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Synthesis and antimalarial activity of E-2-quinolinylbenzocycloalcanones

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Abstract

A series of E-2-quinolinylbenzocycloalcanones **5**–**21** were prepared and evaluated for their activity to inhibit β-hematin formation and the hydrolysis of hemoglobin in vitro. Positive compounds for both assays were also tested for their efficacy in rodent *Plasmodium berghei*. Compounds **6**, **16**, **19**, and **20**, were the most promising. Inhibition of β-hematin formation was minimal when a hydrogen or methoxy groups were present on the position 8 of the quinoline and position 4′ of the indanone ring as it appeared for compounds **5**, **7**–**15**, **17**, **18**, and **21**, and greatest with compounds (52%) and (90%) with a substitution of methoxy on position 6 and 7 or methyl on position 8 of the quinoline nucleus and methoxy or methyl groups on position 4′ of the indanone. The most active compound to emerge from this study is 2-chloro-8-methyl-3-[(4′-methoxy-1′-indanoyl)-2′-methyliden]-quinoline **20** effective as antimalarial that target β-hematin formation and the inhibition of the hydrolysis of hemoglobin in vitro together with a good survival in a murine malaria model, which should help delay the rapid onset of resistance to drugs acting at only a single site. Results with these assays suggest that quinolinylbenzocycloalcanones exert their antimalarial activity via multiple mechanisms.

Keywords: Antimalarial; *Plasmodium berghei*; Haem; Hemoglobin

1. Introduction

Despite years of continued efforts, malaria is still the number one infectious disease in the world and remains a serious endemic disease in more than 90 countries in Africa, Asia and South America, with 300 and 500 million new infections each year. It causes more than 1 million deaths annually, mainly children and pregnant women [1]. The situation regarding control and treatment of malaria has progressively worsened with the spread of insecticide-resistant mosquito vectors and drug resistant parasites [2]. Therefore, there is an urgent need for new agents active against multi-resistant *Plasmodium* strains, through the identification of new targets, which are critical for the disease process or essential for the survival of the parasite [3,4]. The design of chemical agents specifically affecting these targets could lead to the availability of better drugs for treatment of malaria. The potential antimalarial activity of chalcones has generated a great interest [5–9]. The antimalarial activity of chalcones was first noted when licochalcone A **1**, a natural product isolated from Chinese liquorice roots, was reported to exhibit potent antimalarial activity [5]. Subsequently, a synthetic analogue 2,4-dimethoxy-4′-buthoxy chalcone **2**, was reported to have antimalarial activity [6]. Antimalarial chalcones are widely thought to act against a malarial cysteine protease [7]. Several oxygenated chalcones were reported to have antimalarial and antileishmanial activities **3**, the study showed that different physicochemical and structural requirements exist for the antimalarial and antileishmanial activities of chal-

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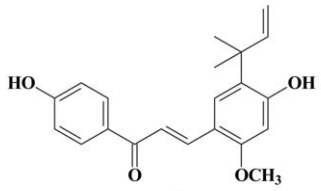
cones. Conventional structure–activity relationships show that antileishmanial activity is favored by chalcones with more hydrophilic character, with the most active members found among 4′-hydroxychalcones. In contrast, good antimalarial activity is found among alkoxylated chalcones with polar B rings, in particular those substituted with electron withdrawing groups or replaced by quinoline rings [8]. We have reported the synthesis and antimalarial activity of some quinolinyl chalcone analogues **4**, however, the potencies against falcipain, were somewhat lower than those seen for related quinolinyl chalcones [7,9]. Although some of the quinolinyl chalcones inhibited falcipain, their most potent antimalarial effects was independent of the inhibition of this enzyme and was evident that the substituted group in the benzoyl ring plays a significant role in determining the antimalarial activity.

2. Structures

In the present study, a series of quinolinylbenzocycloalcanones **5**–**21**, have been synthesized and evaluated as antimalarial in vitro, firstly against haem polymerization and secondly as inhibitors of hemoglobin hydrolysis. The best compounds were tested in vivo against *Plasmodium berghei* infected mice.



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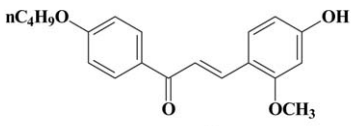

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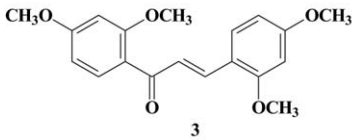

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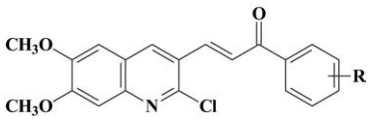

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3. Chemistry

Compounds **5**–**21** (Table 1) were synthesized by a base-catalyzed Claisen-Schmidt condensation of the 2-chloro-3-formyl quinoline derivatives with the appropriate 1-indanones [10] or commercially available 1-tetralones (Fig. 1). Theoretically, E and Z geometric isomers can be equally formed during the reaction. However, Z configuration is highly unfavorable due to its steric interactions between the chloro and carbonyl groups. Perhaps due to diamagnetic anisotropy of carbonyl group, where vinyl proton of E isomer gives a signal with a greater chemical shift than the vinyl proton of Z isomer, observation that has been noted previously [11,12]. It is important to mention that ^{1}H NMR spectra of these compounds, the protons of the β and 3′ positions absorbed as a triplet around 8.1 ppm and a doublet around 3.8 ppm, respectively, with coupling constants *J* ranging 0.9–1.2 Hz, clearly indicating the appearance of allylic coupling among these positions. Additional supports for these structures were obtained from ^{13}C NMR. The main shift of carbonyl carbons has a slight variation at 187 ppm in the tetralone series, the ring strain indanone series increase this value 192 ppm. The enhancement in the CO shift is due to a moderate polarization, resulting from the localization of CO bond in the non planar enone moiety.

4. Biological results and discussion

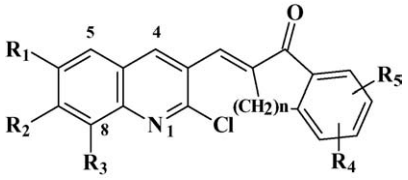
Previous report has shown that chalcones exhibited potent antimalarial activities [13]. Seventeen analogs of those derivatives were tested for their effects as inhibitors of β-hematin formation, inhibition of hemoglobin hydrolysis in vitro and their efficacy in a murine model (Tables 1 and 2; Fig 2).

The presence of an alcoxy group as substituents in the aromatic ring appeared to be favorable for antimalarial activity, since most of compounds possessing this group showed measurable levels of inhibition of β-hematin formation, regardless of the nature of the substitutions in the aromatic ring.

To evaluate the antimalarial activity of 2-quinolinyl-benzocycloalcanones derivates **5**–**21**, we tested the ability of the compounds to inhibit haem polymerization, considering that haem can polymerize spontaneously under acid and low oxygen conditions found in the food vacuole of the parasite [14]. Results showing more than 50% of inhibition of haem polymerization were considered significative (compounds **6**, **16**, **19** and **20**, Table 1).

Consequently these compounds were tested for their capacity of inhibiting globin hydrolysis, in an in vitro assay which uses trophozoyte-rich extract to digest the native hemoglobin of mice. The electrophoretic analysis indicated that only two compounds (**6** and **16**) were effective inhibiting the degradation of hemoglobin (intact band at 14.4 kDa). On the other

Table 1
Derivatives of E-2-quinolinyl-benzocycloalcanones **5–21**, and inhibition of biological activities against Haem polymerization and hemoglobin degradation in vitro. % IHP: percentage of inhibition of haem polymerization. IGH: Inhibition of globin hydrolysis. +: Undegraded globin as seen on the electrophoretical analysis (Fig. 2)



Number	R_1	R_2	R_3	R_4	R_5	% IHP	IGH
5	OCH_3	OCH_3	H	H	H	< 5	
6	OCH_3	OCH_3	H	4′-CH_3	H	52	+
7	OCH_3	OCH_3	H	4′-OCH_3	H	< 5	
8	OCH_3	OCH_3	H	5′-OCH_3	H	9	
9	OCH_3	OCH_3	H	6′-OCH_3	H	< 5	
10	OCH_3	OCH_3	H	7′-OCH_3	H	< 5	
11	OCH_3	OCH_3	H	4′-OCH_3	5′-OCH_3	< 5	
12	OCH_3	OCH_3	H	5′-OCH_3	6′-OCH_3	< 5	
13	OCH_3	OCH_3	H	4′-OCH_3	7′-OCH_3	< 5	
14	OCH_3	OCH_3	H	5′,6′-OCH_2O		< 5	
15	OCH_3	OCH_3	H	H	H	< 5	
16	OCH_3	OCH_3	H	5′-OCH_3	H	61	+
17	OCH_3	OCH_3	H	6′-OCH_3	H	< 5	
18	OCH_3	OCH_3	H	7′-OCH_3	H	< 5	
19	H	H	CH_3	4′-CH_3	H	90	–
20	H	H	CH_3	4′-OCH_3	H	79	–
21	H	H	CH_3	5′,6′-OCH_2O		< 5	

5–14, **19–21** (*n*: 1); **15–18** (*n*: 2).

hand, compounds **19** and **20** showed moderate inhibition due to the presence of some intact hemoglobin (Fig. 2).

Compounds **6**, **16**, **19**, **20** were tested in mice infected with *P. berghei* ANKA, a chloroquine-susceptible strain of murine malaria. Mice were given the compound (chloroquine or **6**, **16**, **19** and **20** in 20 mg kg^{-1}, i.p. once daily) for 4 consecutive days (days 0–4 postinfection). At day fourth postinfection, the parasitemia was determined, the survival days were monitored and compared with control mice receiving saline (untreated mice). Control mice died between days 6 and 7 post-infection, compound **6** only slightly increased the survival time, while **16** and **19** prolonging that time for 7 and 8 days, respectively. Particular attention was paid to compound **20** which prolonged the survival time of the infected mice to 12 days (Table 2). These compounds were able to reduce and delay the progression of malaria but did not eradicate the infection (Table 2).

Compounds E-2-quinolinylbenzocycloalcanones were not tested as falcipain inhibitor in vitro, however, the mechanism of action of these compounds on hemoglobin degradation

Table 2
The effects of 2-quinolinylbenzocycloalcanones derivatives **6**, **16**, **19**, **20** (20 mg kg^{-1}) on *P. berghei* infected mice The results are expressed as the media ± S.E.M. $n = 6$ (number of treated mice). ‡ The chloroquine-treated group (25 mg kg^{-1}) was observed until day number 30 post-infection, and they remained alive. * $P < 0.001$ comparing to control group. † $P < 0.001$ comparing to six- and 16-treated mice

Group	Post-infection	Days of survival over control
Control	33.66 ± 1.49	–
6	21.83 ± 1.14 *	1.66 ± 0.42
16	14.83 ± 0.83 *	6.5 ± 0.5
19	15.16 ± 0.79 *	9.66 ± 1.31
20	9.16 ± 0.70 *	12.16 ± 0.31 †
Chloroquine	4.5 ± 0.56	‡

could be related to the blockade of this protease because of the presence of intact globin band (Fig. 2).

We could observe some rigidity of the ketone group of these structures a cooperative process seems to be necessary for a better activity during the inhibition of hemoglobin degradation as well as a noticeable decrease of the parasitemia of infected mice of the rodent malaria model as could be seen in previous results [9]. The monosubstitution of indanoyl or tetraloyl fragment on position 4′ or 5′ with methyl or methoxy groups, respectively, appeared to possess an important role in the antimalarial activity too. According with these results, we have observed when methoxy and methyl groups have been incorporated to the quinoline ring at positions 6–8 their antimalarial activity have improved a great deal [9]. In addition, this type of compounds highlights the cooperative action of cystein proteases in hemoglobin degradation by malaria parasites.

These results offer new possibilities for further improvements about the antimalarial performance of quinolinylbenzocycloalcanones derivatives. Although this initial study involved only a limited number of compounds, it has provided structure–activity relationships that are well worth studying further. A number of new antimalarial compounds will likely to be needed over the coming years, so it is important to pursue multiple strategies for drug discovery.

5. Experimental

5.1. Chemistry

Melting points were determined on a Thomas micro hot stage apparatus and are uncorrected. Infrared spectra were determined as KBr pellets on a Shimadzu model 470 spectrophotometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded using a JEOL Eclipse 270 MHz spectrometer and are reported in ppm downfield from TMS (tetramethylsilane)

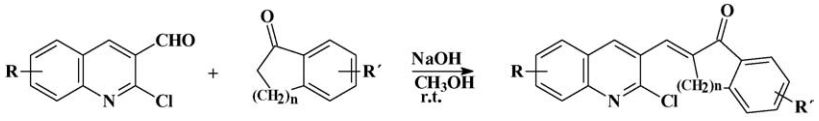


Fig. 1. Synthesis of E-2-quinolinylbenzocycloalcanones **5–21**.

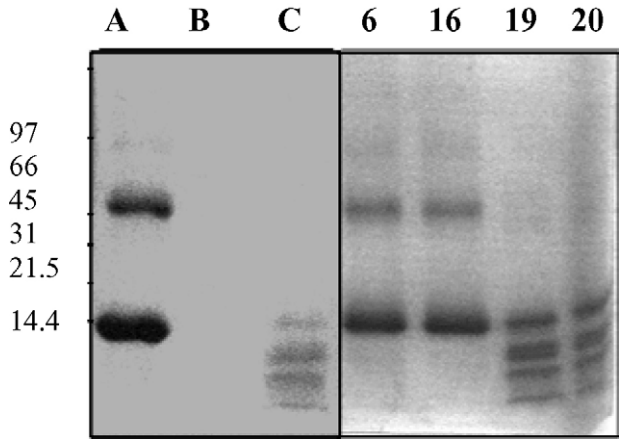


Fig. 2. Effects on hemoglobin hydrolysis of E-2-quinolinylbenzocycloalcanones derivatives. The samples were solubilized in SDS-sample buffer containing β-mercapto ethanol and boiled before electrophoresis in 15% SDS-PAGE gels. The gels were stained with Coomassie blue. The positions of molecular weight (MW) standards are shown in kilodaltons (kDa). Undegraded globin appears as a dimmer at 14–16 kDa; A: control, without enzyme; B: control, without hemoglobin; C: control, enzyme with hemoglobin; 2-chloro-6,7-dimethoxy-3-[(4′-methyl-1′-indanoyl)-2′-methyliden]-quinoline **6**, 2-chloro-6,7-dimethoxy-3-[(5′-methoxy-1′-tetralonyl)-2′-methyliden]-quinoline **16**, 2-chloro-8-methyl-3-[(4′-methyl-1′-indanoyl)-2′-methyliden]-quinoline **19**, 2-chloro-8-methyl-3-[(4′-methoxy-1′-indanoyl)-2′-methyliden]-quinoline **20** compounds.

as internal standard. Elemental analyses were performed by Atlantic Microlab, Norcross, GA, USA, results were within ±0.4% of predicted values for all compounds. Chemical reagents were obtained from Aldrich Chemical Co., USA. All solvents were distilled and dried in the usual manner.

*5.1.1. General procedure for the synthesis of 2-chloro-6,7-dimethoxy or 8-methyl-3-[(1′-indanoyl or 1′-tetranoyl)-2′-methyliden]-quinoline (**5–21**)*

A mixture of 2-chloro-3-formylquinoline respective (1 mmol), the respective 1-indanone or 1-tetralone (1 mmol), and sodium methoxide (catalytic) in methanol (8 ml) was stirred at room temperature over night. Water was added; the resulting precipitate was collected off by filtration, washed with water and recrystallized from ethyl acetate.

*5.1.1.1. 2-Chloro-6,7-dimethoxy-3-[(1′-indanoyl)-2′-methyliden]-quinoline (**5**).* Yield 92%; m.p. 282–284 °C; IR (KBr) cm^{-1}: 1696 (CO). ^1H NMR $CDCl_3$: δ 4.0 (d, 2H, $H_{3'}$, *J*: 1.76 Hz), 4.02 (s, 3H, OCH_3), 4.04 (s, 3H, OCH_3), 7.11 (s, 1H, H_5), 7.34 (s, 1H, H_8), 7.44 (t, 1H, $H_{5'}$, *J*: 1.3, 7.42 Hz), 7.54 (d, 1H, $H_{4'}$, *J*: 7.42 Hz), 7.63 (t, 1H, $H_{6'}$, *J*: 1.27, 7.42 Hz), 7.93 (d, 1H, $H_{7'}$, *J*: 7.63 Hz), 8.06 (t, 1H, Hv, *J*: 1.76 Hz), 8.27 (s, 1H, H_4). ^{13}C NMR: 32.06, 56.29, 56.44, 105.24, 107.40, 122.54, 124.60, 124.80, 125.97, 126.18, 128.90, 134.96, 136.12, 136.28, 137.54, 137.99, 144.81, 149.35, 150.65, 154.46, 193.86.

Anal. $C_{21}H_{16}NO_3Cl$: C, 68.95; H, 4.41; N, 3.83. Found: C, 69.16; H, 4.35; N, 3.60%.

*5.1.1.2. 2-Chloro-6,7-dimethoxy-3-[(4′-methyl-1′-indanoyl)-2′-methyliden]-quinoline (**6**).* Yield 78%; m.p. 260–261 °C; IR (KBr) cm^{-1}: 1702 (CO). ^1H NMR $CDCl_3$: δ 2.42 (s, 3H, CH_3), 3.80 (d, 2H, $H_{3'}$, *J*: 1.73 Hz), 4.0 (s, 3H, OCH_3), 4.06 (s, 3H, OCH_3), 7.12 (s, 1H, H_5), 7.31 (s, 1H, H_8), 7.32 (t, 1H, $H_{6'}$, *J*: 7.18 Hz), 7.42 (d, 1H, $H_{5'}$, *J*: 7.18 Hz), 7.73 (d, 1H, $H_{7'}$, *J*: 7.42 Hz), 8.01 (t, 1H, Hv, *J*: 1.76 Hz), 8.23 (s, 1H, H_4). ^{13}C NMR: 18.16, 30.67, 56.33, 56.41, 105.32, 107.32, 122.07, 122.52, 125.87, 128.22, 128.74, 135.35, 135.55, 136.04, 137.69, 144.64, 148.32, 149.37, 150.63, 154.24, 193.69.

Anal. $C_{22}H_{18}NO_3Cl$: C, 69.57; H, 4.78; N, 3.69. Found: C, 69.87; H, 4.75; N, 3.87%.

*5.1.1.3. 2-Chloro-6,7-dimethoxy-3-[(4′-methoxy-1′-indanoyl)-2′-methyliden]-quinoline (**7**).* Yield 83%; m.p. 265–266 °C; IR (KBr) cm^{-1}: 1705 (CO). ^1H NMR $CDCl_3$: δ 3.87 (d, 2H, $H_{3'}$, *J*: 1.98 Hz), 3.93 (s, 3H, OCH_3), 3.99 (s, 3H, OCH_3), 4.05 (s, 3H, OCH_3), 7.06 (d, 1H, $H_{5'}$, *J*: 7.92 Hz), 7.12 (s, 1H, H_5), 7.29 (s, 1H, H_8), 7.48 (d, 1H, $H_{7'}$, *J*: 7.42 Hz), 7.73 (t, 1H, $H_{6'}$, *J*: 7.67 Hz), 8.01 (t, 1H, Hv, *J*: 1.98 Hz), 8.26 (s, 1H, H_4). ^{13}C NMR: 28.86, 55.68, 56.34, 56.40, 105.46, 107.28, 115.35, 116.38, 122.56, 125.67, 128.88, 129.43, 136.15, 137.23, 137.97, 139.33, 144.65, 149.49, 150.58, 154.27, 156.63, 193.44.

Anal. $C_{22}H_{18}NO_4Cl$: C, 66.75; H, 4.58; N, 3.54. Found: C, 67.03; H, 4.28; N, 3.28%.

*5.1.1.4. 2-Chloro-6,7-dimethoxy-3-[(5′-methoxy-1′-indanoyl)-2′-methyliden]-quinoline (**8**).* Yield 73%; m.p. 248–250 °C; IR (KBr) cm^{-1}: 1705(CO). ^1H NMR $CDCl_3$: δ 3.93 (s, 3H, OCH_3), 3.97 (d, 2H, $H_{3'}$, *J*: 1.73 Hz), 3.99 (s, 3H, OCH_3), 4.03 (s, 3H, OCH_3), 6.92 (dd, 1H, $H_{6'}$, *J*: 1.98, 8.41 Hz), 6.95 (s, 1H, $H_{4'}$), 7.07 (s, 1H, H_5), 7.13 (d, 1H, $H_{7'}$, *J*: 8.41 Hz), 7.31 (s, 1H, H_8), 7.94 (t, 1H, Hv, *J*: 1.76 Hz), 8.20 (s, 1H, H_4). ^{13}C NMR: 31.85, 55.90, 56.28, 56.40, 105.23, 107.28, 109.85,117.84, 122.54, 126.01, 126.16, 127.62, 131.33, 135.98, 137.88, 138.14, 144.46, 149.36, 150.56, 154.12, 163.27, 191.73.

Anal. $C_{22}H_{18}NO_4Cl$: C, 66.75; H, 4.58; N, 3.54. Found: C, 66.51; H, 4.55; N, 3.44%.

*5.1.1.5. 2-Chloro-6,7-dimethoxy-3-[(6′-methoxy-1′-indanoyl)-2′-methyliden]-quinoline (**9**).* Yield 86%; m.p. 292–294 °C; IR (KBr) cm^{-1}: 1692 (CO). ^1H NMR $CDCl_3$: δ 3.86 (s, 3H, OCH_3), 3.97 (d, 2H, $H_{3'}$, *J*: 1.83 Hz), 4.02 (s, 3H, OCH_3), 4.03 (s, 3H, OCH_3), 7.09 (s, 1H, H_5), 7.34 (s, 1H, H_8), 7.36 (d, 1H, $H_{4'}$, J: 8.16 Hz), 7.38 (d, 1H, $H_{7'}$, *J*: 1.78 Hz), 7.41 (d, 1H, $H_{5'}$, *J*: 8.16 Hz), 8.04 (t, 1H, Hv, *J*: 1.83 Hz), 8.26 (s, 1H, H_4). ^{13}C NMR: 31.41, 55.72, 56.25, 56.33, 105.34, 106.32, 107.51, 122.59, 124.22, 126.03, 126.17, 128.80, 136.06, 138.46, 139.36, 142.15, 144.81, 149.51, 150.78, 154.45, 160.07, 193.13.

Anal. $C_{22}H_{18}NO_4Cl$: C, 66.75; H, 4.58; N, 3.54. Found: C, 66.68; H, 4.67; N, 3.78%.

*5.1.1.6. 2-Chloro-6,7-dimethoxy-3-[(7′-methoxy-1′-indanoyl)-2′-methyliden]-quinoline (**10**).* Yield 71%; m.p. 254–255 °C; IR (KBr) cm^{-1}: 1686 (CO). ^1H NMR $CDCl_3$: δ 3.75 (d, 2H, $H_{3'}$, *J*: 1.83 Hz), 3.95 (s, 3H, OCH_3), 4.01 (s, 3H,

OCH_3), 4.02 (s, 3H, OCH_3), 6.82 (d, 1H, $H_{4'}$, *J*: 8.16 Hz), 7.05 (d, 1H, $H_{6'}$, *J*: 8.41 Hz), 7.08 (s, 1H, H_5), 7.29 (s, 1H, H_8), 7.52 (t, 1H, $H_{5'}$, *J*: 8.16 Hz), 7.92 (t, 1H, Hv, *J*: 1.83 Hz), 8.19 (s, 1H, H_4). ^{13}C NMR: 32.12, 55.79, 56.27, 56.41, 105.24, 107.30, 109.64, 115.42, 125.97, 126.01, 126.51, 127.51, 135.97, 136.68, 137.92, 139.40, 144.83, 150.01, 150.74, 154.71, 159.03, 191.19.

Anal. $C_{22}H_{18}NO_4Cl$: C, 66.75; H, 4.58; N, 3.54. Found: C, 66.70; H, 4.29; N, 3.54%.

*5.1.1.7. 2-Chloro-6,7-dimethoxy-3-[(4′,5′-dimethoxy-1′-indanoyl)-2′-methyliden]-quinoline (**11**).* Yield 69%; m.p. 258–260 °C; IR (KBr) cm^{-1}: 1699 (CO). ^{1}H NMR $CDCl_3$: δ 3.96 (d, 2H, $H_{3'}$, *J*: 1.98 Hz), 3.97 (s, 3H, OCH_3), 3.98 (s, 3H, OCH_3), 4.01 (s, 3H, OCH_3), 4.06 (s, 3H, OCH_3), 7.01 (d, 1H, $H_{6'}$, *J*: 8.41 Hz), 7.15 (s, 1H, H_5), 7.31 (s, 1H, H_8), 7.68 (d, 1H, $H_{7'}$, *J*: 8.41 Hz), 7.96 (t, 1H, Hv, *J*: 1.98 Hz), 8.27 (s, 1H, H_4). ^{13}C NMR: 28.85, 56.33, 56.35, 56.41, 60.79, 105.43, 112.80, 107.30, 121.54, 122.57, 125.87, 128.18, 131.96, 131.96, 136.10, 136.10, 137.84, 144.58, 145.14, 149.40, 150.57, 154.19, 157.83, 191.88.

Anal. $C_{23}H_{20}NO_5Cl$: C, 64.87; H, 4.73; N, 3.29. Found: C, 64.57; H, 4.91; N, 3.48%.

*5.1.1.8. 2-Chloro-6,7-dimethoxy-3-[(5′,6′-dimethoxy-1′-indanoyl)-2′-methyliden]-quinoline (**12**).* Yield 87%; m.p. 298–300 °C; IR (KBr) cm^{-1}: 1689 (CO). ^{1}H NMR $CDCl_3$: δ 3.95 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3), 3.98 (d, 2H, $H_{3'}$, *J*: 1.98 Hz), 4.0 (s, 3H, OCH_3), 4.02 (s, 3H, OCH_3), 6.92 (s, 1H, $H_{4'}$), 7.04 (s, 1H, H_5), 7.28 (s, 1H, H_8), 7.29 (s, 1H, $H_{7'}$), 7.94 (t, 1H, Hv, *J*: 1.98 Hz), 8.19 (s, 1H, H_4). ^{13}C NMR: 31.75, 55.75, 56.24, 56.33, 56.37, 105.14, 105.28, 107.13, 107.33, 122.53, 126.03, 127.29, 131.06, 135.95, 138.30, 144.52, 144.67, 149.37, 149.88, 150.60, 154.14, 155.74, 192.17.

Anal. $C_{23}H_{20}NO_5Cl$: C, 64.87; H, 4.73; N, 3.29. Found: C, 65.09; H, 4.61; N, 3.30%.

*5.1.1.9. 2-Chloro-6,7-dimethoxy-3-[(4′,7′-dimethoxy-1′-indanoyl)-2′-methyliden]-quinoline (**13**).* Yield 69%; m.p. 280–282 °C; IR (KBr) cm^{-1}: 1686 (CO). ^{1}H NMR $CDCl_3$: δ 3.87 (d, 2H, $H_{3'}$, *J*: 2.23 Hz), 3.89 (s, 3H, OCH_3), 3.93 (s, 3H, OCH_3), 4.01 (s, 3H, OCH_3), 4.05 (s, 3H, OCH_3), 6.79 (d, 1H, $H_{5'}$, 8.66 Hz), 7.04 (d, 1H, $H_{6'}$, *J*: 8.66 Hz), 7.13 (s, 1H, H_5), 7.33 (s, 1H, H_8), 7.98 (t, 1H, Hv, *J*: 2.23 Hz), 8.26 (s, 1H, H_4). ^{13}C NMR: 28.74, 56.04, 56.24, 56.32, 56.40, 105.42, 107.33, 110.37, 117.25, 122.60, 126.10, 128.21, 136.00, 137.75, 139.52, 144.74, 149.51, 149.98, 150.56, 152.89, 154.17, 191.39.

Anal. $C_{23}H_{20}NO_5Cl$: C, 64.87; H, 4.73; N, 3.29. Found: C, 64.60; H, 4.67; N, 3.53%.

*5.1.1.10. 2-Chloro-6,7-dimethoxy-3-[(5′,6′-methylendioxy-1′-indanoyl)-2′-methyliden]-quinoline (**14**).* Yield 61%; m.p. 298–300 °C; IR (KBr) cm^{-1}: 1689 (CO). ^{1}H NMR $CDCl_3$: δ 3.92 (d, 2H, $H_{3'}$, *J*: 1.98 Hz), 4.02 (s, 3H, OCH_3), 4.06 (s, 3H, OCH_3), 6.09 (s, 2H, OCH_2O), 6.90 (s, 1H, $H_{4'}$) Hz), 7.09 (s, 1H, H_5), 7.29 (s, 1H, H_8), 7.34 (s, 1H, $H_{7'}$), 7.95 (t, 1H, Hv, *J*: 1.98 Hz), 8.22 (s, 1H, H_4). ^{13}C NMR: 32.12, 56.43, 56.57, 105.30, 107.64, 115.03, 122.38, 126.30, 126.51, 128.33, 131.47, 136.98, 137.85, 139.44, 144.92, 149.57, 150.74, 151.49, 154.71, 155.74, 192.19.

Anal. $C_{22}H_{16}NO_5Cl$: C, 64.48; H, 3.94; N, 3.42. Found: C, 64.66; H, 3.60; N, 3.48%.

*5.1.1.11. 2-Chloro-6,7-dimethoxy-3-[(1′-tetralonyl)-2′-methyliden]-quinoline (**15**).* Yield 62%; m.p. 217–218 °C; IR (KBr) cm^{-1}: 1673 (CO). ^{1}H NMR $CDCl_3$: δ 2.98 (m, 2H, $H_{3'}$), 3.04 (m, 2H, $H_{4'}$), 4.0 (s, 3H, OCH_3), 4.01 (s, 3H, OCH_3), 7.04 (s, 1H, H_5), 7.24 (d, 1H, $H_{5'}$, *J*: 7.41 Hz), 7.34 (s, 1H, H_8), 7.37 (dd, 1H, $H_{6'}$, *J*: 7.67, 1.48 Hz), 7.49 (dd, 1H, $H_{7'}$, *J*: 7.42, 1.48 Hz), 7.89 (s, 1H, H_4), 7.92 (brs, 1H, Hv), 8.14 (dd, 1H, $H_{8'}$, *J*: 7.67, 0.99 Hz). ^{13}C NMR: 27.56, 29.00, 105.03, 107.35, 122.24, 126.43, 127.25, 128.39, 128.42, 132.04, 133.23, 133.65, 136.59, 137.80, 143.24, 144.47, 148.55, 150.49, 153.49, 187.30.

Anal. $C_{22}H_{18}NO_3Cl$: C, 69.57; H, 4.78; N, 3.69. Found: C, 69.70; H, 4.71; N, 3.45%.

*5.1.1.12. 2-Chloro-6,7-dimethoxy-3-[(5′-methoxy-1′-tetralonyl)-2′-methyliden]-quinoline (**16**).* Yield 70%; m.p. 190–191 °C; IR (KBr) cm^{-1}: 1660 (CO). ^{1}H NMR $CDCl_3$: δ 2.92 (m, 2H, $H_{3'}$), 3.02 (m, 2H, $H_{4'}$), 3.84 (s, 3H, OCH_3), 4.0 (s, 3H, OCH_3), 4.01 (s, 3H, OCH_3), 7.03 (dd, 1H, $H_{6'}$, *J*: 7.92, 0.74 Hz), 7.04 (s, 1H, H_5), 7.31 (t, 1H, $H_{7'}$, *J*: 7.92 Hz), 7.33 (s, 1H, H_8), 7.79 (dd, 1H, $H_{8'}$, *J*: 7.92, 0.99 Hz), 7.87 (brs, 1H, Hv), 7.89 (s, 1H, H_4). ^{13}C NMR: 26.83, 28.80, 55.83, 56.25, 56.37, 105.05, 107.33, 114.65, 120.00, 122.26, 126.47, 127.41, 131.65, 132.34, 134.20, 136.64, 137.86, 144.43, 148.59, 150.46, 153.77, 156.50, 187.60.

Anal. $C_{23}H_{20}NO_4Cl$: C, 67.40; H, 4.92; N, 3.42. Found: C, 67.03; H, 4.67; N, 3.55%.

*5.1.1.13. 2-Chloro-6,7-dimethoxy-3-[(6′-methoxy-1′-tetralonyl)-2′-methyliden]-quinoline (**17**).* Yield 65%; m.p. 238–239 °C; IR (KBr) cm^{-1}: 1651 (CO). ^{1}H NMR $CDCl_3$: δ 2.94 (m, 2H, $H_{3'}$), 3.01 (m, 2H, $H_{4'}$), 3.85 (s, 3H, OCH_3), 4.0 (s, 3H, OCH_3), 4.01 (s, 3H, OCH_3), 6.69 (d, 1H, $H_{5'}$, *J*: 2.23 Hz), 6.87 (dd, 1H, $H_{7'}$, *J*: 8.91, 2.72 Hz), 7.04 (s, 1H, H_5), 7.34 (s, 1H, H_8), 7.87 (s, 1H, H_4), 7.91 (brs, 1H, Hv), 8.13 (d, 1H, $H_{8'}$, *J*: 8.66 Hz). ^{13}C NMR: 27.60, 29.40, 55.56, 56.23, 56.36, 105.02, 107.34, 112.49, 113.59, 122.26, 126.63, 126.82, 130.97, 131.39, 136.57, 137.99, 144.40, 145.76, 148.58, 150.46, 153.74, 163.90, 186.14.

Anal. $C_{23}H_{20}NO_4Cl$: C, 67.40; H, 4.92; N, 3.42. Found: C, 67.48; H, 4.80; N, 3.38%.

*5.1.1.14. 2-Chloro-6,7-dimethoxy-3-[(7′-methoxy-1′-tetralonyl)-2′-methyliden]-quinoline (**18**).* Yield 59%; m.p. 199–200 °C; IR (KBr) cm^{-1}: 1655 (CO). ^{1}H NMR $CDCl_3$: δ 2.91 (m, 2H, $H_{3'}$), 3.02 (m, 2H, $H_{4'}$), 3.87 (s, 3H, OCH_3), 4.0 (s, 3H, OCH_3), 4.01 (s, 3H, OCH_3), 7.04 (s, 1H, H_5), 7.09 (dd, 1H, $H_{6'}$, *J*: 8.41, 2.72 Hz), 7.16 (d, 1H, $H_{5'}$, *J*: 8.41 Hz), 7.35

(s, 1H, H_8), 7.64 (d, 1H, $H_{8'}$, *J*: 2.72 Hz), 7.90 (s, 1H, H_4), 7.91 (brs, 1H, Hv). ^{13}C NMR: 27.78, 28.20, 55.66, 56.25, 56.39, 105.01, 107.35, 110.41, 121.98, 122.24, 126.48, 129.66, 132.08, 134.05, 135.96, 136.59, 137.79, 144.47, 148.56, 150.49, 153.80, 158.85, 187.25.

Anal. $C_{23}H_{20}NO_4Cl$: C, 67.40; H, 4.92; N, 3.42. Found: C, 67.35; H, 4.83; N, 3.63%.

*5.1.1.15. 2-Chloro-8-methyl-3-[(4′-methyl-1′-indanoyl)-2′-methyliden]-quinoline (**19**).* Yield 87%; m.p. 212–214 °C; IR (KBr) cm^{-1}: 1702 (CO). ^{1}H NMR $CDCl_3$: δ 2.40 (s, 3H, CH_3), 2.76 (s, 3H. CH_3), 3.86 (d, 2H, $H_{3'}$, *J*: 1.78 Hz), 7.35 (t, 1H, H_6, *J*: 7.42 Hz), 7.42 (m, 1H, $H_{6'}$), 7.46 (m, 1H, $H_{5'}$), 7.51 (d, 1H, $H_{7'}$, *J*: 7.92 Hz), 7.61 (d, 1H, H_7, *J*: 7.67 Hz), 7.75 (d, 1H, H_5, *J*: 7.67 Hz), 8.04 (t, 1H, Hv, *J*: 1.78 Hz), 8.36 (s, 1H, H_4). ^{13}C NMR: 17.75, 18.09, 30.52, 112.16, 125.96, 126.92, 127.25, 127.42, 127.98, 128.67, 128.71, 131.59, 130.23, 135.42, 135.63, 136.89, 138.26, 138.62, 138.65, 146.48, 148.41, 150.19, 193.61.

Anal. $C_{21}H_{16}NOCl$: C, 75.56; H, 4.83; N, 4.20. Found: C, 75.43; H, 4.87; N, 3.80%.

*5.1.1.16. 2-Chloro-8-methyl-3-[(4′-methoxy-1′-indanoyl)-2′-methyliden]-quinoline (**20**).* Yield 90%; m.p. 208–210 °C; IR (KBr) cm^{-1}: 1705 (CO). ^{1}H NMR $CDCl_3$: δ 2.75 (s, 3H. CH_3), 3.88 (d, 2H, $H_{3'}$, *J*: 1.92 Hz), 3.93 (s, 3H, OCH_3), 7.07 (d, 1H, $H_{5'}$, J:7.92 Hz), 7.39 (t, 1H, H_6, *J*: 7.67 Hz), 7.47 (m, 1H, $H_{6'}$), 7.51 (m, 1H, $H_{7'}$), 7.59 (d, 1H, H_7, J:6.68 Hz), 7.74 (d, 1H, H_5, *J*: 8.16 Hz), 8.03 (t, 1H, Hv, *J*: 1.90 Hz), 8.39 (s, 1H, H_4). ^{13}C NMR: 17.42, 28.74, 55.63, 115.69, 116.39, 126.09, 126.95, 127.75, 127.38, 128.63, 128.71, 131.61, 136.81, 138.14, 138.38, 139.30, 146.46, 150.29, 156.70, 193.40.

Anal. $C_{21}H_{16}NO_2Cl$: C, 72.10; H, 4.61; N, 4.01. Found: C, 71.97; H, 4.39; N, 3.89%.

*5.1.1.17. 2-Chloro-8-methyl-3-[(5′,6′-metylendioxy-1′-indanoyl)-2′-methyliden]-quinoline (**21**).* Yield 86%; m.p. 266–268 °C; IR (KBr) cm^{-1}: 1689 (CO). ^{1}H NMR $CDCl_3$: δ 2.76 (s, 3H, CH_3), 3.90 (d, 2H, $H_{3'}$, *J*: 1.93 Hz), 6.09 (s, 2H, OCH_2O), 6.89 (s, 1H, $H_{4'}$), 7.26 (s, 1H, $H_{7'}$), 7.42 (t, 1H, H_6, *J*: 7.42 Hz), 7.59 (d, 1H, H_7, *J*: 7.18 Hz), 7.70 (d, 1H, H_5, *J*: 8.41 Hz), 7.95 (t, 1H, Hv, *J*: 1.93 Hz), 8.31 (s, 1H, H_4). ^{13}C NMR: 17.75, 31.82, 102.38, 103.47, 105.53, 125.86, 126.94, 127.39, 127.40, 131.52, 132.86, 136.87, 138.16, 138.37, 138.99, 146.46, 148.81, 150.37, 154.55, 191.91.

Anal. $C_{21}H_{14}NO_3Cl$: C, 69.33; H, 3.88; N, 3.85. Found: C, 69.41; H, 3.86; N, 4.01%.

5.2. Biological assays

5.2.1. Inhibition of haem polymerization

The haem polymerization assay was performed according to [14], briefly, a solution of hemin chloride (50 μl, 4 mM), dissolved in DMSO (5.2 mg ml^{-1}), was distributed in 96-well micro plates. Different concentrations (100–5 μM) of the compounds dissolved in DMSO, were added in triplicate in test wells (50 μl). Controls contained either water (50 μl) or DMSO (50 μl). β-Hematin formation was initiated by the addition Acetate buffer (100 μl 0.2 M, pH 4,4). Plates were incubated at 37 °C for 48 h to allow completion of the reaction and centrifuged (4000 rpm × 15 min, IEC-CENTRA, MP4R). After discarding the supernatant, the pellet was washed twice with DMSO (200 μl) and finally, dissolved in NaOH (200 μl, 0.2 N). The solubilized aggregates were further diluted 1:2 with NaOH (0.1 N) and absorbances recorded at 405 nm (Microplate Reader, BIORAD-550). The results were expressed as a percentage of inhibition of flavoprotein (FP) polymerization.

5.2.2. Parasite, experimental host and strain maintenance

Male Balb-C mice, weighing 18–22 g were maintained on a commercial pellet diet and housed under conditions approved by Ethics Committee. *P. berghei* (ANKA strain), a rodent malaria parasite, was used for infection. Mice were infected by i.p. injection with 1×10^6 infected erythrocytes diluted in phosphate buffered saline solution (PBS, 10 mM, pH 7.4, 0.1 ml). Parasitemia was monitored by microscopic examination of Giemsa stained smears.

5.2.3. Parasite extracts

Blood of infected animals, at a high level of parasitemia (30–50%), was collected by cardiac puncture with a heparinized syringe and the blood pool was centrifuged (500 × *g* × 10 min, 4 °C). Plasma and buffy coat were removed and the red blood cells (RBC) pellet was washed twice with chilled PBS-glucose (5.4%). The washed RBC pellet was centrifuged on a discontinuous percoll gradient (80–70% percoll in PBS-glucose, 20,000 × *g* × 30 min × 4 °C) [15]. The upper band (mature forms) was removed by aspiration, collected in eppendorf tubes and washed twice with chilled PBS-glucose and the infected erythrocytes were lysed with the non-ionic detergent saponin (0.1% in PBS × 10 min). One milliliter of cold PBS was added and the samples were centrifuged (13,000 × *g* × 5 min, 4 °C) to remove erythrocyte cytoplasm content (including erythrocyte hemoglobin). The free parasites were mixed PBS-glucose (5.4%), and subjected to three freeze–thaw cycles (–70 °C/+37 °C). The final homogenate was used in the hemoglobin hydrolysis inhibition assay [16].

5.2.4. Mice native hemoglobin

Native hemoglobin from non-infected mice was obtained by treating one volume of pellet erythrocytes with two volumes of water. The resulting solution was used as the substrate in the inhibition of the hemoglobin hydrolysis assay.

5.2.5. Inhibition of hemoglobin hydrolysis

The proteolytic effect of the parasite extract on the native mice hemoglobin was assayed using 96-wells tissue culture plate (Greiner Bio-One). The assay mixture contained: mice native hemoglobin (10 μl), parasite extract (50 μl), GSH (10 μl, 10 μM), and acetate buffer (0.2 M, pH 5.4) to a final volume

of 100 μl. The compounds (1–100 μM) were incorporated in the incubation mixture dissolved in DMSO. The incubations were carried out at 37 °C for 18 h and the reactions were stopped by addition of reduced sample buffer. The degree of digestion was evaluated electrophoretically by SDS-PAGE [16] by visual comparison the globin bands (14 kDa). A DMSO control was electrophoresed at the same time.

5.2.6. Four-days suppressive test

Balb-C mice (18–23 g) were infected i.v. (using caudal vein) with 10^6 infected RBC with *P. berghei* ($n = 6$). Two hours after infection, treatment began with the best compounds tested in the in vitro assays. These were dissolved in DMSO (0.1 M), diluted with Saline-Tween 20 solution (2%). Each compound (20 mg kg^{-1}) was administered once by i.p. for 4 days. At day 4, the parasitemia was counted by examination of Giemsa stained smears. Chloroquine (25 mg Kg^{-1}) was used as a positive control. The survival time beyond the control group (saline treated) was recorded. The results were expressed as percentage of parasitemia (% of parasitemia) and survival days of each compound treated-group over the control (saline treated group) [17].

6. Data analysis

Data were statistically analyzed using one-way ANOVA and *t*-tests for specific group comparisons; assuming 95% of confidence according to GraphPad Prism 3.02.

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