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Synthesis and Insight into the Structure–Activity Relationships of Chalcones as Antimalarial Agents

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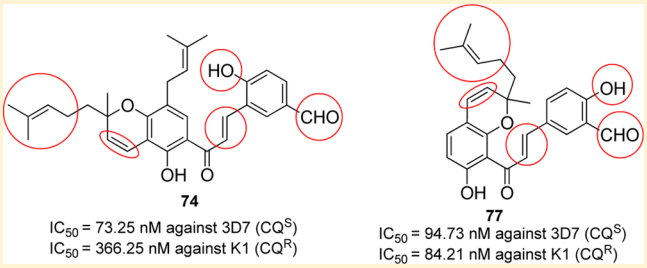
Supporting Information

ABSTRACT: Licochalcone A (**I**), isolated from the roots of Chinese licorice, is the most promising antimalarial compound reported so far. In continuation of our drug discovery program, we isolated two similar chalcones, medicagenin (**II**) and munchiwarin (**III**), from *Crotalaria medicagenia*, which exhibited antimalarial activity against *Plasmodium falciparum*. A library of 88 chalcones were synthesized and evaluated for their in vitro antimalarial activity. Among these, **67**, **68**, **74**, **77**, and **78** exhibited good in vitro antimalarial activity against *P. falciparum* strains 3D7 and K1 with low cytotoxicity. These chalcones also showed reduction in parasitemia and increased survival time of Swiss mice infected with *Plasmodium yoelii* (strain N-67). Pharmacokinetic studies indicated that low oral bioavailability due to poor ADME properties. Molecular docking studies revealed the binding orientation of these inhibitors in active sites of falcipain-2 (FP-2) enzyme. Compounds **67**, **68**, and **78** showed modest inhibitory activity against the major hemoglobin degrading cysteine protease FP-2.



INTRODUCTION

About 3.3 billion people, almost half of the world's population, are at risk of malaria. Every year, this leads to about 250 million cases and nearly one million deaths. People living in poor countries are the most vulnerable, particularly children below five years of age and pregnant women. Malaria is especially a serious problem in Africa, where it is responsible for one in every five (20%) childhood deaths.[1] An African child has on average 1.6–5.4 episodes of malaria fever each year and every 30 seconds a child dies from malaria. Malaria-endemic countries in South East Asia account for 15% (34 million) of the total estimated cases worldwide, the second highest number after Africa. India, Indonesia, and Myanmar comprise most of the region's reported cases (94%) in South East Asia. According to WHO, India contributes about 70% of the total malaria cases in the South East Asian region.[2] One of the major hurdles to control malaria is resistance of malaria parasites to most of the commonly used antimalarials. Hence, new antimalarials are urgently needed to combat the disease.

Chalcones are one of the major classes of natural products with widespread distribution in fruits, vegetables, spices, tea, and soy-based food products. They have been recently subjects of great interest for their pharmacological activities.[3] Some of the chalcones have been reported to possess many biological properties including anticancer,[4] antimalarial,[5] antiinflammatory,[6] antileishmanial,[7] antituberclulosis,[8] nitric oxide inhibition,[6,9] antimitotic,[10] analgesic,[6] antioxidant,[6] antifungal,[11] anti-HIV,[12] and antiprotozoal activities.[13] They are also reported to be gastric protectant,[14] antimutagenic, and antitumorogenic.[15] Licochalcone A (**I**), an oxygenated chalcone isolated from the roots of Chinese licorice,[16] is the most promising antimalarial compound reported so far. Natural chalcone **I** have been shown to inhibit the development of both chloroquine-susceptible (CQ^S) 3D7 and chloroquine-resistant (CQ^R) Dd2 *Plasmodium falciparum* strains. The growth of the parasites at all stages was inhibited by compound **I**. The chalcone **I** administered intraperitoneally (IP) or orally for 3–5 days also protected mice from lethal *Plasmodium yoelii* infection. Hence, the antimalarial activity of chalcone **I** has stimulated interest in antimalarial potential of other chalcones from natural and synthetic sources.[5,17] Chalcones are thought to act against

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malarial papain-like cysteine proteases as well,[17] which degrade hemoglobin to generate amino acids for erythrocytic stage development of the parasite. Chalcones can be readily synthesized, and contrasting substitution pattern in the two rings (Figure 1) could be envisaged to produce a large number of potential analogues. A thorough assessment of structural requirements for antimalarial activities of chalcones is vital to develop and optimize drug design efforts aimed at chalcones.

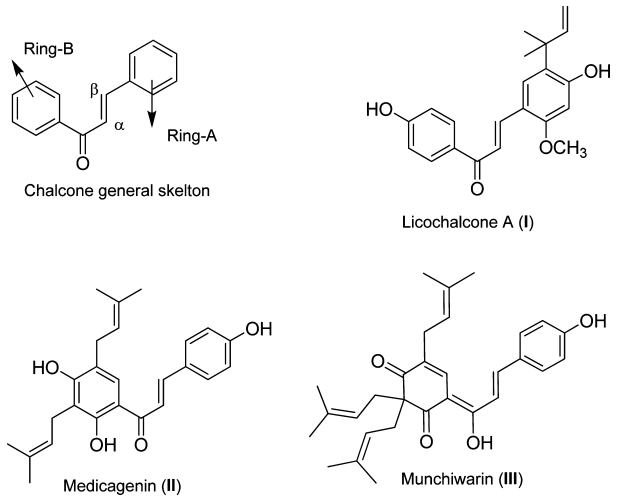


Figure 1. General skeleton of chalcone and naturally occurring antimalarial chalcones **I**–**III**.

In continuation of our drug discovery program on antimalarial agents from Indian medicinal plants, we isolated a diprenylated chalcone, known as medicagenin (**II**),[18] and a triprenylated chalcone, known as munchiwarin (**III**)[19] (Figure 1), from the roots of *Crotalaria medicagenia*. These two chalcones exhibited in vitro antimalarial activity (MIC = 5.10 and 4.34 μM, respectively) against *P. falciparum*.[18,19] This motivated us to synthesize various analogues of these lead molecules to obtain more active compounds. A library of 88 chalcones with various structural features were synthesized and evaluated for their in vitro antimalarial activity. Several of these compounds were found active in the in vitro screening, which were further evaluated for in vivo antimalarial activity in a Swiss mice model. Herein, we report a comprehensive assessment of antimalarial activity, structure–activity relationship (SAR) analyses, pharmacokinetics (PK), mode of action, and molecular docking studies of promising chalcones.

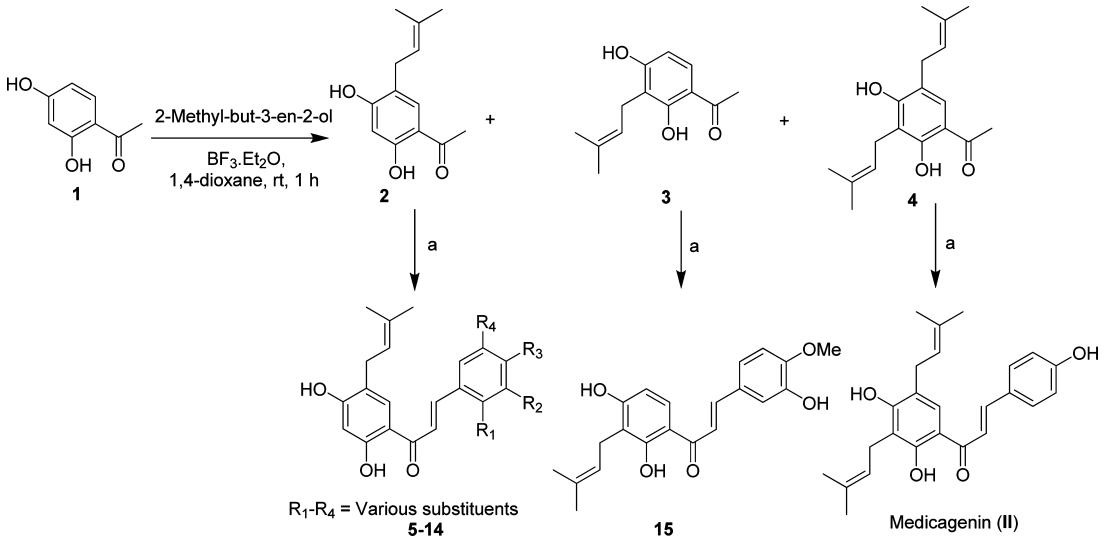
RESULTS AND DISCUSSION

Chemistry. *Synthesis of Prenylated Chalcones.* Prenylated chalcones **5**–**15** and **II** were prepared from 2,4-dihydroxyacetophenone (**1**). Prenylation of **1** with 2-methyl-but-3-en-2-ol in the presence of $BF_3{\cdot}OEt_2$ in dry dioxane resulted in the mixture of three compounds: 2,4-dihydroxy-5-*C*-prenylacetophenone (**2**), 2,4-dihydroxy-3-*C*-prenylacetophenone (**3**), and 2,4-dihydroxy-3,5-*C*-diprenylacetophenone (**4**).[19,20] The prenylacetophenones **2**–**4** and various substituted benzaldehydes were subjected to Claisen–Schmidt condensation[21] using aqueous KOH in ethanol to afford the corresponding prenylated chalcones **5**–**15** and **II** (Scheme 1).

Synthesis of Chromanochalcones. We wanted to study the cyclization effect of prenyl or geranyl group of chalcones; therefore, few chromanochalcones in which the *C*-prenyl or *C*-geranyl groups are involved in cyclization with phenolic hydroxyl group were synthesized. Initially, chromanochalcones **18**–**22** were prepared through a short and convenient route developed by us, which involves the one-pot synthesis of acetyl chromans **16** and **17** by carrying out a reaction between **1** and isoprene using $BF_3{\cdot}Et_2O$ (Scheme 2)[22] and Claisen–Schmidt condensation of resultant chromans **16** and **17** with various substituted aromatic aldehydes. The chromanochalcones **25** and **26** were synthesized by pyridine-catalyzed condensation between **1** and citraldimethylacetal to provide the acetylchromene **23**.[23] Then, the intermediate **23** was smoothly reduced to acetyl chroman **24** with Pd/C[24] and subsequently subjected to Claisen–Schmidt condensation with aromatic aldehydes to form the desired chromanochalcones **25** and **26** (Scheme 2).

Synthesis of Chromenochalcones. A large number of chromenochalcones were prepared to find out the role of the

Scheme 1. Synthesis of Chalcones Containing *C*-Prenyl Substituents on Ring-B (5–15 and II)



Reagents and Conditions: a) Appropriate aldehyde, aq. KOH, ethanol, rt, 48 h

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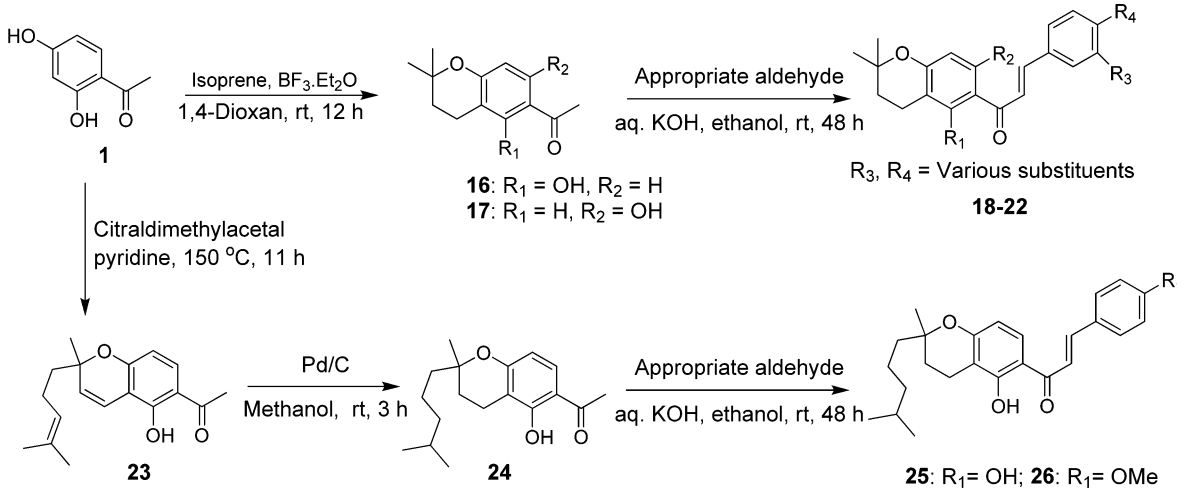
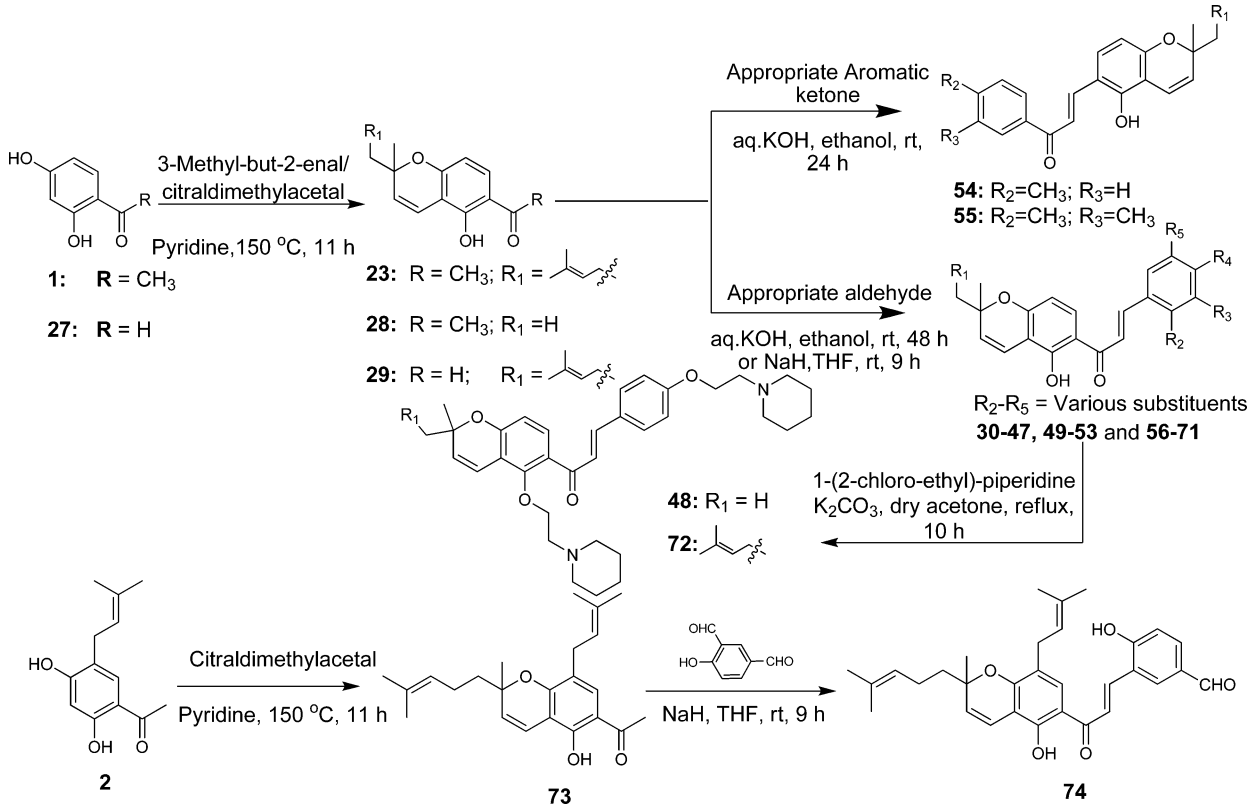
Scheme 2. Synthesis of Chromanochalcones (18−22, 25, and 26)



Scheme 3. Synthesis of Chromenochalcones (30−72) and Prenylated Chromenochalcone (74)



olefinic bond in benzopyran moiety. The acetyl/carboxaldehyde chromenes **23**, **28**, and **29** were synthesized using pyridine-catalyzed condensation between **1** or **27** and 3-methyl-but-2-enal/citraldimethylacetal.[23] The resultant acetyl chromenes **23** and **28** and substituted aromatic aldehydes or *p*-hydroxy-benzene-1,3-dicarbaldehyde[25] and chromene carboxaldehyde **29** and appropriate acetophenones were subjected to Claisen−Schmidt condensation using either aqueous KOH in ethanol or NaH in dry THF at room temperature to furnish the corresponding chromenochalcones **30**−**47** and **49**−**71** in good yields (Scheme 3).[24] To improve the bioavailability of **33** and **52**, the aminoalkyl groups were introduced as in **48** and **72** (see Tables 3 and 4). The synthesis of chalcones **48** and **72** was accomplished by the replacement of the hydrogen of the phenolic hydroxyl group of the chromenochalcones **33** and **52** with the alkyl part of the corresponding amines using K_2CO_3 in dry acetone (Scheme 3).[26] We also synthesized a prenylated-chromenochalcone **74**, which has an extra prenyl group on benzopyran moiety. The intermediate prenylatedacetylchromene **73** was synthesized using pyridine-catalyzed condensation between **2** and citraldimethylacetal. The intermediate **73** and *p*-hydroxy-benzene-1, 3-dicarbaldehyde were subjected to Claisen−Schmidt condensation using NaH in dry THF at room temperature to give the desired chalcone **74** (Scheme 3).

Chromenochalcones **77** and **78**, which are isomeric to **67** and **68** (Table 4), and a dichromenochalcone **81** were also

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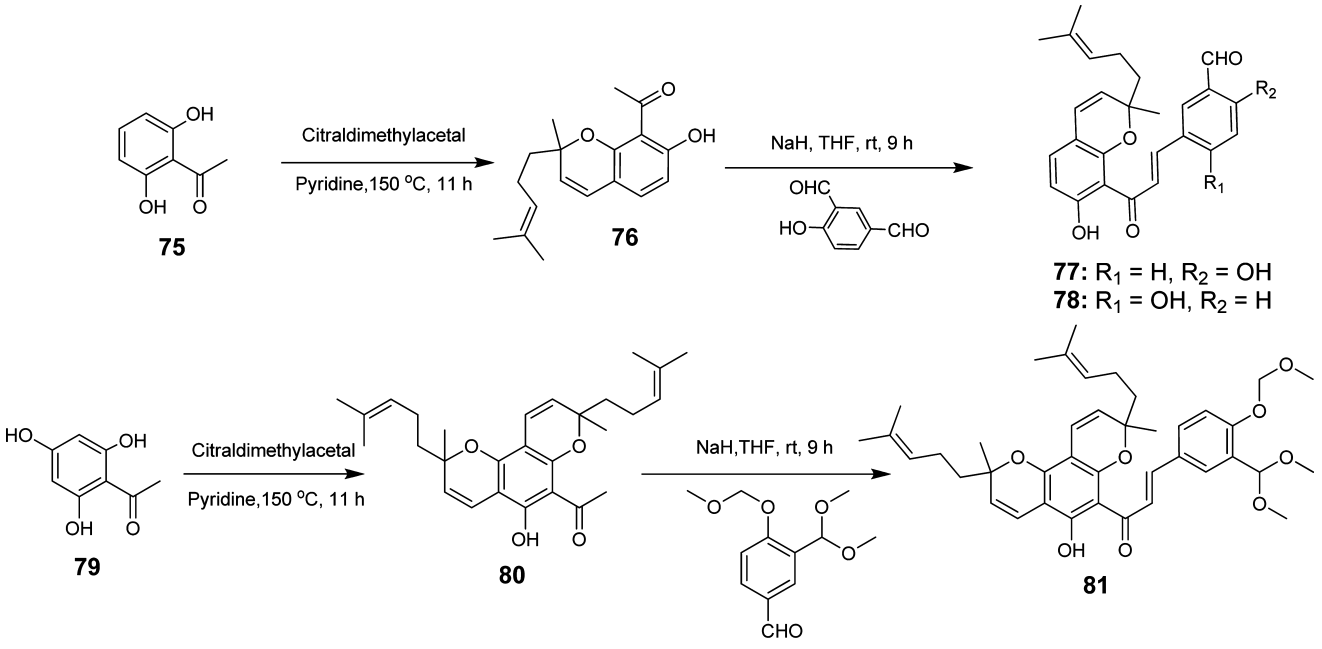
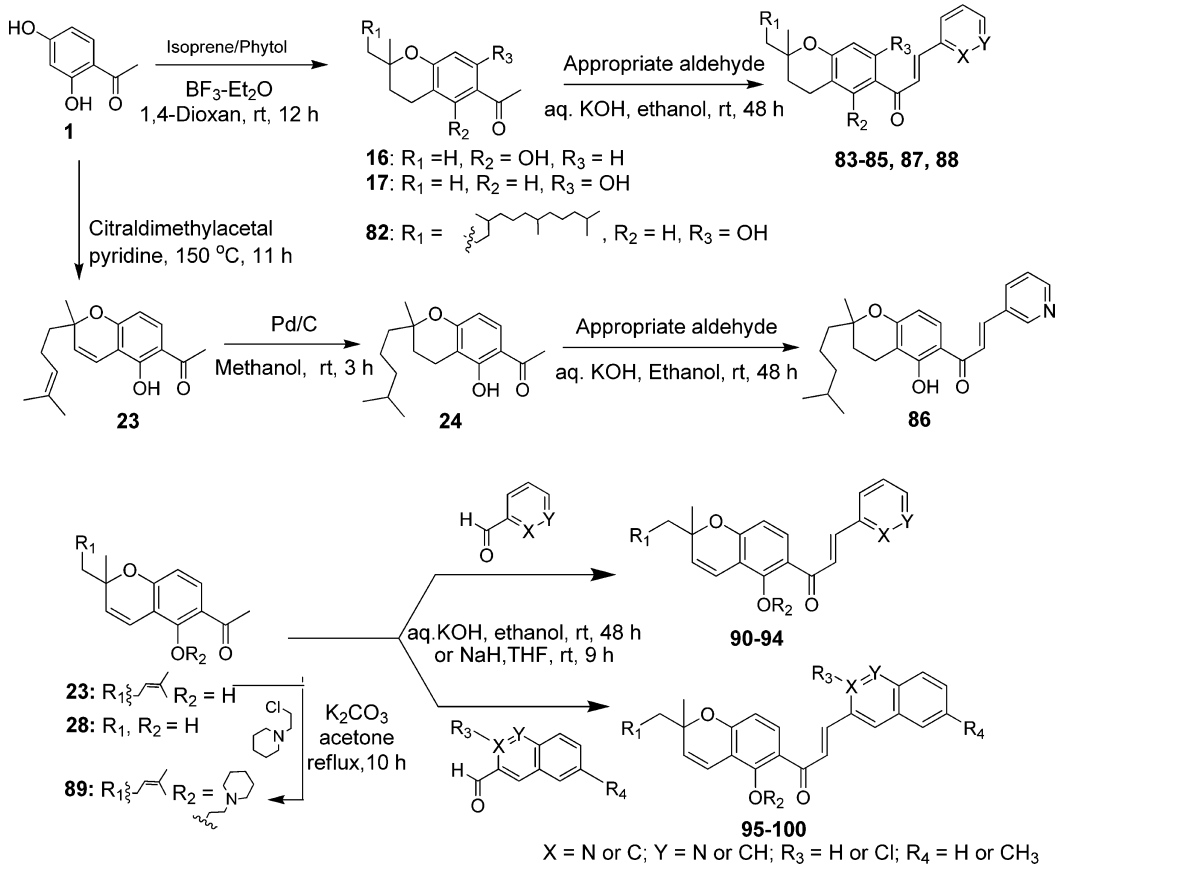
Scheme 4. Synthesis of Chromenochalcone (77, 78, and 81)



Scheme 5. Synthesis of Chromanochalcones 83−88 and Chromenochalcones Containing Hetero Atoms in Ring-A (90−100)



synthesized as outlined in Scheme 4 from 2,6-dihydroxyacetophenone (**75**), 2,4,6-trihydroxyacetophenone (**79**), and citraldimethylacetal via intermediates **76** and **80**, respectively.

Synthesis of Chromeno and Chromanochalcones with Hetero Atoms in Ring-A. Chromanochalcones **83−88** and chromenochalcones **90−100** which have hetero atoms in ring-A (Figure 1) were synthesized using Claisen−Schmidt condensation from acetyl chromans[22] **16**, **17**, **24**, and **82** and acetyl chromens[23] **23**, **28**, and **89**, respectively, and various heteroarylaldehydes as shown in Scheme 5.

Synthesis of Chromenodihydrochalcones. The synthesis of chromenodihydrochalcones **109−114** were carried out to

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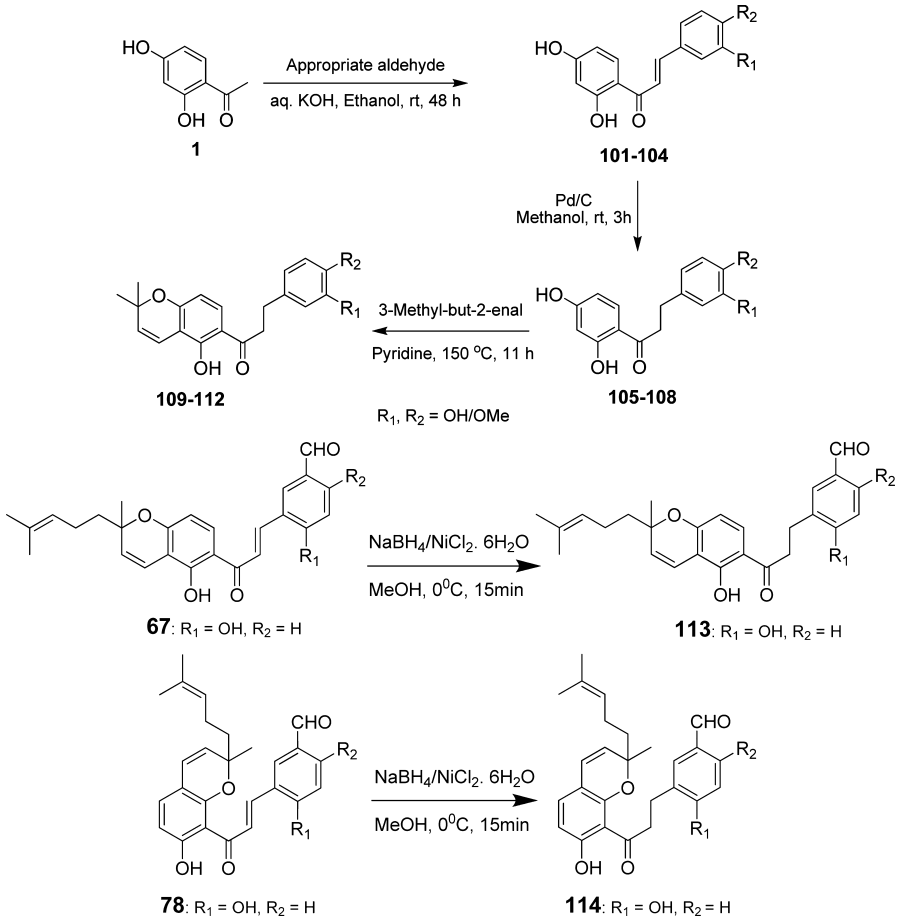
Scheme 6. Synthesis of Dihydrochromenochalcones (109−114)



determine the role of the $\alpha-\beta$ olefinic bond, which connects the ring-A and carbonyl carbon (Figure 1). Initially, the chalcones **101−104** were prepared by Claisen−Schmidt condensation between **1** and substituted benzaldehydes. The resultant chalcones were hydrogenated using Pd/C to give dihydrochalcones **105−108** and subsequent chromenylation using 3-methyl-but-2-enal in presence of pyridine provided the chromenodihydrochalcones **109−112** (Scheme 6).[23] Compounds **113** and **114** were synthesized by regioselective hydrogenation of **67** and **78** by using $NaBH_4/NiCl_2{\cdot}6H_2O$ (Scheme 6).

Biological Activity. As a part of our ongoing interest in developing new antiparasitic agents, we have recently reported the in vitro antimalarial activity of prenylated chalcones **II** and **III** (Figure 1) from the roots of *C. medicagenia*.[18,19] On the basis of these optimistic results, a library of 88 chalcones were prepared and their antimalarial activity was evaluated[27−29] in the following protocols:

1. All of these chalcones were preliminarily screened for their in vitro antimalarial activity against *P. falciparum* chloroquine sensitive (CQ^S) strain 3D7 at three concentrations (2, 10, and 50 μg/mL) to determine the lowest concentration of the compound that inhibits 100% parasitemia (minimum inhibitory concentration, MIC) and the respective activity results presented in μM. The compounds activity profile of this library was defined on the basis of their MIC values (MIC > 155 μM, inactive; MIC = between 50 and 155 μM, poor activity; MIC = between 6 and 50 μM, moderate activity; MIC = between 0.25 and 6 μM, good activity).
2. The compounds, which have MIC = 0.25−1.2 μM, were further studied to determine their IC_{50} values against CQ^S strain (3D7) as well as CQ^R strain (K1)[27] with chloroquine (CQ) and arteether as a reference drug.
3. The compounds with IC_{50} values between 70 and 350 nM were assessed for cytotoxicity,[30] in vivo efficacy,[31] pharmacokinetic studies, inhibition of the *P. falciparum* cysteine proteases falcipain-2 and falcipain-3,[32,33] and molecular docking studies.[34,35]

Determination of MIC of 88 Compounds Library. *In Vitro Antimalarial Activity of Prenylated Chalcones.* Initially, a series of simplified analogues **5−15** of **II** were synthesized (Scheme 1) and evaluated for their in vitro antimalarial activity and the results are shown in Table 1. Chalcones **5−15**, which contain either the electron-donating groups (EDGs) or halogen substituents on ring-A and a prenyl group on ring-B, showed moderate activity (MIC = 25.12−29.58 μM) with the exception of **8** and **14** (MIC = 142.45 and 146.19 μM, respectively; Table 1), which demonstrated that the monoprenylated compounds are less potent than diprenylated medicagenin **II** (MIC = 5.10 μM).[18,19]

In Vitro Antimalarial Activity of Chromanochalcones. To find out the role of the effect of cyclization of a prenyl/geranyl group in the form of a chroman ring (dihydrobenzopyran moiety), chromanochalcones **18−22**, **25**, and **26** were prepared (Scheme 2) and screened for their in vitro antimalarial activity (Table 2). Chalcones **18−22**, which have the electron-donating

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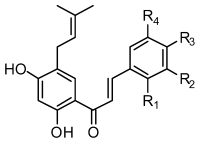
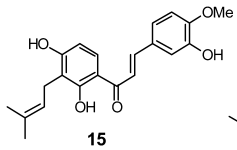
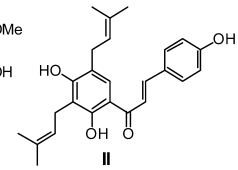
Table 1. In Vitro Antimalarial Activity of Chalcones Containing *C*-Prenyl Substituents on Ring-B (5−15 and II)



5-14



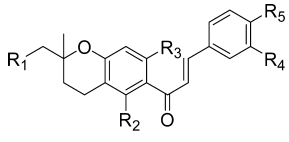
15



II

Compd	R_1	R_2	R_3	R_4	MIC (μM)
5	OMe	H	H	H	29.58
6	H	H	OMe	H	29.58
7	H	H	OEt	H	28.40
8	H	H	NMe_2	H	142.45
9	H	H		H	27.17
10	H	OMe	OH	H	28.24
11	H	OH	OMe	H	28.24
12	H	OMe	OMe	H	27.17
13	OMe	H	OMe	OMe	25.12
14	H	H	Cl	H	146.19
15	-	-	-	-	28.24
II	-	-	-	-	5.10

Table 2. In Vitro Antimalarial Activity of Chromanochalcones (18−22, 25, and 26)



18-22, 25 and **26**

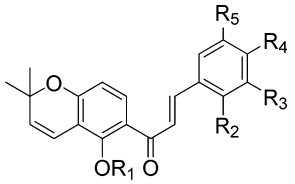
Compd	R_1	R_2	R_3	R_4	R_5	MIC (μM)
18	H	OH	H	H	NMe_2	>28.49[a]
19	H	H	OH	F	F	>29.06[a]
20	H	H	OH	Cl	Cl	>26.59[a]
21	H	H	OH	NO_2	H	>28.16[a]
22	H	H	OH	H	NO_2	>28.32[a]
25		OH	H	H	OH	126.90
26		OH	H	H	OMe	122.54

[a]Not inhibited 100% parasitemia at 10 μg/mL concentration and they were not tested at 50 μg/mL concentration.

groups (EDGs), electron-withdrawing groups (EWGs), and halogen groups on ring-A, appear to be less potent (MIC >25 μM: these compounds were not tested at a higher concentration). Chalcones **25** (MIC = 126.90 μM) and **26** (MIC = 122.54 μM), which contain an extra alkyl chain on benzopyran core also exhibited poor activity (Table 2). Diminished antimalarial activity of chromanochalcones (Table 2: **18−22**, **25**, and **26**) compared to *C*-prenylated chalcones (Table 1: **5−15**) indicated that cyclization of a prenyl/geranyl group has a negative impact on antimalarial activity.

In Vitro Antimalarial Activity of Chromenochalcones. A large number of chromenochalcones (**30−48**; Table 3) were synthesized to find out the activity profile of chromene (benzopyran) moiety instead of chroman. Chromenochalcones **30−36** contain the EDG's on ring-A. Out of these, **30−33** and **35** exhibited poor activity (MIC = 126−155 μM range), whereas **34** (MIC = 27.47 μM) and **36** (MIC = 25.25 μM) have moderate antimalarial activity. Except chromenochalcone **42** (MIC = 5.34 μM), all other chalcones (**37−41**), which contain either mono- or 3,4-dihalogen substituents on ring-A, have poor activity (MIC = 105−155 μM). Chromenochalcone **43** (MIC = 135.86 μM) and its regioisomer **44** (>135.86 μM), which have the combination of EDGs and halogen substituents on ring-A, exhibited poor activity. Compounds **45** and **46**, which contain the EWGs, were also showed poor activity, while **47**, in which the ring-A is substituted with combination of EDGs and EWGs, showed moderate activity (MIC = 28.57 μM). The presence of an aminoalkyl substituent as in **48** (MIC = 3.67 μM) on the hydroxyl group of **33** (MIC = 155.27 μM) significantly increased the activity profile (Table 3).

Table 3. In Vitro Antimalarial Activity of Chromenochalcones (30−48)



30-48

Compd	R_1	R_2	R_3	R_4	R_5	MIC (μM)
30	H	OMe	H	H	H	>148.80[a]
31	H	H	H	OMe	H	>148.80[a]
32	H	H	H	OEt	H	>142.85[a]
33	H	H	H	OH	H	155.27
34	H	H	H	OAc	H	27.47
35	H	H	OMe	OMe	H	136.61
36	H	OMe	H	OMe	OMe	25.25
37	H	H	H	F	H	154.32
38	H	H	H	Cl	H	147.05
39	H	H	H	Br	H	130.20
40	H	F	H	F	H	>146.19[a]
41	H	H	F	F	H	146.19
42	H	Cl	H	Cl	H	5.34
43	H	OEt	H	F	H	135.86
44	H	H	OEt	F	H	>135.86[a]
45	H	H	H	CN	H	>151.05[a]
46	H	H	H	NO_2	H	>142.45[a]
47	H	OH	H	H	CHO	28.57
48		H	H		H	3.67

[a]Not inhibited 100% parasitemia even at 50 μg/mL concentration.

Another series of chromenochalcones **49−72** were also synthesized in which benzopyran core contains an additional alkenyl substituent (prenyl/C-5 unit: Table 4). Of these, **49−57** contain EDGs on ring-A. Compounds **49−51**, **53**, and **56** exhibited poor activity, and **52**, **54**, **55** and **57** showed moderate activity (MIC = 21.55−25.77 μM: Table 4). It is noteworthy to mention here that the activity was retained even after shifting the pyran moiety to ring-A as in **54** (MIC = 25.77 μM) and **55** (MIC = 24.87 μM). Chalcones **58−63**, which contain either

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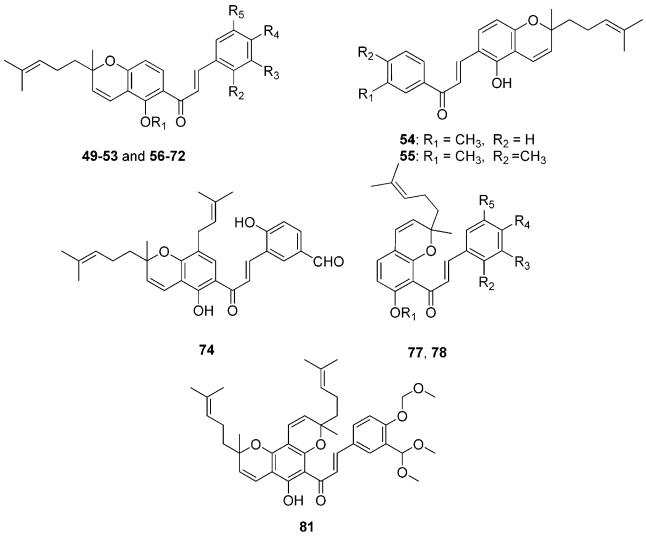
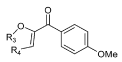
Table 4. In Vitro Antimalarial Activity of Chromenochalcones (49−72, 74, 77−78, and 81)



Compd	R_1	R_2	R_3	R_4	R_5	MIC (μM)
49	H	OMe	H	H	H	123.76
50	H	H	H	OMe	H	>123.76 [a]
51	H	H	H	OEt	H	119.61
52	H	H	H	OH	H	25.64
53	H	H	H	NMe_2	H	>119.90 [a]
54	-	-	-	-	-	25.77
55	-	-	-	-	-	24.87
56	H	H	OMe	OMe	H	115.20
57	H	H	OMe	OMe	OMe	21.55
58	H	H	H	F	H	127.55
59	H	H	H	Cl	H	>122.54 [a]
60	H	H	H	Br	H	>110.61 [a]
61	H	H	F	F	H	24.39
62	H	Cl	H	Cl	H	>113.12 [a]
63	H	H	Cl	H	Cl	113.12
64	H	H	H	CN	H	5.01
65	H	H	H	NO_2	H	119.33
66	H	H	NO_2	H	H	4.77
67	H	OH	H	H	CHO	1.19
68	H	H	CHO	OH	H	0.59
69	H	H	CHO		H	21.64
70	H	H			H	19.30
71	H	H			H	>91.24 [a]
72		H	H		H	3.26
74	-	-	-	-	-	0.25
77	H	H	CHO	OH	H	0.39
78	H	OH	H	H	CHO	0.31
81	-	-	-	-	-	15.19

[a]Not inhibited 100% parasitemia even at 50 μg/mL concentration.

mono- or dihalogen sustituents on ring-A, were poorly active or inactive, with the exception of **61** (MIC =22.62 μM). The chalcones **64** (MIC = 5.01 μM) and **66** (MIC = 4.77 μM), which contained the EWDs (4-cyano and 3-nitro, respectively) on ring-A, exhibited good activity except **65** (4-nitro, MIC = 119.33 μM). Chalcone **64** (Table 4; MIC = 5.01 μM) was more active than its lower homologue **45** (Table 3; >151.05 μM). Chalcones **67** and **68**, which have the combination of hydroxyl and aldehyde substituents on ring-A, showed good in vitro activity (MIC = 1.19 and 0.59 μM, respectively: Table 4). Masking the hydroxyl group of **68** with methyl-oxy-methyl (MOM) substituent as in **69** dramatically decreased the activity (**69**, MIC = 21.64 μM, versus **68**, MIC = 0.59 μM). The chalcones **70** and **71**, in which the hydroxyl at 4-position and aldehyde at 3-position of ring-A were masked in the form of furan (fused to aromatic ring), showed moderate to poor activity (MIC = 19.30 and 91.24 μM, respectively). These results indicated that aldehyde and hydroxyl groups in ring-A have a pivotal role in antiparasitic activity. The presence of an aminoalkyl substituents on the hydroxyl group as in **72** led to improvement in activity (see **72**, MIC = 3.26 μM, versus **52**, MIC = 25.64 μM; Table 4). A careful review of the in vitro antiparasitic activities presented in Tables 3 and 4 and their structural features suggested that both hydroxyl and aldehyde substituents on ring-A an extra alkenyl (prenyl or C-5 unit) substituent on benzopyran core and an aminoalkyl substituent on hydroxyl groups are playing an important role for good antimalarial activity. Because the chromenochalcones **67** and **68** exhibited good activity (MIC = 1.19 and 0.59 μM, respectively), we prepared chalcone **74** with an additional prenyl substituent on ring-B of **67**, as outlined in Scheme 3, and screened for in vitro antimalarial activity, which led to improved activity (MIC = 0.25 μM: Table 4).

Two chromenochalcones (**77** and **78**) that are isomeric to **67** and **68** (Table 4) and a dichromenochalcone (**81**) were synthesized as outlined in Scheme 4. Compounds **77** and **78** also showed good antimalarial activity (MIC = 0.39 and 0.31 μM, respectively) similar to those of **67** and **68**, which again supports the role of hydroxyl, aldehyde substituents on ring-A, and an extra alkenyl (prenyl or C-5 unit) substituent on a benzopyran core in antiparasitic activity. However, the dichromene **81** showed moderate activity (MIC = 15.19 μM).

In Vitro Antimalarial Activity of Chromano and Chromenochalcones with Hetero Atoms in Ring-A. Chromanochalcones **83−88** and chromenochalcones **90−100**, which have the heteroatoms in ring-A, were prepared (Scheme 5) and screened for their in vitro antimalarial activity (Table 5). The chalcones with heteroatom at 3-position in ring-A with alkenyl (prenyl or C-5 unit) side chain on chroman **86** (MIC = 5.27 μM) or chromene **93** (MIC = 5.33 μM) and **94** (MIC = 4.11 μM)) showed good antiparasitic activity and thus indicated that 3-pyridyl ring and extra alkenyl substituents (prenyl or C-5 unit) are required for antimalarial activity (Table 5). The chromenochalcones **95−100** with substituted 3-quinolyl ring (ring-A) exhibited poor activity (105.70−140.05 μM), with the exception of **98** (MIC = 23.52 μM). In vitro activities of **95−100** indicated that methyl or chloro substituents have a negative effect, whereas an extra alkenyl (prenyl or C-5 unit) side chain on the benzopyran moiety (**98** versus **95**) has a positive effect on activity (Table 5).

In Vitro Antimalarial Activity of Dihydrochromenochalcones. To determine the role of the α−β olefinic bond, chromenodihydrochalcones **109−114** were synthesized (Scheme 6). The chalcones **109−112**, in which the α−β unsaturated bond is in reduced form with a monohydroxyl (**109**: MIC = 30.86 μM)) or dihydroxyl (**111**: MIC = 29.41 μM)) system on ring-A exhibited moderate activity, whereas their *O*-methyl derivatives (**110** and **112**) were less active (MIC >147.92 and >135.86 μM; Table 6). It is noteworthy to mention here that the compound **113** and **114**, which are dihydroderivatives of **67** and **78**, showed moderate activity with MIC of 10.3 and 9.12 μM, respectvely. These results supported the importance of Michael acceptor (α,β-unsaturated ketone) moiety in the inhibition of parasitemia.

Determination of IC_{50} for Antimalarial Chalcones with MIC = 0.251−1.196 μM. The most active compounds, **67**, **68**,

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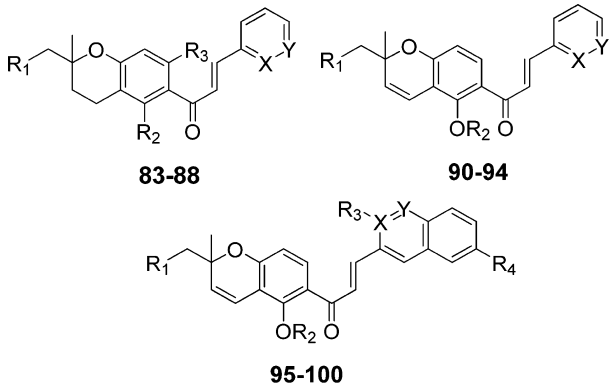
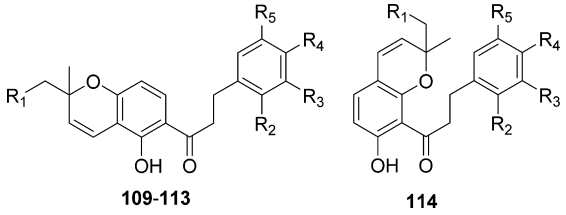
Table 5. In Vitro Antimalarial Activity of Chromano and/or Chromenochalcones with Hetero Atoms on Ring-A (83−88, 90−100)



Compd	R_1	R_2	R_3	R_4	X	Y	MIC (μM)
83	H	OH	H	-	CH	N	32.36
84	H	H	OH	-	N	CH	>32.36 [a]
85	H	H	OH	-	CH	N	32.36
86		OH	H	-	CH	N	5.27
87		H	OH	-	CH	N	>19.26 [a]
88		H	OH	-	N	CH	>19.26 [a]
90	H	H	-	-	N	CH	32.57
91	H	H	-	-	CH	N	32.57
92		H	-	-	N	CH	26.66
93		H	-	-	CH	N	5.33
94			-	-	CH	N	4.11
95	H	H	H	H	C	N	>140.05 [b]
96	H	H	Cl	H	C	N	127.87
97	H	H	Cl	Me	C	N	123.45
98		H	H	H	C	N	23.52
99		H	Cl	H	C	N	108.93
100		H	Cl	Me	C	N	105.70

[a]Not inhibited 100% parasitemia at 2 and 10 μg/mL concentrations and they were not tested at 50 μg/mL concentration. [b]Not inhibited 100% parasitemia even at 50 μg/mL concentration.

Table 6. In Vitro Antimalarial Activity of Dihydrochromenochalcones (109−114)



Compd.	R_1	R_2	R_3	R_4	R_5	MIC (μM)
109	H	H	H	OH	H	30.86
110	H	H	H	OMe	H	>147.92[a]
111	H	H	OH	OH	H	29.41
112	H	H	OMe	OMe	H	>135.86[a]
113		OH	H	H	CHO	10.30
114		OH	H	H	CHO	9.12

[a]Not inhibited 100% parasitemia even at 50 μg/mL concentration.

74, **77**, and **78** of this library, were further subjected to determine their IC_{50} values against *P. falciparum* CQ^S (3D7) and CQ^R (K1) strains (Table 7). The chalcones **67**, **68**, **74**, **77**, and **78** have an IC_{50} of 347.36, 125.35, 73.25, 94.73, and 87.55 nM, respectively, against the CQ^S strain (3D7) and an IC_{50} of 935, 1093.37, 366.25, 84.21, and >1196.17 nM, respectively, against CQ^R strain (K1). The marketed drug chloroquine has an IC_{50} of 17 nM against CQ^S and 443.57 nM against CQ^R strains, where as arteether has an IC_{50} of 1.37 nM against CQ^S and 1.08 nM against CQ^R strain. It is noteworthy to mention here that chalcone **77** was almost equally effective against both (CQ^S and CQ^R) strains (IC_{50} = 94.73 nM against 3D7 strain and 84.21 nM against K1 strain) and exhibited better in vitro activity profile than chloroquine against CQ^R strain.

Toxicity Studies of Potent Antimalarial Chalcones of Library. Toxicity is an important consideration in any drug development program, therefore we studied cytotoxicity of these compounds against (Table 7) Vero cell lines (C1008; monkey kidney fibroblast).[30] Given the reasonably good potencies of the compounds against malaria, the low cytotoxicities are encouraging, especially for **74** and **77**. In fact, **74** and **77** have a very good "selectivity index" (cytotoxicity/efficacy) of 1210, 334 for 3D7 strain and 242, 376 for K1 strain, respectively. Chalcones **67**, **68**, and **78** also showed good activity against 3D7 strain with good selectivity index.

In Vivo Activity against the CQ Resistant P. yoelii (N-67 strain) in Swiss Mice. The compounds (**67**, **68**, **74**, **77**, and **78**), which showed good potency and low cytotoxicity (Table 7), were evaluated in Swiss mice infected with *P. yoelii* (N-67 strain: CQ resistant).[31] The aqueous suspensions of test compounds was administered from day 0 to day 3 at 50 mg/kg/day by IP/oral route, and parasitemia levels were recorded from thin blood smears on day 4 (Table 8). The results indicated a rapid parasite clearance (71−98%), however, none of the treated animals was completely cured. The mean survival time of these animals was increased from 12.33 ± 1.45 to 18.40 ± 2.73 days (Table 8). The compound **67** exhibited 98% parasitemia suppression on day four, while compounds **68**, **74**, **77**, and **78** suppressed parasitemia by 89%, 71%, 88%, and 87% (Table 8), respectively. However, a curative effect was not obtained at the tested dose. The compound **67** when administered by oral route showed very poor in vivo efficacy compared to IP route. It suppressed only 23% parasitemia on day 4, and mean survival time decreased to 9.23 ± 0.81 due to rapid parasite recrudescence. The marketed drugs chloroquine (oral administration) and arteether (intramuscular administration) suppressed 99 and 100% parasitemia at a dose of 20 and 3 mg/kg body weight, respectively, and all the animals survived up to 28 days in both experiments.

In Vivo Oral Pharmacokinetic Studies. In vivo oral pharmacokinetic study was performed in male Sprague−Dawley rats (n = 5, weight range 220−240 g) to find out the reason for poor antiparasitic activity by oral route. Chalcone **67** was administered as a single oral dose of 50 mg/kg, and analysis was done using LC-MS/MS method to get the plasma concentration−time profile. Along with the plasma samples, quality control samples were distributed among calibrators and unknown samples. Results showed its slow (T_{max}, 3.0 h) and poor absorption with a peak plasma level of 78.27 ± 5.92 ng/mL (C_{max}) (Table 9). The plasma concentration of the compound **67** decreased drastically after 6 h of administration, and it was detectable in plasma up to 11 h. The overall systemically available area under curve$_{0-t}$ [AUC_{0-t}] of the compound was 389.18 ± 17.08 h·ng/mL (Figure 2). The plasma elimination half-life and clearance were 2.32 ± 0.81 h

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Table 7. IC_{50} of Chalcones, 67, 68, 74, 77, and 78 against *P. falciparum* Strains 3D7 and K1

compd	IC_{50} (nM) 3D7 (CQ^S)	IC_{50} (nM) K1 (CQ^R)	cytotoxicity[a] CC_{50} (nM)	IC_{50} ratio (K1/3D7)	selectivity index 3D7	selectivity index K1
67	347.36	935	22200	2.6	64	23.7
68	125.35	1093.37	9904	8.7	79	9.0
74	73.25	366.25	88621	5.0	1210	242
77	94.73	84.21	31674	0.9	334	376
78	87.55	>1196.17	102679	ND	1173	ND
chloroquine	17.00	443.57	235109	25.2	13393	530
arteether	1.37	1.08	136057	0.79	98720	124857

[a]Cytotoxicity against Vero cell line (C1008; Monkey kidney fibroblast). ND: not determined.

Table 8. In Vivo Antimalarial Efficacy of Chromenochalcones in *P. yoelii* N-67 (Resistant to CQ)[a]

compd	dose mg/kg/day (3 days)	route of administration	no. of treated mice	% parasitemia reduction vs controls; day 4	mean survival time (± SE) (days)
controls			5		9.20 ± 0.44
67	50	IP	5	98	17.40 ± 2.50
67	50	oral	5	23	9.20 ± 0.81
68	50	IP	5	89	12.33 ± 1.45
74	50	IP	5	71	12.60 ± 0.87
77	50	IP	5	88	16.75 ± 1.55
78	50	IP	5	87	18.40 ± 2.73
chloroquine	20	oral	5	99	>28
arteether	3	IM	5	100	>28

[a]IP: Intraperitoneal; IM: Intramuscular.

Table 9. Pharmacokinetic Parameters of 67 after Single Administration at 50 mg/kg (Oral) in Male Sprague–Dawley Rats

parameter	oral at 50 mg/kg
AUC_{0-t} (h·ng/mL)	389.18 ± 17.08
T_{max} (h)	3.00
C_{max} (ng/mL)	78.27 ± 5.92
$T_{1/2}$ (h)	2.32 ± 0.81
V_d/F (L/kg)	433.45 ± 163.81
CL/F (L/h/kg)	128.53 ± 5.49
MRT (h)	3.89 ± 0.72

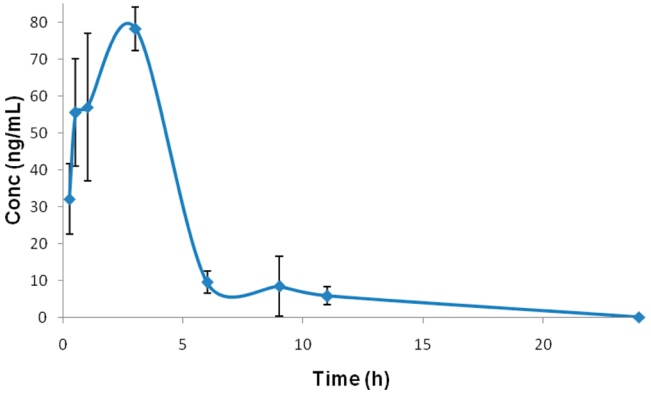

Figure 2. Mean plasma concentration–time profile of **67** in male Sprague–Dawley rats (220–240 g) after a single oral administration of **67** (50 mg/kg). Results are presented as mean ± SEM.

and 128.53 ± 5.49 L/h/kg, respectively. Thus the pharmacokinetic study indicated very low oral bioavailability of compound **67** could be the reason for its poor in vivo antimalarial activity to clear 100% parasitemia in experimental animals. The compound **67** was also tested in simulated gastric fluid and simulated intestinal fluid and found to be stable in both.

Cysteine Protease Inhibition Studies. Because chalcones are known to inhibit the cysteine proteases, **67**, **68**, and **78** were evaluated for their inhibitory activity against the cysteine proteases falcipain-2 (FP-2) and falcipain-3 (FP-3), which are validated drug targets for developing new antimalarial chemotherapy.[32] Recombinant FP-2 (1 nM) and FP-3 (2 nM) were used to determine cysteine protease inhibiting activity. Compounds **67** and **78** did not inhibit FP-3 and, rather, activated the enzyme at higher concentrations. The compound **68** showed marginal inhibition of FP-3 at lower concentrations but, intriguely, stimulated the enzyme activity at higher concentrations. However, compound **67**, **68**, and **78** inhibited FP-2 with an IC_{50} of 13.5, 5.5, and 4.6 μM, respectively, which correlates with their in vitro antimalarial activity (IC_{50} of 347.3, 125.3, and 87.5 nM). The FP-2 inhibiting activity of these compounds is comparable with that of the chalcones reported in the literature (Table 10).[33]

Docking of Chromenochalcones to Falcipin-2 Protein Crystal Structure. Chalcones have been reported to act by competitive inhibition at the malarial cysteine proteases (FP-2). We therefore carried out docking studies to find out the binding orientations of these inhibitors at active sites of amino

Table 10. Chalcones 67, 68, and 78's Falcipain-2 Inhibiting Activity and Docking Studies against Falcipain-2 Protein Crystal Structure

compd	IC_{50} of falcipain-2 inhibiting activity (μM)	docking scores (Flexi X total score)[a]
67	13.5	−13.3
68	5.5	−14.00
78	4.6	−15.55

[a]Higher the score means strong binding interactions with Falcipain-2.

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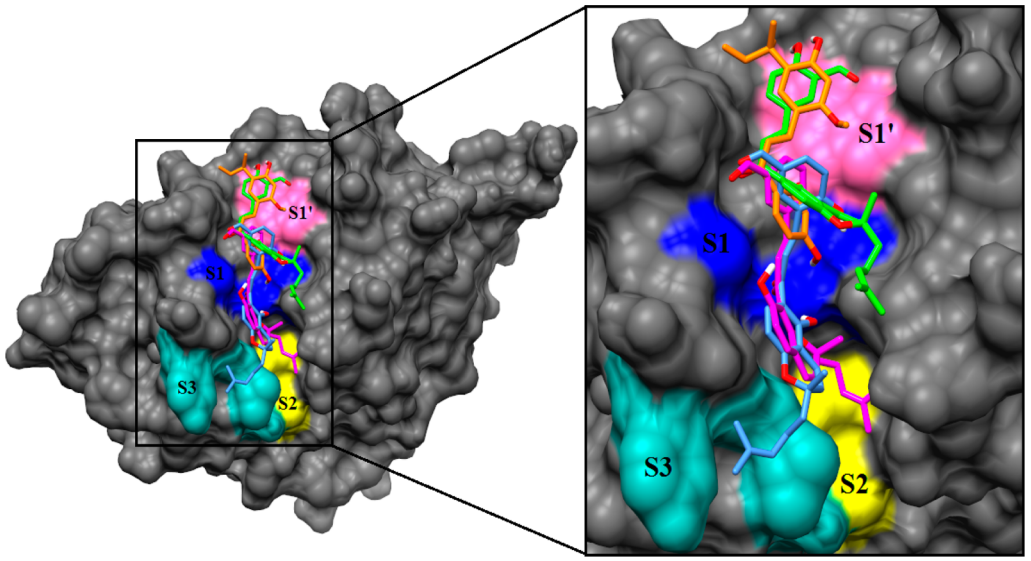

Figure 3. Surface representation of falcipain-2 (FP-2) showing subsites occupied by docked compounds with enlarged view (right). Only polar hydrogen atoms of compounds are shown. FP-2 has surface representation colored in gray.

acid residues of falcipain-2 enzyme (Figure 3; Table 10). The binding pockets of cysteine protease, falcipain-2 (FP-2), is well explored and its crystal structure (3BPF and 2GHU) has also been reported with small molecule inhibitors.[34,35] However, crystal structure of FP-2 with chalcone-based inhibitor is not available in structural database. In this context, in silico study provides valuable insight into binding mode of these inhibitors. FP-2 active site is divided into four subsites: S1, S1′, S2, and S3.[36,37] Any nonsubstrate molecule interacting with these subsites may have potential to become FP-2 inhibitor. In docking simulation by Flex X, we observed two populations of docked conformations of chalcones (Figure 4), one consisting of **67** and **78**, has shown H-bond interactions with residues Tyr206 of S1′, Gln36 (except compound **67**), Cys39, Cys42, and His174 of S1. In the S3 subsite, only **67** formed H-bond with Gly83. The alkenyl (prenyl or C-5 unit) unit of **67** was found to be in close proximity to hydrophobic residues Trp78 and Gly83 of the S3 subsite. However, the alkenyl (prenyl or C-5 unit) unit of **78** optimally occupied the hydrophobic cavity formed by Leu84 and Ile85 in S3 and S2 subsites, respectively. Another population consisted of compounds **68** and Licochalcone (**I**) occupying mainly S1′ and S1 subsites. Compounds **68** appeared to form H-bond with Gln36, Cys39, His174, Trp206, and Gln209. In this population, the alkenyl (prenyl or C-5 unit) unit of **68** was away from the hydrophobic S2 subsite and ring-A was involved in $\pi-\pi$ interaction with Trp206. Licochalcone A (**I**) showed weak H-bonding only with Cys39, Cys42, and His174 and $\pi-\pi$ interactions with Trp206.

Overlay of docked conformations of all compounds revealed the interesting point that one aryl ring (ring-A of **67** and **78** and ring-B of **68** and **I**) and carbonyl group (formyl group of **67** and **78** and ketone of **68** and **I**) of these chalcones clustered at almost the same position (Figure 5). We hypothesized that aryl ring clustering was observed due to the presence of hydrophobic residue Trp206 and carbonyl group clustering was due to formation of H-bond with main chain of Cys39. Recently similar docking result was reported by Sahu and co-workers,[38] where they have shown the role of Cys39 and Gln36 in chalcone binding. Overall, compound **78** (Flex X total score: −15.5) and **68** (Flex X total score: −14.00) showed better interaction than **67** (Flex X total score: −13.41) in terms of H-bonding. These docking results correlate with the experimental FP-2 inhibition results (IC_{50} of **78**, 4.6 μM; **68**, 5.5 μM; and **67**, 13.5 μM) and their in vitro antimalarial activity. Further **67**, **68**, and **78** have better interactions than Licochalcone A (Flex X total score: −10) in our docking studies (Table 10).

■ CONCLUSIONS

In summary, a library of 88 chalcones with various structural features such as prenylated chalcones, chromanochalcones, chromenochalcones, and chromenodihydrochalcones were synthesized based on the natural products lead molecule. Our SAR studies indicated that both the hydroxyl and aldehyde substituents on ring-A, an extra alkenyl (prenyl or C-5 unit) substituent on the benzopyran core (as in **67**, **68**, **74**, **77**, **78**), aminoalkyl substituent on hydroxyl groups (as in **48** and **72**), and a heteroatom at the 3-position in ring-A (as in **86**, **93**, **94**) are required for good antimalarial activity of chalcones. Several compounds of this synthetic series are equipotent to natural products, and a few of them showed several-fold (>20) higher order of in vitro activity. Some of these compounds also suppressed the parasitemia and increased survival time of Swiss mice infected with the chloroquine resistant *P. yoelii* N-67 strain. PK Studies revealed that very low oral bioavailability due to poor absorption. Inhibition of recombinant FP-2 by compounds **67**, **68**, and **78** suggested that hemoglobin degradation is one of the mechanisms of antimalarial activity of these compounds. Molecular docking studies against protein crystal structure of FP-2 revealed, binding of these inhibitors at active sites of enzyme and these results correlate with the experimental FP-2 studies data and in vitro antimalarial activity. The correlation between in vitro and in vivo was not observed during our studies that might be due to varied pharmacokinetic properties of tested compounds in in vivo system. Further optimization studies are in progress in our laboratory to improve bioactivity of these compounds to achieve better curative effect and oral bioavailability.

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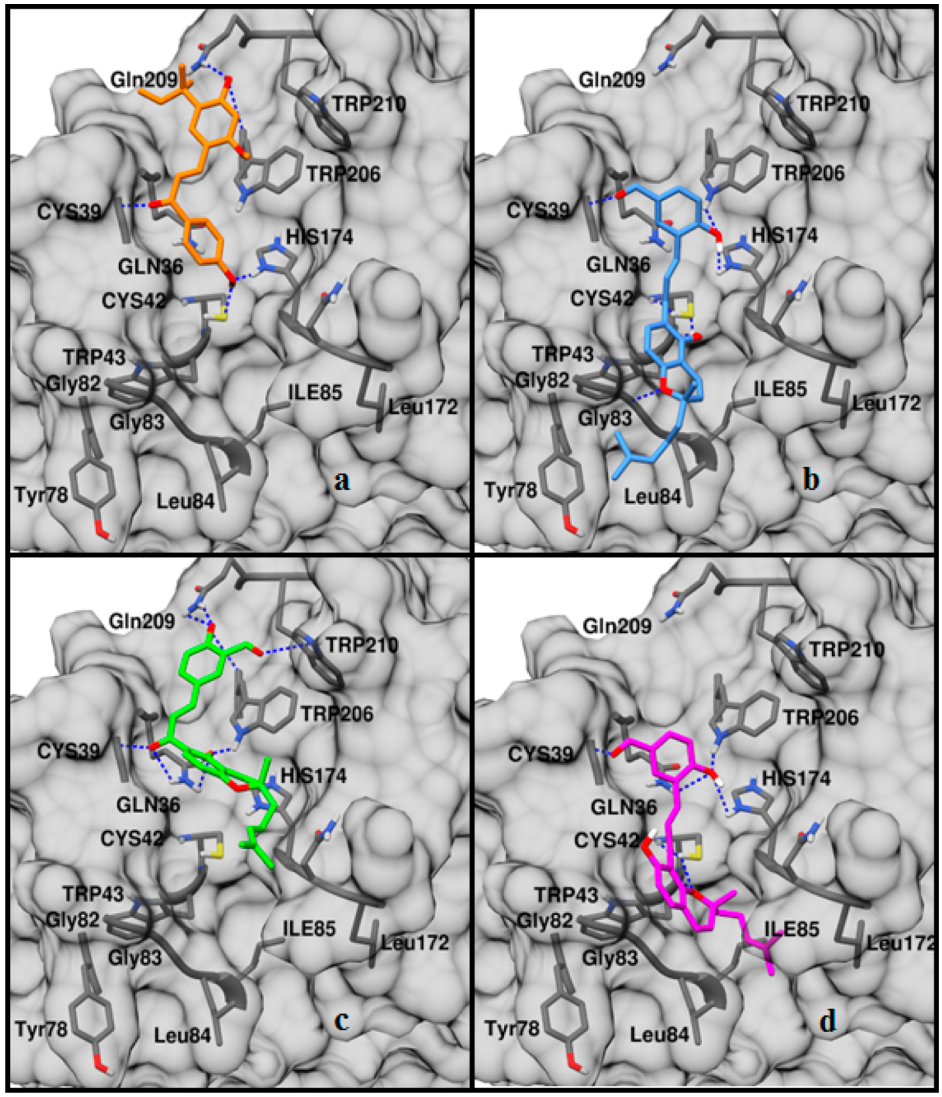

Figure 4. Predicted bound conformation of **I** (a), **67** (b), **68** (c), and **78** (d) in the active site of FP2 (mixed surface, ribbon-and-stick representation in gray color). For clear appearance, only polar hydrogens are shown in both FP2 and docked compounds. Residues were labeled using 2D label option in Chimera. Hydrogen bonds are shown in blue dotted line. Some H-bonds may not be visible (for detail, see Supporting Information).

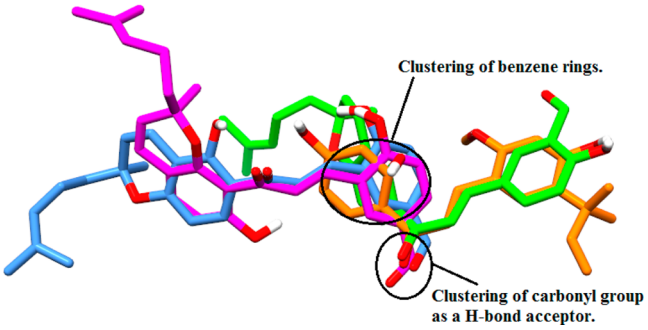

Figure 5. Overlay of docked conformations of **67** (cornflower blue), **68** (green), **78** (magenta), and **I** (orange). Molecules were colored individually for clear understanding.

EXPERIMENTAL SECTION

General Methods. Melting points were recorded on Buchi-530 capillary melting point apparatus and are uncorrected. IR spectra were recorded on Perkin-Elmer AC-1 spectrometer. ^{1}H NMR spectra were recorded on Bruker Avance DPX 200 FT, Bruker Robotics and Bruker DRX 300, spectrometers at 200, 300 MHz (^{1}H), and 50, 75 MHz (^{13}C). Experiments were recorded in $CDCl_3$, CD_3OD, pyridine-d_5, D_2O, and DMSO-d_6 at 25 °C. Chemical shifts were given in parts per million (ppm) downfield from internal standard Me_4Si (TMS). ESI mass spectra were recorded on JEOL SX 102/DA-6000. Chromatography was executed with silica gel (60−120 or 230−400 mesh) using mixtures of ethyl acetate and hexane as eluants. Reactions, which required the use of anhydrous, inert atmosphere techniques, were carried out under an atmosphere of nitrogen. 1,4-Dioxane was distilled over sodium. Commercially available reagents, solvents, and starting materials were used without further purification. Elemental analyses were performed on a Vario EL-III C, H, N, S analyzer (Germany), and values were within ±0.5% of the calculated values; therefore, these compounds meet the criteria of >95% purity. Analytical HPLC analyses were performed on a Shimadzu 10ATVP HPLC instrument, Zorbax C18 column (150 mm × 4.6 mm, 5 μm). A purity of ≥95% has been established for compounds, which showed good in vitro and in vivo activity.

Synthesis of Prenyl Acetophenones (2−4). To a magnetically stirred solution of **1** (1.0 g, 6.5 mmol) in anhydrous 1,4-dioxane (15 mL) maintained under nitrogen atmosphere was added gradually $BF_3{\cdot}Et_2O$ (0.8 g, 6.5 mmol) at rt. When the solution acquired pink−red, a solution of 2-methyl-but-3-en-2-ol (790 mg, 7.9 mmol) in anhydrous 1,4-dioxane (10 mL) was added and the whole solution was

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stirred for 1 h at rt. After dilution with diethyl ether (100 mL), the solution was washed with water (3 × 50 mL) to discharge the color. The combined ethereal solution obtained after extraction was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. Then the crude product was chromatographed on silica gel to afford **2** (307 mg, 27%), **3** (183 mg, 16%), and **4** (397 mg, 21%) as white solids.

*1-[2,4-Dihydroxy-5-(3-methyl-but-2-enyl)-phenyl]-ethanone (**2**).* Melting point 145−146 °C. FT-IR (KBr, cm^{-1}) 3340, 1635. ^1H NMR ($CDCl_3$, 200 MHz) δ 7.43 (s, 1H), 6.35 (s, 1H), 5.29 (t, J = 7.1 Hz, 1H), 3.29 (d, J = 7.2 Hz, 2H), 2.54 (s, 3H), 1.78 (s, 6H). MS (FAB) (m/z) 221 $(M + H)^+$.

*1-[2,4-Dihydroxy-3-(3-methyl-but-2-enyl)-phenyl]-ethanone (**3**).* Melting point 154−157 °C. FT-IR (KBr, cm^{-1}) 3330, 1630. ^1H NMR ($CDCl_3$, 200 MHz) δ 7.53 (d, J = 8.8 Hz, 1H), 6.37 (d, J = 8.8 Hz, 1H), 5.25 (t, J = 7.2 Hz, 1H), 3.43 (d, J = 7.2 Hz, 2H), 2.55 (s, 3H), 1.82 (s, 3H), 1.76 (s, 3H). MS (FAB) (m/z) 221 $(M + H)^+$.

*1-[2,4-Dihydroxy-3,5-bis-(3-methyl-but-2-enyl)-phenyl]-ethanone (**4**).* Melting point 110 −112 °C. FT-IR (KBr, cm^{-1})1633. ^1H NMR (200 MHz, $CDCl_3$) δ 7.21 (s, 1H), 6.14 (s, 1H), 5.18 (m, 2H), 3.34 (d, J = 7.0 Hz, 2H), 3.20 (d, J = 7.0 Hz, 2H), 2.46 (s, 3H), 1.74 (s, 3H), 1.70 (s, 3H), 1.68 (s, 6H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 203.1, 161.4, 160.1, 135.5, 135.1, 129.9, 122.2, 121.8, 119.3, 114.6, 113.8, 29.4, 26.6, 26.2 (2C), 22.2, 18.3 (2C). MS (FAB) (m/z) 289.0 $(M + H)^+$.

Representative Procedure for the Synthesis of 1-[2,4-Dihydroxy-5-(3-methyl-but-2-enyl)-phenyl]-3-(4-methoxy-phenyl)-propenone (6). To a stirred solution of **2** (500 mg, 2.2 mmol) in aqueous KOH solution in ethanol (5 mL) was added 4-methoxybenzaldehyde (615 mg, 4.5 mmol). The whole reaction mixture was stirred for 48 h at rt and quenched in ice-cold water, acidified with 1 N HCl, and extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed with water, brine solution, dried over anhydrous Na_2SO_4, and the solvent evaporated under reduced pressure. The crude product was subjected to silica gel column chromatography using hexane/ethyl acetate as mobile phase to afford the prenylated chalcone **6** (307 mg, 40%). Melting point 158−160 °C. FT-IR (KBr, cm^{-1}) 3315, 1637. ^1H NMR ($CDCl_3$, 200 MHz) δ 13.61 (s, 1H), 7.84 (d, J = 15.4 Hz, 1H), 7.60 (d, J = 8.6 Hz, 2H), 7.43 (d, J = 15.4 Hz, 1H), 6.94 (d, J = 8.6 Hz, 2H), 6.41 (s, 1H), 6.12 (s, 1H), 5.32 (t, J = 6.9 Hz, 1H), 3.86 (s, 3H), 3.34 (d, J = 6.9 Hz, 2H), 1.79 (s, 6H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 192.3, 165.2, 162.1, 161.9, 144.5, 135.6, 131.4, 130.7 (2C), 128.0, 122.0, 119.2, 118.9, 118.4, 114.8 (2C), 104.4, 55.8, 29.5, 26.1, 18.3. MS (FAB) (m/z) 339 $(M + H)^+$.

Representative Procedure for the Synthesis of Acetylchromans 16 and 17. To a stirred solution of **1** (1.0 g, 6.6 mmol) in dry 1,4-dioxane (15 mL) was added gradually $BF_3{\cdot}Et_2O$ (0.8 g, 6.6 mmol) at rt. When the solution acquired a pink−red, solution of isoprene (450 mg, 6.6 mmol) in dry 1,4-dioxane (10 mL) was added and the whole solution was stirred for 12 h at rt. After dilution with moist ether (100 mL), the solution was washed with water (3 × 50 mL) to discharge the color. The combined ethereal solution obtained after extraction was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The crude mixture was isolated on column chromatography to afford the desired acetyl chromans **16** (521 mg, 36%). Melting point 72−73 °C. FT-IR (KBr, cm^{-1}) 3429, 1629. ^1H NMR (200 MHz, $CDCl_3$) δ 13.11 (s, 1H), 7.49 (d, J = 8.9 Hz, 1H), 6.33 (d, J = 8.9 Hz, 1H), 2.68 (t, J = 6.7 Hz, 2H), 2.54 (s, 3H), 1.80 (t, J = 6.7 Hz, 2H), 1.34 (6H, s). MS (FAB) m/z 220 $(M)^+$, 221 $(M + H)^+$, and **17** (530 mg, 37%), mp 116−118 °C. FT-IR (KBr, cm^{-1}) 3429, 1642. ^1H NMR (200 MHz, $CDCl_3$) δ 12.32 (s, 1H), 7.43 (s, 1H), 6.31 (s, 1H), 2.73 (t, J = 6.7 Hz, 2H), 2.53 (s, 3H), 1.82 (t, J = 6.7 Hz, 2H), 1.34 (s, 6H). MS (FAB) (m/z) 220 $(M)^+$, 221 $(M + H)^+$.

Representative Procedure for the Synthesis of 1-(5-Hydroxy-2-methyl-2-(4-methylpent-3-enyl)-2*H*-chromen-6-yl) Ethanone (23). To a magnetically stirred solution of **1** (12.0 g, 79 mmol) in dry pyridine (8.04 mL) was added gradually citraldimethylacetal (15.6 g, 79 mmol) at rt. The whole reaction mixture was refluxed for 4 h at 150 °C, and an additional equivalent of citraldimethylacetal (15.6 g, 79 mmol) was added and refluxed for further 6 h. Excess pyridine in the reaction mixture was evaporated by rotary evaporator under reduced pressure. The crude product was subjected to silica gel column chromatography using hexane/ethyl acetate as mobile phase to afford the desired compound **23** (14.45, 64%). Semisolid; FT-IR (neat, cm^{-1}) 3436, 1695. ^1H NMR ($CDCl_3$, 200 MHz) δ 6.48 (d, J = 8.8 Hz, 1H), 6.74 (d, J = 10.1 Hz, 1H), 6.31 (d, J = 8.8 Hz, 1H), 5.51 (d, J = 10.1 Hz, 1H), 5.08 (t, J = 8.1 Hz, 1H), 2.51 (s, 3H), 2.07 (q, J = 7.4 Hz, 2H), 1.69−1.80 (m, 2H), 1.67 (s, 3H), 1.59 (s, 3H), 1.40 (s, 3H). MS (FAB) (m/z) 287 $(M + H)^+$.

Representative Procedure for the Synthesis of 1-(5-Hydroxy-2-methyl-2-(4-methylpentyl)-chroman-6-yl) Ethanone (24). To a solution of **23** (575 mg, 2 mmol) in methanol (10 mL) was added a catalytic amount of 10% Pd/C. The reaction mixture was shaken in hydrogenation assembly under hydrogen gas at 50 lbs for 2 h. After replacement of air by nitrogen, Pd/C was filtered off and methanol was evaporated under reduced pressure. The crude product was subjected to silica gel column chromatography using hexane/ethyl acetate as mobile phase to afford the title compound **24** (375 mg, 64%); semisolid. FT-IR (neat, cm^{-1}) 3430, 1714. ^1H NMR ($CDCl_3$, 200 MHz) δ 7.48 (d, J = 8.9 Hz, 1H), 6.33 (d, J = 8.9 Hz, 1H), 2.66 (t, J = 6.8 Hz, 2H), 2.53 (s, 3H), 1.79 (t, J = 6.4 Hz, 2H), 1.35−1.60 (m, 5H), 1.29 (s, 3H), 1.18−1.21 (m, 2H), 0.88 (s, 3H), 0.85 (s, 3H). MS (FAB) (m/z): 291 $(M + H)^+$.

Representative Procedure for the Synthesis of 1-[2-Methyl-2-(4-methyl-pent-3-enyl)-5-(2-piperidin-1-yl-ethoxy)-2*H*-chromen-6-yl]-3-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-propenone (72). To a stirred solution of chalcone, **52** (250 mg, 1.0 mmol) in dry acetone (20 mL) were added anhydrous K_2CO_3 (2.85 g, 20.8 mmol), and 1-(2-chloro-ethyl)-piperidine hydrochloride (953 mg, 5.2 mmol), and the reaction mixture was refluxed for 5 h. The mixture was filtered off under suction, and solvent was evaporated under reduced pressure. The crude product was subjected to silica gel column chromatography using hexane/ethyl acetate as mobile phase to afford the compound **72**. Yield: 51%. FT-IR (neat, cm^{-1}) 1652. ^1H NMR ($CDCl_3$, 200 MHz) δ 7.67 (d, J = 15.6 Hz, 1H), 7.58 (d, J = 8.6 Hz, 1H), 7.53 (d, J = 8.5 Hz, 2H), 7.42 (d, J = 15.6 Hz, 1H), 6.91 (d, J = 8.5 Hz, 2H), 6.80 (d, J = 10.0 Hz, 1H), 6.61 (d, J = 8.6 Hz, 1H), 5.63 (d, J = 10.0 Hz, 1H), 5.12 (d, J = 6.0 Hz, 1H), 4.14 (t, J = 6.2 Hz, 2H), 3.93 (t, J = 6.3 Hz, 2H), 2.79 (t, J = 6.2 Hz, 2H), 2.62 (t, J = 6.3 Hz, 2H), 2.52−2.38 (m, 12H), 1.73−1.25 (m, 21H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 191.4, 161.1, 158.1, 155.6, 143.2, 132.3, 131.6, 130.5 (2C), 129.7, 128.3, 126.4, 124.6, 124.2, 117.8, 115.3 (3C), 112.8, 79.6, 73.9, 59.0, 58.2, 55.4 (2C), 55.2 (2C), 41.8, 27.1, 26.2 (6C), 24.6 (2C), 23.1, 18.0. MS (FAB) (m/z) 613 $(M + H)^+$.

Representative Procedure for the Synthesis of 2-Hydroxy-5-{3-[7-hydroxy-2-methyl-2-(4-methyl-pent-3-enyl)-2*H*-chromen-8-yl]-3-oxo-propenyl}-benzaldehyde (77). To a stirred solution of **76** (575 mg, 2 mmol) in anhydrous THF (5 mL) was added portionwise NaH (120 mg, 5 mmol) and stirred for 20 min at rt under nitrogen. Then *p*-hydroxy-benzene-1, 3-dicarbaldehyde (300 mg, 2 mmol) in 2 mL of THF was added to the reaction mixture and stirred for 8 h at rt. The mixture was poured into ice-cold water, acidified with 1 N HCl, and extracted with ethyl acetate (3 × 50 mL). The combined organic layers were washed with water and brine solution, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The crude product was subjected to silica gel column chromatography using hexane/ethyl acetate as mobile phase to afford the chromenochalcone **77** (210 mg, 25%). FT-IR (neat, cm^{-1}) 3493, 3392, 2923, 2854, 2359, 1730, 1599, 1554, 1478, 1358, 1209, 1096, 760. ^1H NMR (300 MHz, $CDCl_3$) δ 13.06 (s, 1H), 11.23 (s, 1H), 9.92 (s, 1H), 7.94 (d, J = 15.7 Hz, 1H), 7.77 (d, J = 15.7 Hz, 1H), 7.74 (m, 2H), 7.08 (s, 1H), 7.05 (d, J = 8.2 Hz, 1H), 6.47 (d, J = 8.2 Hz, 1H), 6.33 (d, J = 9.8 Hz, 1H), 5.48 (d, J = 9.8 Hz, 1H), 5.07 (t, J = 6.7 Hz, 1H), 2.15 (m, 2H), 1.85(m, 2H), 1.63 (s, 3H), 1.47 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 196.7, 194.3, 164.7, 163.5, 155.4, 141.2, 136.2, 134.7, 134.1, 132.8, 128.1, 127.1, 125.4, 123.3, 121.1, 119.1, 113.2, 110.9, 110.1, 81.2, 42.2, 30.1, 27.4, 26.0, 23.4, 18.0. MS (FAB) (m/z) 419 $(M + H)^+$.

Representative Procedure for the Synthesis of 4-Hydroxy-3-(3-(5-hydroxy-2-methyl-2-(4-methylpent-3-enyl)-2*H*-chromen-6-yl)-3-oxopropyl) benzaldehyde (113). To a magnetically stirred

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solution of **67** (50 mg, 0.11 mmol) in methanol (5 mL) was added gradually $NiCl_2 \cdot 6H_2O$ (28 mg, 0.11 mmol) at rt. The whole reaction mixture was brought to 0 °C, and $NaBH_4$ (2 mg, 0.05 mmol) was added portionwise. After addition of $NaBH_4$, the whole solution was stirred for 15 min at 0 °C. Methanol was removed by vacuum, and then the reaction mixture was dissolved in ethyl acetate and neutralized with 10% HCl solution, and the organic layer was washed with water, dried over anhyd Na_2SO_4, and evaporated under reduced pressure. Then the crude product was chromatographed on silica gel to afford the desired compound **113** (30 mg, yield 60%): FT-IR (neat, cm^{-1}) 3434, 2925,2885, 1632, 1489, 1447,1376, 1020, 770, 671. ^1H NMR (CDCl3, 300 MHz) δ 12.38 (s, 1H), 9.87(s, 1H), 7.74 (s, 1H), 7.69 (d, J = 8.2 Hz, 1H), 7.57 (d, J = 8.8 Hz, 1H), 7.04 (d, J = 8.2 Hz, 1H), 6.76 (d, J = 10.0 Hz, 1H), 6.36 (d, J = 8.8 Hz, 1H), 5.56 (d, J = 10.0 Hz, 1H), 5.10 (t,1H), 3.45 (t, J = 5.5 Hz, 2H), 3.10 (t, J = 5.5 Hz, 2H), 2.11 (m, 2H), 1.81 (m, 2H), 1.68 (s, 3H), 1.63 (s, 3H), 1.59 (s, 3H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 204.4, 190.9, 161.0, 160.5, 159.6, 132.4, 131.9, 131.2, 131.1, 128.2, 127.2, 123.6, 117.7, 116.0, 114.0, 112.8, 109.1, 108.8, 80.6, 41.7, 38.9, 27.2, 25.6, 23.2, 22.7, 17.6. MS (FAB) m/z: 421 $(M + H)^+$.

In Vitro Antimalarial Assay. The in vitro antimalarial activity of the compounds was assessed against chloroquine sensitive (CQ) 3D7 strain of *P. falciparum* and compared with that of chloroquine. The schizontocidal activities (MIC) as well as 50% inhibitory concentration (IC_{50}) were obtained as per the method we described earlier,[27,28] respectively. In brief, the parasites were maintained in vitro in RPNI medium[29] supplemented with gentamycin at 40 μg/mL (Sigma), Fungizone at 0.25 μg/mL (Gibco), and 10% fetal bovine serum (pH 7.2) at 37 °C in a CO_2 incubator.

Compounds were dissolved in DMSO at 5 mg/mL, and required dilutions were made in a template plate with RPMI medium. Then 20 μL from each dilution was transferred, in duplicate, in the test plate and two wells receiving 20 μL of vehicle were kept as untreated control. For evaluation of schizontocidal activity, parasite culture was synchronized using 5% D-sorbitol to obtain ring stages only and 180 μL of 3% cell suspension containing 1% parasitized cells was added to each well containing test compounds. The plates were incubated at 37 °C in CO_2 incubator for more than 40 h, after which thin smears were prepared from each well on grease-free glass slides. These were fixed in methanol, stained with Giemsa's stain, and examined under light microscope, 100× oil immersion.

The minimum inhibitory concentration (MIC) of test compound was designated as the minimum concentration required producing 100% inhibition of schizont maturation.

$$\text{percent inhibition of maturation} = \left(\frac{\text{CS} - \text{TS}}{\text{CS}}\right) \times 100$$

CS: Number of schizonts in untreated culture.

TS: Number of schizonts in treated culture.

For evaluation of IC_{50} of the compounds, SYBR Green I-based fluorescence (MSF) assay was used. For the assays, fresh dilutions of all compounds in screening medium were prepared and 50 μL of highest starting concentration was dispensed in duplicate wells in row "B" of 96-well tissue culture plate. Subsequently, 2-fold serial dilutions (seven concentrations) were prepared up to row "H" and finally 50 μL of 2% parasitized cell suspension containing 0.8% parasitemia was added to each well except four wells in row "A" received noninfected cell suspension. After incubating the plates for 72 h, 100 μL of lysis buffer containing 2× concentration of SYBR Green-I was added to each well and incubated for 1 h at 37 °C. The plates were examined for the relative fluorescence units (RFUs) per well using the fluorescence plate reader (FLX800, Biotek). The 50% inhibitory concentration (IC_{50}) was determined using Logit regression analysis of dose–response curves.

Cytotoxicity Assay. Cytotoxicity assays of the compounds were carried out using Vero cell line (C1008; monkey kidney fibroblast).[30] The cells were incubated with different dilutions of test agents for 72 h and MTT was used for detection of cytotoxicity. 50% cytotoxic concentration (CC_{50}) was determined using nonlinear regression analysis. Selectivity index (SI) was calculated as: $SI = CC_{50}/IC_{50}$.

In Vivo Antimalarial Efficacy Assay. The in vivo drug response was evaluated in Swiss mice infected with *P. yoelii* (N-67 strain), which is innately resistant to chloroquine (CQ). Mice (22 ± 2 g) were inoculated with 1 × 106 parasitized RBC on day 0, and treatment was administered to a group of five mice from day 0–3, once daily. The aqueous suspensions of compounds were prepared with a few drops of Tween 80. Initially, the efficacy of test compounds was evaluated at 50 mg/kg/day and the required daily dose was administered in 0.2 mL volume via oral and/or intraperitoneal (IP) route. Parasitemia levels were recorded from thin blood smears between day 4. The mean value determined for a group of five mice was used to calculate the percent suppression of parasitemia with respect to the untreated control group. Animals were followed till day 28 to determine curative effect of the length of survival. Mice treated with chloroquine (CQ) and arteether served as reference controls.[31]

In Vivo Oral Pharmacokinetic Studies. In vivo oral pharmacokinetic study was performed in male Sprague–Dawley rats (n = 5, weight range 220–240 g). Compound to be studied was administered as a single oral dose of 50 mg/kg, and blood samples were collected from the retro-orbital plexus of rats under mild ether anesthesia in microfuge tubes containing heparin as an anticoagulant at 0.25, 0.50, 1, 3, 6, 9, 11, 24, 30, and 48 h postadministration. Plasma was separated and stored frozen at −70 ± 10 °C until analysis. Each plasma sample (100 μL) was processed using a protein precipitation method employing 200 μL of acetonitrile as a protein precipitant, and analysis was done using LC-MS/MS method to get the plasma concentration–time profile. Along with the plasma samples, quality control samples were distributed among calibrators and unknown samples.

Assays with Recombinant Falcipains. Recombinant falcipain-2 (FP-2) and falcipain-3 (FP-3) were produced as described earlier.[38] FP-2 and FP-3 concentrations were determined by active site titration using E64. Briefly, enzymes were incubated with DMSO (1% final) or varying concentrations of E64 in sodium acetate buffer (100 mM sodium acetate, 10 mM DTT, pH 5.5) for 30 min at room temperature, Z-LR-AMC was added (25 μM final) to the reactions, and enzyme activity was determined by monitoring the release of AMC upon substrate hydrolysis over 30 min at 37 °C using a microplate reader (excitation 355 nm; emission 460 nm; SpectraMax M5 (Molecular Devices)). Enzyme activities as the relative fluorescence units/min (RFU) for DMSO and inhibitor-containing reactions were plotted against inhibitor concentrations and analyzed using the ORIGIN program (OriginLab) to determine enzyme concentrations.

To determine if antiparasitic effects of chalcones are due to inhibition of hemoglobin degrading cysteine proteases, compounds were assessed for inhibition of the major hemoglobin degrading cysteine proteases FP2 and FP3. FP2 (1 nM) or FP3 (2 nM) was incubated with DMSO (1%) or with varying concentrations of the compounds in sodium acetate assay buffer for 30 min at room temperature. Z-LR-AMC was added to the reactions (25 μM final), and enzyme activity was measured by monitoring substrate hydrolysis for 30 min at 37 °C as described above. Enzyme activities of inhibitor-containing reactions were compared with those of DMSO-containing reactions, expressed as percent inhibition, and plotted against inhibitor concentrations using nonlinear regression analysis (Graph Pad Prism) to determine IC_{50} values.[32]

Docking of Chromenochalcones to Falcipain-2. Docking of the active compounds **67**, **68**, and **78** to crystal structure of FP-2 (PDB 3BPF) was carried out with FlexX[39] module of Sybyl7.1.[40] FlexX uses incremental approach while docking of compounds. Initially these compounds were sketched and then minimized using 200 steps of steepest descent and finally by conjugate gradient up to convergence. In FlexX active site was constructed by selecting all residues lying within 5 Å radius of reported chalcones binding residues. Total 30 docked conformations were generated for each compound. Final visualization and image processing was done using UCSFchimera1–6[41] and Sybyl7.1

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■ ASSOCIATED CONTENT

Supporting Information

Final compounds characterization data and NMR spectra of the selected chalcones and molecular docking results of **67**, **68**, **74**, **77**, and **78**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

SAR, structure−activity relationship; PK, pharmacokinetics; IP, intraperitoneally; MIC, minimum inhibitory concentration; IC_{50}, half-maximal inhibitory concentration; CQ, chloroquine; CQ^S, chloroquine-sensitive; CQ^R, chloroquine-resistant; EDG's, electron-donating groups; EWG's, electron-withdrawing groups; MOM, methyl-oxy-methyl; ADME, adsorption, distribution, metabolism and excretion; FP, falcipian

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