiety will be worth investigating for its antimalarial potency (Fig. 1). Hence in this communication, a series of 3-cinnamoyl-4-hydroxy-2H-chromen-2-ones was synthesized and assessed for their antimalarial properties in vitro against chloroquine susceptible and chloroquine resistant strains.

Chemistry

The synthesis of the 3-cinnamoyl-4-hydroxy-2H-chromen-2-ones was accomplished by a two step procedure outlined

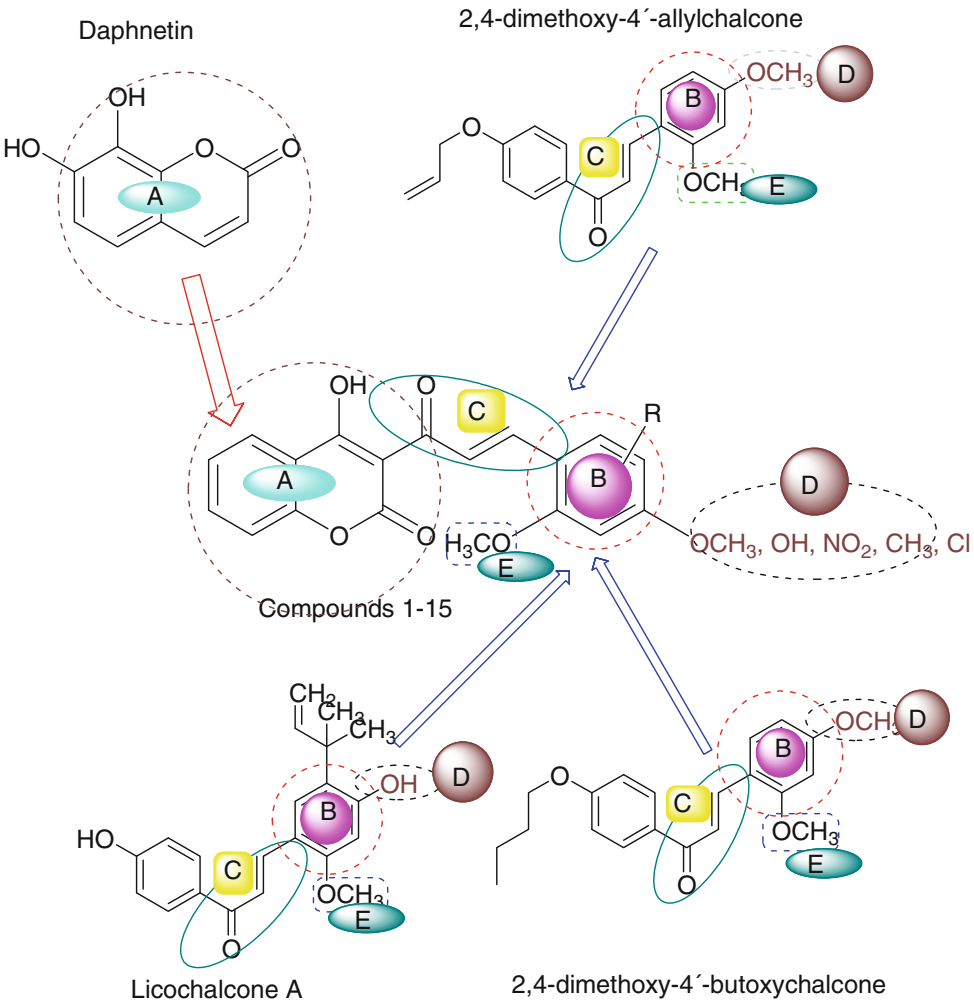


Fig. 1 Pharmacophore similarity of synthesized compounds with existing active compounds (coumarin and chalcone derivatives)

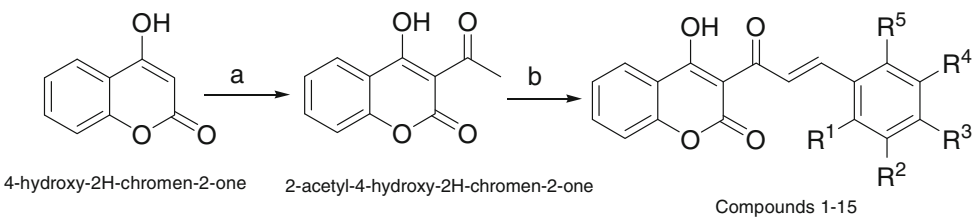
in the Scheme 1. The experimental procedure and spectral data of the compounds are given in our earlier communication (Patel *et al*., 2011). The first step involves synthesis of the precursor 4-hydroxy-3-acetyl coumarin by reacting 4-hydroxy coumarin with phosphorous oxychloride and glacial acetic acid and the second step includes Knoevenagel condensation between the 4-hydroxy-3-acetyl coumarin and substituted benzaldehydes in chloroform in the presence of piperidine catalyst.

Results and discussions

The antiplasmodial efficacy of the synthesized 3-cinnamoyl-4-hydroxy-2H-chromen-2-ones was ascertained by conventional in vitro parasite culture method (Trager and Jensen, 1976; Nivsarkar *et al*., 2005), using chloroquine sensitive (3D7) and chloroquine resistant strain (W2) of *P. falciparum*. In addition to antiplasmodial properties, the in vitro toxicity of 10 representative compounds of the series towards K562 cell line was also evaluated by following Sulphorhodamine B (SRB)-based protocol (Skehan *et al*., 1990; Vichai and Kirtikara, 2006; Solomon *et al*., 2009). Chloroquine di phosphate was used as the reference drug. The antimalarial activity and cytotoxicity shown by the synthesized compounds is summarized in Table 1.

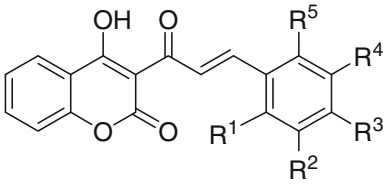
The effect of structural variation in the antimalarial activity exhibited by 3-cinnamoyl-4-hydroxy-2H-chromen-2-one (**1**) was explored by varying the substituents on the aromatic ring as illustrated in Table 1. On perusal of the table, it is evident that meta and para substitution in the phenyl ring are mostly favored as shown by the low IC_{50} values ($IC_{50} < 10$ μg/ml). The unsubstituted 3-cinnamoyl-4-hydroxy-2H-chromen-2-one (**1**) has antimalarial activity at 22.1 μg/ml but substitution with chlorine either in ortho position of the phenyl ring (**2**) or in the ortho and para position of the phenyl ring (**5**) abolished the antimalarial activity. However, it may also be observed that compounds with substitution of chlorine atom in the meta position (**3**) and para position (**4**) have significant activity at low concentration. Hence, it can be concluded that ortho substitution in phenyl ring is not well tolerated for antimalarial activity shown by the title compounds. Amongst the para substituted compounds, nitro substitution on the phenyl ring (**7**) showed highest antimalarial potency ($IC_{50} < 5$ μg/ml) against both chloroquine sensitive and resistant strains and the potency decreases in the order $NO_2 > Cl > CH_3 > OCH_3 > N(CH_3)_2$. It is also interesting to note that there is only a moderate difference in activity values on moving from a more a polar nitro group (**7**) to a less polar methyl group (**9**). In case of meta position in the phenyl ring, chloro group seems to be a substituent of choice as indicated by low IC_{50} values (<5 μg/ml). In contrast to para substituted compounds, nitro substitution in meta position of phenyl ring did not favour antimalarial activity. Furthermore, the methoxy group alone in the para position (**10**) and along with ortho and/or meta position (**12**, **13**) of the phenyl ring also show significant activity at lower concentrations. The replacement of methoxy group with hydroxyl group (**11**) in the para position decreased the activity two fold than the methoxy substitution (**12**). The dimethylamino substitution in the para position of the ring (**8**) has antimalarial activity five times lower than the nitro group containing compound. The methoxy group in the para, meta and meta′ position (**14**) is devoid of antimalarial activity. Replacement of furan ring in place of phenyl ring (**15**) resulted in loss of activity (>50 μg/ml). It is also worth mentioning that most of compounds (**3**, **4**, **7** and **9**) which showed significant inhibitory activity against chloroquine sensitive strain 3d7 also exhibited comparable potencies against chloroquine resistant strain. Cytotoxicity studies conducted on K562 cell line suggest that the studied compounds are devoid of any significant toxicity. Only two of the 10 compounds (Compound **4** and **13**) exhibit cytotoxicity at less than 50 micromolar concentration. The most potent anti malarial compound of the studied series (7) did not show any toxicity against K562 cell line even at concentrations greater than 100 μg/ml. Altogether, the results of SAR studies establish the 3-cinnamoyl-4-hydroxy-2H-chromen-2-one nucleus as a chemical structure endowed with antiplasmodial properties.

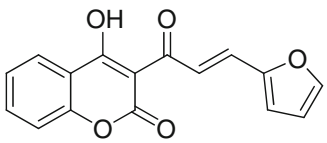
In conclusion, a series of 3-cinnamoyl-4-hydroxy-2H-chromen-2-ones were synthesized and evaluated for their antimalarial activity against chloroquine sensitive and chloroquine resistant plasmodial strain. Compounds 3, 4, 7 and 9 exhibited good antimalarial potency against both



Scheme 1 Synthesis of the 3-cinnamoyl-4-hydroxy-2H-chromen-2-ones. Reagents and conditions: *a* Glacial acetic acid, $POCl_3$, reflux; *b* Substituted benzaldehydes, $CHCl_3$, Piperidine (Catalytic), reflux

Table 1 Chemical structure, antimalarial activity and human cell toxicity of the synthesized compounds



S. no	R^1	R^2	R^3	R^4	R^5	IC_{50}^{3D7} AACSS (μg/ml)	IC_{80}^{3D7} AACSS (μg/ml)	IC_{50}^{W2} AACRS (μg/ml)	IC_{50} (μg/ml) cytotoxicity against K562 cell lines	SI = IC_{50} K562/ IC_{50}^{3D7}	SI = IC_{50} K562/ IC_{80}^{3D7}	SI = IC_{50} K562/IC_{50}^{W2}
1	H	H	H	H	H	22.1	56.3	11	99.2	4.49	1.76	9.02
2	Cl	H	H	H	H	>50	ND	ND	123.5	ND	ND	ND
3	H	Cl	H	H	H	4.2	9.0	6.2	ND	ND	ND	ND
4	H	H	Cl	H	H	5.1	14.2	6.5	32.1	6.29	2.26	4.94
5	Cl	H	Cl	H	H	>50	ND	ND	149	ND	ND	ND
6	H	NO_2	H	H	H	15.6	25.2	54.1	151.7	9.72	6.02	2.80
7	H	H	NO_2	H	H	3.1	7	4	154	49.68	22.00	38.50
8	H	H	$N(CH_3)_2$	H	H	17.2	22.1	68.2	156.5	9.10	7.08	2.29
9	H	H	CH3	H	H	6.1	15	5.1.	ND	ND	ND	ND
10	H	H	OCH_3	H	H	7.2	6.2	35.8	ND	ND	ND	ND
11	H	OCH_3	OH	H	H	11.2	27.2	61.3	ND	ND	ND	ND
12	H	OCH_3	OCH_3	H	H	6.2	15.1	22	71.4	11.52	4.73	3.25
13	OCH_3	OCH_3	OCH_3	H	H	8.5	20.3	56.1	46.6	5.48	2.30	0.83
14	H	OCH_3	OCH_3	OCH_3	H	>50	ND	ND	103.2	ND	ND	ND
15						>50	ND	ND	ND	ND	ND	ND
16	Chloroquine					22.3[a]	40.1[a]	230.1[a]	43.8	1.96	1.09	0.19

ND not determined

AACSS activity against chloroquine sensitive strain

AACRS activity against chloroquine resistance strain

SI selectivity index

[a] ng/ml

chloroquine sensitive and chloroquine resistant plasmodial strains. The preliminary structure–activity relationship study revealed para and meta position in the phenyl rings are ideal points for structural manipulation for developing potent antimalarial 3-cinnamoyl-4-hydroxy-2H-chromen-2-ones. The findings of study successfully establish 3-cinnamoyl-4-hydroxy-2H-chromen-2-ones as a novel lead for the development of novel antimalarial agents that can overcome resistance.

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