An *In Silico* 3D Pharmacophore Model of Chalcones Useful in the Design of Novel Antimalarial Agents

A.K. Bhattacharjee[a,*], D.A. Nichols[a], L. Gerena[a], N. Roncal[a] and C.E. Gutteridge[b]

[a]*Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Silver Spring, MD 20910-7500, USA;*
[b]*Department of Chemistry, United States Naval Academy, Annapolis, MD 21402, USA*

Abstract: Malaria, the most important of the human parasitic diseases, causes about 500 million infections worldwide and over 1 million deaths every year. The search for novel drug candidates against specific parasitic targets is an important goal for antimalarial drug discovery. Recently the antimalarial activity of chalcones has generated great interest. These compounds are small non-chiral molecules with relative high lipophilicity (clogP ~ 5-7), have molecular weights in the range of 300 to 600 g/mol, and possess *in vivo* efficacy against both *P. berghei* and *P. yeolii*. Preliminary data on our on-going chalcone synthesis project indicate that these compounds are active *in vitro* against *P. falciparum,* but are rapidly metabolized in liver microsome assays. Structurally-related compounds not including the enone linker are found to be much more metabolically stable and yet have comparable *in vitro* efficacy. In this study, we have utilized the efficacy data from an in-house on-going chalcone project to develop a 3D pharmacophore for antimalarial activity and used it to conduct virtual screening (*in silico* search) of a chemical library which resulted in identification of several potent chalcone-like antimalarials. The pharmacophore is found to contain an aromatic and an aliphatic hydrophobic site, one hydrogen bond donor site, and a ring aromatic feature distributed over a 3D space. The identified compounds were not only found to be potent *in vitro* against several drug resistant and susceptible strains of *P. falciparum* and have better metabolic stability, but included one with good *in vivo* efficacy in a mouse model of malaria.

Key Words: *In silico* 3D pharmacophore, virtual screening, malaria, chalcone, design, novel antimalarial agents, quantitative structure activity relationship (QSAR).

INTRODUCTION

Malaria is one of the most prevalent diseases in tropical and subtropical countries and affects 300-500 million people a year [1]. Despite years of efforts, the disease is still estimated to be responsible over 1 million deaths worldwide, mostly of children under the age of five in developing countries. Even in the United States, more than a thousand people are affected annually [2]. Currently about two billion people are exposed to malaria with two-thirds of the world's population living in endemic regions [3]. The situation is rapidly worsening due to limited availability of effective drugs, the development of drug resistance by the parasite and by frequent visitation of non-immune people to areas where malaria is frequently transmitted [4]. Chloroquine, mefloquine, and other frontline drugs for the treatment and prevention of malaria are becoming increasingly ineffective (Fig. (**1**)) [5]. Artemisinin analogs such as artesunate and arteether were later introduced and found to be quite effective, particularly against drug-resistant *Plasmodium falciparum*, but observations of drug-induced and dose-related neurotoxicity in animals have raised concern about the safety of these compounds for human use [5]. Therefore, much effort and attention are needed for discovery and development of new and less toxic antimalarial drugs.

*Address correspondence to this author at the Department of Medicinal Chemistry, Division of Experimental Therapeutics, Walter Reed Army Institute of Research, 503 Robert Grant Avenue, Silver Spring, MD 20910, USA; Tel: 301-319-9043; Fax: 301-319-9449;
E-mail: apurba.bhattacharjee@na.amedd.army.mil

A major initiative in this direction is to find new targets that are critical to the disease process or essential for the survival of the parasite. Identification and design of novel chemical entities specifically affecting these targets could lead to better drugs for the treatment of malaria. The antimalarial activity of the chalcone class of compounds is well established [6-12]. The naturally occurring glycosidic dihydrochalcone Phlorizidin, isolated from *Micromelum tephrocarpum* (Rutaceae), was one of first chalcones shown to possess antiplasmodial activity, and appears to induce permeability to various substrates in *Plasmodium*-infected erythrocytes [11]. More promising is licochalcone A, first isolated from *Glycyrrhiza glabra* (Fabaceae). It possesses *in vitro* and *in vivo activity* against different parasites including *P. falciparum*, *L. donovani* and *L. major* and has undergone intensive preclinical study [7]. Several groups have studied the relationship between the structure of chalcone analogs and their antimalarial activity [8-10]. Since the emerging relationship does not correlate with that between structure and the inhibition of any known antimalarial target, the mechanism by which the chalcones act appears to be distinct from the established mechanisms [12]. In addition to antiparasitic activity the chalcones possess a wide range of other biological activity [8-10].

Results from our on-going chalcone project [13] indicate that chalcones are rapidly metabolized by liver microsomes. Since preliminary studies suggested that analogs in which the enone linker of the chalcone is modified have significantly improved metabolic stability, we sought to identify compounds sharing the antimalarial properties of the chal-

1573-4064/07 $50.00+.00

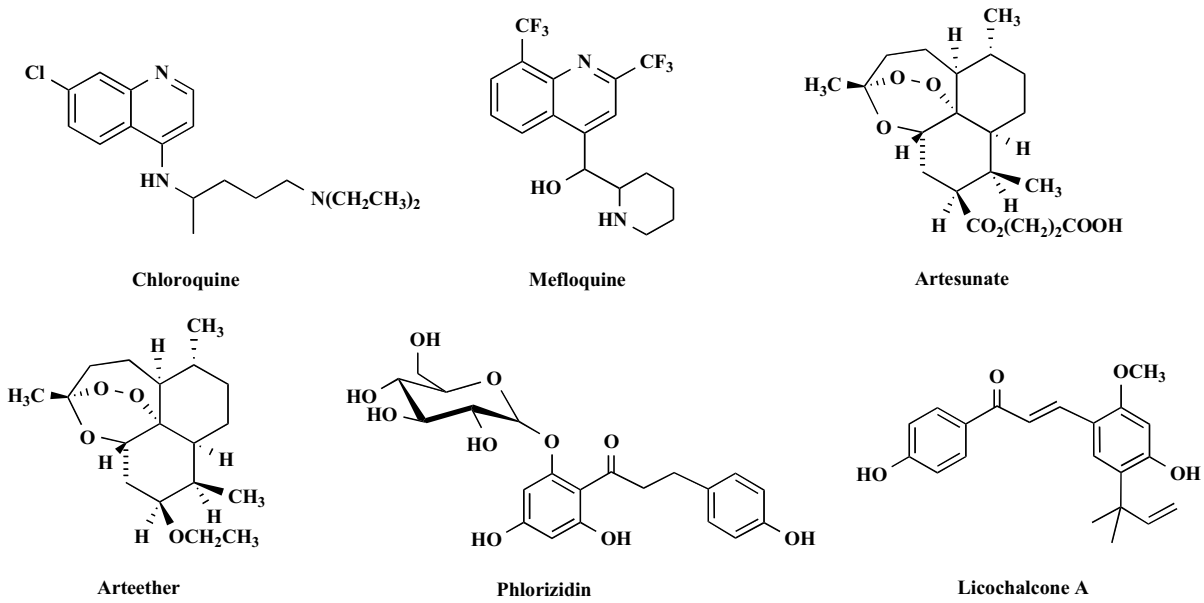


Fig. (1). Known antimalarial agents.

cones, but lacking the enone structure. Despite some understanding of the structural basis for the antimalarial activity of the chalcones, specific knowledge of the pharmacophore was unclear at the outset of the study described herein. Information about the pharmacophore will provide a better understanding of the structural requirements for antimalarial activity. It will be a valuable tool to enable identification of novel candidate compounds through virtual screening of databases and will facilitate the design of novel analogs.

In a continuation of our efforts to develop new antimalarial agents using computational methodologies [14], we report herein an *in silico* three-dimensional chemical function based pharmacophore model for the antimalarial activity of the chalcones and demonstrate the application of this model in virtual screening of a compound library to identify novel, potent antimalarial agents.

MATERIALS AND METHODS

Chemicals

The following chalcones and chalcone-like compounds were obtained and identified through virtual screening from the in-house chemical library at the CIS-WRAIR [15] which includes both commercially available compounds and others submitted by various laboratories. Their structures are shown in Figs. (**2**) and (**3**). *N9*,*N9*-Diethyl-12*H*-benzo[a]phenoxazine-5,9-diamine (Compound **1**) [16], (*E*)-1-(2,5-dichlorophenyl)-3-quinolin-4-yl-propenone (Compound 2) [8], (*E*)-1-(5-fluoro-2-methoxyphenyl)-3-quinolin-3-yl-propenone (Compound **3**) [13], (*E*)-3-(2,5-difluorophenyl)-1-(2,4-dimethoxyphenyl)propenone (Compound **4**) [17], (*E*)-1-(2,4-dimethoxyphenyl)-3-pyridin-2-yl-propenone (Compound **5**) [18], (*E*)-1-(4-butoxyphenyl)-3-(4-hydroxy-2-methoxyphenyl)-propenone (Compound **6**) [17], 1,3-bis-(4-chlorophenyl)propane-1,3-dione (Compound **7**) [19], 1,3-bis-(3,4,5-trimethoxyphenyl)-propan-1-one (Compound **8**) [20], *N*-ethyl-*N*-phenyl-2-amino-1-phenylethanone (Compound **9**) [21], 2,3-dichloro-3-(4-chlorophenyl)-1-(3-chlorophenyl)-propan-1-one (Compound **10**) [22], 2-(4-chlorobenzyl)-3-(4-chlorophenyl)propylamine (Compound **11**) [23], (*E*)-1,3-bis-(4-chlorophenyl)propenone (Compound **12**) [17], (*E*)-3-(2-chlorophenyl)-1-(2,4-dimethylphenyl)propenone (Compound **13**) [17], 2,*N*-bis-(4-chlorophenyl)-2-ethoxyacetamide (Compound **14**) [24], (*E*)-3-(2-chloroquinolin-3-yl)-1-phenylpropenone (Compound **15**) [25], (*E*)-1-(4-hydroxyphenyl)-3-quinolin-4-yl-propenone (Compound **16**) [26], 5-[4-(3-chlorobenzylamino)phenyl]-6-ethylpyrimidine-2,4-diamine (Compound **17**) [27], *N6*-(3-bromobenzyl)-quinazoline-2,4,6-triamine (Compound **18**) [28], 4-amino-*N*-(4-trifluoromethylphenyl)benzenesulfonamide (Compound **19**) [29], 3-hydroxynaphthalene-2-carboxylic acid (4-pentylphenyl)-amide (Compound **20**) [30], *N*-(4-*tert*-butylbenzyl)-*N*-methyl-2-amino-1-phenylpropan-1-ol (Compound **21**) [31], 1-(4-amino-2-methyl-quinolin-6-yl)-3-(4-ethoxyphenyl) urea (Compound **22**) [32], 1-(4-butylphenyl)-3-(4-fluorophenyl) urea (Compound **23**) [33], 1-(4-ethylphenyl)-3-phenylurea (Compound **24**) [33], 1-(4-ethoxyphenyl)-3-(4-fluorophenyl) urea (Compound **25**) [33], *N*-(4-chloro-3-trifluoromethylphenyl)-*N′*-(4-hydroxy-6-methylpyrimidin-2-yl)guanidine (Compound **26**) [34], 1-(4-ethylphenyl)-3-*m*-tolylurea (Compound **27**) [33], 1-(4-ethylphenyl)-3-*o*-tolylurea (Compound **28**) [33], 1-(4-chlorophenyl)-3-(3-ethoxyphenyl)urea (Compound **29**) [33].

Biological Testing

In vitro efficacy testing was conducted by the Department of Parasitology, WRAIR, against *P. falciparum* W2 (resistant to chloroquine, sulfadoxine, pyrimethamine, and quinine but susceptible to mefloquine), D6 (resistant to mefloquine, but susceptible to chloroquine, sulfadoxine, pyrimethamine, and quinine), TM91C235 (a multi drug resistant isolate from Thailand), RCS (a chloroquine resistant isolate from Brazil) and TM90C2A (an chloroquine and mefloquine resistant isolate from Thailand) strains. The semiautomated microdilution technique of Desjardins, *et al*. [35] and Chu-

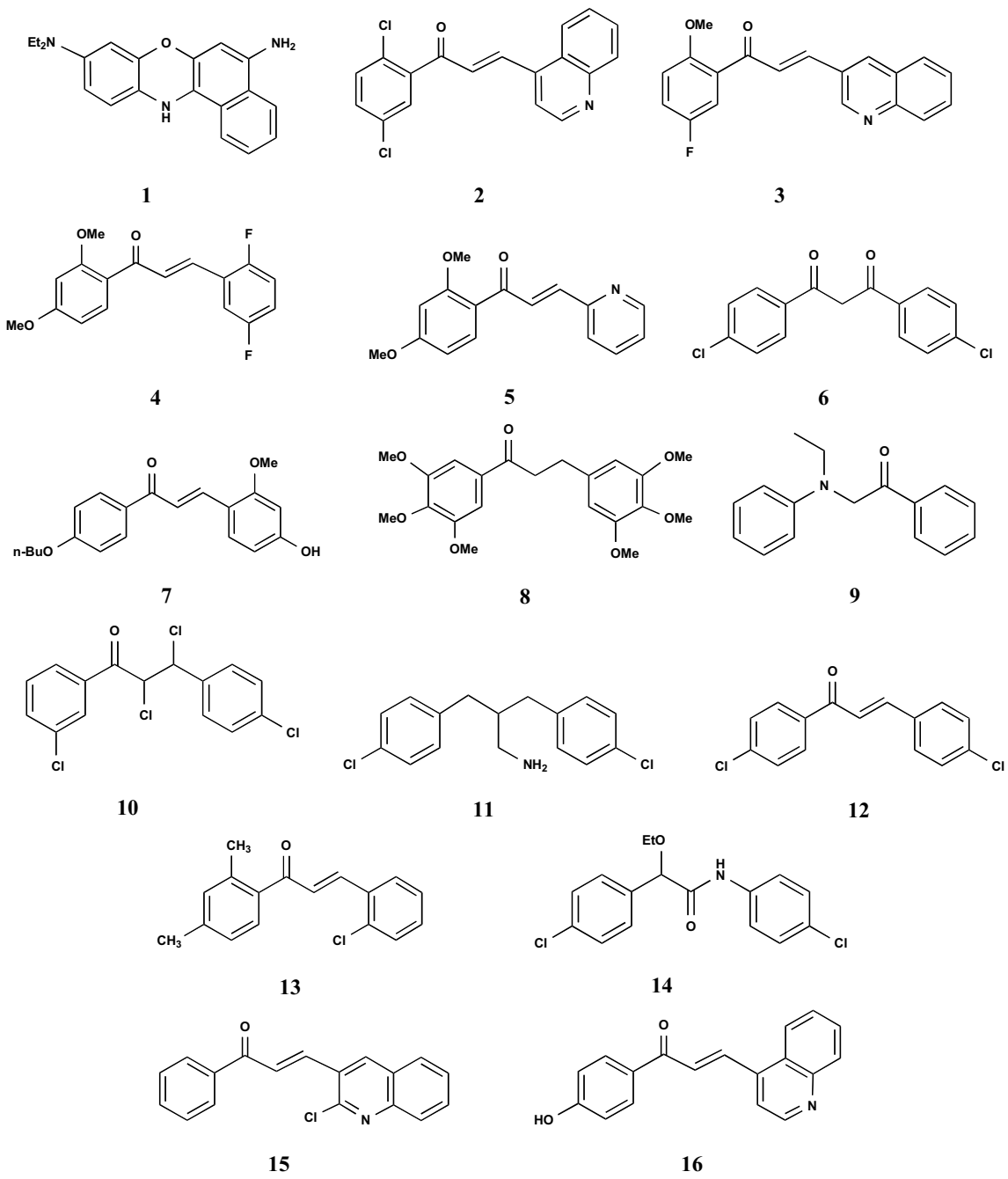


Fig. (2). Structures of compounds in the training set.

lay, *et al*. [36] was used. The test compounds were dissolved in DMSO and then diluted 400-fold in RPMI 1640 culture medium supplemented with 25mM Hepes, 32 mM $NaHCO_3$ and 10% Albumax I (GIBCO BRL, Grand Island, NY). These solutions were subsequently serially diluted 2-fold with a Biomek 2000 (Beckman, Fullerton, CA) over 11 different concentrations. The parasites were exposed to serial dilutions of each compound for 48 h and incubated at 37 ºC with 5% O_2, 5% CO_2, and 90% N_2 prior to the addition of [^{3}H]-hypoxanthine. After a further incubation of 18 h, parasite DNA was harvested from each microtiter well using a Packard Filtermate 196 Harvester (Meriden, CT) onto glass filters. Uptake of [^{3}H]-hypoxanthine was measured with a Packard topcount scintillation counter. Concentration-response data were analyzed by a nonlinear regression logistic dose-response model and the IC_{50} values (50% inhibitory concentrations) for each compound were determined.

In vivo efficacy testing of the majority of compounds identified (Compound **17**-**29**) had already been performed by

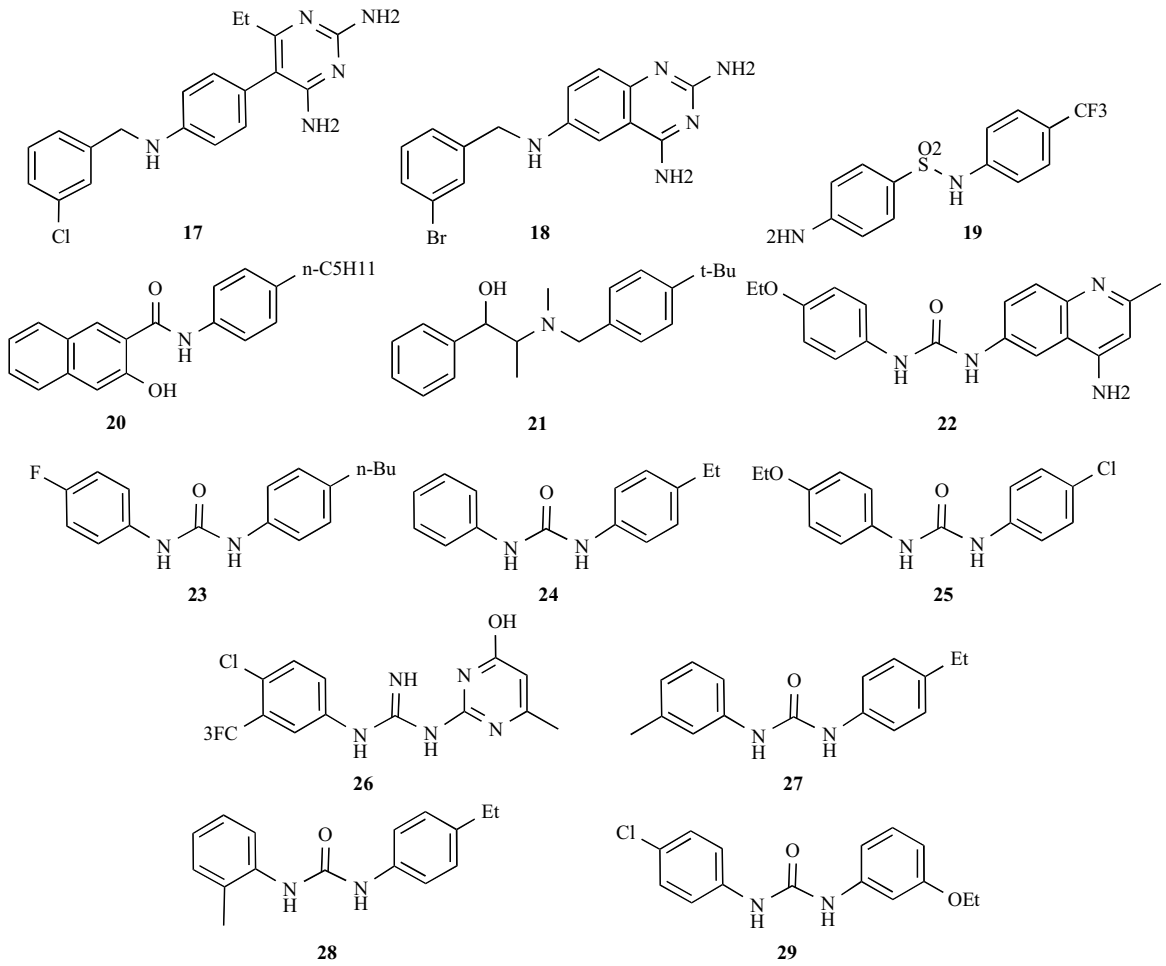


Fig. (3). Structure of compounds identified from a compound library using the pharmacophore.

the Division of Experimental Therapeutics, WRAIR in the early 1970s. The protocol used extended over 14 days, with Day 0 being when the test mice were infected with the malaria parasite, a subcutaneous dose of test compound of 640 mg/kg was administered once daily on Days 1 to 3. The presumptive casual prophylactic test determines if test compounds have activity against either the sporozite or exoerythrocyte stages of *Plasmodium yoelii*.

COMPUTATIONAL METHODS

Procedure for Development of the 3D QSAR Pharmacophore Model

The three-dimensional QSAR study was performed using CATALYST 4.9 software [37]. The algorithm treats molecular structures as templates comprised of chemical functions localized in space that will bind effectively with a complementary function on the respective binding protein(s). The most relevant biological features are extracted from a small set of compounds that cover a broad range of activity.

The CATALYST procedure uses the structure-activity data for a set of lead compounds to generate a pharmacophore characterizing the activity of the lead set. In this case activity was the *in vitro* efficacy against the W2 strain of *P. falciparum*. In order to obtain a reliable model, adequately describing the interaction of ligands with high predictability, the procedure recommends beginning with a collection of 15-25 chemically diverse molecules with biological activity covering 4-5 orders of magnitude for the training set. The structures of the sixteen chalcone and chalcone-like compounds (**1**-**16**) selected for this study are shown in Fig. (**2**). We consider chalcone-like compounds as being those possessing a non-enone, three-atom spacer between two aryl moieties. The *in vitro* efficacies of this training set of sixteen compounds, shown in Table **1**, cover a reasonably broad range.

We used the HypoGen algorithm to identify the pharmacophores that are common to the 'active' molecules in the training set but are absent in the 'inactives' [38]. The structures of the sixteen compounds (**1**-**16**) were edited within CATALYST, minimizing energy to the closest local minimum using the generalized CHARMM-like force field as implemented in the program. The most relevant chemical features were extracted from this set of compounds [39]. Molecular flexibility was taken into account by considering each compound as an ensemble of conformers representing

Table 1. Compounds in the Training Set

Cmpd.	Experimental IC_{50} (ng/mL)	Predicted IC_{50} (ng/mL)	Error*
1	24	32	1.3
2	74.5	380	5.1
3	318	590	1.9
4	385	480	1.3
5	454	340	-1.3
6	468	1800	3.7
7	502	280	-1.8
8	637.6	280	-2.3
9	752.5	2400	3.2
10	905	620	-1.5
11	1256	440	-2.9
12	1457	1300	-1.1
13	2385	1700	-1.4
14	3465	830	-4.2
15	7247	6100	-1.2
16	18856	15000	-1.2

*A value that increases as the rms (root-mean square) difference between predicted and experimental activities for the training set compounds increases. This factor is designed to favor models for which the activity correlation is better. The standard deviation of this parameter is given by the uncertainty parameter as described under Materials and Methods in the computational methods section.

different accessible areas in a three dimensional space. The "best searching procedure" was applied to select representative conformers within 10 kcal/mol of the global minimum [40].

Conformational models of the sixteen compounds were generated in such a way to emphasize representative coverage within a range of permissible Boltzman population with significant abundance (within 10.0 kcal/mol) of the calculated global minimum. This conformational model, used for pharmacophore generation within CATALYST, aims to identify the best three-dimensional arrangement of chemical functions (such as hydrophobic regions, hydrogen bond donor, hydrogen bond acceptor, and positively and/or negatively ionizable sites) which explains the variation in activity among the compounds in the training set. The hydrogen bonding features in CATALYST [37] are represented as vectors, whereas all other functions are represented as points. Pharmacophore generation was carried out using the default parameters in the automatic generation procedure of CATALYST including function weight = 0.302, mapping coefficient = 0, resolution = 280 pm (2.8 Å), and activity uncertainty "Δ" = 3. An uncertainty in the CATALYST paradigm indicates an activity value lying somewhere in the interval from "activity divided by Δ" to "activity multiplied by Δ". The statistical relevance of the pharmacophore obtained was then assessed on the basis of the cost relative to the null hypothesis and the correlation coefficient, along with CatScrambled confidence level of the pharmacophore [37]. The pharmacophore was then used to estimate the activities of the compounds in the training set. These activities are derived from the best conformation generation mode of the conformers displaying the smallest root-mean square (RMS) deviations when projected onto the pharmacophore. HypoGen considers each feature (e.g. hydrogen-bond acceptor, hydrogen-bond donor, hydrophobic regions, positive ionizable group, etc.) in the pharmacophore to contribute equally when estimating activity. Also, each chemical feature in the HypoGen pharmacophore requires a match to a corresponding ligand atom within the same distance of tolerance [38]. The method has been documented to perform better than structure-based pharmacophore generation [40].

Docking Calculations

Docking calculations with heme were performed using the Affinity module of the InsightII package [41], adopting the ESFF (electrostatic force field) force field for assigning the potentials of both the ligands and of heme. Affinity is a collection of programs for automatic docking of a ligand to a receptor (host-heme). For a given assembly, consisting of a ligand molecule and a receptor molecule, docking procedures in Affinity optimizes the structure of ligand/receptor complex based upon the energy of the complex. This energy-driven method [42] is particularly useful in situations where the experimentally determined structure of a protein-ligand

complex is unavailable. It uses a combination of Monte Carlo and Simulated Annealing procedures to dock the guest molecule to the host. A key feature is that the "bulk" of the receptor, defined as atoms not in the binding (active) site specified, is held rigid during the docking process, while the binding site atoms and ligand atoms are allowed to move. Non-bonded interactions in Affinity are calculated using either a grid based approach developed by Luty, *et al*. [43], a cell multipole approach, a group based cutoff approach, or a hard sphere steric method without electrostatics [42]. Furthermore, Affinity allows incorporation of solvation effects by the method of Stouten, *et al*. [44]. In addition to non-bonded interactions, various empirical penalty terms, such as a distance based H-bond term, a ligand confining term, and a simple tethering term are included in the calculations to aid in the process of docking.

We present here an overview of the docking procedure. Firstly, a roughly docked complex of Compound **1** and heme is generated manually. Affinity is then used to perform an energy minimization to obtain an initial structure. This step should remove bad contacts and poor internal geometry in this structure resulting in a reasonable starting point for subsequent searches. The ligand is then moved by a random combination of translation, rotation, and torsional changes. The random movement of the ligand samples both the conformational space of the ligand and its orientation with respect to the receptor. This has the advantage of overcoming any energy barrier on the potential energy surface. Subsequently the method checks the energy of the resulting randomly moved structure. If it is within the a specified energy tolerance parameter of the previous minimized structure, it is considered to have passed the first step and the structure is then subjected to energy minimization, the second step for fine-tuning the docking. The final minimized structure is accepted or rejected based on the Metropolis energy criterion as implemented in the software and its similarity to structures found before. The Metropolis criterion is found to be best suited for finding a very small number of docked structures with very low energies, while the other energy range criterion is designed to find more and diverse structures. In checking structure similarity, the RMS distances between the current structure and structures found so far are computed for ligand atoms. Since the Affinity module has the ability to employ different docking methodologies, such as simulated annealing and dynamics in conjunction with Monte Carlo minimization, after generating initial structures by the above method, we further refine these structures with these procedures.

Quantum Chemical Calculations

Binding affinities for the interaction between a sodium ion and the π-electrons of various aromatic rings were calculated using the 6-31G** basis set as implemented in the Gaussian98 package [42] (using d- & p-orbital polarized functions). Complete optimization of geometry of each complex was carried out using the above basis set. Similar calculations were performed on the uncomplexed molecular fragments and sodium ion separately, at the same level of theory. The choice of the 6-31G** basis set was documented as adequate enough for the study, since substantially higher levels of theory produce similar trends [43]. The initial structure of the complex was built by placing the sodium ion above the aromatic ring centroid. The molecular electrostatic potential (MEP) profiles were calculated on the optimized geometry of the molecules using SPARTAN [44]. The MEPs were sampled over the entire accessible surface of a molecule (corresponding roughly to the van der Waals contact surface) providing a measure of charge distribution from the point of view of an approaching reagent. The regions of negative potential indicate areas of excess negative charge (represented by red color, deepest red being the most negative potential) so the deepest red region is the most nucleophilic site in the molecule. Regions of positive potential indicate areas of excess positive charge (represented by blue color, deepest blue being the most positive potential), so the deepest blue region is the most electrophilic site in the molecule.

RESULTS AND DISCUSSION

3D Pharmacophore and its Correlation with Experimental Activity

Pharmacophores are spatial arrangements of key chemical features associated with a set of known compounds that are responsible for a specific biological activity of the compounds. They are mainly useful when, as is the case for the chalcones, active compounds have been identified, but a three dimensional structure of the biological target is unknown. We utilized data for a training set of sixteen chalcone and chalcone-like compounds (**1**-**16**) (Fig. (**2**)) to develop a 3D pharmacophore model for the *in vitro* efficacy of chalcones against *P. falciparum* W2.

In generating the pharmacophore, chemical features were selected from the feature dictionary, including hydrogen bond acceptor, hydrogen bond donor, aliphatic hydrophobe, aromatic hydrophobe, and positive ionizable features. The HypoGen algorithm in CATALYST was run using default values for all parameters. The log file obtained from the HypoGen calculations indicated a successful run for the generation of the pharmacophore containing the important parameters well within the appropriate range as recommended by the methodology. By performing a Fischer randomization as implemented in the CatScramble module of the CATALYST [37], we observed an approximately 85-95% confidence level for our model. During the pharmacophore development, the molecules were mapped to the features with their predetermined conformations generated using the “fast fit” algorithm in CATALYST. The conformational energy range for developing the model was between 0 to 20 kcal/mol. The procedure appeared to run successfully and resulted in the generation of 10 alternative pharmacophores for activity of the compounds.

The correlation coefficient for the statistically most significant pharmacophore was good (R = 0.85). The structural elements, which are shown in Fig. **4a**, comprise an aromatic hydrophobic and an aliphatic hydrophobic site, one hydrogen bond donor site, and a ring aromatic feature. The corresponding binding site in the target protein presumably possesses complementary features to these, leading to binding.

The correlation between the experimental and predicted efficacies of the training set compounds is presented in Fig. (**5**). The more potent compounds in the training set are found

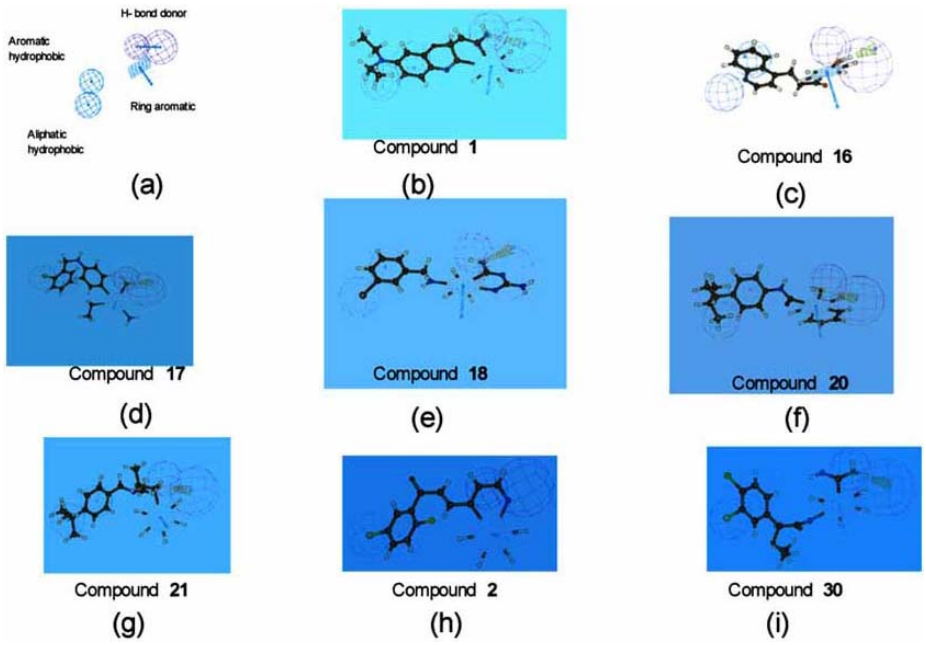


Fig. (4). (a) Pharmacophore for the antimalarial activity of the chalcones.

(b) Mapping of the pharmacophore with a potent compound (**1**); (c) Mapping of the pharmacophore with a less potent compound (**16**); (d) Mapping of the pharmacophore with the identified Compound (**17**); (e) Mapping of the pharmacophore with the identified Compound (**18**); (f) Mapping of the pharmacophore with the identified Compound **(20**); (g) Mapping of the pharmacophore with the identified Compound (**21**); (h) Mapping of the pharmacophore with compound (**2**), which matches three features; (i) Mapping of the pharmacophore with compound (**30**), a structure designed to matches all four features.

to map all the features of the pharmacophore (e.g. Compound **1**, Fig. (**4b**)), whereas the less potent compounds fail to map all the features (e.g. Compound **16**, Fig. (**4c**)).

In addition to the CatScramble method of statistical validation, we further cross-validated the pharmacophore model by using it to virtually screen an in-house chemical library

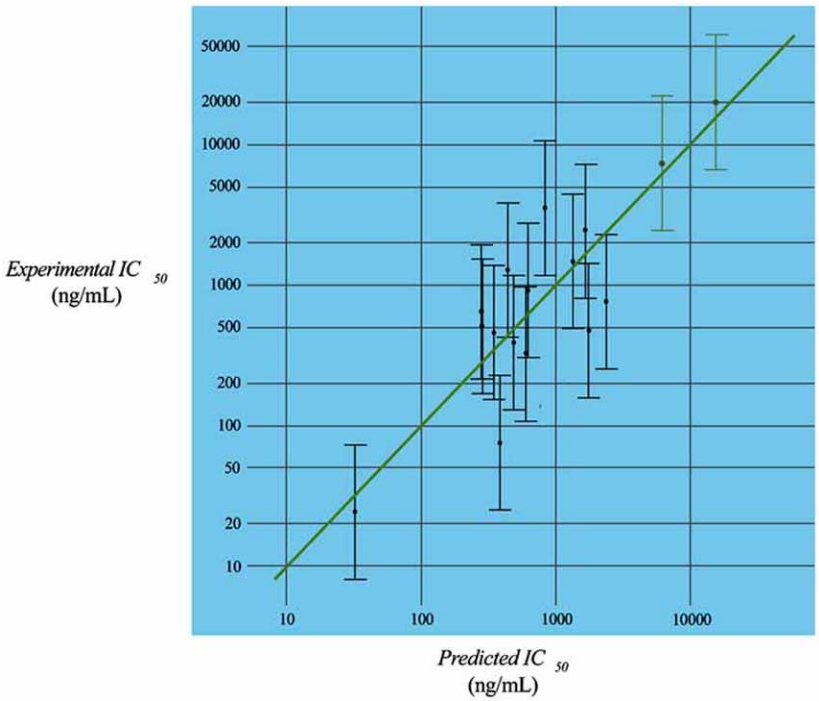


Fig. (5). Correlation of experimental activity with those predicted *in silico.*

[15]. In so doing, we should identify new compounds which possess the chalcone antimalarial pharmacophore. To this end, the pharmacophore was mapped onto a potent analog and the spatial positions of the chemical features were converted into a 3D shape-based single template which was used to search for new compounds in the library. The generated template has both shape (to account for the steric factors) and the features (necessary for binding to the active site) for antimalarial activity. The structures of the most interesting compounds identified (**17**-**29**), are shown in Fig. **3**, and their antimalarial properties in Table **2**. The potent compounds (IC_{50} < 100 ng/mL against both W2 and D6 strains) all map well on the features of the pharmacophore; mappings for Compounds **17**, **18**, **20**, and **21** are shown in Figs. (**4d**-**4g**). Two of these, compounds **17** and **18,** are highly potent *in vitro* against all five strains of *P. falciparum* tested. Moreover, compound **18** showed significant potency in a malaria-infected mouse model. The other compounds, which are less potent *in vitro*, do not map to all the features of the pharmacophore. The data is not shown, but some of these compounds (**17**-**29**) were found to have improved metabolic stability compared to the chalcones. In considering which hits to pursue, ADME and toxicity properties were considered, in part by undertaking a rapid *in silico* screening using Cerus2-ADME and TOPKAT methodologies [48].

One compound that we elected to further investigate is Compound **18**. It is known to be a folate antagonist and is a highly potent antimalarial agent [49]. We are currently using the pharmacophore and other literature describing the structural requirements for inhibition of dihydrofolate reductase [50-52] to design new antimalarial compounds based upon Compounds **18**, which lack antifolate activity, yet possess antimalarial activity. The results of these studies will be published shortly [53].

The pharmacophore model was also used in an effort to enhance the potency of the chalcone-like compounds in our program. Fig. (**4h**) shows the mapping of the pharmacophore onto one of our most potent compounds to date, Compound **2**. It maps three of the four features in the pharmacophore: the two phenyl rings provide the aromatic hydrophobe and aromatic ring features; and the 3-chloro substituent the aliphatic hydrophobe. Since it lacks the hydrogen bond donor function, we felt it will be possible to design more highly potent compounds. One such compound is the amidine, compound **30**, shown in Figs. (**4i**) and (**6**), which maps all four features in the pharmacophore. Additionally, the amidine functional group is found in many potent antimalarial compounds [54]. The results of this study will be published later.

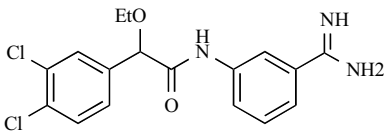
Since potent chalcones are reported to participate in the inhibition of hemozoin formation and antimalarial activity has been suggested to be due to the inhibition of heme formation [55], we performed docking calculations with heme to assess the role of electron transfer to the heme iron from the compounds. By adopting the docking procedure as implemented in the affinity module of InsightII [41], we calculated the minimum energy of heme and the docked structure for the most potent analog in the training set, Compound **1**, along with that for two of the less potent analogs, Compounds **2** and **5**. The interaction energy values of the docked structures were found to be as follows: Compound **1** = 364.3 kcal/mol, Compound **2** = 113.6 kcal/mol, and Compound **5** = 101.9 kcal/mol. Clearly, the more potent analogs seem to have a stronger interaction with heme, thus inhibition of

Table 2. Antimalarial Activity of Compounds Identified from a Compound Library Using the Pharmacophore

Cmpd.	IC_{50} (ng/mL) D6	IC_{50} (ng/mL) W2	IC_{50} (ng/mL) TM91C235	IC_{50} (ng/mL) RCS	IC_{50} (ng/mL) TM90C2A	*In vivo* activity*
17	<0.9	5.7	57.6	6.6	26.5	Not tested
18	0.14	4.17	19	1.7	5.8	Curative
19	17.4	>12500	>500	>500	>500	Not tested
20	38.8	28.6	128.4	27.7	134.6	Inactive
21	96.4	31.8	296	30.6	235.4	Inactive
22	165.8	175.7	nd	nd	nd	Inactive
23	225.9	640.8	nd	nd	nd	Inactive
24	291.2	1060.9	nd	nd	nd	Inactive
25	360.8	436.3	nd	nd	nd	Inactive
26	410.2	1090	nd	nd	nd	Inactive
27	425.1	732.9	nd	nd	nd	Inactive
28	516.7	2590.2	nd	nd	nd	Inactive
29	679	593.5	nd	nd	nd	Inactive

* Based upon survivability of test mice, dosed subcutaneously 640 mg/kg/day for 3 days. Compounds are curative when all 5 test mice survive the 14 days of the assay and suffer no reemergence of infection. Compounds are inactive when test mice have comparable survivability to control mice, which receive only vehicle and typically die during days 6-9 of the assay.

heme formation may be involved in the mechanism of antimalarial action of these compounds.



30

Fig. (6). Structure of compound **30**, a chalcone-like compound designed using the pharmacophore.

The interaction between the π-electrons of the aromatic moiety and a positively charged entity in the binding site is proposed to be involved in the antimalarial activity of the chalcones [8]. In order to analyze such a possibility, we assessed the binding affinity of the π-electron in the A ring in three chalcones using the sodium ion as a probe test positive charge: Compound **1**, the most potent analog in the training set; Compound **2**, a moderately potent; and Compound **5**, a less potent analog from the training set. The calculated stabilization energies due to sodium complexation for the three compounds are: Compound **1** = 28.0 kcal/mol; Compound **2** = 26.0 kcal/mol; and Compound **5** = 52.0 kcal/mol. The results indicate that the π-electrons of A ring in Compound **5** are almost two fold more stabilized by complexation than the corresponding A rings in Compound **1** (stabilized by 28.0 kcal/mol) and Compound **2** (stabilized by 26.0 kcal/mol). Therefore interaction between the aromatic ring and a positively charged entity in the binding site does not appear to have a role in the antimalarial activity of these compounds. However, the molecular electrostatic potential (MEP) profile of uncomplexed Compound **1** indicates the strongest nucleophilic center at the nitrogen atom of the heterocycle. Thus, protonation at this center could be involved in the mechanism of antimalarial activity of these compounds, either by enhancing interaction with the biological target or by concentrating the compound in the acidic food vacuoles of the parasites [56].

CONCLUDING REMARKS

The study demonstrates how the chemical features of a set of diverse chalcone and chalcone-like compounds can be organized to develop a pharmacophore for antimalarial activity. An aromatic and an aliphatic hydrophobic site, one hydrogen bond donor site, and a ring aromatic feature distributed over a three dimensional space, appear to be the functional features required for potent antimalarial activity of this class of compounds. The activity of the compounds estimated by this pharmacophore was found to correlate well with those determined experimentally. The pharmacophore was used to conduct a virtual screening of a compound library to identify new classes of compound with potential as antimalarial candidates. The validity of the pharmacophore extends to structurally different classes of compounds, and thereby provides a powerful template by which novel drug candidates may be designed. This three-dimensional QSAR pharmacophore should assist in the design of novel antimalarial agents directed against the (as yet unknown) target of the chalcones.

ACKNOWLEDGEMENTS

Material has been reviewed by the Walter Reed Army Institute of Research. There is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the authors and are not to be construed as official, or reflecting true views of the Department of the Army or the Department of Defense. The authors also acknowledge the funding support for this work by the Military Infectious Diseases Research Program, the Office of Naval Research and the United States Naval Academy.

REFERENCES

[1] Malaria Foundation International, www.malaria.org, and links therein; (b) Hoffman, S.L.; Subramanian, G.M.; Collins, F.H.; Venter, J.C. *Nature*, **2002**, *415*, 702.
[2] Jerrard, D.A.; Broder, J.S.; Hanna, J.R.; Colletti, J.E.; Grundmann, K.A.; Geroff, A.J.; Mattu, A. *J. Emerg. Med.,* **2002**, *23*, 23.
[3] Trigg, P.I.; Kondrachine, A.V. In *Malaria Parasite Biology, Pathogenesis and Protection: The current global malaria situation*, Sherman I.W., Ed.; ASM: Washington, DC, **1998**, pp. 11-22.
[4] White, N.J. *Br. Med. Bull.* **1998**, *54*, 703.
[5] Vroman, J.A.; Gaston, M.A.; Avery, M.A. *Curr. Pharm. Des.,* **1999**, *5*, 101.
[6] Chen, M.; Theander, T.G.; Christensen, B.S.; Hiviid, L.; Zhai, L.; Kharazmi, A. *Antimicrob. Agents Chemther.,* **1994**, *38*, 1470.
[7] Chen, M.; Christensen, S.B.; Zhai, I.; Rasmussen, M.H.; Theander, T.G.; Frokjaer, S.; Steffansen, B.; Davidson, J.; Kharazmi, A. *J. Infect. Dis.,* **1997**, *176*, 1327.
[8] Li, R.; Kenyon, G.L.; Cohen, F.E.; Chen, X.; Gong, B.; Dominguez, J.N.; Davidson, E.; Kurzban, G.; Miller, R.; Nuzum, E.O.; Rosenthal, P.J.; McKerrow, J.H. *J. Med. Chem.,* **1995**, *38*, 5031.
[9] Feldbaeck, N.S.; Brogger, C.S.; Cruciani, G.; Kharazami, A.; Liljefors, T. *J. Med. Chem.,* **1998**, *41*, 4819.
[10] Liu, M.; Wilairat, P.; Go, M.L. *J. Med. Chem.,* **2001**, *44*, 4443.
[11] Kayser, O.; Kiderlen, A.F.; Croft, S.L. *Stud. Natl. Prod. Chem.,* **2002**, *26*, 779.
[12] Go, M.-L.; Liu, M.; Wilairat, P.; Rosenthal, P.J.; Saliba, K.J.; Kirk, K. *Antimicrob. Agents Chemother.,* **2004**, *48*, 3241; b) Ziegler, H.L.; Hansen, H.S; Staerk, D.; Christensen, S.B.; Hägerstrand, H.; Jaroszewski, J. W. *Antimicrob. Agents Chemother.,* **2004**, *48*, 3241.
[13] Gutteridge, C.E.; Nichols, D.A.; Curtis, S.M.; Thota, D.S.; Vo, J.V.; Gerena, L.; Montip, G.; Asher, C.O.; Diaz, D.S.; DiTusa, C.A.; Smith K.S.; Bhattacharjee, A. K. *Bioorg. Med. Chem. Lett*., **2006**, *16*, 5682.
[14] Bhattacharjee, A.K.; Kyle, D.E.; Vennerstrom, J.L.; Milhous, W.K. *J. Chem. Info. Comput. Sci.,* **2002**, *42*, 1212; (b) Bhattacharjee, A.K.; Skanchy, D.J.; Jennings, B.; Hudson, T.H.; Brendle, J.J.; Werbovetz, K.A. *Bioorg. Med. Chem*., **2002**, *10*, 1979; (c) Bhattacharjee, A.K.; Geyer, J. A.; Woodard, C.L.; Kathcart, A.K ; Nichols, D.A.; Prigge, S.T.; Li, Z.; Mott, B.T; Waters, N.C. *J. Med. Chem.*, **2004**, *47*, 5418.
[15] Chemical Information System, Division of Experimental Therapeutics, Walter reed Army Institute of Research, Silver Spring, MD.
[16] Ni, F.; Feng, H.; Gorton, L.; Cotton, T.M. *Langmuir,* **1990**, *6*, 66.
[17] Accessible *via* the procedure described in [13].
[18] Cohen, F.E.; McKerrow, J.H.; Kenyon, G.L.; Li, Z.; Chen, X.; Gong, B.; Li, R. WO Patent 9630004, **1996**; *Chem. Abstr.,* **1996**, *126*, 14741.
[19] Franek, W. *Monatshefte fuer Chemie*, **1996**, *127*, 895.
[20] Bhat, B.A.; Dhar, K.L.; Puri, S.C.; Saxena, A.K.; Shanmugavel, M.; Qazi, G.N. *Bioorg. Med. Chem. Lett*., **2005**, *15*, 3177; b) Saikia, A.; Barthakur, M.G.; Boruah, R.C. *Synlett*, **2005**, *3*, 523.
[21] Galons, H.; Girardeau, J.F.; Farnoux, C.C.; Miocque, M. *J. Heterocycl Chem.*, **1981**, *18*, 561.
[22] Accessible *via* the procedure described in Abell, R.D.; Siddall, W. *J. Chem. Soc.*, **1953**, 2804.
[23] Komrsova, H.; Farkas, J. *Chemicke Listy pro Vedu a Prumysl*, **1958**, *52*, 454.

[24] Accessible *via* the procedure described in Maki, T.; Ishihara, K.; Yamamoto, H. *Org. Lett.,* **2005**, *7*, 5043.
[25] Datta, N.J.; Khunt, R.C.; Parikh, A.R. *Oriental J. Chem.*, **2002**, *18*, 131.
[26] Cheng, M.S.; Li, R.S.; Kenyon, G. *Chin. Chem. Lett.*, **2000**, *11*, 851.
[27] Kosogof, C.; Liu, B.; Liu, G.; Liu, M.; Nelson, L.T. j.; Serby, M.D.; Sham, H.L.; Szczepankiewicz, B.G.; Xin, Z.; Zhao, H. U.S. Patent 2005070712, **2005**; *Chem. Abstr.,* **2005**, *142*, 336390.
[28] Davoll, J.; Johnson, A. M.; Davies, H.J.; Bird, O.D.; Clarke, J.; Elslager, E.F. *J. Med. Chem.,* **1972**, *15*, 812.
[29] Schmidt, L.H.; Sesler, C.L. *J. Pharmacol.*, **1946**, *87*, 313.
[30] Ootsu H.; Higeta S. Japanese Patent 59204160, **1984**, *Chem. Abstr.,* **1984**, *102*, 133558.
[31] Accessible *via* the procedure described in Pfanz, H.; Muller, H. *Arch. Pharm.*, **1955**, *288*, 11.
[32] Peng, C.-T.; Daniels, T.C. J. *Am. Chem. Soc*., **1956**, *76,* 3703.
[33] Accessible *via* the procedure described in Hutchison, A.J.; Yuan, J. Worldwide Patent 2006049941, **2006**; *Chem. Abstr.,* **2006**, *144*, 467912.
[34] Elslager, E.F.; Werbel, L.M.; Curry, A.; Headen, N.; Johnson, J. *J. Med. Chem.,* **1974**, *17*, 75.
[35] Desjardins, R.E.; Canfield, C.J.; Haynes, D.E.; Chulay, J.D. *Antimicrob. Agents Chemther.,* **1979**, *16*, 710.
[36] Chulay, J.D.; Haynes, J.D.; Diggs, C.L. *Exp. Parasitol.,* **1983**, *55*, 138.
[37] CATALYST Version 4.9; Accelrys Inc., San Diego, CA, **2001**.
[38] Gunner O.A. In *Pharmacophore, perception, development, and use in drug design*, Ed.; University International Line: San Diego, **2000**, pp. 17-20.
[39] Grigorov, M.; Weber, J.; Tronchet, M.J.; Jefford, C.W.; Milhous, W.K.; Maric, D.A. *J. Chem. Inf. Comput. Sci*., **1995**, *35*, 285.
[40] Greenridge, P.A.; Weiser, J. *Mini Rev. Med. Chem.,* **2001**, *1*, 79.
[41] InsightII, Accelrys Inc., San Diego, CA, **2001**.
[42] Kuntz, I.D.; Meng, E.C.; Shoichet, B.K. *Acc. Chem. Res.*, **1994**, *27*, 117.
[43] Luty, B.A.; Wasserman, Z.R.; Stouten, P.F.W.; Hodge, C.N.; Zacharias, M.; McCammon, J.A. *J. Comp. Chem.*, **1995**, *16*, 454.
[44] Stouten, P.F.W.; Frommel, C.; Nakamura, H.; Sander, C. *Mol. Simul.*, **1993**, *10*, 97.
[45] Frisch, M.J.; Trucks, G.W.; Schlegel, H.B.; Scuseria, G.E.; Robb, M.A.; Cheeseman, J.R.; Zakrzewski, V.G.; Montgomery, J.A.; Stratmann, R.E.; Burant, J.C.; Dapprich, S.; Millam, J.M.; Daniels, A.D.; Kudin, K.N.; Strain, M.C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G.A.; Ayala, P.Y.; Cui, Q.; Morokuma, K; Malick, D.K.; Rabuck, A.D.; Raghavachari, K.; Foresman, J.B.; Cioslowski, J.; Ortiz, J.V.; Stefanov, B.B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, R.; Gomperts, R.; Martin, R.L.; Fox, D.J.; Keith, T.; Al-Laham, M.A.; Peng, C.Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P.M.W.; Johnson, B.G; Chen, W.; Wong, M.W.; Andres, J.L.; Head-Gordon, M.; Replogle, E.S; and Pople, J.A. Gaussian 98 (Revision A.1), Gaussian, Inc., Pittsburgh PA. **1998**.
[46] Mecozzi, S.; West, A.P.; Dougherty, D.A. *J. Am. Chem. Soc*., **1996**, *118*, 2307.
[47] SPARTAN, 5.0, Wavefunction, Inc.: Irvine, CA, **2002**.
[48] Cerus2-ADME and TOPKAT, Accelrys Inc., **2001**.
[49] Parenti, M.D.; Pacchioni, S.; Ferrari, A.M.; Rastelli, G. *J. Med. Chem.,* **2004**, *47,* 4258.
[50] Roth, B.; Cheng, C.C. *Prog. Med. Chem.*, **1982**, *19*, 269.
[51] Ghose, A.K.; Crippen, G.M. *J. Med. Chem.,* **1984**, *27,* 901.
[52] Rydon, H.N.; Undheim, K. *J. Chem. Soc.,* **1962**, 468.
[53] Gutteridge, C.E.; Hoffman, M.M.; Bhattacharjee, A.K.; Gerena, L. Manuscript in press, **2006**.
[54] Leban, J. Pegoraro, S.; Dormeyer, M.; Lanzer, M.; Aschenbrenner, A.; Kramer, B. *Bioorg. Med. Chem. Lett*., **2004**, *14*, 1979.
[55] Dominguez, J.N.; Leon, C.; Rodrigues, J.; de Dominguez, N.G.; Gut, J.; Rosenthal, P.J. *J. Med. Chem.,* **2005**, *48*, 3654.
[56] Dyke, S.F.; Kinsman, R.G. In *Properties and reactions of isoquinoline and their hydrogenated derivatives. In Heterocyclic Compounds Isoquinolines*, Grethe, G., Ed.; John Wiley & Sons: New York, **1981**; pp. 3-10.

Received: 08 September, 2006 Revised: 03 October, 2006 Accepted: 04 October, 2006