Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Medicinal Chemistry 39 (2004) 59–67
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
www.elsevier.com/locate/ejmech

Original article

Structure-activity relationship study of antimalarial indolo [2,1-b]quinazoline-6,12-diones (tryptanthrins). Three dimensional pharmacophore modeling and identification of new antimalarial candidates

Apurba K. Bhattacharjee *, Mark G. Hartell, Daniel A. Nichols, Rickey P. Hicks, Benjamin Stanton, John E. van Hamont, Wilbur K. Milhous

Department of Medicinal Chemistry, Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Silver Spring, MD 20910-7500, U.S.A.

Received 5 August 2003; received in revised form 7 October 2003; accepted 9 October 2003

Abstract

A widely applicable three-dimensional QSAR pharmacophore model for antimalarial activity was developed from a set of 17 substituted antimalarial indolo[2,1-b]quinazoline-6,12-diones (tryptanthrins) that exhibited remarkable in vitro activity (below 100 ng/mL) against sensitive and multidrug-resistant *Plasmodium falciparum* malaria. The pharmacophore, which contains two hydrogen bond acceptors (lipid) and two hydrophobic (aromatic) features, was found to map well onto many well-known antimalarial drug classes including quinolines, chalcones, rhodamine dyes, Pfmrk cyclin dependent kinase inhibitors, malarial FabH inhibitors, and plasmepsin inhibitors. The phamacophore allowed searches for new antimalarial candidates from multiconformer 3D databases and enabled custom designed synthesis of new potent analogues.

Keywords: QSAR; Pharmacophore; Malaria

1. Introduction

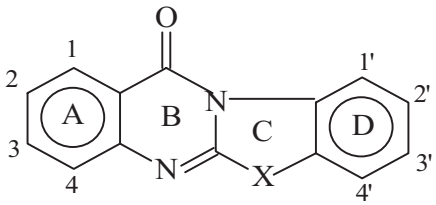
The current global situation with respect to malaria indicates that about two billion people are exposed to the disease and more than 1 million people die from it every year [1]. The situation is rapidly worsening mainly due to non-availability of effective drugs and development of drug resistance in areas where malaria is frequently transmitted [2]. Chloroquine, mefloquine, and other frontline drugs for the treatment and prevention of malaria, are becoming increasingly ineffective [3]. Artemisinin analogues such as artesunate and arteether were later introduced and found to be quite effective, particularly against drug-resistant *P. falciparum,* but observations of drug-induced and dose-related neurotoxicity in animals have raised concern about the safety of these compounds for human use [3,4]. Therefore, much effort is needed for discovery and development of new and less toxic antimalarial drugs.

Tryptanthrin (indolo[2,1-b]quinazoline-6,12-dione, **6**), an alkaloid isolated from the Taiwanese medicinal plant *Strobilanthes cusia*, and its substituted derivatives (Table 1) were recently revisited for a screening program conducted by the Walter Reed Army Institute of Research, Silver Spring, Maryland, U.S.A.[5-8]. The compounds displayed remarkable in vitro antimalarial activity against *P. falciparum*, both sensitive and multidrug-resistant strains [9]. The more potent analogs exhibit IC_{50} values in the range 0.43 to 10 ng/mL, about one one-thousandth of the concentrations necessary to inhibit bacteria. Furthermore, the compounds are also found to be highly potent against strains of *P. falciparum* that are up to 5000-fold resistant to atovoquone, 50-fold resistant to chloroquine, and 20-fold resistant to mefloquine. Thus, this novel class of compounds has opened a new chapter for study in the chemotherapy of malaria.

* Corresponding author.
E-mail address: apurba.bhattacharjee@na.amedd.army.mil (A.K. Bhattacharjee).

doi:10.1016/j.ejmech.2003.10.004

Table 1
Structure of tryptanthrins



compd	1-	2-	3-	4-	X	1'	2'	3'	4'
Training set:									
1	CH	CH	CH	-N=	C=O	CH	CH	C-CI	CH
2	CH	CH	C-F	-CH	C=O	CH	CH	C-CI	CH
3	CH	-N=	CH	CH	C=O	CH	CH	$C\text{-}CH_2CH_3$	CH
4	CH	CH	$C\text{-}N<(CH_2)_4>N\text{-}CH_3$	CH	C=O	CH	CH	C-CI	CH
5	CH	-N=	$C\text{-}CH_3$	-N=	C=O	CH	CH	CH	CH
6	CH	CH	CH	-CH	C=O	CH	CH	CH	CH
7	CH	-N=	CH	-CH	C=O	CH	CH	$CCHC_7H_{16}$	CH
8	-N=	CH	CH	-N=	C=O	CH	CH	CH	CH
9	CH	CH	CH	$C\text{-}OCH_3$	C=C-phenyl	CH	CH	C-F	CH
10	CH	CH	C-F	CH	C=O	CH	$C\text{-}N<(CH_2)_4>N\text{-}CH_3$	C-F	CH
11	CH	CH	CH	CH	-O-	CH	CH	CH	CH
12	CH	-N=	CH	CH	-S-	CH	CH	CH	CH
13	CH	CH	CH	-N=	C=O	CH	C-Cl	CH	CH
14	-N=	C-OH	-N=	C-OH	C=O	CH	CH	C-I	CH
15	CH	CH	CH	CH	C=indole	CH	CH	CH	CH
16	CH	CH	CH	CH	C=C-C=C-phenyl	CH	CH	CH	CH
17	CH	CH	CH	CH	C-dioxane	CH	CH	C-Br	CH
Test set:									
1*	CH	-N=	CH	CH	C=O	CH	CH	$C\text{-}C_8H_{17}$	CH
2'	CH	CH	CH	-N=	C=O	CH	CH	C-CI	CH
3'	CH	CH	CH	CH	C=O	CH	CH	CH	C-CI
4'	CH	CH	$C\text{-}S\text{-}C_2H_4OH$	CH	C=O	CH	CH	C-CI	CH
5'	CH	-N=	CH	CH	C=O	CH	CH	$C\text{-}C_4H_9$	CH
6'	CH	-N=	CH	CH	C=O	CH	CH	HC_2H_5	CH
7'	CH	CH	CH	$C\text{-}OCH_3$	C=O	CH	CH	CH	CH
8'	CH	-N=	CH	CH	C=O	CH	CH	$C\text{-}CHOCH_3C\text{-}(CH_3)_2$	CH
9'	CH	CH	CH	CH	C=O	CH	CH	$C\text{-}OCF_3$	CH
10'	CH	CH	CH	-N=	C=O	CH	CH	C-I	CH
11'	CH	CH	CH	$C\text{-}OCH_3$	C=O	CH	CH	C-I	CH
12'	CH	CH	C-piperidine	CH	C=O	CH	CH	C-CI	CH
13'	CH	-N=	CH	CH	C=O	CH	CH	C-Br	CH
14'	CH	CH	$CNCH_3(CH_2)_2OH$	CH	C=O	CH	CH	C-CI	CH
15'	CH	$C\text{-}CH_3$	CH	CH	C=O	CH	CH	CH	CH

Tryptanthrins have a long history [5-8] and are known to possess activity against a variety of pathogenic bacteria, particularly the causative agent of tuberculosis [8]. To further improve its in vitro efficacy, a series of additional azatryptanthrin analogs were synthesized incorporating one or two nitrogen atoms in the A ring (Table 1), and tested for antimalarial activity. Surprisingly, many of these compounds showed improved efficacy against *P. falciparum* and *P. vivax* [5,9]. Thus, in continuation of our efforts to design new antimalarial therapeutic agents from structure-activity relationship studies [10-14], a three-dimensional chemical feature based pharmacophore model with applicability over different classes of antimalarials is presented here to provide a foundation for 3D database searches to help identify new potential antimalarial candidates.

2. Results and discussion

The 3D-QSAR pharmacophore for antimalarial activity was found to contain two hydrogen bond acceptor (lipid) functions and two aromatic hydrophobic functions at a specific geometric orientation (Fig. 1). It was developed from a set of 17 substituted tryptanthrin derivatives including the parent compound, shown in Table 1. The biological activity of the 17 tryptanthrin compounds covers a broad spectrum of activity, ranging from an IC_{50} of 0.4 ng/mL to 50000 ng/mL. Although two *P. falciparum* malaria parasite clones, designated as Sierra Leone (D6) and Indochina (W2) were used in the susceptibility testing, we used the IC_{50} values obtained from the W2 clones (W2 is resistant to chloroquine, pyrimethamine, sulfadoxine, and quinine whereas, D6 is resis-

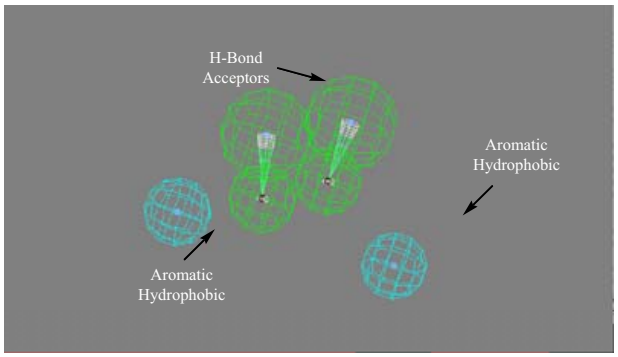


Fig. 1. Pharmacophore for antimalarial activity of the tryptanthrins. (*Light blue color:* represents aromatic hydrophobic groups. This mesh sphere is a location constraint that represents the volume in which a matching feature must be located when the pharmacophore is mapped to a candidate molecule; *Green color*: represents hydrogen bond acceptor functions. Hydrogen bond acceptor functions include a position in the space for the heavy (nonhydrogen) atom and a position in the space (the projected point) representing the point from which the participating hydrogen will extend).

tant to mefloquine) as the activity parameter to develop the pharmacophore model since the D6 clone results closely paralleled the W2 clones.

The pharmacophore was developed using the CATALYST methodology [15] by placing suitable constraints on the number of available features such as, aromatic hydrophobic or aliphatic hydrophobic interactions, hydrogen bond donors, hydrogen bond acceptors, hydrogen bond acceptors (lipid), and ring aromatic sites to describe the antimalarial activity of the tryptanthrin compounds. Earlier reported [12] quantum chemical calculations on the stereoelectronic properties of these compounds provided guidance for selection of these physico-chemical features. During pharmacophore development, the molecules were mapped to the features with their pre-determined conformations generated using the "fast fit" techniques in the CATALYST. The procedure resulted in the generation of 10 alternative pharmacophores for antimalarial activity of the compounds and appeared to perform quite well for the training set. The statistical relevance of the various pharmacophores (hypotheses) so obtained is assessed on the basis of their cost relative to the null hypothesis and their correlation coefficients [15, 16]. Ideally, the difference between the fixed cost and the null cost should be greater than or equal to 60 bits [15]. The correlation coefficients were found to be between 0.89 to 0.87 for six of the ten models, and the RMS values ranged between 1.47 and 1.71. The total costs of the pharmacophores varied over a narrow range between 88 to 95 bits and the difference between the fixed cost and the null cost is 77.0, satisfying the acceptable range recommended in the cost analysis of the CATALYST procedure [15,17]. Significantly, the best pharmacophore characterized by two hydrogen bond acceptor (lipid) functions and two aromatic hydrophobic functions (Fig. 1) is also statistically the most relevant pharmacophore. The predicted activity values along with the experimentally determined IC_{50} values for antimalarial activity of the compounds are presented in Table 2. A plot of the experimentally determined IC_{50} values versus the calculated activities demonstrates a good correlation (R=0.89) within the range of uncertainty 3, indicating the predictive power of the pharmacophore (Fig. 2). The highly potent analogues of the series map all the functional features of the best hypothesis with high scores, whereas the less potent compounds either do not map at all or map fewer of the features.

For example, the more potent analogs of the training set such as **1**&**4** map well with the statistically most significant pharmacophore (Fig. 3a & 3b) whereas, the less potent analogues such as **11** & **16** do not map adequately with the hypothesis (Fig. 3c & 3d). Two critical sites such as one hydrogen bond acceptor site and one aromatic hydrophobic site appear to be missing in the less potent analogues (Fig. 3c & 3d).

Table 2
Predicted and experimentally determined activity values of the training set and the test set compounds. (IC_{50} values are given in ng/mL)

Training set (compd)	IC_{50} (experimental)	IC_{50} (predicted)	Error*	Test set (compd)	IC_{50} (experimental)	IC_{50} (predicted)	Error*
1	0.43	1.3	2.9	**1'**	7.6	19.0	2.5
2	0.73	1.6	2.1	**2'**	262.9	170.0	-1.5
3	1.8	17.0	9.4	**3'**	403.0	320.0	-1.2
4	2.7	2.3	-1.2	**4'**	1.9	1.6	-1.2
5	11.2	70.0	6.3	**5'**	8.5	17.0	2.0
6	69.0	260	3.7	**6'**	7.2	17.0	2.3
7	120.0	23.0	-5.2	**7'**	126.0	180.0	1.4
8	354.3	3900	11.0	**8'**	3.8	19.0	5.0
9	572.9	740.0	1.3	**9'**	11.5	11.0	-1.0
10	734.3	170.0	-4.4	**10'**	1.7	1.9	1.2
11	50000	13000	-3.9	**11'**	1.8	1.6	-1.2
12	15626	12000	-1.3	**12'**	7.7	2.7	-2.8
13	263.0	170.0	-1.5	**13'**	34.0	51.0	1.5
14	2589	190.0	-13.0	**14'**	6.3	1.8	-3.5
15	8780	12000	1.4	**15'**	263.8	560.0	2.1
16	4423	12000	2.7				
17	6902	210.0	-33.0				

* Values in the error column represent the ratio of the estimated activity to measured activity, or its negative inverse if the ratio is less than one.

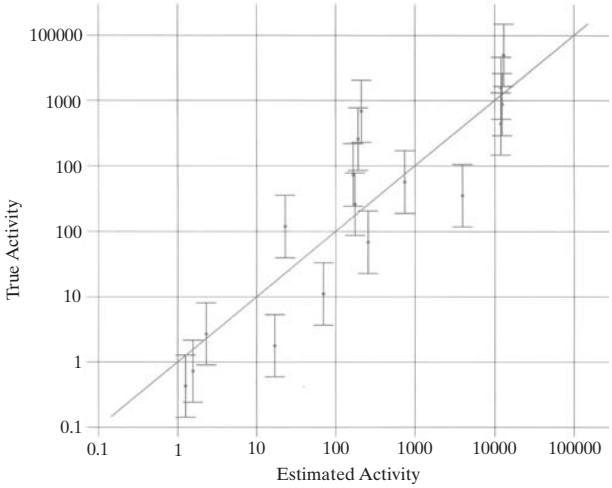


Fig. 2. Correlation (R = 0.89) line displaying the observed versus estimated IC_{50} values (ng/mL) of the training set by using the statistically most significant hypothesis derived form the W2 activities.

To cross-validate the observed correlation we prepared a "test set" of 15 additional substituted tryptanthrin compounds (Table 1) that were tested for in vitro antimalarial activity against D6 and W2 clones of *P. falciparum* identical to the original training set. However, this set was not used for automatic generation of the pharamacophore and thus, the test set of the tryptanthrin compounds was not used in determining the features of the pharmacophore generated from the original training set.

Interestingly, a better correlation (R= 0.92) than the original training set was observed when a regression analysis was performed by mapping this test set onto the features of the pharmacophore. The predicted and the experimental IC_{50} values for the test set tryptanthrins along with the respective error ratios are also shown in Table 2. The actual activity values are within the limits of uncertainty 3 (Table 2), thus demonstrating the predictive power of the original pharmacophore. As observed in the training set, the more potent analogues of the test set such as **4' & 11'** map well with the pharmacophore whereas, the less potent analogues of the test set do not map adequately.

To examine the validity of the pharmacophore against other commonly used antimalarial drugs and to derive some insights about the possible target of the tryptanthrin compounds, we performed computer simulations with eight antimalarial drugs that are currently used in the United States [3]; viz., quinine, chloroquine, mefloquine, primaquine, hydroxychloroquine, pyrimethamine, sulfadoxine, and doxycycline. The "best-fit scores", the predicted & experimental activity, and the conformational energy required for the anti-

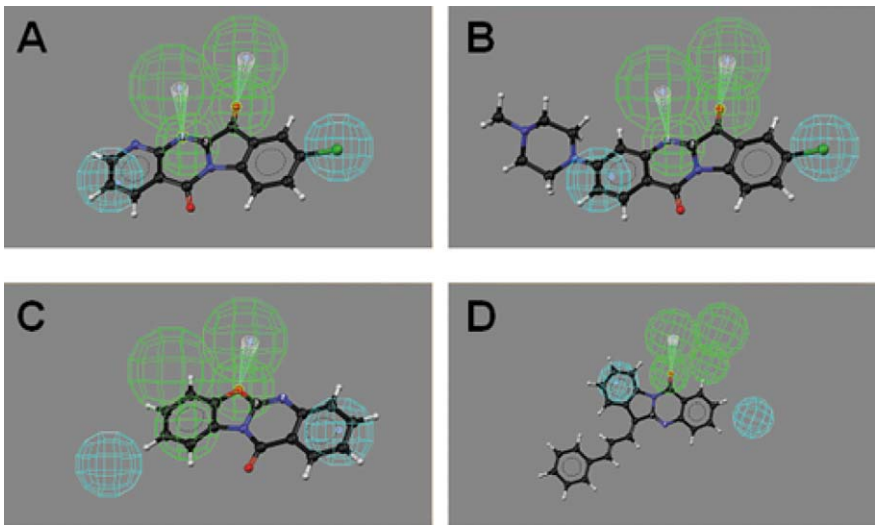


Fig. 3. Mapping of the more potent analogues: (a) **1** & (b) **4**, and less potent analogues: (c) **11** & (d) **16**, onto the pharmacophore model.

Table 3
"Best-Fit" Scores, Predicted Activity and Conformational Energies of Commonly Used Antimalarial Drugs in the United States by Mapping on the Pharmacophore

Drug	Best-Fit Score	Predicted Activity (D6) (ng/mL)	Experimental Activity (D6) (ng/mL)	Conformational Energy (kcal/mol)
Quinine	8.6	1.3	5.0	0.0
Chloroquine (CQ)	6.5	140.0	4.2	11.2
Mefloquine	7.0	50.0	4.7	5.1
Primaquine	6.8	82.0	1200	2.6
Hydroxy-CQ	7.3	23.0	12.0	14.6
Pyrimethamine	4.6	12000.0	0.03	0.0
Sulfadoxine	7.0	49.0	12.2	6.3
Doxycycline	6.3	23.0	498.0	0.0

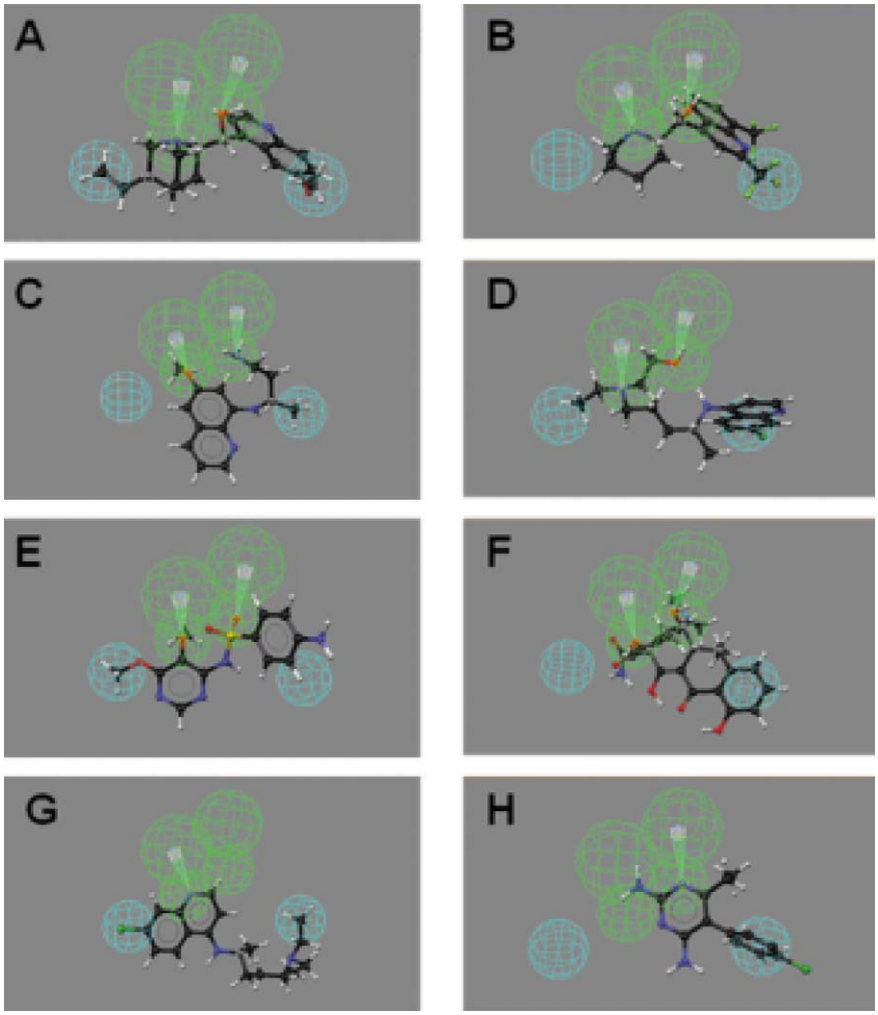


Fig. 4. Mapping of the pharmacophore onto eight commonly used antimalarial drugs in the United States: (a) quinine, (b) mefloquine, (c) primaquine, (d) hydroxychloroquine, (e) sulfadoxine, (f) doxycycline, (g) chloroquine, and (h) pyrimethamine.

malarial agents to fit the tryptanthrin pharmacophore model are presented in Table 3 and Fig. 4.

Surprisingly, quinine maps (has the required spatial distribution of the four essential features) completely with the pharmacophore (Fig. 4a) and the other drugs map in varying degrees. Thus, mefloquine (Fig. 4b), primaquine (Fig. 4c), hydroxychloroquine (Fig. 4d), sulfadoxine (Fig. 4e), and doxycycline (Fig. 4f) map the two hydrogen-bond acceptor sites and one of the two hydrophobic sites, whereas chloroquine (Fig. 4g) and pyrimethamine (Fig. 4h) map only one of the two hydrogen-bond acceptors and neither of the hydrophobic sites. It may be worthwhile to mention here that quinine and other quinoline-containing antimalarials including chloroquine have shown varying capacity to inhibit malaria heme polymerase extracted from *P. falciparum* tropozoites [18]. In particular, the interaction of quinine with heme has been well documented [10]. Since the pharmacophore maps well on quinine and in varying degrees on the other quinoline-containing antimalarials, it may be reasonable to speculate that the tryptanthrin compounds may target heme polymerase from the *P. falciparum* tropozoites. In addition, we have also mapped the pharmacophore on a few recently reported potent antimalarials such as the chalcones [19] and rhodacyanine dyes [20]. Surprisingly again, the more potent chalcone analogues such as, 4- trifluoromethyl-2',3',4'–trimethoxy and 4-ethyl-2',4'–dimethoxy chalcones (Fig 5a & 5b) and the rhodacyanine dye MKT-077 and its para- analogue map well on the tryptanthrin pharmacophore (Fig 6a & Fig 6b).

Since the target protein for the antimalarial activity of the tryptanthrins is as yet unknown, we mapped the pharmacophore on known inhibitors of several malarial proteins in order to utilize the information for identifying the target and to design more potent inhibitors. The pharmacophore maps well onto several potent inhibitors of *P. falciparum* and *P. vivax* [21] plasmepsins such as the *ortho*- and *para*-phenoxyldiphenylurea analogues (Fig. 7a & 7b), moderately potent Pfmrk cyclin-dependent kinase inhibitors [22] such as roscovitine and 2-(2-hydroxyethylamino)-6-(3-chloroanilino)-9-isopropylpurine (Fig. 8a & 8b), to a varying degree onto known inhibitors for malarial β–ketoacyl-ACP synthase III, (KASIII or FabH) such as thiolactomycin [23], and triclosan [24] for Enoyl acyl Carrier Protein Reductase (ENR or FabI). This information led to a search of multicon-

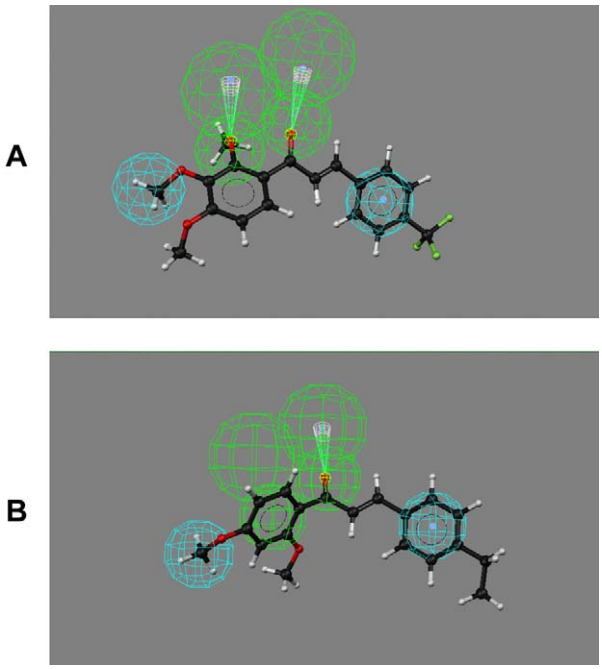


Fig. 5. Mapping of the pharmacophore on (a) 4,-trifluoromethyl-2',3',4'–trimethoxychalcone, and (b) 4-ethyl-2',4'-dimethoxychalcone.

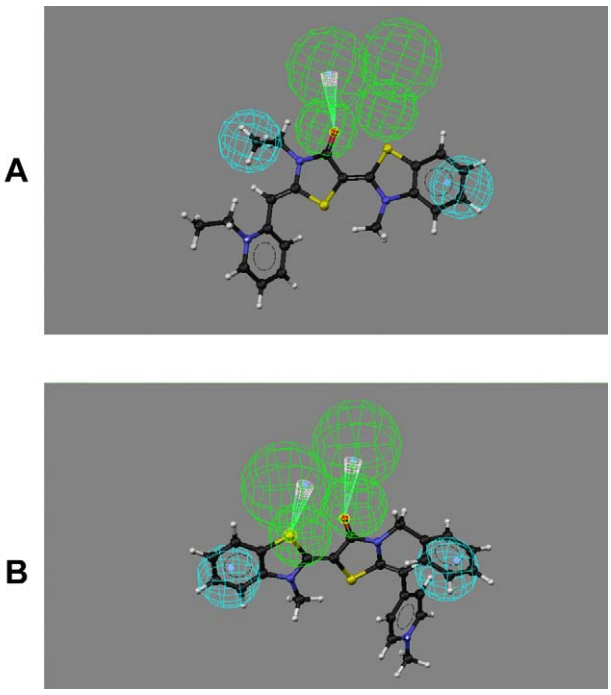


Fig. 6. Mapping of the pharmacophore on (a) rhodacyanine dye MKT-077 and (b) *para*-rhodacyanine dye MKT-077.

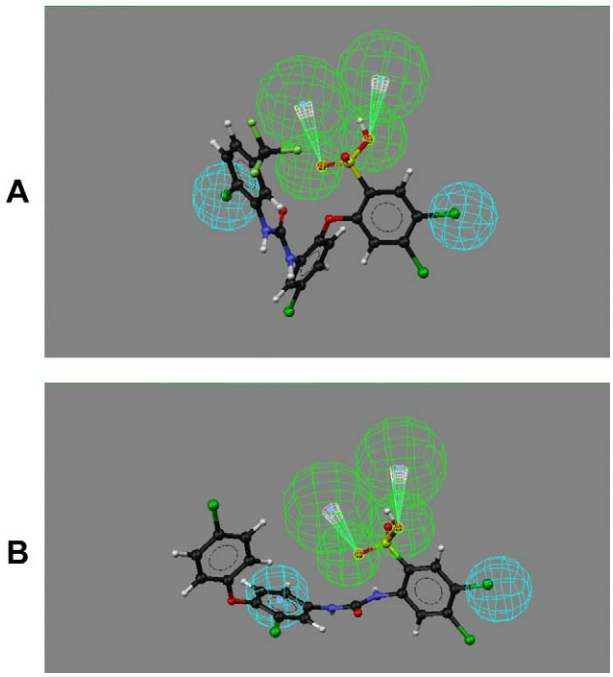


Fig. 7. Mapping of the pharmacophore on known Plasmepsin inhihitors: (a) *ortho*- and (b) *para*- phenoxyldiphenylurea analogues.

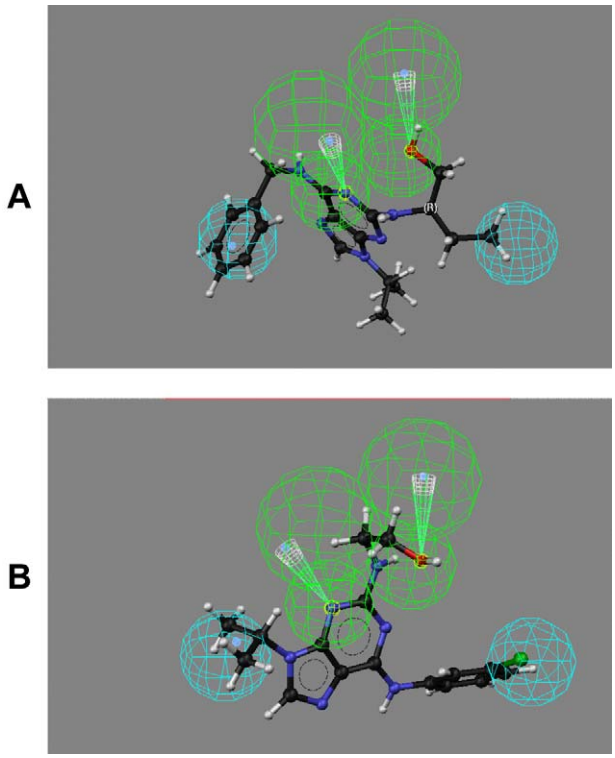


Fig. 8. Mapping of the pharmacophore on known Pfmrk cyclin dependent protein kinase (CDK) inhibitors: (a) roscovitine and (b) 2-(2-hydroxyethylamino)-6-(3-chloroanilino)-9-isopropylpurine.

former 3D databases which identified one fairly potent plasmepsin inhibitor 6-diethylamino-2,3-diphenylbenzofuran (Fig. 9), two excellent Pfmrk inhibitors from the tryptanthrin classes, such the 8-nitro- and 7-chloro- 4-azatryptanthrins, and a moderately good inhibitor, 4-(benzenesulfonamido)-salicylic acid for the FabH (KASIII) protein (Fig. 10).

Thus, the tryptanthrin pharmacophore model could be successfully used as a search template for 3D database searches, and we have further demonstrated the validity of the model by identifying five new aminoquinazoline derivatives from our in-house Chemical Information System [25] database as promising candidates for further study. All five of these compounds have shown potent in vivo activity in a

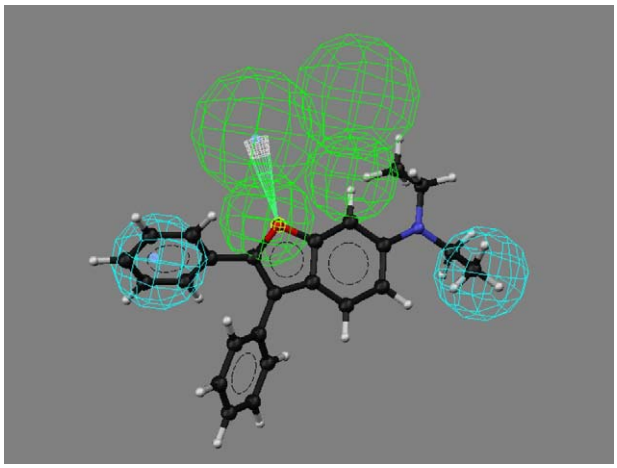


Fig. 9. Mapping of the pharmacophore on the newly identified Plasmepsin inhibitor through database search: 6-diethylamino-2,3-diphenylbenzofuran.

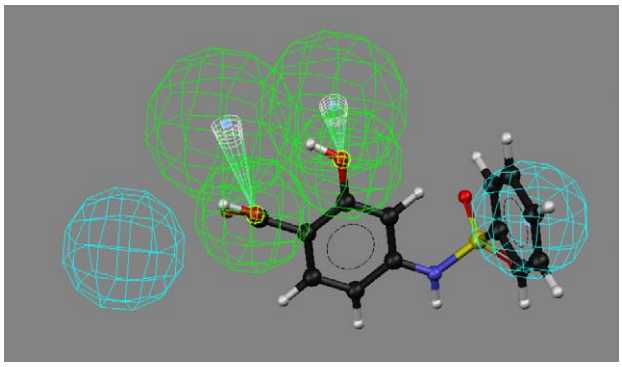


Fig. 10. Mapping of the pharmacophore on a newly identified inhibitor for malarial β–ketoacyl-ACP synthase III (KASIII or FabH) through database search: 4-(benzenesulfonamido)salicylic acid.

mouse malarial screening test. The Chemical Information System [25] database has over 240,000 compounds and it was transformed into a multiconformer database in CATALYST using the catDB utility program as implemented in the software [15]. The catDB format allows a molecule to be represented by a limited set of conformations thereby permitting conformational flexibility to be included during the search of the database.

Although the tryptanthrins possess outstanding in vitro activity against *P. falciparum* (both W2 & D6 clones) and have reasonably well tolerated properties for promising antimalarial drug candidates, the compounds have yet to display in vivo activity in a *P. berghei* mouse model system (Thompson test) [26]. We are currently trying to synthesize analogs with vastly superior aqueous solubility to overcome the in vivo efficacy problem. We are continuing to explore new strategies to translate the potent intrinsic activity of the compounds into new candidates with superior bioavailability profiles for treatment of malaria.

3. Conclusion

The study demonstrates how the molecular characteristics from a set of diverse tryptanthrin derivatives may be organized to be both statistically and mechanistically significant for potent antimalarial activity that may have universal applicability. In addition, the resulting model can be used to unravel a possible rationale for the target-specific antimalarial activity of these compounds. The chemically significant molecular characteristics disposed in a three dimensional space generated a pharmacophore that is found to be quite satisfactory in correlating true activity with the estimated activity of the compounds. Two hydrogen bond acceptors and two aromatic hydrophobic sites appear to be the required functional features for potent antimalarial activity of many classes of compounds. The validity of the pharmacophore extends to structurally different classes of compounds, and thereby provides a powerful template from which novel drug candidates may be identified for extended study. Since the identity of the target for antimalarial activity of the tryptanthrin class of compounds remains unknown, this three-dimensional QSAR pharmacophore should aid in the design of well-tolerated target-specific antimalarial agents.

4. Experimental

4.1. Chemicals

Indolo[2,1-b]quinazoline-6,12-dione (**6**, tryptanthrin) and its derivatives were obtained from PathoGenesis Corporation, Seattle, WA, USA (now owned by Chiron, Emeryville, CA). In general, these compounds can be synthesized by base-catalyzed condensation of substituted isatins and substituted isatoic anhydrides through a convenient one-step flexible synthesis as previously reported [5]. The remaining compounds mentioned herein were obtained from the in-house Chemical Information System repository [25].

4.2. Biological testing

All biological testing was provided by courtesy of the Department of Parasitology in our Institute. Tryptanthrin and its derivatives were examined for IC_{50} values against the *P. falciparum* W2 (Indochina) or D6 (Sierra Leone) clones in vitro as previously reported [9]. Briefly, the in vitro assays were conducted by using the semiautomated microdilution technique of Desjardins et al. [26] and Chulay et al. [27]. The W2 clone is susceptible to mefloquine, but resistant to chloroquine, sulfadoxine, pyrimethamine, and quinine. The D6 clone is resistant to mefloquine, but susceptible to chloroquine, sulfadoxine, pyrimethamine, and quinine. The clones were derived by direct visualization and micromanipulation from the patient isolates. Test compounds were initially dissolved in DMSO and 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 1000 (Beckman, Fullerton, CA) over 11 dif-

ferent concentrations. The parasites were exposed to serial dilutions of each compound for 48 h and incubated at 37 °C with 5% O_2, 5% CO_2, and 90% N_2 prior to the addition of [^{3}H]-hypoxanthine. After a further incubation 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.

4.3. Procedure for development of the 3D QSAR pharmacophore model

The three-dimensional QSAR study was performed using CATALYST 4.6 software [15]. It enables the use of structure and activity data for a set of lead compounds to generate a pharmacophore characterizing the activity of the lead set. At the heart of the software is the HypoGen algorithm that allows identification of pharmacophores that are common to the 'active' molecules in the training set but are absent in the 'inactives' [17]. Structures of tryptanthrin (**6**) and 16 analogues (**1 – 17**) were edited within CATALYST and energy minimized to the closest local minimum using the generalized CHARMM-like force field as implemented in the program. The CATALYST model treats molecular structures as templates comprising chemical functions localized in space that will bind effectively with complementary functions on the respective binding proteins. The most relevant chemical features were extracted from a small set of compounds that cover a broad range of activity [28]. Molecular flexibility was taken into account by considering each compound as an ensemble of conformers representing 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 [29].

Conformational models of the training set of tryptanthrins were generated that emphasize representative coverage within a range of permissible Boltzman population with significant abundance (10 kcal/mol) of the calculated global minimum. This conformational model was used for pharmacophore generation within CATALYST, which 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 distributed over a three dimensional space explaining the activity variations among the training set. The hydrogen bonding features are vectors, whereas all other functions are points. Pharmacophore generation was carried out by setting the default parameters in the automatic generation procedure in CATALYST such as function weight 0.302, mapping coefficient 0, resolution 260 pm, and activity uncertainty 3. An uncertainty "Δ" 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 obtained pharmacophore is assessed on the basis of the cost relative to the null hypothesis and the correlation coefficient [15, 16]. The pharmacophores are then used to estimate the activities of 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 a pharmacophore that contains features with equal weights and tolerances. Each feature (e.g. hydrogen-bond acceptor, hydrogen-bond donor, hydrophobic regions, positive ionizable group, etc.) contributes equally to estimate the activity. Similarly, each chemical feature in the HypoGen pharmacophore requires a match to a corresponding ligand atom to be within the same distance of tolerance [16]. The method has been documented to perform better than a structure-based pharmacophore generation [28].

Acknowledgements

The work was funded under a Military Infectious Disease Research Program grant (A40016_03_WR). The opinions expressed herein are the private views of the authors and are not to be considered as official or reflecting the views of the Department of the Army or the Department of Defense.

References

[1] P.I. Trigg, A.V. Kondrachine, in: I.W. Sherman (Ed.), Malaria Parasite Biology, Pathogenesis and Protection: The current global malaria situation, ASM, Washington, DC, 1998, pp. 11–22.
[2] N.J. White, Br. Med. Bull. 54 (1998) 703–715.
[3] J.A. Vroman, M.A. Gaston, M.A. Avery, Curr. Pharm. Design 5 (1999) 101–138.
[4] A.K. Bhattacharjee, J.M. Karle, Chem. Res. Toxicol. 12 (1999) 422–428.
[5] W.R. Baker, L.A. Mitscher, 1995 U.S. Patent 5 441 955.
[6] S. Eguchi, H. Takeuchi, Y. Matsushita, Heterocycles 33 (1992) 153–156.
[7] G. Honda, M. Tabata, J. Med. Plant Res. Planta Medica 36 (1979) 85–86.
[8] L.A. Mitscher, W.C. Wong, T. DeMeulenaere, J. Sulko, S. Drake, Heterocycles 15 (1981) 1017–1021.
[9] K.K. Pitzer, D.E. Kyle, L. Gerena, 2001 Patent 6 284 772.
[10] S.R. Meshnick, in: I.W. Sherman (Ed.), Malaria Parasite Biology, Pathogenesis and Protection: From quinine to qinghaosu: historical perspectives, ASM, Washington, DC, 1998, pp. 341–353.
[11] A.K. Bhattacharjee, D.E. Kyle, J.L. Vennerstrom, W.K. Milhous, J. Chem. Info. Comput. Sci. 42 (2002) 1212–1220.
[12] A.K. Bhattacharjee, D.J. Skanchy, B. Jennings, T.H. Hudson, J.J. Brendle, K.A. Werbovetz, Bioorg. Med. Chem. 10 (2002) 1979–1989.
[13] A.K. Bhattacharjee, J.M. Karle, J. Med. Chem. 39 (1996) 4622–4629.
[14] M.A. Riel, D.E. Kyle, A.K. Bhattacharjee, W.K. Milhous, Antimicrob Agents Chemother. 46 (2002) 2627–2632.
[15] CATALYST Version 4.5 software, Accelrys Inc., San Diego, CA, 2000.
[16] R. Fischer, The Design of Experiments, Hafner Publishing:, New York, 1966 Chapter 2.

[17] O.A. Gunner, Pharmacophore, perception, development, and use in drug design, University International Line, San Diego, 2000, pp. 17–20.

[18] A.F.G. Slater, A. Cerami, Nature 355 (1992) 167–169.

[19] M. Liu, P. Wilairat, M.L. Go, J. Med. Chem. 44 (2001) 4443–4452.

[20] K. Takasu, H. Inoue, H.K. Kim, M. Suzuki, T. Shishido, Y. Wataya, M. Ihara, J. Med. Chem. 45 (2002) 995–998.

[21] S. Jiang, S.T. Prigge, L. Wei, Y.E. Gao, T.H. Hudson, L. Gerena, J.B. Dame, D.E. Kyle, Antimicrob. Agents Chemother. 45 (2001) 2577–2584.

[22] Z. Xiao, N.C. Waters, C.L. Woodward, Z. Li, P.K. Li, Bioorg. Med. Chem. Lett. 11 (2001) 2875–2878.

[23] R.A. Daines, I. Pendrak, K. Sham, G.S. Van Aller, A.K. Konstantinidis, J.T. Lonsdale, X.Q. Janson, M. Brandt, S.S. Khandekar, C. Silverman, M.S. Head, J. Med. Chem. 46 (2003) 5–8.

[24] N. Surolia, A. Surolia, Nature Medicine 2 (2001) 167–173.

[25] Chemical Information System. Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Silver Spring, MD, U.S.A.

[26] R.E. Desjardins, C.J. Canfield, D.E. Haynes, J.D. Chulay, Antimicrob. Agents Chemther. 16 (1979) 710–718.

[27] J.D. Chulay, J.D. Haynes, C.L. Diggs, Exp. Parasitol. 55 (1983) 138–146.

[28] M. Grigorov, J. Weber, M.J. Tronchet, C.W. Jefford, W.K. Milhous, D. A. Maric, J. Chem. Inf. Comput. Sci. 35 (1995) 285–304.

[29] P.A. Greenridge, J. Weiser, Mini Reviews in Medicinal Chemistry 1 (2001) 79–87.