In Vitro Antimalarial Activity of Chalcones and Their Derivatives[1]

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A series of chalcones and their derivatives have been synthesized and identified as novel potential antimalarials using both molecular modeling and *in vitro* testing against the intact parasite. A large number of chalcones and their derivatives were prepared using one-step Claisen–Schmidt condensations of aldehydes with methyl ketones. These condensates were screened *in vitro* against both chloroquine-sensitive and chloroquine-resistant strains of *Plasmodium falciparum* and shown to be active at concentrations in the nanomolar range. The most active chalcone derivative, 1-(2,5-dichlorophenyl)-3-(4-quinolinyl)-2-propen-1-one (**7**), had an IC_{50} value of 200 nM against both a chloroquine-resistant strain (W2) and a chloroquine-sensitive strain (D6). The resistance indexes for all compounds were substantially lower than for chloroquine, suggesting that this series will be active against chloroquine-resistant malaria. Structure–activity relationships (SAR) of the chalcones in the context of a homology-based model structure of the malaria trophozoite cysteine protease, the most likely target enzyme, are presented.

The World Health Organization estimates that 280 million people are infected with malaria,[2] and approximately one million deaths are reported annually.[3] While various classes of antimalarial agents are available, chloroquine (1, Chart 1) and its derivatives remain the mainstay of therapy against malaria. Unfortunately, the emergence of malarial parasite strains resistant to chloroquine has eroded its efficacy.[4] This increases the urgency of the search for novel and cost-effective agents to treat chloroquine-resistant malaria.

Extensive programs are underway to screen natural products and synthetic derivatives for new agents to treat chloroquine-resistant malaria.[5–8] Except for the natural product artemisinin and its derivatives,[9] most of the agents are chemically related to chloroquine (Chart 1). Pyronaridine (**2**), a (hydroxyanilino)benzonaphthyridine derivative synthesized in 1970, is an antimalarial compound with an acceptable toxicity profile that is effective against *Plasmodium falciparum*.[5] Acridinedione (**3**) and its derivatives have been studied extensively in both rodents and primates.[6] Recently, a series of bisquinoline (**4**) derivatives were reported as potential agents against chloroquine-resistant malaria.[7,8] Although the mechanism of action is unknown, these bisquinolines retain the basic side chain of chloroquine and presumably are concentrated in the parasite's food vacuole and interfere with the heme polymerization process.[10]

Chart 1. Novel Antimalarials Chemically Related to Chloroquine

1, Chloroquine

2, Pyronaridine

3, Acridinedione

4, Bisquinoline

5, Mefloquine

In our previous reports,[11,12] we described a structure-based approach to inhibitor design for antimalarial drug development using models of a malaria trophozoite cysteine protease structure. Here, we report our recent findings of another class of chemical entities, chalcone (1,3-diphenyl-2-propen-1-one) and its derivatives, as potential novel antimalarials that are active against chloroquine-resistant strains of *P. falciparum*. While preparation of this paper was in progress, a paper describing licochalcone A, isolated from Chinese licorice roots, inhibiting the *in vitro* growth of both chloroquine-sensitive and chloroquine-resistant *P. falciparum* strains was published.[13]

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Scheme 1. "Lead" Optimization by Chemical Modification

original lead found by DOCK

(**6**)

vary size of rings
vary size of linker

change to N-containing heterocycles

R_1 = H, OH, OCH_3
R_2 = H, OH, OCH_3
R_3 = H, OH, OCH_3
R_4 = H, Cl
R_5 = H, CH_3
R_6 = H, CH_3, CH_2Ar
X, Y or Z = N or CH

metabolic stability
tighter binding

R_1 = H, Cl, OH, OCH_3
R_2 = H, Cl, OH, OCH_3
R_3 = H, CH_3
R_4 = H, Cl
R_5 = H, OCH_3
R_6 = H, OCH_3, imidazole
X, Y or Z = N or CH

vary size of rings

R_1—R_{10} = H, F, Cl, Br, NO_2, CF_3, OH, OCH_3, $N(CH_3)_2$
R_{11} = H, CH_3

Figure 1. A putative binding orientation of the lead compound bound to the active site of the malarial cysteine protease. Key residues and the binding subsites of the protease are colored as cyan ($S_{1'}$ site), yellow (S_1/catalytic site), and green (S_2/S_3 site); for lead compound, carbon is shown in gray, oxygen in red, and nitrogen in blue.

Design Strategy and Lead Optimization

The erythrocytic malarial life cycle, which is responsible for the majority of the clinical manifestations of malaria, is initiated by the invasion of the host erythrocytes by free merozoites. Erythrocytic trophozoites degrade hemoglobin as a principal source of amino acids. Extensive evidence suggests that malarial cysteine and aspartyl proteases mediate hemoglobin degradation.[14] The cysteine protease inhibitor E-64 [L-*trans*-epoxysuccinylleucylamido(4-guanidino)butane] and the aspartyl protease inhibitor pepstatin have both been shown to block further growth and development of *P. falciparum*.[15-18] Chloroquine evidently does not act by these mechanisms.[10] Thus, the malarial cysteine protease is a promising target for the development of treatments for chloroquine-resistant malaria.

We reported the identification of a lead compound, oxalic bis((2-hydroxy-1-naphthylmethylene) hydrazide) (**6**), as the product of a computational search of a database of commercially available small molecules for compounds that could fit a model of the active site of the malarial cysteine protease as shown in Scheme 1 and Figure 1.[12] The DOCK[19] program placed this lead compound **6** into the enzyme's active site, filling three of the substrate side-chain specificity pockets (subsites S_2, $S_{1'}$, and to a lesser extent S_1). Beginning with compound **6** as shown in Scheme 1, the following chemical modifications were made in an attempt to identify more active agents: (i) The length of the backbone linking the aromatic rings of **6** was shortened via the construction of asymmetric acyl hydrazides, which could have less conformational heterogeneity than the symmetric hydrazides, yet still could fill at least two of the three subsites. Compounds can be constructed by attaching a third aryl group to the center aromatic moiety to fill all three subsites. (ii) Heterocyclic acyl hydrazides were generated by incorporating nitrogen atoms into aromatic rings both to improve water solubility of the compounds and to enhance electrostatic interactions with His67 in the S_2 site. (iii) To increase the chemical/metabolic stability of the compounds,[20] a four-atom hydrazide linker was replaced with a three-atom α,β-unsaturated ketone bridge. (iv) Naphthalene, quinoline, or isoquinoline rings were exchanged for substituted phenyl rings on the α,β-unsaturated ketone backbone to explore the effective size and electronic character of the subsite specificity pocket.

Chemistry and Antimalarial Activity

A procedure based on a Claisen–Schmidt condensation was developed for syntheses of all the chalcones and their derivatives. In a screw-capped vial, substituted aromatic aldehydes and methyl ketones were condensed to form chalcones using solid sodium hydroxide as a catalyst in methanol at room temperature. These conditions were found to be optimal whereas organic bases such as triethylamine or piperidine generally gave lower yields under the same reaction conditions. Sodium methoxide did not work as well as the sodium hydroxide pellet. In most cases, products were formed immediately after addition of the sodium hydroxide pellet to the stirred mixture of aldehyde and

methyl ketone. To ensure the formation of the solid, a minimal amount of methanol must be used. If the starting materials are insoluble in methanol, either tetrahydrofuran (THF) or 1,4-dioxane can be used as cosolvents. Products of the formation of the α,β-unsaturated ketones almost always yielded the *trans*-alkene (*E*-form) as judged by ^{1}H-NMR spectroscopy. Yields ranged from ~55% to quantitative and were not always optimized. α,β-Saturated chalcone derivatives were prepared by catalytic hydrogenation of the corresponding chalcone using Adam's platinum oxide catalyst.[21]

Antimalarial activities of chalcone derivatives were evaluated using an *in vitro* screen based on modifications of the procedures of Desjardins and coworkers.[22-24] The system is limited to the assessment of the intrinsic activity against the erythrocytic asexual life cycle (blood schizontocides). Two *P. falciparum* clones, CDC/Indochina III (W2) and CDC/Sierra Leone I (D6),[25] were used for all assays. W2 is resistant to chloroquine, quinine, and pyrimethamine and susceptible to mefloquine (**5**). D6 is resistant to mefloquine and susceptible to chloroquine, quinine, and pyrimethamine. The resistance indexes are defined as ratios of the IC_{50} of a compound against W2 to the IC_{50} of the same compound against D6. This index is used as a factor to evaluate whether novel antimalarials are potential agents against chloroquine-resistant parasites. Chloroquine and mefloquine were used as controls in the assays.

Structure–Activity Relationships of Chalcones

From the *in vitro* antimalarial results of the first set of chalcone derivatives synthesized, we observed that (i) chalcones with chloro or fluoro substitution on the A ring and with electron-donating substitution (e.g., methoxy, imidazole, etc.) on the B ring have better antimalarial activities (submicromolar IC_{50} as shown in Table 1) and (ii) when the A ring was exchanged with the B ring, antimalarial activity was decreased by 5–10-fold as seen by comparison of either **17** with **24** or **22** with **25** (see Table 1).

On the basis of antimalarial evaluation of over 200 chalcone derivatives, we have concluded the following SAR (see examples in Table 1). (i) In most cases, dichloro or difluoro substitution on the A ring at the 2,3- or 2,4-positions is favored for the activity; monochloro substitution at the *para* position (**13**) also gives rise to higher potencies. (ii) Both chloro-substituted and non substituted quinolinyl groups in the A ring (**7**, **9**, **10**, and **14–16**) yield compounds with good activity no matter which substitution pattern was displayed on the B ring. (iii) Di- or trisubstituted methoxy groups on the B ring increase activity regardless of substitution positions. (iv) The α,β-unsaturated ketone bridge is essential for activity in the series since α,β-saturated compounds 1-(2,4-dimethoxyphenyl)-3-(2,4-dichlorophenyl)-1-propanone (**26**) and 1-(2,4-dimethoxyphenyl)-3-(3,4-difluorophenyl)-1-propanone (**27**), generated from **17** and **23**, respectively, showed a decrease of at least 10-fold in antimalarial activity. At 15 μM, compounds **26** and **27** showed no antimalarial activity against either chloroquine-resistant or chloroquine-sensitive strains. (v) Steric constrants appear to limit substituents in the bridge portion of the chalcone series. Compound **28**, the α-methyl derivative of **17**, showed a 3–8-fold reduction in antimalarial activity for both chloroquine-resistant and chloroquine-sensitive strains. (vi) Consistent with our initial observation, compound **29** with 2,4-dichlorophenyl (A ring) swapped with 2,4-dimethoxyphenyl (B ring) of **28** showed at least a 6-fold decrease in antimalarial activity.

We also tested some of these compounds for inhibition of a mammalian cysteine protease, cathepsin B. The majority of these compounds showed lower inhibition of cathepsin B, with estimated IC_{50} values in the micromolar range, than that of malarial parasites. For example, compound **9** had an estimated IC_{50} of 50 μM against cathepsin B (unpublished results). Three compounds showed submicromolar inhibition against cathepsin B, even though they had lower potency in the antimalarial assay.

Discussion

Even though the specific mechanism of chloroquine in inhibiting malaria parasite growth is not known, it has been generally agreed upon that chloroquine is involved in the heme polymerization process.[10] The observed resistant indexes for our inhibitors are substantially lower than that of chloroquine, suggesting that the mechanism of action for our compounds is different from that of chloroquine. Such results are consistent with proposed mechanism for our inhibitors, inhibition of hemoglobin degradation.

In general, electron-withdrawing groups on the A ring should favor the Michael addition of the chalcones to an available nucleophilic side chain on the enzyme. Although our active chalcones, in most cases, possess an electron-withdrawing A ring (at least relative to a methoxyphenyl B ring), the cysteine protease assay has shown that our chalcones are reversible inhibitors of Cruzain, a parasitic cysteine protease from *Trypanosoma cruzi* with 33% homology to malarial cysteine protease (unpublished results). This allays concerns that our chalcones are potential Michael acceptors of the cysteine protease target, a possible source of toxicity. This is consistent with the observed low inhibition of these inhibitors against a mammalian cysteine protease, cathepsin B.

Chalcones with a three-atom linker share some similarity with the acyl hydrazide series reported earlier.[12] They have roughly the same overall size and contain similar aromatic groups on both ends (i.e., a "barbell" shape). Using the DOCK program, modeling studies suggest that both the acyl hydrazides and chalcones could occupy similar positions in the active site of the malarial cysteine protease and adopt similar binding modes. However, since inhibition of the malarial cysteine protease most likely occurs in the acidic food vacuole of the malaria parasite,[17] the relative acid stability of the α,β-unsaturated ketone linker of chalcones offers an advantage over the more potentially labile hydrazide linker in the acyl hydrazide series.[12] Such an effect could be responsible, at least in part, for the observed higher potency of chalcones relative to the corresponding acyl hydrazides. The α,β-unsaturated ketone linker also provides conjugation between aromatic groups on both ends. The presence of such a conjugative effect has been shown to be critical. Indeed, compounds **26** and **27**, whose α,β-unsaturated ketone linker has been reduced to a saturated ketone linker,

Table 1. Physicochemical and Antimalarial Activity of Chalcones and Their Derivatives

Compounds	A	B	m.p. (°C)	Recryst. Solvents[a]	IC_{50} (μM)[b] W2	IC_{50} (μM)[b] D6	Resistance Indexes[c]
Chloroquine					0.24±0.13 (7)	0.013±0.004 (3)	18
7			160—161	C	0.23±0.01 (3)	0.19±0.01 (3)	1.2
8			108—109	C	0.36±0.22 (3)	0.43±0.34 (3)	0.8
9			150	C	0.51±0.28 (3)	0.72±0.21 (3)	0.7
10			268—270	C	0.71±0.20 (4)	2.53±0.79 (4)	0.3
11			115—116	C	0.75±0.14 (4)	0.68±0.18 (4)	1.1
12			119—120	A	0.75±0.42 (3)	0.92±0.46 (3)	0.8
13			114—115	C	0.77±0.45 (3)	1.24±0.59 (3)	0.6
14			146—148	C	0.80±0.11 (3)	1.69±0.07 (3)	0.5
15			163—165	C	1.00±0.27 (3)	1.29±0.14 (3)	0.8
16			174—175	C	1.00±0.15 (4)	0.98±0.06 (4)	1.0
17			152—153	A	0.90±0.45 (5)	0.77±0.38 (5)	1.2
18			118—119	A	0.92±0.45 (3)	1.10±0.41 (3)	0.8
19			128—129	A	1.14±0.86 (6)	1.96±0.71 (5)	0.6
20			176—177	D	1.18±0.48 (6)	0.90±0.25 (5)	1.3
21			144—145	A	1.38±0.79 (5)	1.29±0.75 (5)	1.1
22			107—108	E	1.90±1.12 (5)	1.95±1.07 (5)	1.0
23			132—133	B	2.09±0.98 (5)	2.10±0.79 (5)	1.0
24			108—109	A	3.8 (1)	3.4 (1)	1.1
25			109—111	A	11.1 (1)	19.1 (1)	0.6
26			97—99	e	>15 (NI[d])	>15 (NI)	—
27			65—66	e	>16 (NI)	>16 (NI)	—
28			oil	e	2.46±0.16 (3)	6.26±0.89 (3)	0.4
29			109—110	A	>14 (NI)	>14 (NI)	—

[a] Recrystallization solvents: (A) CH_3OH/CH_3CN (1:1); (B) CH_3OH/DMF (2:1); (C) CH_3OH; (D) CH_3OH/CH_3CN (1:4); (E) CH_3OH/CH_3CN/DMF (2:2:1). [b] Number of experiments in parentheses. [c] IC_{50} of W2/IC_{50} of D6. [d] NI: no inhibition. [e] Purified by column chromatography (eluent, CH_2Cl_2).

show dramatically lower potencies against the malaria parasites (IC_{50} > 15 μM) when compared to their chalcone counterparts, compounds **17** and **23**. From the DOCK program and modeling studies, it appears that the chalcones are more rigid and adopt a more extended conformation because of the conjugated linker. This generates a linear, nearly planar structure which evidently fits well into the long cleft of the active site of

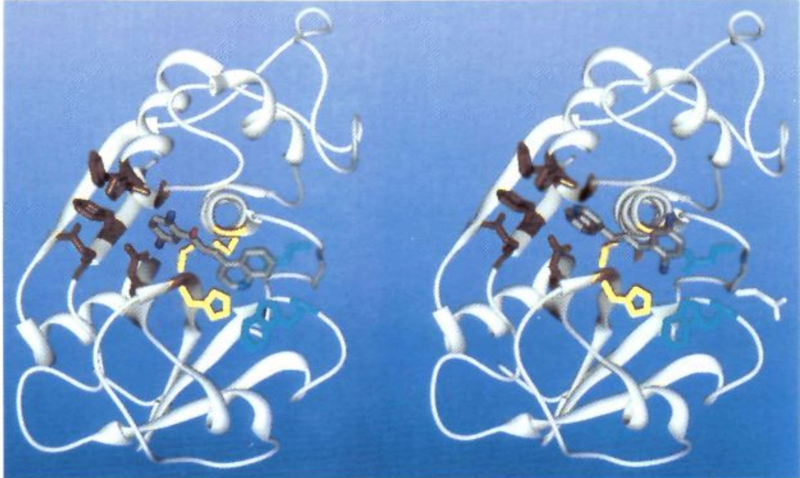

Figure 2. Two potential binding orientations of compound **7** in the active site of the malarial cysteine protease based on DOCK studies. Color schemes are the same as described in Figure 1 with additional color purple for chlorine.

the malarial cysteine protease. However, its saturated counterpart would be expected to adopt a less extended conformation because of the sp^3 hybridization of the two saturated carbons in the linker, thereby likely giving rise to a less favorable interaction with the protease. Saturated analogues may also suffer an entropic penalty upon binding to the protease due to loss of rotational freedom about the carbon–carbon single bond.

The differential inhibition specificities exhibited by these compounds against the malarial cysteine protease (unpublished results) and mammalian cysteine protease cathepsin B is consistent with the structural difference between these two proteases and the results of DOCK studies. The DOCK studies suggest that these compounds bind to subsites $S_{1'}$ through S_3 of the proteases. Key residues in the S_2/S_3 site are not well conserved, resulting in a substantial difference in the inhibitor binding specificity between these two proteases.

The quinolinyl group in the A ring of chalcones probably increases antimalarial activity for several reasons. (i) Protonation of these nitrogen-containing heterocycles under weakly acidic conditions may enhance their interactions with the protease at His67 in the active sites or else protonated His67 (free histidine has a pK_a of ~6[26]) may form a hydrogen-bond with the inhibitors. (ii) These nitrogen-containing heterocycles may be somewhat concentrated in the food vacuoles of malarial parasites at pH ~5 since pK_a values of quinoline and isoquinoline are 4.94 and 5.40 in water at 20 °C, respectively.[27] On the basis of DOCK results, the two most likely binding orientations of compound **7**, the best inhibitor in the series, are shown in Figure 2.

In conclusion, we have identified novel potential antimalarials using the malarial cysteine protease model as a target. Antimalarials in this study should be inexpensive to produce using the convenient chemistry that we have developed. Cost of production is a critically important consideration if the resulting compounds are ever to be developed into therapeutic agents for the world's developing countries. Our preliminary SAR data and molecular efforts will further direct efforts to find even more potent antimalarials in the future. According to our SAR data, for example, we might expect those chalcone derivatives with hydroxyl functionality on the B ring and with some other appropriate substitutions on the A rings to be even better antimalarials. Recently, we have begun development of methodologies for the solid-phase synthesis of chalcone analogue libraries and will report the results of these studies at a later date.

Experimental Section

Melting points were measured on a Thomas-Hoover Unimelt apparatus and are uncorrected. Thin-layer chromatography (TLC, silica gel 60 GF_{254}, Merck, Darmstadt) was used to monitor reactions and check product homogeneity. Nuclear magnetic resonance (NMR) spectra at 300 M Hz (tetramethylsilane as internal standard) were recorded on a General Electric QE-300 spectrometer. LSIMS (liquid secondary ion mass spectrometry) and CIMS (chemical ionization mass spectrometry) spectra were obtained at the UCSF Mass Spectrometry Facility, A. L. Burlingame, Director. Elemental analyses were performed by University of California, Berkeley, Microanalytical Laboratory and were within ±0.4% of the theoretical values. All starting materials were purchased from Aldrich Chemical Company, Inc.

General Procedures To Prepare Chalcone Derivatives 7–25, 28, and 29. In a screw-capped vial (normally a glass scintillation counting vial), a substituted aldehyde (1 mmol) and a substituted methyl ketone (1 mmol) were dissolved in a minimum amount of methanol (normally 2–4 mL) with stirring. Into it was added a single NaOH pellet (about 100 mg). In most cases, off-white to bright yellow solids were formed within a few minutes to 24 h. The solids were collected on a filter and washed three times with cold methanol. The product was recrystallized from appropriate solvents whenever necessary. Column chromatography was used to purify the product if the condensation yielded an oil instead of a solid.

General Procedures To Prepare α,β-Saturated Chalcone Derivatives 26 and 27. In a reaction bottle of the hydrogenation apparatus was placed a solution of the chalcone derivative (1.0 g) in ethyl acetate (15 mL), and 20 mg of Adam's platinum oxide catalyst was then added. The air in the apparatus was displaced with hydrogen (~25 psi), and the mixture was shaken for about 2 h or until hydrogen was no longer being consumed. The platinum was filtered off, and solvent was removed under reduced pressure. The product was obtained by column chromatography (TLC grade silica gel/ CH_2Cl_2). The yield of pure product ranged from 72% to 78%.

1-(2,5-Dichlorophenyl)-3-(4-quinolinyl)-2-propen-1-one (7): 180 mg, 55% yield; ^1H NMR ($CDCl_3$) δ 6.72–9.43 (m, 11H); LSIMS m/z (M + H) 328.1; HR EIMS calcd for $C_{18}H_{11}NClO$ 327.0218, found 327.0208.

2,4-Dichloro-2′,3′,4′-trimethoxychalcone (8): 316 mg, 86% yield; ^1H NMR ($CDCl_3$) δ 3.88 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 6.74 (d, J = 8.8 Hz, 1H), 7.25 (d, J = 7.9 Hz, 1H), 7.42–

7.62 (m, 3H), 7.64 (d, J = 8.5 Hz, 1H), 7.96 (d, J = 15.8 Hz, 1H, β-H); LSIMS m/z (M + H) 367.0. Anal. ($C_{18}H_{16}Cl_2O_4$) C, H.

1-(3,4-Dimethoxyphenyl)-3-(3-(2-chloroquinolinyl))-2-propen-1-one (9): 129 mg, 68% yield; ^{1}H NMR ($CDCl_3$) δ 4.02 (s, 6H, 2OCH$_3$), 6.99 (d, J = 8.4 Hz, 1H, 5'-H), 7.63–8.19 (m, 7H), 8.20 (d, J = 15.7 Hz, 1H, β-H), 8.52 (s, 1H, 4-H); LSIMS m/z (M + H) 354.1. HR EIMS calcd for $C_{20}H_{16}NClO_3$, 353.0819; found, 353.0807. Anal. ($C_{20}H_{16}NClO_3{\cdot}H_2O$) C, H, N; H: calcd, 4.88; found, 4.29.

1-(3-Pyridinyl)-3-(3-(2-chloroquinolinyl))-2-propen-1-one (10): 102 mg, 56% yield; ^{1}H NMR ($CDCl_3$) δ 6.72–9.14 (m, 9H), 8.07 (d, J = 15.2 Hz, 1H, β-H); LSIMS m/z (M + H) 295.1. Anal. ($C_{17}H_{11}N_2ClO$) C, H, N.

4-(Trifluoromethyl)-2',4'-dimethoxychalcone (11): 201 mg, 60% yield; ^{1}H NMR ($CDCl_3$) δ 3.90 (s, 3H, OCH$_3$), 3.94 (s, 3H, OCH$_3$), 6.30 (d, J = 2.2 Hz, 1H, 3'-H), 6.52 (dd, J = 2.1 and 8.8 Hz, 1H, 5'-H), 7.13 (d, J = 8.0 Hz, 2H), 7.42 (d, J = 8.1 Hz, 2H), 7.69 (d, J = 15.5 Hz, 1H, α-H), 7.73 (d, J = 15.9 Hz, 1H, β-H), 7.82 (d, J = 8.7 Hz, 1H, 6'-H); LSIMS m/z (M + H) 237.1. Anal. ($C_{18}H_{15}F_3O_3$) C, H.

2,4-Dichloro-2',5'-dimethoxychalcone (12): 283 mg, 84% yield; ^{1}H NMR ($CDCl_3$) δ 3.78 (s, 3H, OCH$_3$), 3.84 (s, 3H, OCH$_3$), 6.91 (d, J = 9.0 Hz, 1H), 7.00–7.42 (m, 5H), 7.76 (d, J = 8.5 Hz, 1H), 7.93 (d, J = 15.9 Hz, 1H, β-H); LSIMS m/z (M + H) 337.2. Anal. ($C_{17}H_{14}Cl_2O_3$) C, H.

4-Chloro-2',5'-dimethoxychalcone (13): 248 mg, 82% yield; ^{1}H NMR ($CDCl_3$) δ 3.75 (s, 6H, 2OCH$_3$), 6.59 (d, J = 8.5 Hz, 2H), 6.89 (d, J = 16.1 Hz, 1H, α-H), 7.22–7.41 (m, 4H), 7.42 (d, J = 8.5 Hz, 2H); LSIMS m/z (M + H) 303.1. Anal. ($C_{17}H_{15}ClO_3$) C, H.

1-(3,4-Dichlorophenyl)-3-(4-quinolinyl)-2-propen-1-one (14): 200 mg, 61% yield; ^{1}H NMR ($CDCl_3$) δ 6.71–8.75 (m, 10H), 8.77 (d, J = 4.5 Hz, 1H, 2-H); LSIMS m/z (M + H) 328.1. Anal. ($C_{18}H_{11}NClO$) C, H, N.

1-(2,4-Dimethoxyphenyl)-3-(3-(2-chloroquinolinyl))-2-propen-1-one (15): 140 mg, 73% yield; ^{1}H NMR ($CDCl_3$) δ 3.89 (s, 3H, OCH$_3$), 3.93 (s, 3H, OCH$_3$), 6.54 (d, J = 2.1 Hz, 1H, 3'-H), 6.61 (dd, J = 2.2 and 8.6 Hz, 1H, 5'-H), 7.59–8.03 (m, 6H), 8.04 (d, J = 15.7 Hz, 1H, β-H), 8.42 (s, 1H, 4-H); LSIMS m/z (M + H) 354.1. Anal. ($C_{20}H_{16}NClO_3$) C, H, N.

1-(2,4-Difluorophenyl)-3-(3-(2-chloroquinolinyl))-2-propen-1-one (16): 109 mg, 57% yield; ^{1}H NMR ($CDCl_3$) δ 6.84 (d, J = 2.0 Hz, 1H, 3'-H), 6.87 (dd, J = 2.0 and 9.1 Hz, 1H, 5'-H), 7.49–8.25 (m, 6H), 8.26 (d, J = 15.7 Hz, 1 H, β-H), 8.50 (s, 1H, 4-H); LSIMS m/z (M + H) 330.1. Anal. ($C_{18}H_{10}NClF_2O$) C, H, N.

2,4-Dichloro-2',4'-dimethoxychalcone (17): 280 mg, 83% yield; ^{1}H NMR (DMSO-d_6) δ 3.86 (s, 3H, OCH$_3$), 3.90 (s, 3H, OCH$_3$), 6.65 (d, J = 8.1 Hz, 1H, 5'-H), 6.69 (br s, 1H, 3'-H), 7.49 (d, J = 8.1 Hz, 1H, 5-H), 7.62 (d, J = 15.6 Hz, 1H, α-H), 7.64–7.73 (m, 2H), 7.75 (d, J = 15.8 Hz, 1H, β-H), 7.96 (d, J = 8.5 Hz, 1H, 6'-H); LSIMS m/z (M + H) 337.0. Anal. ($C_{17}H_{14}Cl_2O_3$) C, H.

2,3-Dichloro-2',5'-dimethoxychalcone (18): 263 mg, 78% yield; ^{1}H NMR ($CDCl_3$) δ 3.79 (s, 3H, OCH$_3$), 3.84 (s, 3H, OCH$_3$), 6.92 (d, J = 9.0 Hz, 1H), 7.03d (dd, J = 3.1 and 9.0 Hz, 1H), 7.18–7.23 (m, 2H), 7.36 (d, J = 15.9 Hz, 1H, α-H), 7.45 (d, J = 7.8 Hz, 1H), 7.57 (d, J = 8.0 Hz, 1H), 8.00 (d, J = 15.8 Hz, 1H, β-H); LSIMS m/z (M + H) 337.2. Anal. ($C_{17}H_{14}Cl_2O_3$) C, H.

2,3-Dichloro-3',4'-dimethoxychalcone (19): 273 mg, 81% yield; ^{1}H NMR ($CDCl_3$) δ 3.97 (s, 6H, 2OCH$_3$), 6.93 (d, J = 8.3 Hz, 1H, 5'-H), 7.26 (dd, J = 7.9 Hz, 1H, 5-H), 7.44–7.68 (m, 5H), 8.14 (d, J = 15.7 Hz, 1H, β-H); CIMS m/z (M + H) 337.0. Anal. ($C_{17}H_{14}Cl_2O_3$) C, H.

2,4-Dichloro-4'-imidazol-1-ylchalcone (20): 220 mg, 64% yield; ^{1}H NMR ($CDCl_3$) δ 7.26–7.50 (m, 4H), 7.47 (d, J = 15.5 Hz, 1H, α-H), 7.55 (d, J = 8.5 Hz, 2H, 3'- and 5'-H), 7.71 (d, J = 8.4 Hz, 1H, 5-H), 7.98 (s, 1H), 8.14 (d, J = 8.6 Hz, 2H, 2'- and 6'-H), 8.15 (d, J = 15.5 Hz, 1H, β-H); LSIMS m/z (M + H) 343.0. Anal. ($C_{18}H_{12}N_2Cl_2O$) C, H, N.

2,3-Dichloro-2',4'-dimethoxychalcone (21): 280 mg, 83% yield; ^{1}H NMR (DMSO-d_6) δ 3.86 (s, 3H, OCH$_3$), 3.91 (s, 3H, OCH$_3$), 6.66 (dd, J = 2.0 and 8.8 Hz, 1H, 5'-H), 6.69 (d, J = 1.8 Hz, 1H, 3'-H), 7.44 (dd, J = 8.0 Hz, 1H, 5-H), 7.56–7.72 (m, 3H), 7.82 (d, J = 15.7 Hz, 1H, β-H), 7.89 (d, J = 7.9 Hz, 1H, 6-H); LSIMS m/z (M + H) 337.0. Anal. ($C_{17}H_{14}Cl_2O_3$) C, H.

2,4-Difluoro-2',4'-dimethoxychalcone (22): 303 mg, 100% yield; ^{1}H NMR ($CDCl_3$) δ 3.88 (s, 3H, OCH$_3$), 3.91 (s, 3H, OCH$_3$), 6.50 (d, J = 2.0 Hz, 1H, 3'-H), 6.57 (dd, J = 2.1 and 8.6 Hz, 1H, 5'-H), 6.83–7.63 (m, 3H), 7.57 (d, J = 15.9 Hz, 1H, α-H), 7.73 (d, J = 16.0 Hz, 1H, β-H), 7.79 (d, J = 8.7 Hz, 1H, 6'-H); LSIMS m/z (M + H) 305.0. Anal. ($C_{17}H_{14}F_2O_3$) C, H.

3,4-Difluoro-2',4'-dimethoxychalcone (23): 305 mg, 87% yield; ^{1}H NMR ($CDCl_3$) δ 3.88 (s, 3H, OCH$_3$), 3.92 (s, 3H, OCH$_3$), 6.50 (d, J = 2.0 Hz, 1H, 3'-H), 6.58 (dd, J = 2.1 and 8.7 Hz, 1H, 5'-H), 7.16–7.45 (m, 3H), 7.45 (d, J = 15.9 Hz, 1H, α-H), 7.58 (d, J = 15.7 Hz, 1H, β-H), 7.79 (d, J = 8.7 Hz, 1H, 6'-H); CIMS m/z (M + H) 305.0. Anal. ($C_{17}H_{14}F_2O_3$) C, H.

2,3-Dimethoxy-2',4'-dichlorochalcone (24): 210 mg, 62% yield; ^{1}H NMR ($CDCl_3$) δ 3.85 (s, 6H, 2OCH$_3$), 6.44 (d, J = 2.1 Hz, 1H, 3-H), 6.52 (dd, J = 2.1 and 8.6 Hz, 1H, 5-H), 7.12 (d, J = 16.1 Hz, 1H, α-H), 7.32–7.51 (m, 4H), 7.70 (d, J = 16.1 Hz, 1H, β-H); LSIMS m/z (M + H) 337.0. Anal. ($C_{17}H_{14}Cl_2O_3$) C, H.

2,4-Dimethoxy-2',4'-difluorochalcone (25): 170 mg, 56% yield; ^{1}H NMR ($CDCl_3$) δ 3.88 (s, 3H, OCH$_3$), 3.89 (s, 3H, OCH$_3$), 6.46 (br s, 1H, 3-H), 6.53 (dd, J = 1.6 and 8.5 Hz, 1H, 5-H), 6.85–7.40 (m, 3H), 7.55 (d, J = 8.6 Hz, 1H, 6-H), 7.82–7.90 (m, 1H), 8.02 (d, J = 15.8 Hz, 1H, β-H); LSIMS m/z (M + H) 305.1. Anal. ($C_{17}H_{14}Fl_2O_3$) C, H.

1-(2,4-Dimethoxyphenyl)-3-(2,4-dichlorophenyl)-1-propanone (26): 78% yield; ^{1}H NMR ($CDCl_3$) δ 3.18 (t, J = 6.9 Hz, 2H, CH$_2$), 3.28 (t, J = 6.9 Hz, 2H, CH$_2$), 3.85 (s, 6H, 2OCH$_3$), 6.44 (d, J = 2.2 Hz, 1H, 3'-H), 6.53 (dd, J = 2.2 and 8.7 Hz, 1H, 5'-H), 7.08–7.21 (m, 2H), 7.31 (dd, J = 1.5 and 7.9 Hz, 1H, 6-H), 7.82 (d, J = 8.7 Hz, 1H, 6'-H); LSIMS m/z (M + H) 339.1. Anal. ($C_{17}H_{16}Cl_2O_3$) C, H.

1-(2,4-Dimethoxyphenyl)-3-(3,4-difluorophenyl)-1-propanone (27): 72% yield; ^{1}H NMR ($CDCl_3$) δ 2.97 (t, J = 7.49 Hz, 2H, CH$_2$), 3.25 (t, J = 7.49 Hz, 2H, CH$_2$), 3.86 (s, 3H, OCH$_3$), 3.88 (s, 3H, OCH$_3$), 6.46 (d, J = 2.1 Hz, 1H, 3'-H), 6.54 (dd, J = 2.2 and 8.7 Hz, 1H, 5'-H), 7.00–7.07 (m, 3H), 7.82 (d, J = 8.7 Hz, 1H, 6'-H); LSIMS m/z (M + H) 307.1. Anal. ($C_{17}H_{16}F_2O_3$) C, H.

1-(2,4-Dimethoxyphenyl)-3-(2,4-dichlorophenyl)-2-methyl-2-propen-1-one (28): 210 mg, 60% yield; ^{1}H NMR ($CDCl_3$) δ 2.06 (s, 3H, CH$_3$), 3.79 (s, 3H, OCH$_3$), 3.86 (s, 3H, OCH$_3$), 6.49 (d, J = 1.8 Hz, 1H, 3'-H), 6.53 (dd, J = 2.0 and 8.4 Hz, 1H, 5'-H), 7.13 (s, 1H, α-H), 7.28–7.42 (m, 4H); LSIMS m/z (M + H) 351.0. Anal. ($C_{18}H_{16}Cl_2O_3$) C, H.

1-(2,4-Dichlorophenyl)-3-(2,4-dimethoxyphenyl)-2-methyl-2-propen-1-one (29): 204 mg, 58% yield; ^{1}H NMR ($CDCl_3$) δ 2.21 (s, 3H, CH$_3$), 3.82 (s, 3H, OCH$_3$), 3.88 (s, 3H, OCH$_3$), 6.49 (d, J = 8.8 Hz, 1H), 6.58 (dd, J = 2.1 and 8.6 Hz, 1H), 7.40–7.60 (m, 4H), 7.88 (d, J = 1.3 Hz, 1H); LSIMS m/z (M + H) 351.0. Anal. ($C_{18}H_{16}Cl_2O_3$) C, H.

***In Vitro* Antimalarial Testing.** The detailed protocol for *in vitro* antimalarial testing has been published previously.[28] Briefly, test compounds were initially dissolved in DMSO and diluted 400-fold in RPMI 1640 culture medium supplemented with 25 mM Hepes buffer, 32 mM $NaHCO_3$, and 10% human plasma. These solutions were subsequently serially diluted 2-fold with a Biomeck 1000 instrument (Beckman, Fullerton, CA) over 11 different concentrations. The parasites were pre-exposed to the compounds for 48 h and incubated at 37 °C with 5% O_2, 5% CO_2, and 90% N_2 prior to the addition of [^{3}H]-hypoxanthine. After a further incubation for 18 h, parasite DNA was harvested from each microtiter well using a MACH II cell harvester (Tomtec, Orange, CT) onto glass filters. Uptake of [^{3}H]hypoxanthine was measured with a 1205 Betaplate liquid scintillation counter (Wallac, Turku, Finland). Concentration–response data were analyzed by a logistic dose–response model, and the IC_{50} values (50% inhibitory concentration) for each compound were calculated. Results are shown in Table 1.

The activity of the cysteine proteases (cruzain and cathepsin B) was assayed by the liberation of the fluorescent leaving

group, 7-amino-4-methylcoumarin (AMC, Enzyme Systems Products, Livermore, CA) from the peptide substrates Z-Phe-Arg-AMC or Z-Arg-Arg-AMC (where Z is benzyloxycarbonyl), upon incubation with the enzyme at 25 °C in 0.1 M sodium acetate, pH 5.5, 5 mM DTT. The assay was adapted to microtiter plates and carried out on an Automated Fluoroskan II (Lab Systems) spectrofluorometer for high throughput.

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References

(1) This work was presented in part at the 210th American Chemical Society National Meeting, MEDI 216, August 20–24, 1995, Chicago, IL.

(2) Gibbons, A. Researchers Fret over Neglect of 600 Million Patients. *Science* **1992**, *256*, 1135.

(3) Walsh, J. A. Disease Problem in the Third World. *Ann. N. Y. Acad. Sci.* **1989**, *569*, 1–16.

(4) World Health Organization Malaria Action Program. Severe and Complicated Malaria. *Trans. R. Trop. Med. Hyg.* **1986**, *80*, 1–50.

(5) Chen, C.; Tang, L. H.; Chun, J. Studies on a New Antimalarial Compound: Pyronaridine. *Trans. R. Soc. Trop. Med. Hyg.* **1992**, *86*, 7–10.

(6) Kesten, S. J.; Cegnan, M. J.; Hung, J.; McNamara, D. J.; Ortwine, D. F.; Uhlendorf, S. E.; Werbel, L. M. Synthesis and Antimalarial Properties of 1-Imino Derivatives of 7-Chloro-3-substituted-3,4-dihydro-1,9(2*H*,10*H*)-acridinediones and Related Structures. *J. Med. Chem.* **1992**, 3429–3447.

(7) Vennerstrom, J. L.; Ellis, W. Y.; Ager, Jr., A. L.; Andersen, S. L.; Gerena, L.; Milhous, W. K. Bisquinolines. 1. *N,N*-Bis(7-chloroquinolin-4-yl)alkanediamines with Potential against Chloquine-Resistant Malaria. *J. Med. Chem.* **1992**, *35*, 2129–2134.

(8) Raynes, K.; Galatis, D.; Cowman, A. F.; Tilley, L.; Deady, L. W. Synthesis and Activity of Some Antimalarial Bisquinolines. *J. Med. Chem.* **1995**, *38*, 204–206.

(9) Acton, N.; Karle, J. M.; Miller, R. E. Synthesis and Antimalarial Activity of Some 9-Substituted Artemisinin Derivatives. *J. Med. Chem.* **1993**, *36*, 2552–2557.

(10) Dorn, A.; Stoffel, R.; Matile, H.; Bubendorf, A.; Ridley, R. G. Malarial Haemozoin/β-haematin Supports Haem Polymerization in the Absence of Protein. *Nature* **1995**, *374*, 269–271.

(11) Ring, C. S.; Sun, E.; McKerrow, J. H.; Lee, G.; Rosenthal, P. J.; Kuntz, I. D.; Cohen, F. E. Structure-based Inhibitor Design by Using Protein Models for the Development of Antiparasitic Agents. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 3583–3587.

(12) Li, Z.; Chen, X.; Davidson, E.; Zwang, O.; Mendis, C.; Ring, C. S.; Roush, W. R.; Fegley, G.; Li, R.; Rosenthal, P. J.; Lee, G. K.; Kenyon, G. L.; Kuntz, I. D.; Cohen, F. E. Anti-malarial Drug Development Using Models of Enzyme Structure. *Chem. Biol.* **1994**, *1*, 31–37.

(13) Chen, M.; Theander, T. G.; Christensen, S. B.; Hviid, L.; Zhai, L.; Kharazmi, A. 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.* **1994**, *38*, 1470–1475.

(14) McKerrow, J. H.; Sun, E.; Rosenthal, P. J.; Bouvier, J. The Proteases and Pathogenicity of Parasitic Protozoa. *Annu. Rev. Microbiol.* **1993**, *47*, 821–853.

(15) Bailly, E.; Jambou, R.; Savel, J.; Jaureguiberry, G. *Plasmodium falciparum:* Different Sensitivity *in vitro* to E-64 (Cysteine Protease Inhibitor) and Pepstatin A (Aspartyl Protease Inhibitor). *J. Protozool.* **1992**, *39*, 593–599.

(16) Dluzewski, A. R.; Rangachari, K.; Wilson, R. J. M.; Gratzer, W. B. *Plasmodium falciparum:* Protease Inhibitors and Inhibition of Erythrocyte Invasion. *Exp. Parasitol.* **1986**, *62*, 416–422.

(17) (a) Rosenthal, P. J.; McKerrow, J. H.; Aikawa, M.; Nagasawa, H.; Leech, J. H. A Malarial Cysteine Protease is Necessary for Hemoglobin Degradation by *Plasmodium falciparum. J. Clin. Invest.* **1988**, *82*, 1560–1566. (b) Rosenthal, P. J.; Lee, G. K.; Smith, R. E. Inhibition of a *Plasmodium vinckei* Cysteine Proteinase Cures Murine Malaria. *J. Clin. Invest.* **1993**, *91*, 1052–1056.

(18) Vander Jagt, D. L.; Hunsaker, L. A.; Campos, N. M. Characterization of a Hemoglobin-degrading, Low Molecular Weight Protease from *Plasmodium falciparum. Mol. Biochem. Parasitol.* **1986**, *18*, 389–400.

(19) Kuntz, I. D. Structure-based Strategies for Drug Design and Discovery. *Science* **1992**, *257*, 1078–1082.

(20) The chemical stability of hydrazides vs chalcones was determined using UV spectroscopy. A UV spectrum of either (2′,4′-dihydroxyphenyl)acyl (2-hydroxy-1-naphthylmethylene) hydrazide (**ZLIV44A**) or chalcone **9** in methanol containing 1% concentrated HCl (pH ~0.4) at room temperature was recorded over time. The half-life ($t_{1/2}$) of **ZLIV44A** is ~20 min while the UV spectrum of chalcone **9** remains unchanged for 13 h under the same conditions.

(21) *Vogel's Textbook of Practical Organic Chemistry*, 4th ed.; Furniss, B. S., Hannaford, A. J., Rogers, V., Smith, P. W. G., Tatchell, A. R., Eds.; Longman Group Limited: New York, 1978; p 796.

(22) Desjardins, R. E.; Canfield, C. J.; Haynes, D. E.; Chulay, J. D. Quantitative Assessment of Antimalarial Activity *In Vitro* by a Semiautomated Microdilution Technique. *Antimicrob. Agents Chemother.* **1979**, *16*, 710–718.

(23) Chulay, J. D.; Haynes, J. D.; Diggs, C. L. *Plasmodium falciparum:* Assessment of *In Vitro* Growth by [^{3}H]-hypoxathine Incorporation. *Exp. Parasitol.* **1983**, *55*, 138–146.

(24) Milhous, W. K.; Weatherly, N. F.; Bowdre, J. H.; Desjardins, R. E. *In Vitro* Activities of and Mechanisms of Resistance to Antifol Malarials. *Antimicrob. Agents Chemother.* **1985**, *27*, 525–530.

(25) Oduola, A. M.; Weatherly, N. F.; Bowdre, J. H.; Desjardins, R. E. *Plasmodium falciparum:* Cloning by Single-erythrocyte Micromanipulation and Heterogeneity *In Vitro. Exp. Parasitol.* **1988**, *66*, 86–95.

(26) *The Merck Index*, 11th ed.; Budavari, S., O'Neil, M. J., Smith, A., Heckelman, P. E., Eds.; Merck & Co. Inc.: Rahway, NJ, 1989; p 746.

(27) Dyke, S. F.; Kinsman, R. G. Properties and Reactions of Isoquinoline and Their Hydrogenated Derivatives. In *Heterocyclic Compounds Isoquinolines;* Grethe, G., Eds.; John Wiley & Sons: New York, 1981; pp 3–10.

(28) Lin, A. J.; Miller, R. E. Antimalarial Activity of New Dihydroartemisinin Derivatives. 6. α-Alkylbenzylic Ethers. *J. Med. Chem.* **1995**, *38*, 764–770.

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