Xanthones as antimalarial agents; studies of a possible mode of action

Marina V. Ignatushchenko[a], R.W. Winter[b,c], Hans Peter Bächinger[d], David J. Hinrichs[b,c], Michael K. Riscoe[a,b,c,*]

[a]*Department of Biochemistry and Molecular Biology, Oregon Health Sciences University, 3181 SW Sam Jackson Park Road, Portland, OR 97201, USA*
[b]*Medical Research Service, 151-0, Department of Veterans' Affairs Medical Center, 3710 SW US Veterans Hospital Road, Portland, OR 97035, USA*
[c]*Interlab Inc., 151 del Prado, Lake Oswego, OR 97223, USA*
[d]*Shriners Hospital for Crippled Children, 3101 SW Sam Jackson Park Road, Portland, OR 97201, USA*

Abstract We recently demonstrated that 2,3,4,5,6-pentahydroxyxanthone (X5) inhibits the in vitro growth of both chloroquine-sensitive and multidrug-resistant strains of *P. falciparum*. To study the molecular basis of its antimalarial action, we tested X5 and selected hydroxyxanthone analogs as inhibitors of in vitro heme polymerization in a low ionic strength phosphate solution at mildly acidic pH. We found that addition of 1 Eq. of X5 resulted in complete inhibition of polymerization in this system whereas addition of up to 40 Eqs. of standard antimalarial compounds (chloroquine, primaquine, quinacrine, artemisinin and methylene blue) had no such effect although these compounds did co-precipitate with heme. The antimalarial potency of the hydroxyxanthones correlated well with their ability to inhibit in vitro heme polymerization in our assay, suggesting that these compounds exert their antimalarial action by preventing hemozoin formation. Based on the observed structure–activity relationships, we propose a model displaying possible interactions between hydroxyxanthones and heme.
© 1997 Federation of European Biochemical Societies.

Key words: Malaria; Chemotherapy; *Plasmodium falciparum*; Digestive vacuole; Heme polymerization; Xanthone

1. Introduction

Malaria is a disease of enormous importance by any standard of measure. Billions of people live in the regions where, according to recent figures from the World Health Organization, malaria causes 100 million clinical episodes and over 1 million deaths per year [1]. The recent emergence and rapid spread of chloroquine-resistant strains of *Plasmodium falciparum* threaten to increase the annual death toll. As a result, there is a great need for development of mechanistically novel antimalarial drugs.

The malarial parasite infects red blood cells, ingesting and degrading hemoglobin in the acidic food vacuole [2]. Proteolysis of hemoglobin yields amino acids for protein synthesis as well as toxic heme [3]. As the parasite cannot enzymatically cleave the porphyrin ring, heme is 'detoxified' by conversion to an insoluble polymer, hemozoin [2]. It has been suggested that hemozoin formation is inhibited by the 4-aminoquinolines, such as chloroquine, quinine and amodiaquin [4,5], although the evidence appears to be controversial [6].

We have recently identified 2,3,4,5,6-pentahydroxyxanthone (X5) as a potent antimalarial drug with equal activity against multidrug-resistant strains of *P. falciparum* [7]. We were, therefore, interested in exploring the mode of action of this novel compound in an effort to identify structural features critical for its antimalarial action. Here, we describe the complexation of X5 with soluble heme, introduce a slight but significant modification of the in vitro heme polymerization assay and demonstrate the ability of X5 and related xanthones to inhibit this process.

2. Materials and methods

2.1. Chemicals and reagents

Hemin chloride, artemisinin, quinacrine dihydrochloride hydrate and primaquine diphosphate were purchased from Aldrich Chemical (Milwaukee, WI, USA). [³H]Ethanolamine was obtained from American Radiolabeled Chemicals (St. Louis, MO, USA). Chloroquine diphosphate was obtained from Sigma Chemical (St. Louis, MO, USA) and quinine sulfate was from Matheson Coleman and Bell (Cincinnati, OH, USA). Xanthone (9-xanthenone) was purchased from Fluka (Buchs, Switzerland). Detailed methods for synthesis of the hydroxylated xanthones will be published elsewhere.

2.2. Culture of P. falciparum

The chloroquine-susceptible D6 clone of *P. falciparum* has been previously described [8]. The parasites were cultured in Group A$^+$ human erythrocytes, suspended at a 2% hematocrit in RPMI-1640 (pH 7.15) which contained 3 g/l glucose, 50 μg/l gentamicin, 10% human serum and maintained at 37°C in a gas mixture of 5% O_2, 5% CO_2 and 90% N_2 [9].

2.3. Drug testing

In vitro antimalarial activity of the test compounds was assessed by following incorporation of [³H]ethanolamine (50 Ci/mmol) into parasite lipids as described by Elabbadi et al. [10] with minor modifications. The experiments were set up in duplicate in 96-well plates with varying concentrations of the xanthone (10^{-9} to 10^{-4} M) across the plate in a total volume of 200 μl and at a final red blood cell concentration of 2% (v/v). An initial parasitemia of 0.2% was attained by addition of normal uninfected red cells to a stock culture of infected cells. Although no special attempts were made to use synchronous cultures, most experiments were begun with cultures in which ≈50% of infected erythrocytes harbored parasites at the mature trophozoite stage of development. Radiolabeled ethanolamine (1 μCi in 20 μl of medium) was then added after 48 h of incubation and the experiment was terminated after 72 h by collecting the cells onto glass fiber filters with an automated Skatron multiwell harvester. Stock solutions of the xanthones were made by dissolving the compounds into dimethylsulfoxide at 10 mM. The concentration of drug giving 50% inhibition of label incorporation (IC_{50}) was calculated from a computer-generated semi-logarithmic dose–response curve.

2.4. Complex formation between heme and X5

The complexation of heme with X5 was tested spectrophotometrically as described by Vossen et al. [11] using a Cary 4 Bio UV/visible scanning spectrophotometer (Varian). A stock solution of 10 mM

*Corresponding author. Medical Research Service, 151-0, Veterans' Affairs Medical Center, 3710 SW US Veterans' Hospital Road, Portland, OR 97201, USA. Fax: (1) 503-273-5135. E-mail: riscoem@ohsu.edu

hemin chloride in 0.1 M NaOH was prepared freshly and incubated at 37°C for at least 30 min to ensure complete dissolution of the mono-mer. A stock solution of 10 mM X5 in dimethylformamide was prepared freshly. The stock solutions were diluted to 25 µM with ice-cold 0.02 M phosphate (pH 5.2). Dimethylformamide (0.25% v/v) was added to the hemin control. Sample and reference tandem cuvettes were filled each with hemin and X5 solutions (0.8 ml) and the base line was recorded (235–500 nm). The temperature of the cell compartment was kept at 5°C to retard the heme polymerization process. Then, the solutions in the sample cuvette were mixed and the UV/visible difference spectra were recorded over 45 min of incubation.

2.5. Syntheses of heme polymers

Hemin stock solution was added to 3 glass beakers containing 1 l of 0.02 M sodium phosphate, 0.02 M sodium acetate and 4 M sodium acetate solutions, respectively, to yield a final concentration of 25 µM. The pH of all solutions was 5.2. After 2 h of incubation at 37°C followed by overnight incubation at room temperature, the precipitates were washed 10× with deionized water, dried in vacuo and characterized by means of differential solubility, elemental analysis and IR spectroscopy.

2.6. In vitro heme polymerization assay

Heme polymerization was carried out in 0.02 M phosphate (pH 5.2) at 37°C in the absence of proteins. A 10 mM stock solution of hemin chloride in 0.1 M NaOH was prepared freshly and incubated at 37°C for at least 1 h. Xanthones were dissolved in dimethylformamide at 10 mM and diluted into 10 ml of pre-warmed phosphate solution to a final concentration of 25 µM. Polymerization was initiated by addition of 25 µl of the hemin stock solution to the test sample to yield a final concentration of 25 µM heme. 25 µl of dimethylformamide was added to the control sample. After 7, 30, 60, 120 and 210 min of incubation at 37°C, a 1-ml aliquot was withdrawn, transferred into an Eppendorf tube and centrifuged at 14000×g for 2 min at room temperature to pellet the polymer. The soluble fraction was then transferred to a semi-microcuvette (polymethylacrylate, VWR) and its absorption was measured at 360 nm against a blank of the test compound in buffer. Control experiments indicated that: (1) the pH of the phosphate solution did not change upon addition of the reagents or during the polymerization process; and (2) the amount of dimethylformamide used in this assay did not significantly affect the rate of polymerization. To estimate the effect of test compounds on heme polymerization at a given time of incubation, the percentage of soluble hemin remaining in the sample was calculated using the following formula:

$$\% \text{ soluble hemin} = \frac{A_{(\text{drug}+\text{hemin})t} - A_{(\text{drug})t}}{A_{(\text{hemin})t=0}} \times 100\%,$$

where $A_{(\text{drug}+\text{hemin})t}$ is the absorption (360 nm) of the soluble fraction in the drug-hemin sample after various times of incubation; $A_{(\text{drug})t}$ is the absorption of the drug alone; and $A_{(\text{hemin})t=0}$ is the absorption of the hemin control sample (25 µM) measured immediately upon addition of the hemin stock solution.

The dose-dependent inhibition of heme polymerization was evaluated as described above, except the concentration of each drug was varied in the range of 0–1 mM. The reactions were allowed to proceed for 2 h in a 37°C waterbath. After incubation, the polymer was pelleted as described above and the absorption (360 nm) of each soluble fraction was measured against a blank containing the drug alone in buffer. The IC_{50} values were determined by non-linear regression analysis of the dose-response curves of percentage inhibition of heme polymerization vs. drug concentration.

3. Results

3.1. Complex formation between heme and X5

Based on structural features of X5, we predicted that it would form a complex with free heme. We used UV/visible
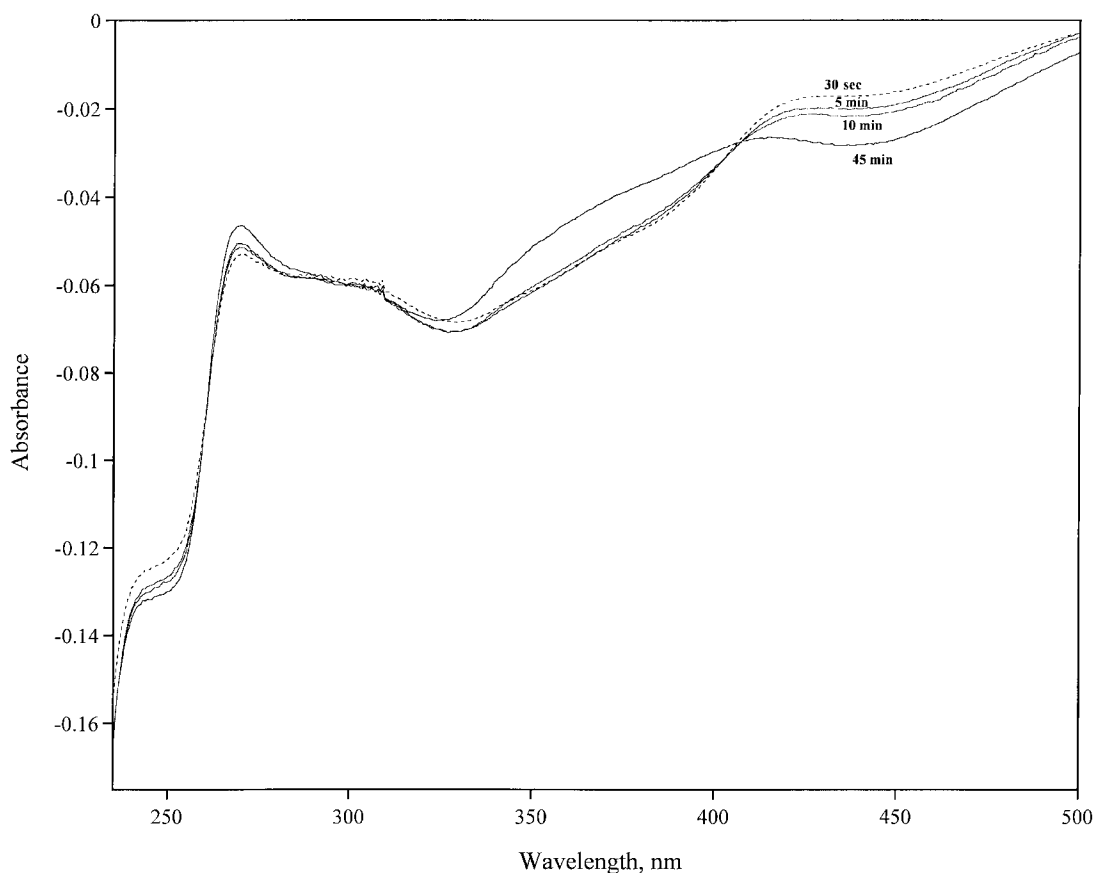

Fig. 1. UV/visible difference spectra induced by binding of X5 to heme in 0.02 M phosphate (pH 5.2) at 5°C for the time points indicated. Scan speed, 75 nm/min.
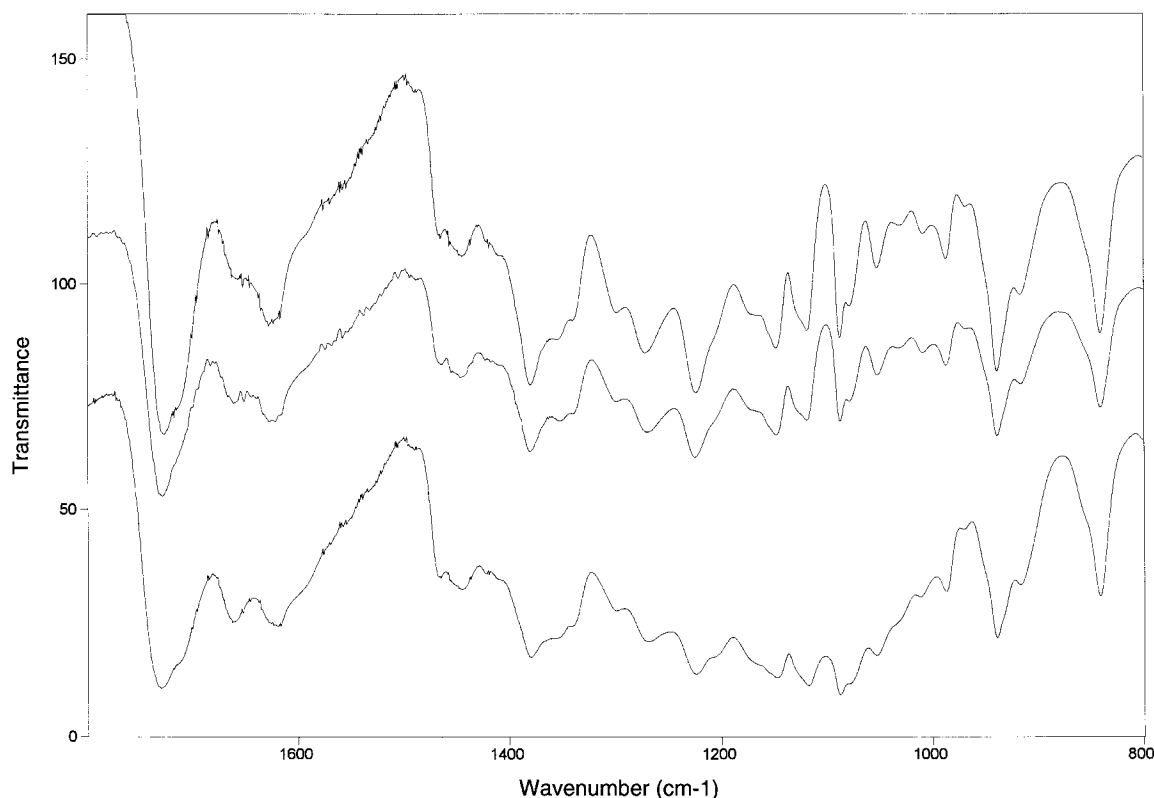
Fig. 2. IR spectra of hematin-acetate (0.02 M) incubation product (top), hematin-phosphate (0.02 M) incubation product (middle) and hematin-acetate (4 M) incubation product (bottom).

difference spectroscopy to measure the optical signal produced upon interaction between heme and X5. Dual tandem cuvettes allowed direct comparison of the same amounts of heme and X5 mixed in the sample cuvette and separated in the reference cuvette. By this experimental design the contributions from slight differences in the heme, X5 and dimethylformamide concentrations to the difference spectra were cancelled, i.e. only the effects of complexation are observed. The method allows the continuous and sensitive monitoring of spectral changes without subsampling from the three different solutions. Fig. 1 shows the family of UV/visible difference spectra induced by binding of X5 to heme over 45 min of incubation. The spectra contain a difference peak at 270 nm which decreased with time, a dip at 327 nm and shoulders at $\approx$250 and $\approx$420 nm which increased with time. These changes are indicative of the red shifts in the UV (240–260 nm) and visible

(320–400 nm) absorbance produced upon formation of the heme–X5 complex. Interestingly, in preliminary experiments in which the samples were kept at 37°C, we detected by visual inspection the formation of a flocculent brown precipitate in the heme control sample within 1 h of incubation, while no such phenomenon was observed in the test sample containing both heme and X5. Substitution of 0.02 M phosphate with 0.02 M acetate at the same pH yielded identical results although at a high acetate concentration (4 M) X5 failed to prevent precipitation.

3.2. Characterization of heme polymers

The precipitates formed as described above (i.e. in 0.02 M phosphate, 0.02 M acetate and 4 M acetate; pH 5.2, 37°C) were characterized by means of differential solubility, elemental analysis and IR spectroscopy. As shown in Table 1, the

Table 1
Physical and chemical properties of heme and heme polymers

Sample	Solubility					Elememtal composition				
	CH_3OH	C_2H_5OH	$CH_3COOH/$ H_2O/CH_3OH $(1.5:0.5:8)$	SDS (2.5%)	Dimethyl sulfoxide	%C	%H	%N	%Fe	%P
Hematin	+	+	+	+	+	64.5	5.3	8.8	8.8	0
Malarial hemozoin[a,b]	–	–	–	–	–	64.6 ± 0.8^c	5.2 ± 0.2^c	8.7 ± 0.2^c	8.7 ± 0.2^c	ND^d
Hematin-phosphate (0.02 M) incubation product	–	–	–	–	–	64.3 ± 0.3	5.4 ± 0.3	8.4 ± 0.3	8.8 ± 0.5	<0.1
Hematin-acetate (0.02 M) incubation product	±	±	±	–	–	64.4 ± 0.3	5.3 ± 0.3	8.4 ± 0.3	8.4 ± 0.5	<0.1
Hematin-acetate (4 M) incubation product	+	+	+	+	+	59.3 ± 0.3	5.2 ± 0.3	7.2 ± 0.3	7.0 ± 0.5	<0.1

[a]Pandey et al. [12]. [b]Slater et al. [13]. [c]Fitch et al. [14]. [d]ND, not determined.
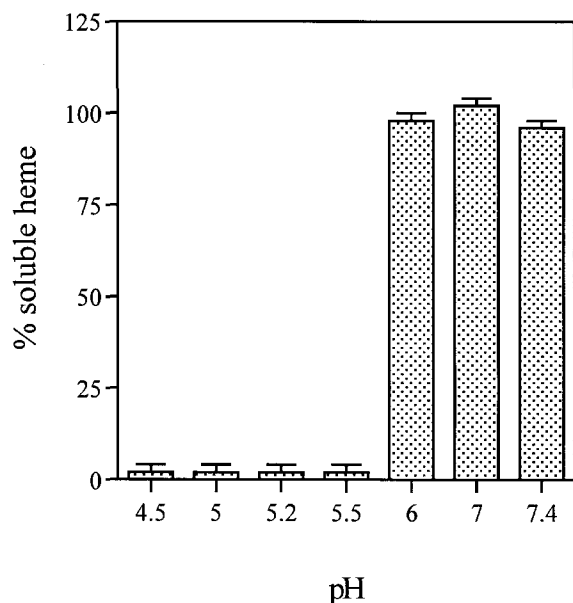
Fig. 3. pH profile for in vitro heme polymerization in 0.02 M phosphate (37°C, 2 h of incubation). Values are the mean of duplicate determinations.

boxylate coordination to iron [13]. In all, these data suggest that the precipitate formed upon incubation of hemin in 0.02 M phosphate solution is a heme polymer chemically analogous to hemozoin and distinct from the product formed upon incubation of hemin in 0.1–4.5M acetate buffers [5,12,15].

3.3. Heme polymerization and its inhibition by X5

Since the preliminary results indicated that X5 inhibited heme polymerization, we developed an assay based on the spectrophotometric detection of soluble heme. Under the conditions of our assay, heme polymerization was pH-dependent (pH 4.5–5.5) (Fig. 3), occurred spontaneously and was >95% complete within 2 h of incubation (Fig. 4). As described in Section 2, the reaction mixture was prepared by dilution of a hemin stock solution into a mildly acidic phosphate solution (pH 5.2).

Addition of 1 Eq. of X5 resulted in complete inhibition of polymerization in a phosphate solution (Fig. 4, panel A). Addition of X5 to polymerized heme did not reverse the process. This, as well as the ability of X5 to alter the spectral properties of heme, strongly suggests that X5 inhibits heme polymerization through the formation of a soluble complex with free heme.

solubility properties of the phosphate (but not 4 M acetate)-derived material were identical to those reported for hemozoin ('malarial pigment') [12,13]. The precipitate formed in 0.02 M acetate solution was partially soluble in alcohols and the methanol/acetic acid/water mixture. Elemental analyses showed that the percentages of carbon, hydrogen, nitrogen and iron in the 0.02 M phosphate and 0.02 M acetate-derived products corresponded closely to the values reported for hemozoin [14]. The 4 M acetate-derived product had an elemental composition consistent with that of a hematin-triacetate adduct. All of the products exhibited increased IR absorbance in the 1600–1650 cm^{-1} region (Fig. 2), indicative of the car-

3.4. Inhibition of heme polymerization by other xanthones

Structure–activity relationships were determined for xanthones as inhibitors of spontaneous heme polymerization (Table 2). The IC_{50} values are the average of at least two independent determinations of full dose–response curves. Xanthone and the tested monohydroxyxanthones did not exhibit any inhibitory activity in our assay. Moderate inhibitory activity (i.e. IC_{50} of ≈ 8–20 μM) was observed for the compounds bearing a single hydroxy group at either 4- or 5-position whereas the greatest activity was observed for xanthones containing hydroxy groups at both positions. For example, 2,3,4-trihydroxyxanthone exhibited an IC_{50} of 16.5 μM, while 2,3,4,5,6-pentahydroxyxanthone (X5) yielded a value of 1.2
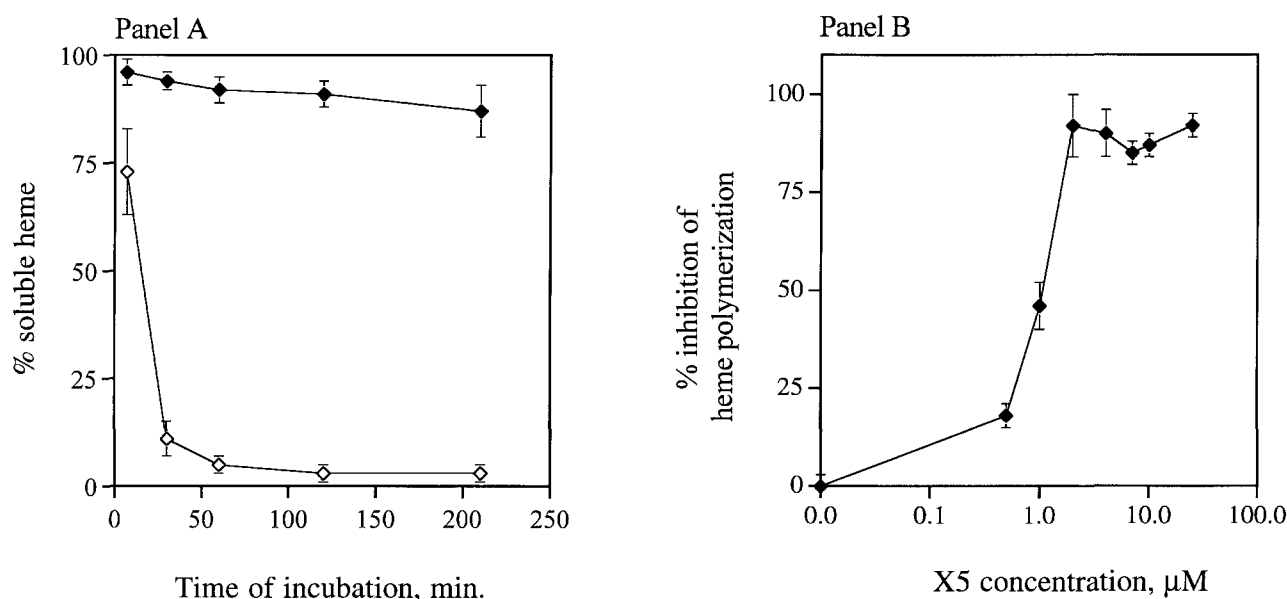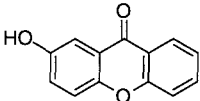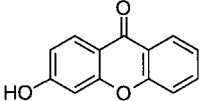
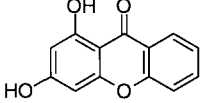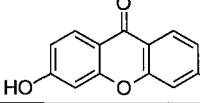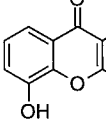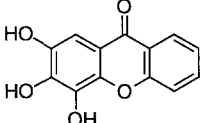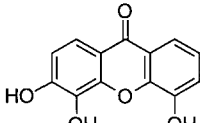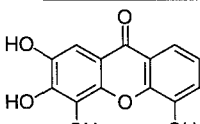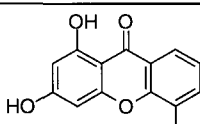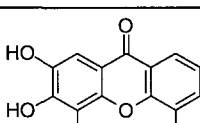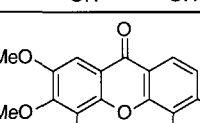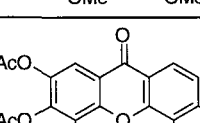

Fig. 4. Panel A: spontaneous heme polymerization in 0.02 M phosphate (pH 5.2) at 37°C in the absence (open diamonds) and presence (black diamonds) of X5. Values are the mean ± S.D. of three independent experiments. Panel B: dose–response effect of X5 on spontaneous heme polymerization in 0.02 M phosphate (pH 5.2) at 37°C. Initial heme concentration is 25 μM.

Table 2
Inhibition of in vitro heme polymerization by xanthones

| Compound name	Compound structure	IC$_{50}$ µM, *P. falciparum* clone D6	IC$_{50}$, µM *in vitro* heme polymerization
2-hydroxyxanthone		50	>1000
3-hydroxyxanthone		>100	>1000
1,3-dihydroxyxanthone		75	>1000
3,6-dihydroxyxanthone		>100	>500
4,5-dihydroxyxanthone		16	14
2,3,4-trihydroxyxanthone		40	17
3,4,5,6-tetrahydroxyxanthone		5	2.5
2,3,4,5,6-pentahydroxyxanthone (X5)		0.4	1.2
1,3,5,6,7-pentahydroxyxanthone		1	9
2,3,4,5,6,7-hexahydroxyxanthone (X6)		0.1	1.4
2,3,4,5,6-pentamethoxyxanthone		>100	>1000
2,3,4,5,6-pentaacetylxanthone		0.075	>1000
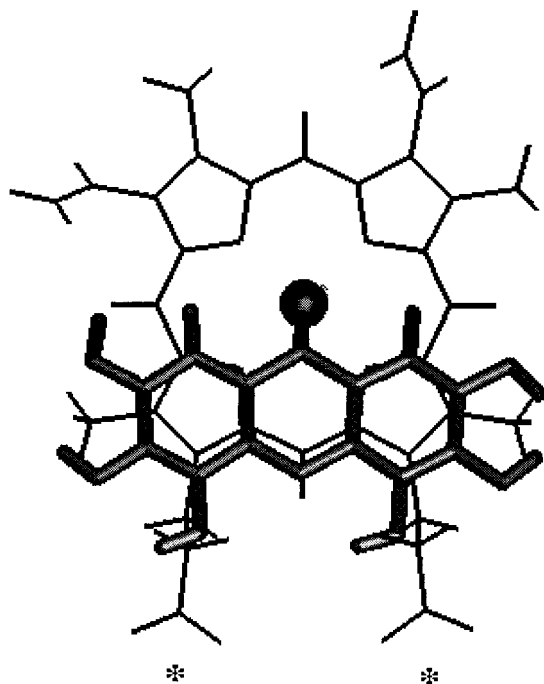
Fig. 5. A model for one possible docking orientation of a symmetrical polyhydroxyxanthone (X6) to heme. Asterisks indicate the heme carboxyl groups.

μM (Fig. 4, panel B). Consistent with this structure–activity profile, the 4,5-hydroxylated xanthones also exhibited the most pronounced in vitro antimalarial activity (Table 2). Furthermore, pentamethoxy–X5 and pentaacetyl–X5 were inactive in this assay though the latter was shown to be a potent antimalarial agent. Presumably, pentaacetyl–X5 is hydrolysable in infected red blood cells by a non-specific esterase whereas pentamethoxy–X5 is not.

3.5. Inhibition of heme polymerization by known antimalarial agents

We evaluated known antimalarials (e.g. chloroquine, primaquine, quinacrine, artemisinin and methylene blue) as inhibitors of heme polymerization under our in vitro assay conditions. As shown in Table 3, we found that the addition of 1–40 Eqs. of these compounds had no effect on the rate of in vitro polymerization as determined spectrophotometrically. We, therefore, decided to investigate the possibility that chloroquine might co-precipitate with the heme polymer as shown previously by Fitch and Kanjananggulpan and, more recently,

by Sullivan et al. [14,16]. We monitored the concentration of chloroquine by measuring its absorption at 340 nm in the presence of an equimolar concentration of polymerizing heme (25 μM). Indeed, we found that the concentration of soluble chloroquine decreased ≈35% after 2 h of incubation, indicative of the chloroquine/heme polymer co-precipitation phenomenon.

Similar spectroscopic studies were then performed with other antimalarial agents. Primaquine, quinacrine and methylene blue, which are positively charged under mildly acidic conditions, co-precipitated with the heme polymer (producing a distinctive change in the color of the polymer in the last case), possibly due to association with free carboxyl groups of the heme polymer and π–π interactions between the aromatic systems.

4. Discussion

The digestive vacuole is an acidic proteolytic compartment central to the metabolism of the Plasmodium parasite and may be considered as its Achilles' heel [3,6]. In this vacuole, hemoglobin is degraded to provide amino acids for parasite growth. Hemoglobinolysis also yields toxic heme, which serves as a reservoir of iron for parasite ferroproteins, although most of the heme is detoxified via polymerization into insoluble hemozoin. The mechanism of heme polymerization remains unknown. Slater and Cerami [17] initially proposed that the formation of hemozoin is an enzyme-mediated process. However, Egan et al. [5] and Dorn et al. [15] have suggested that heme polymerization is a spontaneous process when carried out in acidic acetate solutions (0.1–4.5 M, pH 4.2–5.0). It would appear that acetate will compete with the propionate side-chains of the porphyrin for the iron-centered coordination sites, resulting in formation of heme-acetate adducts [12] and polymer chain termination; hence, the increased solubility of the acetate-derived products ([12] and this report) and the splitting of the IR band (≈ 1650 cm^{-1}). In this report, we describe the development and characterization of an in vitro heme polymerization assay which proceeds under conditions likely to be present in the acidic vacuole. The product of our modified assay appears to be chemically similar to hemozoin. The low-ionic strength (20 mM) phosphate solution was used since this concentration is in the physiological range for most prokaryotic and eukaryotic cells [18]. The only carboxylate moieties present in the reaction mixture were those contributed by heme itself. The optimal pH range for heme polymerization was 4.5–5.5 at 37°C. Polymerization at higher pH did

Table 3
In vitro ability of antimalarial compounds to inhibit heme polymerization and to co-precipitate with the polymerizing heme

Compound name	Inhibition of in vitro heme polymerization	Co-precipitation with heme polymer in vitro[a]
Artemisinin	–	ND[b]
Cloroquine	–	+[c] (24%)
Methylene blue	–	+[d] (37%)
Primaquine	–	+[e] (21%)
Quinacrine	–	+[f] (50%)

[a]Determined as decreased absorbance of the antimalarial compound in the presence of equimolar (25 μM) polimerizing heme after 2 h of incubation; values in parentheses are the mean of duplicate determinations.
[b]ND, not determined; no characteristic UV/visible absorbance peaks (250–750 nm).
[c]Monitored at 328 and 342 nm.
[d]Monitored at 290 and 665 nm; precipate becomes blue-green in color.
[e]Monitored at 258 nm.
[f]Monitored at 275 nm.

not occur. Physical and chemical properties of the reaction product suggest that it is, indeed, a heme polymer with the carboxylate side-chain of one heme linked to the central ferric ion of the next, the structure proposed for β-hematin and hemozoin [6,13,19].

In a previous report, we put forth a 'xanthone hypothesis' to explain the potent antimalarial synergy between oxidant drugs and exifone [7]. We speculated that exifone functions as a prodrug yielding X5 upon free-radical hydroxylation. Based on in vitro characterization of this transformation, we proposed that the cyclodehydration event takes place in the digestive vacuole. We subsequently synthesized X5 and demonstrated its remarkable in vitro antimalarial activity [20].

Herein, we present evidence relating to the mode of action of this xanthone. Our findings suggest that X5 forms soluble complexes with heme monomers or oligomers and interferes with hemozoin formation. Such action may prevent detoxification of free heme, starve the parasite for iron or significantly increase the osmotic pressure within the parasite digestive vacuole. It is to be noted that the polymerization process must sequester all or most of the freed heme, which otherwise would accumulate to a concentration of up to 0.4 M [16]. The relative abilities of X5 and some of its analogs to inhibit in vitro heme polymerization are in good correlation with their in vitro antimalarial activities and are indicative of the following structure–activity relationships: (1) in general, a higher degree of hydroxylation is favored for the inhibitory activity; and (2) hydroxylation at 4- and 5-positions appears to be absolutely critical. Based on these observations, we have developed a model for one possible docking orientation of a symmetrical polyhydroxyxanthone (X6) to a heme monomer (Fig. 5) displaying several significant interactions: (1) between the heme iron and the carbonyl oxygen; (2) between the two planar aromatic systems; and (3) between the carboxylate side-groups of the heme and the 4- and 5-position hydroxyls of the xanthone. Moreover, this model predicts that chemical modifications at the 4- and/or 5-positions which improve association with the heme carboxylate groups will result in even greater antimalarial activity. Xanthone congeners containing positively charged groups (e.g. alkylamines or amidines) at these positions are being prepared to test this model.

Certain antimalarial drugs have been shown to bind free heme in mildly basic phosphate solutions [4,21,22] or to inhibit formation of heme polymers in acidic acetate solutions [5,15,23,24]. In this study, we have evaluated a number of these compounds as inhibitors of in vitro heme polymerization and were unable to observe such effects under our assay conditions (i.e. in a mildly acidic phosphate solution). For example, addition of 10 mM chloroquine (i.e. 400 Eqs.) to the sample containing 25 μM free heme did not affect the rate of polymerization since no detectable levels of soluble heme were observed in the sample after 2 h of incubation. However, we detected co-precipitation of chloroquine and heme which is consistent with the findings of Fitch et al. [14] and Sullivan et al. [16] who have recently studied hemozoin chain extension in the presence of heme substrate and aminoquinolines (chloroquine and quinidine) in acidic acetate solutions and found that these compounds are incorporated into the growing polymer (see Fig. 3 of [16]). None of the

other antimalarials tested in our assay (artemisinin, primaquine, quinacrine or methylene blue) inhibited spontaneous heme polymerization either although primaquine, quinacrine and methylene blue were found to co-precipitate with the polymer. These observations are consistent with the notion that aminoquinolines and other existing antimalarials do not directly inhibit in vivo hemozoin formation to exert their antimalarial action [6,16,23].

Taken together, our data suggest that xanthones act in a unique fashion to kill *Plasmodium* parasites through formation of soluble complexes with heme, thereby inhibiting the process of heme polymerization.

References

[1] World Health Organization Malaria Unit (1993) Bull. WHO 71, 281–284.
[2] I.W. Sherman, Microbiol. Rev. 43 (1979) 453–495.
[3] P. Olliaro, D. Goldberg, Parasitol. Today 11 (1995) 294–297.
[4] G. Blauer, H. Ginsburg, Biochem. Int. 5 (1982) 519–523.
[5] T.J. Egan, D.C. Ross, P.A. Adams, FEBS Lett. 352 (1994) 54–57.
[6] S.R. Meshnick, Ann. Trop. Med. Parasitol. 90 (1996) 367–372.
[7] R.W. Winter, M. Ignatushchenko, K.A. Cornell, L.J. Johnson, D.J. Hinrichs, M.K. Riscoe, Antimicrob. Agents Chemother. 40 (1996) 1408–1411.
[8] A.M.J. Oduola, N.F. Weatherly, J.H. Bowdre, R.E. Desjardins, Exp. Parasitol. 66 (1988) 86–95.
[9] W. Trager, J.B. Jensen, Science 193 (1976) 673–675.
[10] N. Elabbadi, M.L. Ancelin, H.J. Vial, Antimicrob. Agents Chemother. 36 (1992) 50–55.
[11] R.C.R.M. Vossen, M.C.E. Van Dam-Mieras, G. Hornstra, R.F.A. Zwaal, Lipids 28 (1993) 857–861.
[12] A. Pandey, B. Tekwani, FEBS Lett. 393 (1996) 189–192.
[13] A.F.G. Slater, W.J. Swiggard, B.R. Orton, W.D. Flitter, D.E. Goldberg, A. Cerami, G.B. Henderson, Proc. Natl. Acad. Sci. USA 88 (1991) 325–329.
[14] C.D. Fitch, P. Kanjananggulpan, J. Biol. Chem. 262 (1987) 15552–15555.
[15] A. Dorn, R. Stoffel, H. Matlle, A. Bubendorf, R. Ridley, Nature (London) 374 (1995) 269–271.
[16] D.J. Sullivan, I.Y. Gluzman, D.G. Russell, D.E. Goldberg, Proc. Natl. Acad. Sci. USA 93 (1996) 11865–11870.
[17] A.F.G. Slater, A. Cerami, Nature (London) 355 (1992) 167–169.
[18] Mathews, C.K. and Van Holde, K.E. (1990) in: Biochemistry, p. 83, Benjamin/Cummings, New York, NY.
[19] D. Bohle, R. Dinnebier, S. Madsen, P. Stephens, J. Biol. Chem. 272 (1997) 713–716.
[20] Winter, R.W., Ignatushchenko, M.V., Ogundahunsi, O.A.T., Cornell, K.A., Oduola, A.M.J., Hinrichs, D.J. and Riscoe, M.K. (1997, in press) Antimicrob. Agents Chemother.
[21] K. Raynes, M. Foley, L. Tilley, L.W. Deady, Biochem. Pharmacol. 52 (1996) 551–559.
[22] H. Atamna, M. Krugliak, G. Shalmiev, E. Deharo, G. Pescarmona, H. Ginsburg, Biochem. Pharmacol. 51 (1996) 693–700.
[23] W. Asawamahasakda, I. Ittarat, C. Chang, P. McElroy, S. Meshnick, Mol. Biochem. Parasitol. 67 (1994) 183–191.
[24] S.R. Meshnick, R.E. Taylor, S. Kamchonwongpaisan, Microbiol. Rev. 60 (1996) 301–315.