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Research Letter

Antimalarial Activity of Newly Synthesized Chalcone Derivatives *In Vitro*

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Twenty-seven novel chalcone derivatives were synthesized using Claisen-Schmidt condensation and their antimalarial activity against asexual blood stages of *Plasmodium falciparum* was determined. Antiplasmodial IC_{50} (half-maximal inhibitory concentration) activity of a compound against malaria parasites *in vitro* provides a good first screen for identifying the antimalarial potential of the compound. The most active compound was 1-(4-benzimidazol-1-yl-phenyl)-3-(2, 4-dimethoxy-phenyl)-propen-1-one with IC_{50} of 1.1 μg/mL, while that of the natural phytochemical, licochalcone A is 1.43 μg/mL. The presence of methoxy groups at position 2 and 4 in chalcone derivatives appeared to be favorable for antimalarial activity as compared to other methoxy-substituted chalcones. Furthermore, 3, 4, 5-trimethoxy groups on chalcone derivative probably cause steric hindrance in binding to the active site of cysteine protease enzyme, explaining the relative lower inhibitory activity.

Key words: antimalarial activity, Chalcone synthesis, *in vitro*, *Plasmodium falciparum*

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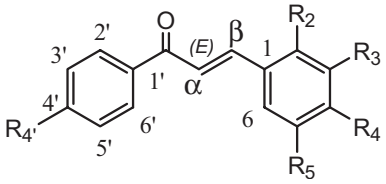
Malaria is a public health concern in countries that are endemic to this disease. These countries constitute over one-fifth of the world population. Approximately 1 million people die annually owing to *Plasmodium falciparum* malaria infections, majority of them in resource poor settings[a] (1). Chloroquine, a synthetic 4-aminoquinoline, is inexpensive, and till recently widely recommended for its effective malaria treatment. Emergence of chloroquine-resistant strains of *P. falciparum* has caused a major setback to *en masse* treatment for malaria and control programs. Artemisinin and its derivatives are now the only drugs available for effective cure of malaria[b]. Synthesis of artemisinin is difficult and expensive. Resistance to artemisinin and its derivatives will also emerge sooner or later. Thus, there is an urgent need for alternate synthetic, stable, affordable, and potent antimalarial compounds, which could be developed as antimalarial drugs for mass administration.

Naturally occurring chalcones (1,3-diaryl-2-propen-1-one) are the key intermediates for various plant metabolites. They are biologically active compounds with known antibacterial (2,3), antifilarial (4), antiviral (5,6), antileishmanial (7), and cytotoxic (8,9) activities. Licochalcone A, a natural compound isolated from Chinese licorice roots, has shown potent antiplasmodial activity (10). The simple structure and low-cost synthesis of chalcones have attracted the attention of chemists to develop different analogs of this novel scaffold for various infectious diseases including malaria. In continuation to our earlier work with potent antimalarial chalcone derivatives (11–13), we have further synthesized 27 chalcones with new substituents on the basic scaffold and evaluated their antiplasmodial activity against human malaria parasite *P. falciparum in vitro.*

Methods and Materials

Compounds **1–27** (Table 1) were synthesized by base-catalyzed Claisen-Schmidt condensation (14,15) using appropriate substituted acetophenone with commercially available methoxy benzaldehyde (Figure 1). Theoretically, E and Z geometric isomers are equally formed during the reaction. However, NMR analysis showed the presence of E form only in all compounds. Yields were variables with approximately in between 50% and 70% and were not optimized. Melting points were recorded by classical approach using capillary methods and were reported uncorrected. ^{1}H NMR spectra given were recorded on Brucker AMX300, Brucker AMX400, Jeol AMX 500 spectrometer; chemical shifts are expressed in δ (ppm) relative to tetra methyl silane. All spectra were recorded in $CDCl_3$. Infrared (IR) spectra were recorded on Perkin Elmer Fourier transform (FT-IR) in KBr pellets and are expressed in per centimeter. All mass were recorded on Macromass G. Elemental analysis was performed on Elementar analysensysteme GmbH VaroiE (CHNSO Laboratory, University Science Instrumentation Centre, University of Delhi). Results were within ±0.4% of predicted values for all compounds. Analytical thin-layer chromatography (TLC)s were run on commercially precoated plates (Merck, silica gel 60 F254, Merck specialities Pvt. Ltd., Mumbai, India) and visualized under UV lamps (254 nm). The progress of the reactions was monitored by TLC with $CHCl_3$ and CH_3OH as eluent. Initially, we synthesized the following

Table 1: Antimalarial activity of chalcones.



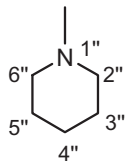
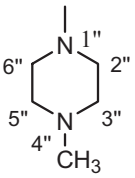
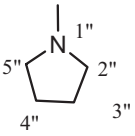
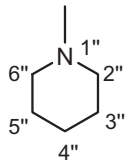
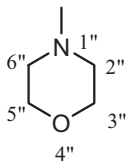
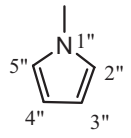
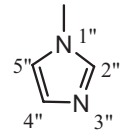
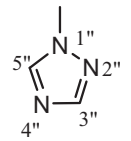
S. No.	$R_{4'}$	R_2	R_3	R_4	R_5	Mean IC_{50} ± SE[a] (μg/mL)
1		H	H	OCH_3	H	6.92 ± 0.25
2		H	H	OCH_3	H	6.9 ± 0.15
3		OCH_3	H	OCH_3	H	2.37 ± 0.22
4		OCH_3	H	OCH_3	H	7.68 ± 0.15
5		OCH_3	H	OCH_3	H	2.95 ± 0.21
6		OCH_3	H	OCH_3	H	5.98 ± 0.22
7		OCH_3	H	OCH_3	H	6.7 ± 0.21
8		OCH_3	H	OCH_3	H	3.38 ± 0.06

Table 1: Continued

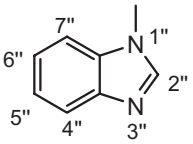
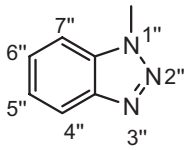
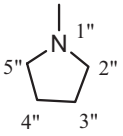
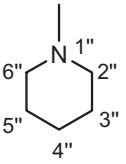
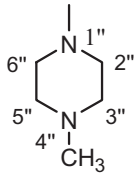
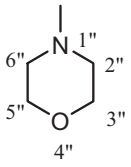
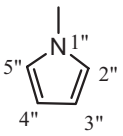
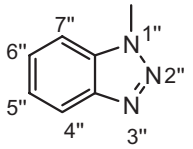
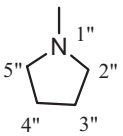
S. No.	$R_{4'}$	R_2	R_3	R_4	R_5	Mean IC_{50} ± SE[a] (μg/mL)
9		OCH_3	H	OCH_3	H	1.1 ± 0.06
10		OCH_3	H	OCH_3	H	7.22 ± 0.29
11		OCH_3	H	H	OCH_3	5.53 ± 0.24
12		OCH_3	H	H	OCH_3	6.13 ± 0.18
13		OCH_3	H	H	OCH_3	10.1 ± 0.31
14		OCH_3	H	H	OCH_3	3.26 ± 0.29
15		OCH_3	H	H	OCH_3	6.36 ± 0.12
16		OCH_3	H	H	OCH_3	12.73 ± 0.32
17		H	OCH_3	OCH_3	H	2.91 ± 0.08

Table 1: Continued

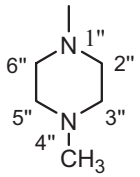
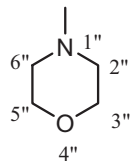
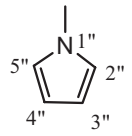
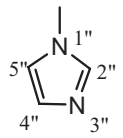
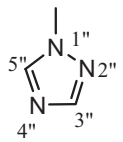
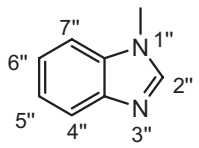
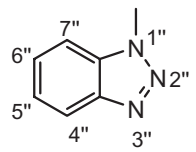
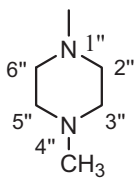
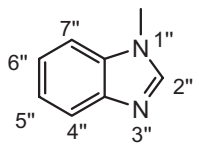
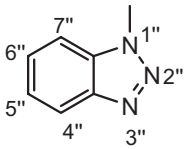
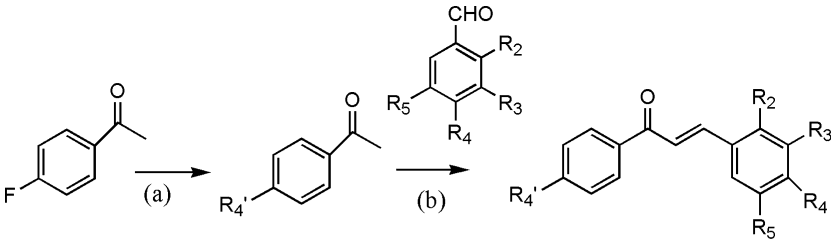
S. No.	$R_{4'}$	R_2	R_3	R_4	R_5	Mean IC_{50} ± SE[a] (μg/mL)
18		H	OCH_3	OCH_3	H	4.96 ± 0.56
19		H	OCH_3	OCH_3	H	5.16 ± 0.32
20		H	OCH_3	OCH_3	H	6.28 ± 0.09
21		H	OCH_3	OCH_3	H	7.34 ± 0.27
22		H	OCH_3	OCH_3	H	5.85 ± 0.07
23		H	OCH_3	OCH_3	H	5.04 ± 0.31
24		H	OCH_3	OCH_3	H	3.5 ± 0.23
25		H	OCH_3	OCH_3	OCH_3	4.7 ± 0.21
26		H	OCH_3	OCH_3	OCH_3	7.6 ± 0.67

Table 1: Continued

S. No.	$R_{4'}$	R_2	R_3	R_4	R_5	Mean IC_{50} ± SE[a] (μg/mL)
27		H	OCH_3	OCH_3	OCH_3	8.15 ± 0.95

[a]Standard error (N = 3).



(a)cyclic amines, K_2CO_3, DMF, 110°C,18 h (b)NaOH, methanol, 16–20 h, RT

i) R_2=H, R_3=H, R_4=OCH_3 , R_5=H
ii) R_2=OCH_3 , R_3=H, R_4=OCH_3 , R_5=H
iii) R_2=OCH_3, R_3=H, R_4=H , R_5=OCH_3
iv) R_2=H , R_3=OCH_3, R_4=OCH_3 , R_5=H
v) R_2=H, R_3=OCH_3, R_4=OCH_3 , R_5=OCH_3

Figure 1: General procedure for the synthesis of chalcone derivatives.

nine substituted acetophenones, which served as intermediate for the synthesis of 27 chalcone derivatives, and the biological activities of these 27 novel compounds are presented in Table 1.

Procedure for the synthesis of 4-benzimidazole acetophenone

To a solution of 4-fluoroacetophenone (2.58 mL, 20 mmol) in 5 mL dry DMF, benzimidazole (2.36 g, 20 mmol) and K_2CO_3 (3.45 g, 25 mmol) were added. The reaction mixture was refluxed for 18 h at 110 °C. On the completion of the reaction, as checked by TLC, the DMF was evaporated in vacuo and redissolved into water (50 mL). The aqueous solution was extracted with chloroform (3 × 50 mL), and the combined organic solution was dried over anhydrous Na_2SO_4 and evaporated in vacuo to yield crude 4-benzimidazole acetophenone. The intermediate was purified by column chromatography using silica gel (2:98 v/v MeOH-$CHCl_3$) and characterized by Macromass G and NMR prior to use in the next step. The remaining intermediates were prepared by the similar method.

Procedure for the synthesis of (4-benzotriazole -1-yl-phenyl)-3-(2, 4-dimethoxy-phenyl)-propen-1-one

NaOH pellet (100 mg) was added to a stirred solution of substituted 4-benzotriazole acetophenone (237.09 mg, 1 mmol) and 2,4-dimethoxybenzaldehyde (166.17 mg, 1 mmol) in minimum amount of methanol (3–5 mL) at ice-cooled flask. The reaction mixture was allowed to draw closer to room temperature and stirred for 18–20 h. Appearance of off-white to yellow solids in solution within few minutes to several hours indicated successful synthesis of chalcone. The product was filtered and washed with ice-cold water (3 × 10 mL). The title compound was purified by column chromatography using chloroform, methanol as eluent and characterized by NMR. The remaining substituted 1,3-diarylpropenone was prepared by the similar method using appropriate reactants.

In vitro *antimalarial assay*

Stock culture of a chloroquine-sensitive malaria parasite *P. falciparum* 3D7 strain and a chloroquine-resistant *P. falciparum* RKL 303 strain was continuously maintained *in vitro* using the candle jar method of Trager and Jensen (16). The *P. falciparum* strains 3D7 and RKL 303 were obtained from International Center for Genetic Engineering and Biotechnology (ICGEB), New Delhi, and National Institute of Malaria Research (NIMR), New Delhi, respectively. Complete culture medium contained RPMI-1640 (with glutamine but without bicarbonate), HEPES buffer, 40 mg of gentamycin, sterile 5% sodium bicarbonate and was finally supplemented with 10% pooled B^+ serum. The parasites were maintained on B^+ human red blood cells. The culture parasitemia was kept between 1% and 5% with hematocrit of 5%.

All the newly synthesized chalcones were individually dissolved in DMSO (Sigma-Aldrich, St. Louis, Mo, USA) to obtain stock solution

of 1 mg/mL. The stock solution was diluted with antibiotic-free complete medium on the day of experiment to obtain the desired concentrations for each test compound. The final concentration of DMSO present in the experiment had no effect on parasite growth.

Fifty percent inhibitory concentration (IC_{50}) of individual compound against *P. falciparum* was determined using dose–response assay in 24-well tissue culture plates (BD Falcon™) in triplicates (17). Synchronous parasites were prepared (18) and challenged to graded concentration of compounds by the modified 48-hour test (19). Medium of each well was changed after 24 h with or without compound. At the end of 48 h, Giemsa stained slides were prepared from each test-well and examined for number of parasites in random adjacent microscopic fields, equivalent to about 3000 erythrocytes at 1000× magnification. Percentage inhibition of parasitemia in relation to control was calculated. Semilog concentration–response graph of percent parasitemia inhibition was plotted for each individual compound to determine IC_{50} values using Microsoft Excel. Figure 2 shows the dose–response curve of Licochalcone A, and compound **9** against *P. falciparum* strain (3D7).

Results and Discussion

Plasmodium is an intracellular parasite for most part of its life cycle in the vertebrate host. The intraerythrocytic parasite grows and reproduces asexually by converting the toxic heme (ferriprotoporphyrin IX) molecules released from hemoglobin catabolism into nontoxic hemozoin (β–hematin) within the acidic food vacuole (20). Inhibition of hemoglobin degradation process proves fatal for the parasite. Clinical manifestations are produced as the merozoites or hemozoin crystals are released into the blood circulation with the rupture of infected erythrocytes. It is predicted that malarial aspartic proteases (plasmepsin) and cysteine proteases (falcipain) mediate the hemoglobin degradation to release amino acids that are required for intraerythrocytic parasite growth and multiplication (20–22). These proteases form an attractive antimalarial drug target (21). Structure-based studies predict antimalarial chalcone derivatives inhibition on trophozoite cysteine protease as the most likely mode of action (14,23). Availability of continuous cultivation of human malaria parasite, *P. falciparum,* (16) provides an interesting *in vitro* system for initial screening to identify antiparasitic action of compounds in the absence of immune components.

We synthesized various substituted chalcones using Claisen-Schmidt condensation method (Table 1). Fluoro function in 4-fluoroacetophenone was substituted systematically with pyrrole, imidazole, benzimidazole, benzotriazole, pyrrolidine, morpholine, piperidine, and *N*-methyl piperazine. These various substituted acetophenones were condensed with methoxy-substituted aldehyde to give rise to various chalcones. Moreover, to broaden our studies, we included all possible combination of methoxy at different positions in benzaldehyde. We chose five series, viz. 4-methoxy; 2, 4-dimethoxy; 2, 5-dimethoxy; 3, 4-dimethoxy; and 3, 4, 5-trimethoxy benzaldehyde. All compounds were analyzed by ^{1}H NMR, ^{13}C NMR, mass spectroscopy, and FT-IR for their authenticity prior to their antiplasmodial evaluation (Details provided as Supporting Information). IC_{50} values of all the compounds were determined against chloroquine-sensitive strain (3D7) of *P. falciparum* (Table 1). Ten compounds showed IC_{50} values <10 μg/mL.

In 4-methoxy series, we evaluated two compounds with piperidine and *N*-methyl piperazine having reasonably good activity. Earlier, we have reported efficient antiplasmodial activity of both pyrrole and methoxy substituents on acetophenone and benzaldehyde with IC_{50} of 1.6 μg/mL, comparable to phytochemical licochalcone A (IC_{50} of 1.43 μg/mL) against chloroquine-sensitive 3D7 strain (12). *In vitro* sensitivity of *P. falciparum* RKL 303 strain to licochalcone A was 1.82 μg/mL.

In 2, 4-dimethoxy series, we evaluated eight different substituents on acetophenone rings while keeping 2, 4-dimethoxy substituent constant on aldehyde and found noteworthy IC_{50} values between 1.1 and 7.68 μg/mL. The compound **9** containing benzimidazole substituent was found to be most active with IC_{50} of 1.1 μg/mL. Thus, the compound **9** was found to be the most potent of about 50 chalcones evaluated by us till now (11,13). In the same series, three compounds (compounds **3**, **5**, and **8**) having pyrrolidine, morpholine, and 1, 2, 4-triazole substituents have also showed profound effect on parasites, with IC_{50} 2.37, 2.95, and 3.38 μg/mL, respectively, while piperidine, pyrrole, imidazole, and benzotriazole substituents showed moderate antiplasmodial activity with IC_{50} between 5.98 and 7.22 μg/mL. Moreover, in this series, we observed very interesting results, when we compared IC_{50} values of benzimidazole and benzotriazole derivative. In 2, 5-dimethoxy series, we evaluated six compounds. Interestingly, morpholine substituent showed good inhibitory activity with IC_{50} of 3.26 μg/mL, while pyrrolidine, piperi-

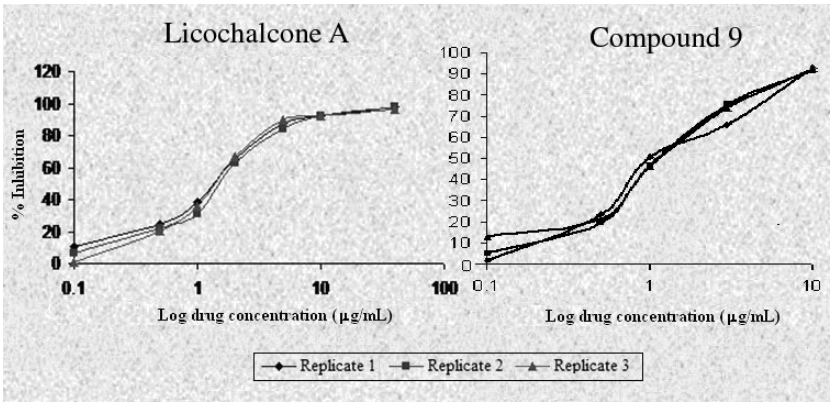


Figure 2: Log dose–response curve of Licochalcone A and newly synthesized compound **9** against blood stages of *Plasmodium falciparum* (3D7 strain).

dine, and pyrrole showed moderate antiplasmodial activity. Further compound **13** and compound **16** having *N*-methyl piperazine and benzotriazole substituents on ring B showed weak activity with IC_{50} 10.1 and 12.73 μg/mL, respectively.

In 3, 4-dimethoxy series, we evaluated eight different substituents. The compound **17** and compound **24** containing pyrrolidine and benzotriazole substituents showed good activity with IC_{50} 2.9 and 3.5 μg/mL, respectively, while rest of compounds showed moderate activity with IC_{50} range from 7.34 to 4.96 μg/mL where piperidine substituent was found to be least active. Interestingly, in 3, 4-dimethoxy series, benzotriazole was potentially effective with IC_{50} 3.5 μg/mL. We presumed that the position of two methoxy groups at position 3 and 4 might be able to accommodate benzotriazole on active site, which was not possible in the case of 2, 4 dimethoxy having weaker activity. Better understanding of the binding mechanism could possibly be revealed by obtaining crystal structures.

In 3, 4, 5-trimethoxy series, all three compounds showed moderate antiplasmodial activity with IC_{50} range from 4.7 to 8.15 μg/mL. Earlier, we have reported good activity of pyrrole and imidazole in ring B of 3, 4, 5-trimethoxy series with IC_{50} of 2.03 and 2.48 μg/mL (13), while rest of the compounds showed either moderate or weaker activity with the enzyme.

Comparison of IC_{50} values between 2,4-dimethoxy; 2,5-dimethoxy and 3,4-dimethoxy series concluded that the methoxy groups at position 2, and 4 in benzaldehyde presented the most appropriate orientation on binding to the active site of the enzyme. The change of one methoxy group from position 4–5 in chalcone derivative disturbed the spacing and enzyme binding as evident from its low IC_{50} value. However, changing the methoxy from position 2–3 also disturbed efficient binding with the enzyme, although it is less effective than the shift from position 4–5 as evident from the IC_{50} values. The result suggests that the chalcone–enzyme binding and their antiplasmodial activity is a multifactorial-dependent phenomenon. *In vitro* sensitivity of *P. falciparum* 3D7 strain to chloroquine was 0.0272 μM.

Conclusion

The chalcone derivative 1-(4-Benzimidazol-1-yl-phenyl)-3-(2,4-dimethoxy-phenyl)-propen-1-one demonstrated the most potent antiplasmodial activity *in vitro* with IC_{50} of 1.1 μg/mL, as compared to licochalcone (1.43 μg/mL). In the series of compounds studied, the presence of two methoxy groups at position 2 and 4 was found to be optimum for antimalarial activity followed by 3, 4 dimethoxy and 2, 5 dimethoxy with moderate activity. Further, 3, 4, 5-trimethoxy series showed weaker activity, presumably due to the stearic hindrance at the binding site of the enzyme. We hypothesize that potent antimalarial activity resulted from the formation of irreversible Michael addition along with interaction of His 67 of the enzyme with benzimidazole nitrogen. Of the 27 compounds, it will be interesting to investigate the potency of compounds **3**, **5**, and **9** in combination with artemisinin.

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Conflict of Interest

Authors have no conflict of interest.

References

1. Phillips R.S. (2001) Current status of malaria and potential for control. Clin Microbiol Rev;14:208–226.
2. Nielsen S.F., Bosen T., Larsen M., Schonning K., Kromann H. (2004) Antibacterial chalcones – bioisosteric replacement of the 4′-hydroxy group. Bioorg Med Chem;12:3047–3054.
3. Nielsen S.F., Larsen M., Bosen T., Schonning K., Kromann H. (2005) Cationic chalcone antibiotics: design, synthesis, and mechanism of action. J Med Chem;48:2667–2677.
4. Awasthi S.K., Mishra N., Dixit S.K., Singh A., Yadav M., Yadav S.S., Rathaur S. (2009) Antifilarial activity of 1, 3-diarylpropen-1-one: effect on glutathione-S-transferase, a phase II detoxification enzyme. Am J Trop Med Hyg;80:764–768.
5. Wood J.E., Munro M.H.G., Blunt J.W., Perry N.B., Walker J.R.C., Ward J.M. (1999) Biologically active compounds from *Ozothamnus leptophyllus*. N Z J Bot;37:167–174.
6. Cheenpracha S., Karalai C., Ponglimanont C., Subhadhirasakul S., Tewtrakal S. (2006) Anti-HIV–1 protease activity of compounds from *Boesenbergia pandurata*. Bioorg Med Chem;14:1710–1714.
7. Liu M., Wilairat P., Croft S.L., Tan A.L., Go M.L. (2003) Structure-activity relationships of antileishmanial and antimalarial chalcones. Bioorg Med Chem;11:2729–2738.
8. Robinson P.T., Hubbard R.B. IV, Ehlers T.J., Arbiser J.L., Goldsmith D.J., Bowen J.P. (2005) Synthesis and biological evaluation of aromatic enones related to curcumin. Bioorg Med Chem;13:4007–4013.
9. Rao Y.K., Fang F.S., Tzeng Y.M. (2004) Differential effects of synthesized 2′-oxygenated chalcone derivatives: modulation of human cell cycle phase distribution. Bioorg Med Chem;122:2679–2686.
10. Chen M., Theander T.G., Christensen B.S., Hviid I., Zhai L., Kharazmi A. (1994) Licochalcone A, a new antimalarial agent, inhibits in vitro growth of the human malaria parasite *Plasmodium falciparum* and protects mice from *P. yoelii* infection. Antimicrob Agents Chemother;38:1470–1475.
11. Mishra N., Arora P., Kumar B., Mishra L.C., Bhattacharya A., Awasthi S.K., Bhasin V.K. (2008) Synthesis of novel substituted

1,3-diaryl propenone derivatives and their antimalarial activity in vitro. Eur J Med Chem;43:1530–1535.
12. Mishra L.C., Bhattacharya A., Bhasin V.K. (2009) Phytochemical licochalcone A enhances antimalarial activity of artemisinin in vitro. Acta Trop;109:194–198.
13. Awasthi S.K., Mishra N., Kumar B., Sharma M., Bhattacharya A., Mishra L.C., Bhasin V.K. (2009) Potent antimalarial activity of newly synthesized substituted chalcone analogs in vitro. Med Chem Res;18:407–420.
14. Li R., Kenyon G.L., Cohen F.E., Chen X., Gong B., Dominguez D.E., Kurzban G., Miller R.E., Nuzum E.O., Rosenthal P.J., McKerrow J.H. (1995) In vitro antimalarial activity of chalcones and their derivatives. J Med Chem;38:5031–5037.
15. Khatib S., Nerya O., Musa R., Shmuel M., Tamir S., Vaya J. (2005) Chalcones as potent tyrosinase inhibitors: the importance of a 2, 4-substituted resorcinol moiety. Bioorg Med Chem;13:433–441.
16. Trager W., Jensen J.B. (1976) Human malaria parasites in continuous culture. Science;193:673–675.
17. Bhattacharya A., Mishra L.C., Sharma M., Awasthi S.K., Bhasin V.K. (2009) Antimalarial pharmacodynamics of chalcone derivatives in combination with artemisinin against *Plasmodium falciparum* in vitro. Eur J Med Chem;44:3388–3393.
18. Lambros C., Vanderberg J.P. (1979) Synchronization of *Plasmodium falciparum* erythrocytic stages in culture. J Parasitol;65:418–420.
19. Nguyen-Dinh P., Payne D. (1980) Pyrimethamine sensitivity in *Plasmodium falciparum*: determination in vitro by a modified 48-hour test. Bull World Health Organ;58:909–912.
20. Olliaro P.L., Goldberg D.E. (1995) The *Plasmodium* digestive vacuole: metabolic headquarters and choice drug target. Parasitol Today;11:294–297.
21. Gamboa de Dominguez N.D., Rosenthal P.J. (1996) Cysteine proteinase inhibitors block early steps in hemoglobin degradation by cultured malaria parasites. Blood;87:4448–4454.
22. Francis S.E., Sullivan D.J., Goldberg D.E. (1997) Hemoglobin metabolism in the malaria parasite, *Plasmodium falciparum*. Annu Rev Microbiol;51:97–123.
23. Li R., Chen X., Gong B., Selzer P.M., Li Z., Davidson E., Kurzban G. *et al.* (1996) Structure-based design of parasitic protease inhibitors. Bioorg Med Chem;4:1421–1422.

Notes

[a]WHO (2001) Antimalarial drug combination therapy. Report of World Health Organisation technical consultation (WHO/CDS/RBM/2001.35) (http://whqlibdoc.who.int/hq/2001/WHO_CDS_RBM_2001.35.pdf).
[b]WHO (1998) The use of artemisinin and its derivatives as antimalarial drugs, Report of a joint CRD/DMP/TDR Informal Consultation (WHO/MAL/98.1086, 1998) (http://www.who.int/malaria/publications/atoz/who_mal_98_1086/en/index.html).

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Characterization, NMR, and ^{13}C spectra of substituted acetophenone and substituted 1, 3-diarylpropenone derivatives.

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