Antimalarial Alkoxylated and Hydroxylated Chalones: Structure−Activity Relationship Analysis

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Chalcones with 2′,3′,4′-trimethoxy, 2′,4′-dimethoxy, 4′-methoxy, 4′-ethoxy, 2′,4′-dihydroxy, and 4′-hydroxy groups on ring B were synthesized and evaluated in vitro against *Plasmodium falciparum* (K1) in a [^{3}H] hypoxanthine uptake assay. The other ring A was quinoline, pyridine, naphthalene, or phenyl rings with electron-donating or electron-withdrawing substituents of varying lipophilicities. Trimethoxy **6** and **27**, dimethoxy **7**, **8**, **29**, and methoxy **31** analogues had good in vitro activities ($IC_{50} < 5\ \mu M$). 3-Quinolinyl ring A derivatives were well represented among the active compounds. Hydroxylated chalcones were less active than the corresponding alkoxylated analogues. When evaluated in vivo, **8** and **208** were comparable to chloroquine in extending the lifespan of infected mice. Multivariate data analysis showed that in vitro activity was mainly determined by the properties of ring B. Quantitative structure−activity relationship models with satisfactory predictive ability were obtained for various B ring chalcones using projections to latent structures. A model with good predictability was proposed for 19 active chalcones. Size and hydrophobicity were identified as critical parameters.

Introduction

Despite years of continual effort, malaria is still one of the most deadly diseases affecting third-world countries, claiming more than 1 million lives annually.[1] The Roll Back Malaria initiative recently established by WHO aims to combat the disease through effective global partnership and cooperation.[2] A major thrust in this initiative is the identification of new targets that are critical to the disease process or essential for the survival of the parasite. The design of novel chemical entities specifically affecting these targets could lead to the availability of better drugs for the treatment of malaria. More recently, there has been strong interest in the potential antimalarial activity of chalcones.[3−6] Chemically, chalcone is 1,3-diphenyl-2-propen-1-one. Depending on the substitution pattern on the two aromatic rings, a wide range of pharmacological activities have been identified for various chalcones. These include antileishmanial,[7] antiinflammatory,[8] antimitotic,[9] and modulation of P-glycoprotein-mediated multidrug resistance.[10]

The antimalarial activity of chalcones was first noted when licochalcone A, a natural product isolated from Chinese liquorice roots, was reported to exhibit potent in vivo and in vitro antimalarial activity.[3] Subsequently, a synthetic analogue, 2,4-dimethoxy-4′butoxychalcone, was reported to have outstanding antimalarial activity.[4] Antimalarial chalcones are widely thought to act against malarial cysteine protease,[5] an enzyme used by the parasite for the degradation of host hemoglobin for its nutritional purposes. Chalcones are very readily synthesized, and various substitution patterns can be attempted on the two aromatic rings to give a large number of potential analogues. A sound understanding of the structural requirements for antimalarial activity in the chalcones is important in guiding and optimizing drug design efforts. However, a review of the literature has shown that no comprehensive structure−activity relationships of chalcones has been reported.

In the present study, a series of 62 ring B alkoxylated and 30 ring B hydroxylated chalcones have been synthesized and evaluated for in vitro antimalarial activity. Selected members were also tested in vivo in *Plasmodium berghei* infected mice. The aim of the study is to derive predictive structure−activity relationships (SAR) based on relevant physicochemical descriptors of the compounds that would be of value in guiding lead compound design. The projections to latent structures (PLS) method was applied to find relationships between in vitro activity and the physicochemical data.

Chemistry and Drug Design

The alkoxylated chalcones were synthesized by a base-catalyzed Claisen Schmidt condensation of an aromatic aldehyde with the appropriate acetophenone (Scheme 1). 2,4-Dimethoxy-4′-butoxychalcone (**41**) was also synthesized as a standard compound for comparison of antimalarial activity because it has been reported to possess outstanding antimalarial activities against both human (in vitro) and rodent (in vivo) parasites.[4]

For the synthesis of the hydroxylated chalcones, protection of the phenolic groups on the acetophenone is necessary for improved product yields. A representative reaction sequence starting with 4-hydroxyacetophenone is shown in Scheme 2. The hydroxyl group on the acetophenone is protected with 2*H*-3,4-dihydropyran,[11] is reacted with aldehyde as described earlier, and is subsequently removed by acid hydrolysis[7] to give the chalcone, which is purified by chromatography and

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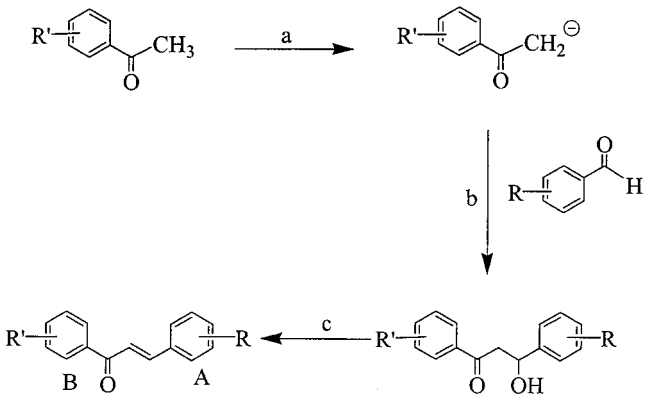
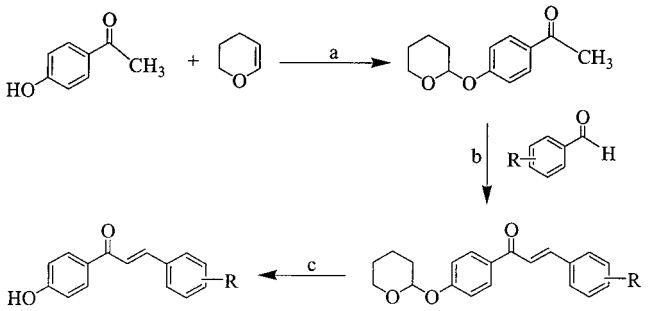
Scheme 1. General Method for the Synthesis of Alkoxylated Chalcones[a]



[a] R′ = 2′,3′,4′-trimethoxy, 2′,4′-dimethoxy, 4′-methoxy, 4′-ethoxy. R = substituents listed in Table 1. Ring A can also be a quinoline or naphthalene ring. (a) 3% w/v NaOH. (b) Carbanion attacks aldehyde by nucleophilic addition. (c) Dehydration.

Scheme 2. Synthesis of 4′-Hydroxychalcones[a]



[a] R = substituents listed in Table 1. Ring A can also be a pyridine, quinoline, or naphthalene ring. (a) Pyridinium *p*-toluenesulphonate in CH_2Cl_2. (b) Base-catalyzed nucleophilic attack on carbonyl carbon of aldehyde. (c) 4 M HCl.

crystallization. The structures of the synthesized chalcones are given in Table 1. The physical and analytical data of these compounds are listed in the Supporting Information (Table 1). Of the compounds listed in Table 1, 38 are novel compounds as ascertained from a search on the Chemical Abstracts databases (1967 to date).

The aromatic rings in the final chalcone are designated A (from the aldehyde) and B (from acetophenone), respectively. The chalcones are divided into six main classes according to the substitution of the B ring: 4′-methoxy (n = 14), 2′,4′-dimethoxy (n = 17), 2′,3′,4′-trimethoxy (n = 18), 4′-ethoxy (n = 13), 4′-hydroxy (n = 19), and 2′,4′-dihydroxy (n = 11). The choice of oxygenated B ring chalcones as a focus for investigation is based on literature findings that this structural feature is associated with good antimalarial activity.[4,5]

The selection of substituents on ring A (aldehyde) was mainly guided by lipophilicity and electronic considerations as defined by the Craig plot.[12] The selected A ring substituents are spread over the four quadrants of the plot. For example, trifluromethyl, halogens, and nitro are representative lipophilic and electron-withdrawing substituents; dimethylamino, methyl, and ethyl are hydrophilic and electron-donating groups; methoxy and hydroxy are hydrophilic and electron-donating groups; and cyano is a representative lipophilic and electron-withdrawing substituent. About a quarter of the 92 chalcones considered in this study have disubstituted A rings, mostly at the 2,4 positions. In the case of 20 chalcones, ring A is an unsubstituted heteroaromatic (quinoline, pyridine) or bicyclic (naphthalene) ring.

A total of 11 molecular descriptors representative of size and electronic and lipophilic characteristics were selected to characterize the chalcones (Table 2 in Supporting Information). The lipophilicity of the chalcones were assessed from experimentally determined capacity factors (log k_w, pH 7.0) and theoretically determined ClogP values for the non-ionized molecule. There are some missing log k_w values, notably among the hydroxylated chalcones, because of difficulties in the experimental determination. A significant correlation exists between log k_w and ClogP (n = 80, r = 0.256, p < 0.05). Both parameters are also significantly correlated to the size parameters (p < 0.05), as seen from the correlation matrix of these descriptors (Table 3 in Supporting Information).

The size characteristics of the chalcones were captured by Connolly volume and area parameters (log V, log A) and the molecular refractivity (MR) parameter. Because a quarter of the chalcones have disubstituted A rings, a dummy parameter "branching" was included to investigate the effect of A ring substitution on activity (branching = 1 for disubstituted A ring, 0 for monosubstituted A ring). Connolly area and volume are well correlated to one another (p < 0.01). Molar refractivity, often described as a parameter with chameleon-like properties, is correlated to both size (log V, log A, p < 0.01) and several electronic parameters (charge on carbonyl oxygen, HOMO, p < 0.01). In contrast, disubstitution of the A ring was only correlated to the charge on the carbonyl oxygen (p < 0.01).

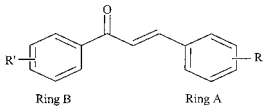
The electronic characteristics of the chalcones were characterized by four parameters (total dipole moment, HOMO, LUMO, charge on carbonyl oxygen), which were determined in silico. In the case of the trimethoxychalcones, an additional electronic parameter, chemical shift of the carbonyl carbon ($\Delta\delta$), was determined experimentally. There are strong correlations among the electronic and steric parameters.

Results

In Vitro Antimalarial Activity. In vitro antimalarial activity was assessed against a strain of chloroquine-resistant human malarial parasite, *P. falciparum* (K1) in a [^{3}H] hypoxanthine uptake assay. The hydroxylated chalcones were generally less active than their alkoxylated counterparts (Table 1). Among the hydroxylated chalcones, the most active compound was 4-chloro-2′,4′-dihydroxychalcone (**211**) with an IC_{50} of 12.3 μM. Six other hydroxylated chalcones (**202**, **203**, **207**, **214**, **227**, **228**) have IC_{50} values below 20 μM. Among the alkoxylated chalcones, 12 compounds (**2**, **3**, **5**−**8**, **19**, **27**, **29**, **31**, **113**) have IC_{50} values below 10 μM and the most active compound was 1-(2′,3′,4′-trimethoxyphenyl)-3-(3-quinolinyl)-2-propen-1-one (**27**), with an IC_{50} of 2 μM.

There is a good representation of active compounds from the trimethoxy, dimethoxy, and methoxy series, but interestingly, none from the ethoxy series. The IC_{50} of 2,4-dimethoxy-4′-butoxy-chalcone (**41**) had previously been reported to be 8.9 and 14.8 μM against a chloroquine-susceptible (3D7) and chloroquine-resistant (Dd2) strain *of P. falciparum*,[4] but in the present in vitro assay, it has an IC_{50} of 108 μM.

Table 1. Structure and in Vitro Antimalarial Activity of Chalcones



compound	R′	R	IC_{50} [a] (μM)	compound	R′	R	IC_{50} [a] (μM)
3	2′,3′,4′-trimethoxy	2,4-dichloro	5.4	**8**[b]	2′,4′-dimethoxy	4-ethyl	2.4
4		4-dimethylamino	18.0	**29**[b,c]		3-quinolinyl	2.2
6[b]		4-trifluoromethyl	3.0	**30**[b,c]		4-quinolinyl	27.0
11[b]		2,4-dimethoxy	16.5	**101**		4-methyl	93.8
12[b]		4-methyl	25.6	**102**		4-methoxy	128.5
13[b]		4-ethyl	16.5	**103**		4-dimethylamino	55.3
27[b,c]		3-quinolinyl	2.0	**104**		4-fluoro	322.0
28[b,c]		4-quinolinyl	60.0	**105**		4-chloro	342.0
35[b]		4-methoxy	25.0	**106**		4-bromo	542.5
36[b]		4-fluoro	9.5	**107**[b]		2-chloro-4-fluoro	600.0
40[b]		4-phenyl	26.2	**108**[b]		3,4-dichloro	297.5
128[b]		2,4-difluoro	18.5	**109**		4-nitro	415.0
129[b]		4-nitro	22.5	**110**[c]		1-naphthalenyl	320.0
130[b]		3,4-dichloro	14.5	**19**	4′-methoxy	4-hydroxy	7.0
131[b]		4-chloro	14.5	**22**[b]		2,4-difluoro	26.8
132[b]		2-chloro	41.5	**23**		4-methoxy	21.7
133[b]		3-chloro	24.4	**31**[b,c]		3-quinolinyl	4.8
134		H	15.8	**32**[b,c]		4-quinolinyl	43.0
41[d]	4′-butoxy	2,4-dimethoxy	108.0	**38**		4-fluoro	14.4
1	2′,4′-dimethoxy	2,4-dichloro	18.8	**111**		2,4-dichloro	16.0
2		4-trifluoromethyl	5.9	**112**[b]		4-trifluoromethyl	19.0
5		2,4-difluoro	6.2	**113**		2,4-dimethoxy	6.4
7		2,4-dimethoxy	2.1	**114**		4-methyl	70.0
115		4-nitro	100.0	**208**[b,c]	2′,4′-dihydroxy	2-naphthalenyl	20.0
116		4-dimethylamino	70.0	**209**[c]		4-pyridinyl	121.6
117		4-cyano	94.5	**210**[b,c]		4-quinolinyl	92.8
135		H	55.5	**211**		4-chloro	12.3
25[b]	4′-ethoxy	2,4-difluoro	28.1	**212**[b,c]	4′-hydroxy	1-naphthalenyl	39.9
26		4-methoxy	33.0	**213**[b,c]		3-quinolinyl	41.0
33[b,c]		3-quinolinyl	24.9	**214**[c]		2-pyridinyl	16.3
34[b,c]		4-quinolinyl	100.0	**215**[c]		4-quinolinyl	51.0
39[b]		4-fluoro	24.1	**216**[b,c]		2-naphthalenyl	27.5
121		2,4-dichloro	96.0	**217**		4-chloro	38.0
122[b]		4-trifluoromethyl	24.0	**218**		2-chloro	61.7
123		2,4-dimethoxy	30.0	**219**		3-chloro	33.1
124[b]		4-methyl	38.0	**220**		4-methoxy	32.2
125		4-nitro	39.0	**221**		4-methyl	25.4
126		4-dimethylamino	30.0	**222**		3-methyl	25.8
127[b]		4-cyano	540.0	**223**[b]		4-butyl	20.9
136		H	43.0	**224**		4-trifluoromethyl	30.4
201	2′,4′-dihydroxy	2,4-dichloro	68.5	**225**		4-nitro	20.4
202[b,c]		3-quinolinyl	16.1	**226**		4-fluoro	21.7
203		2,4-difluoro	16.0	**227**		3,4-dichloro	18.4
204		2,4-dimethoxy	56.4	**228**		4-dimethylamino	17.7
205[c]		1-naphthalenyl	24.8	**229**		2,4-dichloro	24.5
206		4-trifluoromethyl	26.5	**230**		H	29.6
207[c]		2-pyridinyl	19.7				

[a] IC_{50} values for inhibition of [^{3}H] hypoxanthine uptake into *P. falciparum* (K1) in the presence of drug. IC_{50} for chloroquine = 0.265 μM. All readings are the average of two or more separate determinations. [b] Compounds not listed in Chemical Abstract databases (1967 to present). [c] Ring A = heteroaromatic or polycyclic aromatic ring. The nature of the ring is given in R. [d] 2,4-Dimethoxy-4′-butoxychalcone.

In Vivo Antimalarial Activity. The active chalcones identified from in vitro tests (7 hydroxychalcones, IC_{50} < 20 μM; 12 methoxychalcones, IC_{50} < 10 μM) were tested in mice infected with *P. berghei* ANKA, a chloroquine-susceptible strain of murine malaria. The mice were given the drug (100 mg/kg, ip) for 3 consecutive days (days 1−3 postinfection), and their survival times were monitored and compared with control mice receiving DMSO (untreated mice), chloroquine, or **41**. The in vivo test was also carried out on some randomly selected chalcones (**38**, **130**, **201**, **208**) that did not have good in vitro activity. The results of the tests are given in Table 2. The survivability of the mice was assessed from the ratio of the average life span of the drug-treated animal to that of untreated animals ($T/C_{untreated}$), chloroquine-dosed animals (T/C_{CQ}), or **41**-dosed animals (T/C_{41}). A T/C ratio of more than 1 indicates that the drug has increased the survivability of the animal relative to the control group.

Particular attention was paid to compounds that had $T/C_{CQ} > 1$ because these compounds were as good as chloroquine in prolonging the lifespan of the infected mice. Only two compounds fall into this category: **8** and **208**. Both compounds had $T/C_{untreated}$ and T/C_{41} ratios greater than 1.5. There were other compounds that had large $T/C_{untreated}$ and T/C_{41} ratios (>1.5), but none of them had favorable T/C_{CQ} ratios. The activity of **8** may have been anticipated in view of its low IC_{50} (2.4 μM), but the activity of **208** is surprising because it is not particularly active in vitro.

Table 2. Survivability of *P. berghei* ANKA Infected Mice When Treated with Chalcones (100 mg/kg, ip, 3 Days)

compound	IC_{50} [a] (μM)	$T/C_{untreated}$ [b]	T/C_{41} [b]	T/C_{CQ} [b]
27	2.0	1.91	1.91	0.96
7	2.1	0.98	0.98	0.50
29	2.2	1.29	1.29	0.65
8	2.4	2.33,[c] 1.48[c]	2.33,[c] 1.55[c]	1.18,[c] 0.74[c]
6	3.0	1.46	1.46	0.74
31	4.8	0.98	1.03	0.49
3	5.4	0.94	0.94	0.48
2	5.9	1.67	1.67	0.84
5	6.2	1.04	1.04	0.52
113	6.4	0.92	0.97	0.46
19	7.0	1.41	1.47	0.70
36	9.5	1.37	1.37	0.69
211	12.3	1.41	1.48	0.71
38[d]	14.4	2.20,[c] 0.96[c]	1.83,[c] 1.00[c]	1.22,[c] 0.52[c]
130[d]	14.5	1.47	1.53	0.80
203	16.0	1.73,[c] 1.44[c]	1.78,[c] 1.50[c]	1.23,[c] 0.78[c]
202	16.1	1.08	1.11	0.77
214	16.3	0.99	1.03	0.49
228	17.7	0.94	0.99	0.47
227	18.4	1.28	0.81	0.71
207	19.7	1.47	0.93	0.81
208[d]	20.0	1.91,[c] 1.87[c]	1.20,[c] 1.95[c]	1.05,[c] 1.02[c]
201[d]	68.5	1.32	1.36	0.94
chloroquine	0.27	1.81	1.71	
41	108	1.08		0.57

[a] IC_{50} from in vitro tests. [b] $T/C_{untreated}$ = ratio of average life span of drug-treated mice to that of mice receiving DMSO. T/C_{41} = ratio of average life span of drug-treated mice to that of mice treated with **41** (4′-butoxy-2,4-dimethoxychalcone). T/C_{CQ} = ratio of average life span of drug-treated mice to that of mice treated with chloroquine. [c] Results of two separate repeats on groups of three mice. Tests were repeated if $T/C_{CQ} > 1$ in the first instance. [d] Randomly selected chalocones that were not identified as "actives" from in vitro tests.

Multivariate Analysis of Structure−Activity Relationships. An initial stepwise multiple linear regression of in vitro antimalarial activity (−log IC_{50}) of all the compounds against the various descriptors did not yield a significant correlation. This is not unexpected because there are significant correlations among the physicochemical descriptors and missing variables in the data set. These factors would make it difficult for activity to be represented adequately by a single regression equation. In fact, the prevailing conditions would make analysis by multivariate tools very appropriate. Principal component analysis (PCA) can give an overview of the dominant patterns and trends in the data, while partial least-squares projection to latent structures (PLS), which is a regression extension of PCA, will help to quantify and predict the relationships between activity and physicochemical properties.[13]

Principal component analyses (PCA) were carried out separately on the alkoxylated and hydroxylated chalcones. The score plot of the alkoxylated chalcones showed a clustering of the trimethoxychalcones in the upper right quadrant (Figure 1a). Most of the methoxychalcones were found in the lower left quadrant, while members of the ethoxy and dimethoxychalcones were distributed over the upper left and lower right quadrants. The clustering of specific B ring alkoxylated chalcones suggests that there is information in their principal components to explain why these compounds are alike. Such a pattern would also suggest that quantitative structure−actitivity relationship (QSAR) modeling of the alkoxylated chalcones would be of little value. A more sensible approach would be to distinguish

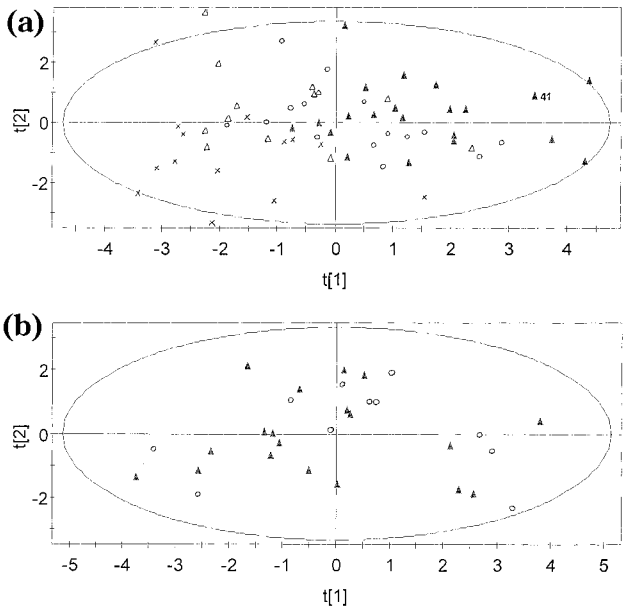


Figure 1. (a) Score plot of principal components t_1 against t_2 for alkoxylated chalcones: 4′-ethoxychalcones (△); 2′,4′-dimethoxychalcones (○); 4′-methoxychalcones (×); 2′,3′,4′-trimethoxychalcones (▲). The location of 2′,4′-dimethoxy-4′butoxychalcone (**41**) is indicated in the score plot. (b) Score plot of principal components t_1 against t_2 for hydroxylated chalcones: 4′-hydroxychalcones (▲); 2′,4′-dihydroxychalcones (○). The ellipse corresponds to the confidence region based on Hotelling T^2 (0.05).

the chalcones according to their clustering patterns and to investigate the QSAR of these clusters separately. Such an approach has been found to be fruitful in other studies.[14] Thus, the relationship between activity and physicochemical properties was explored separately for the methoxy- and trimethoxychalcones and jointly for the ethoxy- and dimethoxychalcones.

In the case of the hydroxylated chalcones, the score plot showed a homogeneous distribution of the hydroxy and dihydroxy members throughout the four quadrants (Figure 1b). Therefore, it is appropriate to consider both series together because they appear to share common characteristics.

(a) Trimethoxychalcones. The trimethoxychalcones series is unusual in many ways. First, the unsubstituted A ring derivative 2′,3′,4′-trimethoxychalcone (**134**) has rather good antimalarial activity (IC_{50} = 15.8 μM). It ranks high (sixth) in terms of in vitro activity among the 18 compounds of this series, indicating that substitution of the A ring, in most instances, resulted in a fall in activity. As to which A ring substituents enhanced activity, a casual examination suggests that these would be electron-withdrawing groups (Table 1). In addition, the substitution pattern of the A ring (**3** vs **130**) and the point of attachment of the ring to the rest of the chalcone skeleton (**27** vs **28**) influenced activity.

Another noteworthy point comes from the score plot of the entire series of the alkoxylated chalcones (Figure 1a). The location of the butoxy derivative **41** (reported to have excellent antimalarial activity[3,4]) in the score plot (upper left quadrant) coincides with that of the trimethoxychalcones. This would suggest that the physicochemical properties of the butoxy analogue **41** is more like that of the trimethoxychalcones than the other alkoxylated series. Therefore, there is good reason to

consider **41** jointly with the trimethoxychalcones in subsequent SAR analysis.

However, the PLS analysis of these compounds together with 11 physicochemical descriptors did not give a significant model (model 1, $r^2 = 0.497$, $q^2 = 0.000$). The $t-u$ score plot, which displays the observations in the projected X (t) and Y (u) space and shows how well the Y space (biological activity) correlates to the X space (descriptors), identified five possible outlier compounds (**4**, **11**, **13**, **28**, **129**). Omission of these compounds resulted in an improved model 2 that could account for 77% and predict 38% of the antimalarial activity of the series (Table 4 in Supporting Information). With 11 descriptors, it is likely that some descriptors would be less important. A measure of the relative importance of the X variables is calculated as VIP (variable importance in the projection), which is available in SIMCA. Each variable has a VIP value that is indicative of its relative importance in accounting for activity, with variables having VIP > 1 making a greater contribution.[13] The omission of variables based on the VIP values was done cautiously because it is possible to omit too many variables and to derive an apparently good model without sound predictive power. The VIP plot was viewed together with the coefficients and loading plots. Natural "thresholds" in the VIP plot was particularly useful in discriminating between important and unimportant parameters. In most cases, a VIP cutoff value of about 0.7 was applied. On the basis of these criteria, the variables branching, molar refractivity, difference in ^{13}C chemical shift of carbonyl carbon, log A, and log V were not found to contribute significantly to model 2, and their removal might result in an improved and simplified model. Of the remaining descriptors, the hydrophobicity parameters (ClogP and log k_w) have comparable VIP values (0.935, 0.911). It was decided that only one parameter would be retained in the final model, and log k_w was arbitrarily chosen. Model 2 was thus reevaluated with a smaller number (five) of physicochemical parameters. An improvement was noted in the new model 3, which had better predictive ability ($q^2 = 0.595$, Table 4 in Supporting Information).

The predictive power of model 3 was further evaluated by selecting a few compounds from this model and using them as the "training set" to predict the activity of the remaining (unselected) compounds. The choice of the training set compounds was made by considering the score plot of model 3 (Figure 1 in Supporting Information). Six compounds were selected to form the training set (model 4, $r^2 = 0.985$, $q^2 = 0.723$), and it was able to predict the activity of the remaining eight compounds reasonably well, as seen from the root-mean-square error of prediction (RMSEP = 0.276). When used to predict the activity of all the trimethoxychalcones ($n = 14$, including five outliers but excluding the training set compounds), the error of prediction rose to 0.514.

A closer look at model 3 showed that electronic parameters are the main contributors to the antimalarial activity of the trimethoxychalcones. The five parameters, in order of increasing importance, were dipole moment < log k_w < charge on carbonyl oxygen < HOMO < LUMO, and they were inversely related to activity (Figure 2). Thus, one would expect good activity in a trimethoxychalcone that has low energies for its lowest empty and highest occupied molecular orbitals, a weakly polarized carbonyl function that would result in a small charge on the carbonyl oxygen, small total dipole moment, and low lipophilicity. The energies of HOMO and LUMO serve as indices of the electron-donating and electron-acceptor abilities of the molecule, respectively. The higher the energy of the HOMO is, the better its electron-donating ability. In the case of LUMO, good electron-acceptor ability is associated with low-energy molecular orbitals. In this case, activity is inversely related to both HOMO and LUMO, which suggests that electron-acceptor ability, rather than electron-donor ability, is important in determining activity. Electron-withdrawing substituents on ring A would make the ring electron-deficient and a better electron acceptor and, according to the present structure–activity relationship, would give rise to a more active antimalarial chalcone. The inductive and mesomeric effect of the electron-deficient ring could be transmitted along the $\alpha\beta$ unsaturated carbonyl chain, resulting in a less polarized carbonyl linkage. This would explain the inverse relationship between activity and the negative charge on the carbonyl oxygen (i.e., good activity is associated with a small negative charge on the carbonyl oxygen).

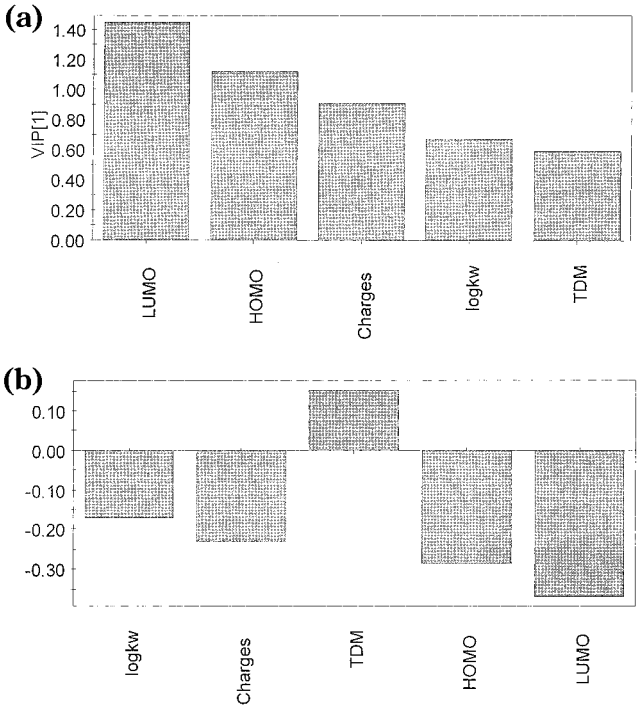


Figure 2. (a) VIP plot from PLS analysis of data from model 3 and (b) coefficients plot from PLS analysis of data from model 3. Parameters with positive coefficient values relate directly to activity, while those with negative coefficients are inversely related to activity. In this plot, only the dipole moment (TDM) is directly related to activity. LUMO = lowest empty molecular orbital; HOMO = highest occupied molecular orbital; charges = charge on carbonyl oxygen; log k_w = hydrophobicity parameter obtained from reversed-phase HPLC, where k_w is the capacity factor in 100% water; TDM = total dipole moment.

(b) Methoxychalcones. The most active compound in this series is 1-(4′-methoxyphenyl)-3-(3-quinolinyl)-2-propen-1-one (**31**), with an IC_{50} of 4.8 μM. The association of the 3-quinolinyl ring with good activity is an interesting recurring feature among all four series of alkoxylated chalcones. The methoxychalcone with an

unsubstituted A ring (**135**) has poor antimalarial activity (IC_{50} = 55.5 μM). Substitution of the A ring with polar, electron-withdrawing groups such as nitro (**115**) and cyano (**117**) results in a further reduction of activity. Substitution with other groups resulted in either improvement or little change in activity, and this aspect was investigated in greater detail with PLS.

A significant PLS model 5 was obtained only after omission of some "outlier" compounds from the series. These outliers were detected visually from the PLS score ($t-u$) of all 14 methoxychalcones as well as from the residual values of the model. In this way, **19**, **31**, **113** were identified as outliers. There is some concern over the omission of **19** and **31** because these compounds are among the three most active compounds (IC_{50} < 10 μM) in the series. However, their omission was necessary to give a significant one-component model 5, which accounted for 63% and predicted 32% of antimalarial activity. As before, the model was improved by omitting less important descriptors identified from the VIP and coefficients plot. The improved model 6 could predict the activity of 11 methoxychalcones using 5 descriptors with a q^2 of 0.585. The predictive power of the model was further confirmed by selecting a training set of five compounds (model 7), and using it to predict activity of the remaining six compounds (RMSEP = 0.283). The training set was also used to predict the activity of all the methoxychalcones (n = 9, including three outliers). Prediction was only satisfactory with an RMSEP of 0.520.

The VIP plot of model 6 identified branching, log k_w, and HOMO as the parameters (in order of decreasing importance) contributing to the activity of the methoxychalcones. Activity was directly related to branching (i.e., disubstitution of A ring) and HOMO, but inversely related to log k_w. The emphasis on the branching parameter may be biased because there are only two compounds (**111**, **113**) with disubstituted A rings in model 5. Moreover, with the omission of **19** and **31** (both active and monosubstituted A ring compounds) as outliers, **113** is the only remaining "active" compound (IC_{50} < 10 μM) in model 6, and this may have led to undue emphasis on the state of substitution of ring A.

The lipophilicity parameter log k_w is important in determining activity. As in the case of the trimethoxychalcones, the relationship is inverse; i.e., lower lipophilicity in the compound favors good antimalarial activity. The direct correlation between activity and HOMO suggests that the electron-donating ability of the chalcone is important for good activity. An interesting observation is the relationship that exists between HOMO and log k_w (r = −0.590, p < 0.05). This would suggest that the electronic parameter may be the single most significant parameter influencing activity.

(c) Dimethoxychalcones and Ethoxychalcones. The initial PCA score plot of the alkoxylated chalcones (Figure 1a) indicated an overlapping distribution of the dimethoxy- and ethoxychalcones. Therefore, there was good reason for considering these two series together in subsequent analyses. However, no significant PLS model could be obtained, and it was decided that they should be evaluated separately.

The ethoxychalcones are outstanding in that no active compound (IC_{50} < 10 μM) has been identified from this series. The unsubstituted A ring ethoxychalcone (**136**) has poor activity (IC_{50} = 43 μM), and activity is further depressed by substitution or replacement with 4-cyano (**127**), 2,4-dichloro (**121**), and 4-quinolinyl (**34**). The most active compounds were the 3-quinolinyl (**33**) and 4-trifluoromethyl (**122**) derivatives, the same A ring substituents that were earlier identified as "actives" for the trimethoxy and methoxy (only for 3-quinolinyl) series. No significant PLS model could be obtained even after removing outliers or reducing the number of descriptors. This means that the present descriptors cannot adequately describe the variation in activity of the ethoxychalcones and that new variables should be found to define activity.

The dimethoxy series is outstanding in having the greatest representation of active alkoxylated chalcones (5 out of 12) in this investigation. A significant PLS model 8 was derived after omitting five outlier compounds (**5**, **103**, **106**, **104**, **110**) (r^2 = 0.694, q^2 = 0.486), and this was further improved when descriptors that contributed little to activity (identified through coefficient and VIP plots) were omitted from the model. The final model 9 accounted for 68% and predicted 61% of activity. As before, a training set (model 10) was selected from these 12 compounds and was used to predict the activity of the remaining 7 compounds. Only a satisfactory prediction was obtained in this case (RMSEP = 0.689). Prediction was poorer (RMSEP = 1.346) when the training set was applied to the entire cohort of dimethoxychalcones (n = 12, including outliers). The main parameters influencing the activity of dimethoxychalcones were the size parameters (log A, log V), which have VIP values greater than 1. The other parameters, in order of decreasing importance, were log k_w, molar refractivity, and HOMO. Except for log k_w, the other parameters affected activity directly.

(d) Hydroxychalcones and Dihydroxychalcones. The 30 hydroxylated chalcones were found to be homogeneous in terms of the principal components summarizing their independent descriptors. This is seen from their PCA score plot (Figure 1b), which showed a smooth integration of compounds of both series within the confines of the four quadrants. Nevertheless, attempts were also made to analyze the two series separately. Both approaches had several similarities. For example, the VIP plots identified dipole moment and a lipophilicity parameter as being important for the activity of each series, whether considered separately or together. However, the small size of the dihydroxy series (n = 11) posed difficulties in developing a training set. Therefore, it was decided that the two series should be considered together.

Applying PLS to the hydroxy and dihydroxychalcones proved to be difficult without eliminating a large number of compounds as outliers. To avoid this, a training set of compounds was directly selected from the 30 hydroxylated chalcones (model 11). The number of descriptors in this model was then reduced, and the final training set comprised 17 compounds and 4 descriptors (dipole moment, branching, molar refractivity, log k_w) (model 12: r^2 = 0.818, q^2 = 0.602). It was able to predict the activity of the remaining 13 compounds with a satisfactory level of accuracy (RMSEP = 0.425).

The coefficient, loadings, and VIP plots of model 12 identified four descriptors as being important determinants of activity. These were dipole moment, branching, molar refractivity, and log k_w, in order of decreasing importance. Of these four descriptors, dipole moment and log k_w influenced activity directly while branching (disubstitution of ring A) and molar refractivity affected activity inversely. Because molar refractivity is significantly correlated ($p < 0.01$) to size parameters for this series, the requirements for good activity in hydroxylated chalcones would be the presence of polar and small-sized substituents, which were different from that observed in the 4′-methoxy and 2′,4′-dimethoxy series.

(e) Active Hydroxylated and Alkoxylated Chalcones. In a final analysis, the active members of the hydroxylated and alkoxylated chalcones (a total of 19 compounds) were gathered to form a separate series. This was prompted by observations from the PLS score plots of the five series prior to weeding out outliers and trimming the number of descriptors. It was noted that some of the active compounds tended to be outliers. In the methoxy series, two out of the three compounds removed as outliers were active compounds ($IC_{50} < 10$ μM). In the dimethoxy series, one active compound was removed because of its outlying position. In addition, when the predictive ability of the training sets of each series was evaluated, it was noted that many of the active compounds had large residual values; i.e., their activities were poorly predicted by the proposed model. Therefore, an analysis of all the active compounds as a series would be useful in revealing if they share common characteristics. A PLS analysis of the 19 active compounds using 10 descriptors (^{13}C chemical shift of the carbonyl carbon was omitted because only four compounds have this descriptor) gave a significant one-component model 13 ($r^2 = 0.677$, $q^2 = 0.563$). This model was further improved by removing descriptors that had VIP values of less than 0.25 (viz. HOMO, LUMO, and branching) to give model 14, which had improved predictive power ($q^2 = 0.598$). A training set of eight compounds was selected from the score plot of model 14, which is depicted in Figure 2a (Supporting Information). The training set (model 15, $r^2 = 0.957$, $q^2 = 0.833$) was able to predict the activity of the remaining active compounds with an RMSEP value of 0.299 (Figure 2b in Supporting Information). The main descriptors important for activity (in order of importance) were the size parameters (log *A*, log *V*, molar refractivity; these parameters are significantly correlated to each other, $p < 0.01$) and log k_w. Therefore, size and lipophilicity are important features of the active chalcones. However, stepwise multilinear regression only identified size (log *A*) as an important parameter for describing in vitro activity of these active chalcones:

$$-\log IC_{50} = (6.35 \pm 1.07)\log A - (10.57 \pm 2.64)$$

$$n = 19, \quad r^2 = 0.690, \quad r_{cv}{}^2 = 0.616, \quad SE = 0.202, \quad SE_{cv} = 0.225, \quad F = 35.567$$

The emphasis on size for good activity must be considered in light of both rings A and B. Among the active chalcones, there is a strong representation from the trimethoxy and dimethoxy series, which have bigger size B rings. This is in compliance with the regression equation. However, bigger size A rings do not always result in more active compounds. For example, although the quinoline ring is comparable in size to the naphthalene ring, there are no active naphthalene A ring derivatives but several active quinolinyl A ring derivatives. Furthermore, only 3-quinolinyl, and not 4-quinolinyl derivatives, are active chalcones. This makes the good activity of the 3-quinolinyl A ring derivatives and chalcones with comparatively small size A rings (e.g., 4-fluorophenyl **36**, 4-trifluoromethylphenyl **2**, **6**, 4-hydroxy **19**) quite exceptional. It may be related to the hydrophobicity parameter log k_w, which was identified as an important parameter from the PLS model 15. On the basis of the correlation matrix drawn up for the descriptors of the active compounds, log k_w is not correlated to any of the size parameters. It may reflect the polarity of the compound because the determination of log k_w depends on its elution from a hydrophobic column with a polar mobile phase.

Discussion

Six series of chalcones, broadly classified according to the substitution pattern of the B ring, have been investigated for in vitro antimalarial activity. Within each series, the other aromatic ring (ring A) is substituted with a wide range of groups of varying lipophilicities and electronic character or is replaced by heteroaromatic or bicyclic ring systems. The physicochemical properties and in vitro activities of these compounds cover a considerable range, a feature that would ensure that a comprehensive and meaningful structure−activity relationship could be carried out.

Nineteen compounds have been identified as "actives" from the present study. These comprise 12 alkoxylated chalcones ($IC_{50} < 10$ μM) and 7 hydroxylated chalcones ($IC_{50} < 20$ μM). The latter series have weaker antimalarial activity when compared to their alkoxylated analogues, and a lower criteria for activity is defined for these compounds. The most active compound to emerge from this study is 1-(2′,3′,4′-trimethoxyphenyl)-3-(3-quinolinyl)-2-propen-1-one (**27**), with an IC_{50} of 2 μM. An interesting observation is the association of good antimalarial activity with the 3-quinolinyl A ring derivatives. This is observed across several ring B series but not with the 4′-hydroxylchalcones. The quinoline ring is a common entity in established antimalarials. It may play a role in facilitating the localization of the drug within the acidic food vacuole of the parasite because of its basic properties. The planar and aromatic quinoline ring would enhance $\pi-\pi$ stacking to the porphyrin rings in heme, which is widely held to be a potential target for quinoline-containing antimalarial agents.[15] The quinoline A ring in the chalcones may have similar roles. A steric element is possibly involved in the interaction because 4-quinolinyl A ring derivatives are significantly less active than their 3-quinolinyl counterparts. It would be of interest to investigate the heme-binding properties of these chalcones to assess the contribution of the quinoline ring to the overall process.

The antimalarial activity of the active chalcones was evaluated in *P. berghei* infected mice. Several of the chalcones increased the survivability of the mice relative to control infected mice treated with either DMSO or **41**, but only a few were comparable to chloroquine in

this respect. Among the active chalcones, the in vivo activity of **8** (2′,4′-dimethoxy-4-ethylchalcone) is noteworthy. More surprising is the good in vivo activity of **208**, 1-(2′,4′-dihydroxy)-3-(4-naphthalenyl)-2-propen-1-one, which was not identified as an active chalcone in view of its high IC_{50} (20 μM). Poor in vitro and in vivo correlation is a problem in the evaluation of antimalarial potency and may be attributed to the pharmacokinetics of the test compound and differences in the strain of plasmodia used for the tests.

Previous structure−activity studies on the antimalarial activity of chalcones have reported a preference for electron-acceptor groups (such as dichloro or difluoro at the 2,3 or 2,4 positions) in the A ring for good activity.[5] Our present study suggests that this is not necessarily so. The substitution of the B ring is important in determining the type of A ring substituents that would give optimum antimalarial activity. For example, 2,4-dichloro substitution of ring A in the trimethoxychalcones gave rise to a very active compound (**3**, IC_{50} = 5.4 uM), but the same substituent gave rise to less active compounds in other series of chalcones (**1**, **111**, **121**, **201**, **229**).

The structure−activity analysis has been carried out using the multivariate data analytical tools PCA and PLS. The distribution of the alkoxylated chalcones in the PCA score plot suggests that each series should be considered separately. Fairly predictive PLS models have been developed for each class of alkoxylated chalcone, with the exception of the ethoxychalcones. The structural requirements of each series are different, confirming our initial observation that the nature of the B ring is critical in determining the overall structural requirements for activity. Thus, size parameters were important for the dimethoxychalcones while electronic characteristics (electron-withdrawing groups) were more important considerations for the methoxy- and trimethoxychalcones. The hydroxy- and dihydroxychalcones were considered together as a class, and in this case, small size and polarity were important considerations for good activity. The PLS models developed for each class have several limitations. For example, satisfactory models could be obtained only upon omission of outlier compounds. This may be due to the inadequate coverage afforded by the descriptors used in the present analysis, as well as the small size of compounds representing each class.

A separate analysis was also carried out on the 19 active compounds because many of them appeared to be outliers in their respective series. Surprisingly, a better PLS model was obtained for the active compounds than for the individual B ring series. This model 14 was able to account for 67% and predict 60% of the activity. A training set (model 15) selected from this model predicted the activity of the remaining compounds with an error (RMSEP) of 0.299. Size and hydrophobicity (log k_w) factors were important parameters for activity. The results suggest that size considerations are dominated by the B ring while the A ring may be more important in influencing hydrophobicity, which in this instance may reflect the polar characteristics of the molecule. Moreover, the association of 3-quinolinyl ring A chalcones with good antimalarial activity would make the ring A/B heterocyclic chalcones particularly attractive candidates for antimalarial activity.

In conclusion, MVA tools have been used in the present study to delineate the structural requirements for antimalarial activity in oxygenated and alkoxylated chalcones. PLS models with reasonably good predictive values have been developed for each series, and the results may be applied to designing drugs with improved antimalarial activity.

Experimental Section

Chemistry. Melting points were uncorrected. Elemental analyses and accurate mass determinations were carried out by the Department of Chemistry, National University of Singapore. Elemental analyses are indicated by symbols (C, H, N) if they are within ±0.4% of the theoretical values. Mass spectra were collected on a VG Micromass 7035 E mass spectrometer by chemical ionization methods. IR spectra (pressed KBr disks or neat) were recorded on a JASCO FTIR-430 instrument. Chemical shifts of ^{1}H NMR spectra, which were obtained on a Bruker (DPX 300 MHz) spectrometer, are reported in δ (ppm) relative to tetramethylsilane as the internal standard. Thin-layer chromatography of the final compounds were done on silica gel sheets (with fluorescent indicator) using $CHCl_3$ or $CHCl_3$/hexane (4:1 or 3:2) as eluting solvents.

Chemical Syntheses. (a) Alkoxylated Chalcones. The general procedure for preparing the alkoxylated chalcones is as follows. In a round-bottom flask, a methanolic solution of NaOH (3% w/v, 10 mL) and the substituted aldehyde (10 mmol in 10 mL) were stirred together at room temperature (28 °C). A methanolic solution of the substituted acetophenone (10 mmol, 10 mL) was added dropwise, and the mixture was stirred for 12−18 h. The alternative method of adding the aldehyde to a stirred solution of the acetophenone in alkaline methanol did not result in any significant difference in yield. In most cases, the product was obtained as a brightly colored precipitate after a short period of stirring. The precipitate was removed by filtration, washed with cold methanol, and recrystallized. When no precipitate was obtained, the solution was diluted with water, neutralized with HCl, and extracted with ethyl acetate. The organic layer was dried with anhydrous Na_2SO_4 and removed by evaporation under reduced pressure to give either a solid or liquid residue. The solid residue is treated as above. The liquid residue is passed through a column of silica gel (230−400 mesh ASTM) and eluted with $CHCl_3$ or $CHCl_3$/hexane. For all compounds, recrystallization was done twice and purity was checked by TLC before characterization by ^{1}H NMR, IR, and accurate mass and elemental analyses. The yields of the synthesized compounds, their melting points, and spectroscopic and elemental analyses data are given in the Supporting Information (Table 1).

(b) Hydroxylated Chalcones. A solution of 4-hydroxyacetophenone or 2,4-dihydroxyacetophenone (10 mmol), pyridinium *p*-toluenesulfonate (0.4 mmol), and 2*H*-3,4-dihydropyran (16 mmol) in methylene chloride (20 mL) was stirred for 4 h at room temperature. A solution of Na_2CO_3 (1 M, 20 mL × 2) was then added to wash the reaction mixture. The organic layer was separated, combined together, dried over anhydrous Na_2SO_4, and concentrated in vacuo to give the crude tetrahydropyranyl ether as a yellow to white product (solid or liquid). The crude tetrahydropyranyl ether was characterized by ^{1}H NMR to confirm its formation and was used without further purification for reaction with the appropriate aldehyde. A methanolic solution of the ketone protected as tetrahydropyranyl ether (10 mmol, 10 mL) was added to a stirred solution of the aldehyde (10 mmol) in methanol (5 mL) at room temperature. This was followed by dropwise addition of a methanolic solution of sodium hydroxide (3% w/v, 10 mL). Stirring was continued for 12−18 h, and the reaction mixture was worked up as described earlier for alkoxylated chalcones, to give the crude protected hydroxylchalcone. Acid (4 M HCl, 4 mL) was added to a stirred solution of the crude product (2

0.2 mmol) in absolute alcohol (10 mL), and the mixture was stirred for 4 h at room temperature and then diluted with water (40 mL). The mixture was extracted with ethyl acetate (50 mL × 3), and the combined organic phases were concentrated in vacuo to give the crude hydroxylated chalcone. The crude product was purified by column chromatography using silica gel (230−400 mesh ASTM) as the stationary phase and $CHCl_3$/hexane as the mobile phase. For all compounds, recrystallization was done twice and purity was checked by TLC before characterization by ^{1}H NMR and accurate mass and elemental analyses. The yields of the synthesized compounds, their melting points, and spectroscopic and elemental analyses data are given in the Supporting Information (Table 1).

Evaluation of in Vitro Antimalarial Activity. The in vitro antimalarial activities of compounds **1**−**92** were evaluated by the method of Desjardins et al.,[16] with modifications. Briefly the assay measures the incorporation of [^{3}H] hypoxanthine by the parasites and the inhibition of the incorporation in the presence of the test compound. A strain of chloroquine resistant (K1) *Plasmodium falciparum* was used in the assay. The test compounds were dissolved in DMSO and serially diluted 10-fold with complete culture media (RPMI-1640, 5% sodium bicarbonate, and 10% normal type "O" human serum) to give a 10^6-fold concentration range. The diluted drugs (25 μL) were transferred to wells in a 96-well microtiter plate, together with 200 μL of parasitized erythrocytes (1−2% parasitemia and 1.5% hematocrit), and the whole was incubated at 37 °C for 24 h in a candle jar. The control well in each plate contained 25 μL of complete medium instead of the test compound. Chloroquine was also tested as a positive control. After 24 h, 25 μL of [^{3}H] hypoxanthine was added, and the plates were incubated for an additional 24 h, after which the cells were filtered onto glass fiber filters (Whatman 934-AH) and counted in a scintillation counter. For each test compound, the concentration−response profile was determined and analyzed by a nonlinear, logistic dose response program to give its IC_{50}, which is the concentration of test compound required to inhibit [^{3}H] hypoxanthine uptake by 50% compared to the control.

Evaluation of in Vivo Antimalarial Activity. The in vivo test measures the survivability of mice following administration of the drug. Swiss albino mice (male, 4 weeks, approximately 25 g) were inoculated intraperitoneally with 10^7 parasitized erythrocytes (*P. berghei* ANKA). The test compound was given intraperitoneally at a daily dose of 100 mg/kg in DMSO for 3 consecutive days after the day of infection (day 0). Each compound was tested against three mice. Three groups of control infected mice were maintained, and they were given chloroquine (52 mg/kg, ip, 0.5% Tween buffer solution, pH 7.4) on day 1 and DMSO or **41** (2,4-dimethoxy-4′-butoxychalcone, 100 mg/kg in DMSO, ip) on days 1−3. Thin blood smears were made from the tail blood of the mice from day 1 to day 14 or until their demise. The blood smears were fixed with 5% Giemsa, examined microscopically, and graded according to WHO protocol for evaluating the degree of parasitemia.[17] Control infected mice treated with DMSO or chloroquine would normally perish within 8 days and 14−16 days, respectively. Mice that received **41** would live on the average for 8−9 days.

Determination of Lipophilicity by Reversed-Phase HPLC. Lipophilicity was determined experimentally from their capacity factors (k') by a reversed-phase HPLC method. Separation was achieved on a LiChrosorb RP-18 (10 μM) stationary phase with a methanol/0.02 M phosphate buffer (pH 7.0) mobile phase. At least four mobile-phase compositions were investigated for each compound, with the methanol content ranging from 50% to 85% w/w for each composition. Determinations were carried out at 30 °C, with the flow rate adjusted to 1.0−1.5 mL/min depending on the mobile-phase composition, with UV detection set at 280 and 330 nm. A stock solution (10 mg/mL) of the compound was prepared in methanol. For each mobile phase composition, equal volumes (20 μL) of the stock solution and an acetone stock solution (10% v/v acetone in the mobile phase) were diluted to 200 μL with the mobile phase and an aliquot (10 μL) was injected for the determination of retention time. Triplicate determinations were done for each concentration of test compound at each mobile-phase composition. The capacity factor (k') was determined from $\log k' = \log[(V_s - V_o)/V_o]$, where V_s and V_o are the retention volumes of the test compound and acetone, respectively. Linear regression of $\log k'$ of each compound against mobile-phase composition and extrapolation to 100% aqueous phase gave $\log k_w$ of the compound at pH 7.0.

Determination of the Chemical Shift of the Carbonyl Carbon. ^{13}C NMR spectroscopy was used to determine the chemical shift of the carbonyl carbon in the trimethoxy series of chalcones. The difference in chemical shift is given by $\Delta\delta = \delta_X - \delta_R$, where δ_X is the chemical shift of the trimethoxychalcone with a substituted A ring and δ_R is that of the reference compound (2′,3′,4′-trimethoxychalcone with no A ring substitution, 189.976 ppm). $\Delta\delta$ is known to be sensitive to the electronic influence of the alkyl/aryl moieties[18,19] and is used here to give a direct assessment of the electronic effects of the A ring, which is attached by conjugation to the carbonyl carbon. The ^{13}C NMR spectra of the chalcones ($CDCl_3$ or dimethyl-d_6 sulfoxide, tetramethylsilane as reference) were determined on a Bruker ACF 300 instrument.

Molecular Modeling Methods. The following parameters were determined from the force-field-minimized geometries of the chalcones using the SYBYL 6.6 force field MMFF94 (Tripos Associates, St Louis, MO), with calculations continued until the rms gradient was less than 0.001 kcal mol^{-1} Å: ClogP, molecular refractivity (MR), total dipole moment (TDM), Connolly surfaces (volume and surface area, calculated from MOLCAD in SYBYL), and negative charge on the carbonyl oxygen using the Gasteiger−Huckel method. Orbital energies for HOMO and LUMO were calculated from MOPAC (QCPE program 455, version 6.0), which is interfaced with SYBYL.

Statistical Methods. Multiple linear regression analyses were carried out using SPSS 10 (SPSS, Inc., Chicago, IL). The following statistical parameters were determined for each regression equation: 95% confidence interval variables, measure of explained variance r^2, Fischer significance ratio F at $P = 0.05$, and standard error SE. Cross-validated r^2 and SE were determined using the QSAR module of SYBYL 6.6.

Multivariate data analyses were performed with SIMCA-P (version 8.0)[20] using default settings.

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Supporting Information Available: Tables containing physical and analytical data of synthesized compounds (Table 1), their physicochemical descriptors (Table 2), correlation maxtrix of descriptors (Table 3) and a summary of PLS models (Table 4) and figures showing the score plots of principal components for alkoxylated chalcones (Figure 1), PLS score plots for actives (Figure 2a), and a plot of predicted vs observed activities of actives (Figure 2b). This material is available free of charge via the Internet at http://pubs.acs.org.

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