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Optimization of the synthesis, in silico ADME/Tox profiling studies, and evaluation of the antimalarial activity of (7-chloroquinolin-4-ylthio) alkylbenzoate derivatives

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Abstract

Optimized reaction conditions are developed to obtain a series of [(7-chloroquinolin-4-yl)sulfanyl] alcohol derivatives as intermediates to prepare a range of (7-chloroquinolin-4-ylthio) alkylbenzoate derivatives. The structures of all the synthesized compounds are confirmed from their infrared and nuclear magnetic resonance spectral data, and by elemental analysis. In silico ADME/Tox profiling studies of the synthesized molecules are undertaken, and the potential antimalarial activity of the products is determined. In vitro, all the prepared compounds significantly reduce heme crystallization with IC_{50} values of $< 10\,\mu M$. In vivo, the reduction in parasitemia levels and survival time increases are marginal.

Keywords

7-chloroquinoline, ADME/Tox, antimalarial, sulfanyl, β-hematin

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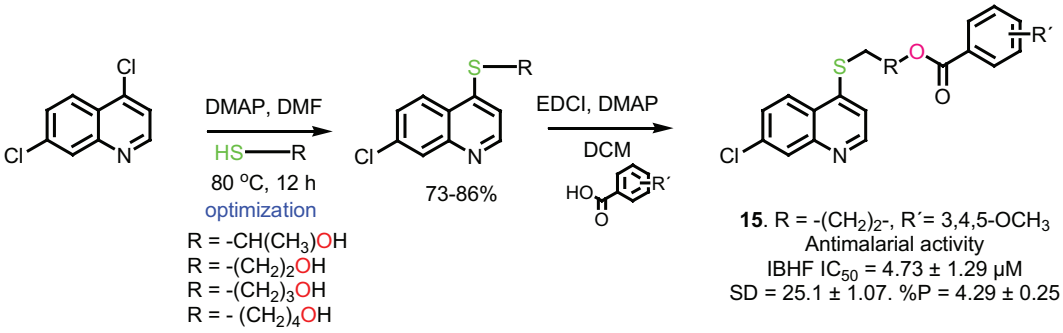

Introduction

Malaria is a tropical infectious disease caused by six species of the genus *Plasmodium: P. falciparum, P. vivax, P. ovale curtisi, P. ovale wallikeri, P. malariae*, and occasionally *P. knowlesi*. Among these, *P. falciparum* and *P. vivax*, the most dangerous species worldwide, are often fatal to humans and are now present in areas containing more than 40% of the world's population.[1] In 2019, 229 million new cases of this disease were reported, resulting in 409,000 deaths.[2] Since the discovery of quinine and the potent antipaludic activity of its quinolinic nucleus, significant efforts have been made to obtain new natural or synthetic structures containing this nucleus to provide novel treatment for the abovementioned

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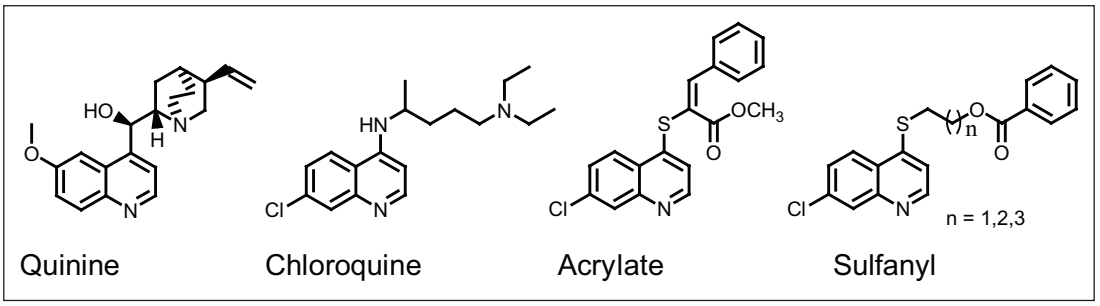


Figure 1. Antimalarial scaffolds with a quinoline moiety.

problem.[3–5] As a privileged fragment, quinoline is a rigid, planar molecule, which is a pharmacophore present in the core of numerous physiologically active agents that display interesting therapeutic properties.[6] Structurally, quinoline can be readily modified with a broad range of substituents to provide the molecular diversity necessary to achieve a library of compounds, among which different members can show different biological effects.[7–11] Similarly, organic compounds displaying chalcogen atoms in their structure, such as sulfur, are well-known and studies have demonstrated efficacious treatments with these types of compounds against disease models associated with β-hematin, adhesion, migration, invasion inhibition, apoptosis induction, oxidative stress, and for their antimalarial and antitubercular actions, as hypocholesterolemic agents and for their antiproliferative activity.[12–16] Because of these facts, we have designed and synthesized new molecules to further optimize chloroquine-based (CQ) antimalarial agents, in which we have selectively modified the side chain of the 4-amino functionality with a sulfur-containing group (Figure 1). Using 4-(dimethylamino)pyridine (DMAP), it was possible to improve the yield of the reaction when compared with previously reported procedures,[16] and a variety of substituted carboxylic acids were incorporated and the antimalarial activities of the products were evaluated.

Results and discussion

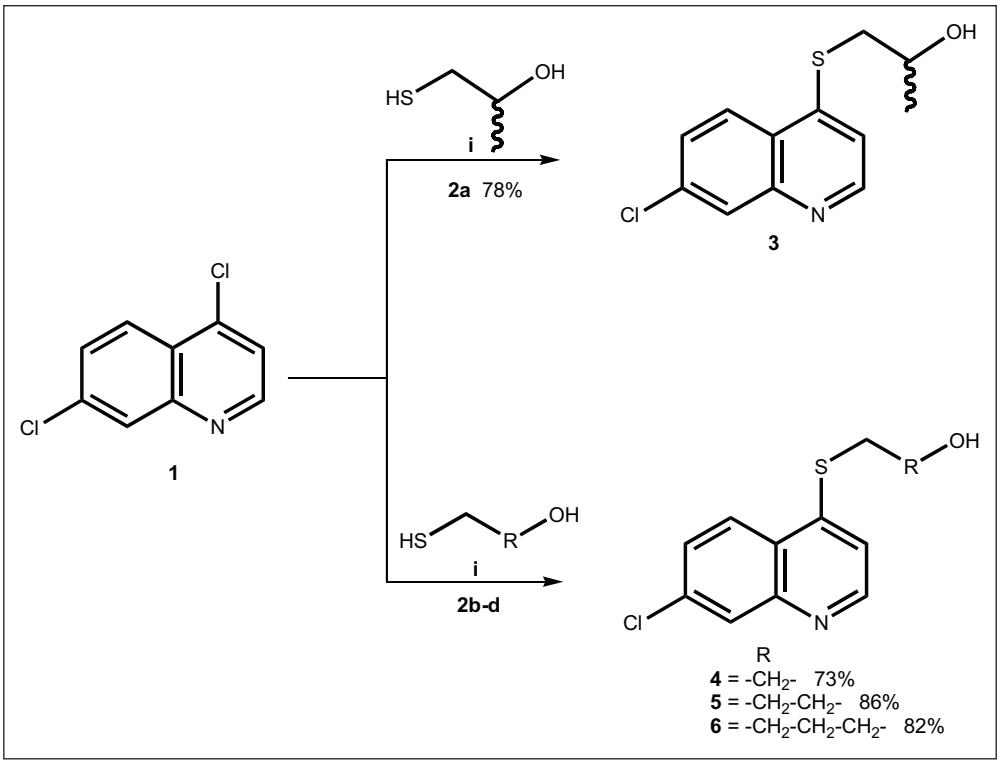
Chemistry

Based on our previous observations on the anticancer and antimalarial activity of 7-chloroquinoline derivatives, we chose to introduce diversity at position 4 by nucleophilic substitution of the chloride derivative **1** with different commercially available hydroxyalkylthiols **2a**–**d**. The yields and other conditions were optimized, for example, the reaction time for the process giving the new and reported intermediates **3**–**6**, and other variables such as the temperature and solvent were studied. When dry acetonitrile, tetrahydrofuran or 1,4-dioxane, and triethylamine were used at reflux temperature for 12 h, products **3**–**6** were not obtained. Changing the solvent to dry EtOH and using an excess of triethylamine and a temperature from room temperature to reflux for 5 days produced the desired products **3**–**6** in yields of 25%, 52%, 71%, and 32%, respectively. The best result was obtained when using an excess of DMAP, dry DMF as the solvent, a temperature of 80 °C, and a reaction time of 12 h, which led to the formation of products **3**–**6** in yields of 78%, 73%, 86%, and 82%, respectively (Scheme 1). Next, the desired products **7**–**18** were synthesized via coupling reactions between **3**–**6** and a series of substituted benzoic acids in the presence of *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDCI) and DMAP in CH_2Cl_2. The products were isolated in good-to-excellent yields (60%–99%) after purification by recrystallization or by column chromatography (Scheme 2).

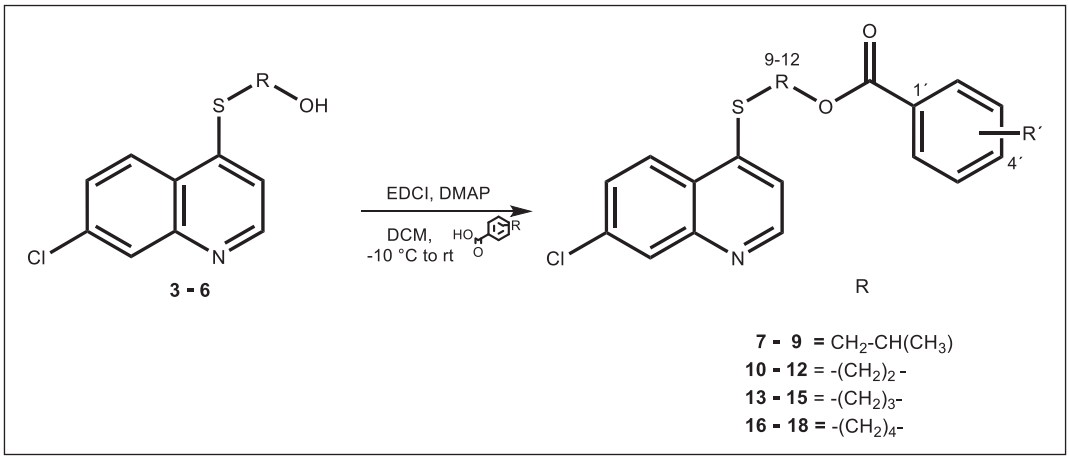
The chemical structures of the newly synthesized compounds were confirmed based on their infrared (IR), nuclear magnetic resonance (NMR), and mass spectral data, and their purity was ascertained by elemental analysis. The IR spectra revealed the presence of an intense stretching band at 3083–2959 cm^{-1} (C–H), along with C=O stretching vibrations at 1765–1669 cm^{-1}. Additional stretching bands around 1243–1200 cm^{-1} were assigned to bending vibrations of the sulfur-containing groups. In the ^1H NMR spectra, the signals of the respective protons of each compound were assigned based on their chemical shifts, multiplicities, and coupling constants. For compounds **7**–**9**, which were obtained as racemic mixtures, doublets centered at 1.56 ppm (d, $J = 6$ Hz) were assigned to CH_3 protons, and two double doublets between 3.27–3.32 and 3.56–3.61 ppm were assigned to the methylene protons 9a, b. The aliphatic signals expected at upfield shifts for compounds **10**–**18** were present between 1.84 and 4.38 ppm, and were assigned to protons 9, 10, 11, and 12. The quinoline moiety protons appeared as a doublet around 6.5 ppm (d, $J = 5$ Hz) assigned to proton H_3, a double doublet around 7.3 ppm (dd, $J = 8$ and 2 Hz) which corresponds to proton H_6, a doublet around 7.5 ppm (d, $J = 8$ Hz) for the proton H_5, a doublet around 7.9 ppm (d, $J = 2$ Hz) that corresponds to proton H_8, and a doublet around 8.5 ppm (d, $J = 5$ Hz) assigned to proton H_2. The aromatic region of the ^1H NMR spectra featured signal patterns ranging from 6.5 to 8.0 ppm, which were characteristic of the substitution pattern of each aromatic ring. The structures of all the target compounds were confirmed using ^{13}C NMR, DEPT-135°, COZY, and HETCOR. The spectroscopic and physicochemical characterizations of compounds **10**–**15** have been previously reported.[16]

Prediction of the ADME/Tox properties of derivatives **3**–**18**

Drug-likeness descriptors selected using the Lipinski and Veber rules were calculated with *SwissADME*.[17] The rule of



Scheme 1. Synthesis of compounds **3**–**6**. Reagents and conditions. **i**: DMAP, dry DMF, 80 °C, 12 h.



Scheme 2. Synthesis of compounds **7**–**18**.
R′. **7** = 4-OMe, **8** = 3,4-di(OMe), **9** = 3,4,5-tri(OMe), **10** = 4-OMe, **11** = 2,4-di(OMe), **12** = 3,4,5-tri(OMe), **13** = 4-OMe, **14** = 3,4-OMe, **15** = 3,4,5-tri(OMe), **16** = 2,4,5-tri(OMe), **17** = 3,4,5-tri(OMe), **18** = 4-CF_3.

five by Lipinski argues that good absorption or permeation is more likely when LogP is < 5, the molecular weight (MW) is < 500 g mol^{-1}, the number of hydrogen bond donors (nHbds) is < 5, and the number of hydrogen bond acceptors (nHbas) is < 10.[18] These results obtained with products **3**–**18** are summarized in Table 1. In general, according to these criteria, compounds **3**–**18** did not violate these rules with the exception of compound **18**. However, the LogSw values for our compounds were predicted to range from −3.26 to −6.46. On the *SwissADME* LogSw scale, compounds with values less than −6 are considered to be poorly soluble, which may compromise the absorption of compounds **9**, **11**, and **18** in in vivo models.[19] Another important descriptor, the topological polar surface area (TPSA) < 140 Å^2, was determined,[20] compounds **3**–**6** with polar surface areas < 140 Å^2 were exceptions and relate to the value presented by CQ of 138 Å^2, suggesting that compounds **7**–**18** would display poor absorption or permeation.

To complement our in silico evaluation, we calculated other molecular descriptors, such as the percentages of compounds **3**–**18** that would be absorbed through the human intestine (%HIA), using the web tool *pkCSM-pharmacokinetics*.[21] The analyses indicated values ranging from 90.41% to 95.00%, and were considered appropriate compared with CQ 89.70%. The descriptors P-glycoprotein

Table 1. In silico evaluation of the physicochemical properties of compounds **3–18**.

No.	Log P	MW	Hb_a	Hb_d	Rot_b	Viol	LogSw	%HIA	P-gp	BBB Per	FU	CLtot	LD_{50}
3	3.03	253.75	3	1	3	0	−3.26	93.31	No	0.211	0.22	0.379	2.732
4	2.97	239.72	3	1	3	0	−3.37	93.18	No	0.280	0.23	0.460	2.372
5	3.05	253.75	4	1	3	0	−3.35	92.82	Yes	0.250	0.19	0.327	2.384
6	3.75	267.77	3	1	5	0	−3.80	92.23	No	0.222	0.15	0.231	2.396
7	4.70	387.88	4	0	7	0	−5.56	94.07	No	−0.04	0	0.237	2.356
8	4.65	417.91	6	0	7	0	−5.63	93.78	No	0.03	0	0.435	2.439
9	4.09	463.97	6	0	9	0	−6.20	94.73	No	−0.38	0	0.632	2.637
10	4.43	373.86	5	0	6	0	−5.61	94.58	No	−0.083	0	0.289	2.348
11	4.36	403.88	6	0	7	0	−6.25	94.30	No	−0.013	0	0.487	2.446
12	4.36	433.91	7	0	8	0	−5.36	95.28	No	−0.418	0	0.684	2.654
13	4.84	387.88	5	0	7	0	−5.84	94.28	No	−0.028	0	0.335	2.368
14	4.70	417.91	6	0	8	0	−5.52	93.54	No	0.019	0.02	0.553	2.504
15	4.68	447.94	7	0	9	0	−5.60	95.00	No	−0.364	0	0.730	2.611
16	5.01	461.96	7	0	11	0	−5.83	94.64	No	0.282	0.27	0.726	2.691
17	4.97	461.96	7	0	10	0	−5.83	94.39	No	−0.410	0	0.641	2.537
18	6.08	439.88	4	0	9	1	−6.46	90.41	No	0.26	0	0.138	2.638
CQ	4.15	319.87	3	1	8	0	−4.55	89.70	Yes	0.383	0.21	0.156	2.833

Log P, partition coefficient. MW, molecular weight. Hb_a, hydrogen bond acceptors. Hb_d, hydrogen bond donors. Rot_b, rotatable bonds. Viol, Lipinski's violations. LogSw: water solubility. %HIA, human intestinal absorption. P-gp, P-glycoprotein. BBB Per, blood–brain barrier permeability. FU, fraction unbound. CLtot, total clearance. LD_{50}, rat acute oral toxicity. TPSA: topological polar surface area > 140 Å^2 for 7–18, but not compounds 3–6 and **CQ**.

(P-gp) substrate, blood–brain barrier (BBB) permeability, and fraction unbound (FU) were used to predict the distribution.[22,23] Only compound **5** was predicted to be a substrate for P-gp, while the BBB permeability values of our compounds were predicted to range from −0.41 to 0.282. The fraction unbound influences renal glomerular filtration and hepatic metabolism and consequently affects the volume of distribution, efficacy, and total clearance of drugs. For the most active compounds in vivo, the FU values are in the order of 0, which indicates that their elimination via the kidneys could be difficult when compared to CQ with a value of 0.21. The hepatotoxicity and oral rat acute toxicity LD_{50} values of compounds **3**–**18** were also predicted,[24] and like CQ, compounds **5**, **9**, **12**, and **15** in vivo could be hepatotoxic.

Biological activity

To identify the potential of the 4-sulfanylquinoline derivatives **3**–**18** as antimalarial agents, the 16 derivatives were tested in vitro as inhibitors of β-hematin formation (βHF) and in vivo in a murine model, and the results are presented in Table 2. All compounds significantly reduced heme crystallization to a half maximal inhibitory concentration of less than 10 μM ($IC_{50} < 10$ μM). Compounds **5**, **9**, **12**, and **15** were found to inhibit heme crystallization with IC_{50} values of 5.23 ± 0.87, 5.17 ± 1.14, 5.83 ± 1.75, and 4.73 ± 1.29 μM, respectively, compared to the IC_{50} value of CQ (0.18 ± 0.03 μM).

This result motivated us to evaluate all compounds in vivo in mice infected with *P. berghei* ANKA, a CQ-susceptible strain of murine malaria. The antimalarial potential of these compounds was determined by the ability of compounds **3**–**18** to increase mouse survival and reduce parasitemia in vivo, as assessed on the fourth day post-infection compared to the untreated control group. Mice were treated intraperitoneally (*ip*) once daily with compounds **3**–**18** (25 mg kg^{-1}) or CQ (25 mg kg^{-1}) following previously reported protocols.[25–28]

Structures **5**, **9**, **12**, and **15** used as monotherapies extended the average survival time of the infected mice to 17.1 ± 1.53, 21.3 ± 1.72, 23.4 ± 2.17, and 25.1 ± 1.07 days, respectively; however, they were not able to decrease or delay the evolution of malaria. CQ prolonged the mouse survival time to 30 days and decreased the development of malaria to 1.40 ± 0.28%. The hemolytic response of compounds **5**, **9**, **12**, and **15** was further determined.[29] Hemolysis was less than 5% in mouse red globules at a concentration of 1 mM, which shows that these compounds do not have a marked lytic action on the RBCs of mice (Table 2). All compounds exhibited activity as βHF inhibitors; however, compounds with a pattern of substitution 3,4,5-OMe had better IC_{50} values than the doubly substituted with OMe groups or mono-OMe substituted compounds. Taking the predictive values obtained in silico as a reference, the marginal activity observed in vivo led us to infer that these compounds may have poor solubility in water, a moderate partition coefficient, a low unbound fraction, strong inhibition of the main cytochromes (CYPs) of the P450 superfamily, and hepatotoxicity at the dose administered to each group of mice.

Conclusion

In summary, we have developed an efficient method to optimize the synthesis of a series of [(7-chloroquinolin-4-yl)sulfanyl]alcohol derivatives, as interesting intermediates for the preparation of (7-chloroquinolin-4-ylthio) alkylbenzoate derivatives **7**–**18** . The reactions were carried out under mild reaction conditions in DMF at 80 °C in the presence of DMAP. The yields of intermediates **3**–**6** were above 70%. In vitro, all compounds significantly reduced heme crystallization with IC_{50} values of < 10 μM; however,

Table 2. The half maximal inhibitory concentrations (IC_{50} values) of the 7-chloroquinoline derivatives **3**–**18** to inhibit the formation of β-hematin (βHF) and the effects on *P. berghei*-infected mice (25 mg kg^{-1}).

No.	IC_{50}[a] (μM)	(%) Hemolysis	Sd[b] (± SEM)[d]	%P[c] (± SEM)[d]	Survival[e]
3	9.17 ± 1.08	ND	15.3 ± 2.19	15.5 ± 2.37	0/6
4	8.14 ± 1.21	ND	13.7 ± 1.61	16.2 ± 1.27	0/6
5	5.23 ± 0.87†	2.41 ± 0.32	17.1 ± 1.53*	10.9 ± 2.48	0/6
6	9.83 ± 0.36	ND	12.9 ± 0.75	16.0 ± 2.21	0/6
7	9.13 ± 0.93	ND	12.2 ± 2.15	13.7 ± 0.81	0/6
8	8.13 ± 2.60	ND	15.3 ± 2.03	10.1 ± 1.32	0/6
9	5.17 ± 1.14†	2.39 ± 0.08	21.3 ± 1.72*	7.18 ± 1.20**	0/6
10	8.26 ± 0.94	ND	13.6 ± 0.25	14.1 ± 2.50	0/6
11	7.21 ± 1.17	ND	16.9 ± 0.95	11.7 ± 0.42	0/6
12	5.83 ± 1.75†	3.87 ± 0.19	23.4 ± 2.17*	6.1 ± 1.45**	0/6
13	9.07 ± 2.01	ND	14.2 ± 1.48	16.4 ± 1.43	0/6
14	6.29 ± 0.44	ND	19.3 ± 2.62	9.8 ± 2.4	0/6
15	4.73 ± 1.29†	2.73 ± 0.61	25.1 ± 1.07*	4.29 ± 0.25**	0/6
16	8.97 ± 1.40	ND	14.3 ± 2.17	13.1 ± 1.72	0/6
17	7.13 ± 1.22	ND	16.2 ± 1.41	9.27 ± 1.12	0/6
18	9.10 ± 1.80	ND	12.5 ± 0.93	13.2 ± 1.11	0/6
CQ	0.18 ± 0.03	ND	30	1.40 ± 0.28	5/6
CiSS	–	–	7.21 ± 1.37	22.3 ± 0.87	0/6

[a]IC_{50}: Half maximal inhibitory concentration (βHF) ($n = 3$). [b]Sd: Survival days. [c]%P: Percentage of parasitemia. [d]SEM: Standard error of the mean. [e]Number of mice that survived until Day 30 post-infection/total number of mice in the group. CQ: Chloroquine. CiSS: Control infected and treated with saline solution. ND: not determined. †$p < 0.001$ compared to chloroquine. *$p < 0.01$, **$p < 0.001$ compared to CiSS. $n = 6$.

in vivo, the reduction in parasitemia and survival time increase were marginal, with only four compounds, **5**, **9**, **12**, and **15**, giving survival times of 17.1 ± 1.53, 21.3 ± 1.72, 23.4 ± 2.17, and 25.1 ± 1.07 days, respectively. The ADME/Tox analysis predictions allowed us to understand the marginal or low activity of our compounds in vivo.

Experimental section

Chemicals

All chemicals and solvents were purchased from different chemical suppliers and were used without further purification unless stated otherwise. For analytical thin-layer chromatography (TLC), pre-coated aluminum sheets (Silica Gel 60 F254, Merck)™ were used, and spots were observed under UV light (254 nm). Column chromatography was performed on Merck Silica Gel 60 (40–63) μm as a stationary phase. Melting points were measured in open capillary tubes using a Thomas Hoover™ apparatus and are uncorrected. IR spectra were determined as KBr pellets on a Shimadzu™ model 470 spectrophotometer and are expressed in cm^{-1}. The ^1H and ^{13}C NMR spectra were recorded on a JEOL Eclipse™ 270 (270 /67.9 MHz) spectrometer using $CDCl_3$ or DMSO-d_6 as the solvent, and are reported in ppm downfield from the residual $CHCl_3$ or DMSO (δ 7.25 or 2.50 for ^1H NMR and 77.0 or 39.8 for ^{13}C NMR, respectively). Signal multiplicities are as follows: singlet (s), doublet (d), doublet of doublets (dd), multiplet (m), quartet (q); coupling constants (*J*) are given in Hz. A Perkin-Elmer™ 2400 CHN elemental analyzer was used to obtain the elemental analyses, and the results were within ± 0.4% of the predicted values.

General procedure for the synthesis of compounds 3–6

To a solution of 4,7-dichloroquinoline (**1**) (5.0 g 25 mmol) in dry DMF (50 mL) was added dropwise mercapto alcohol **2a**–**d** (30 mmol) and DMAP in dry DMF (2.0 mL, 37.5 mmol). The mixture was stirred at 80 °C for 12 h under an N_2 atmosphere and then allowed to cool to room temperature. The solvent was evaporated under reduced pressure. To the resulting solid was added ethyl acetate (150 mL), and the organic layer was subsequently washed with water (100 mL), 10% sodium bicarbonate solution (2 × 20 mL), and saturated NaCl solution (50 mL). Anhydrous sodium sulfate was finally added to the organic layer, which was then filtered and evaporated under reduced pressure. The residues were then purified by recrystallization or column chromatography.

(R,S)-1-(7-Chloroquinolin-4-ylthio)propan-2-ol (**3**): Yellow solid, yield: 78% from ethanol; m.p. 125–127 °C. IR (KBr) cm^{-1}: 3344, 2979, 1601. ^1H NMR (DMSO-d_6, 270 MHz): δ 1.32 (3H, d, $J = 5.9$ Hz, H11), 3.39–3.53 (2H, m, H9), 4.07 (1H, m, H10), 5.10 (1H, br s, OH), 7.87 (1H, d, $J = 5.9$ Hz, H3), 7.91 (1H, dd, $J = 2.2$, 9.2 Hz, H6), 8.35–8.38 (2H, m, H5,8), 8.99 (1H, d, $J = 5.9$ Hz, H2). ^{13}C NMR (DMSO-d_6 67.9 MHz): δ 23.5 (C11), 45.8 (C9), 65.0 (C10), 116.9 (C3), 122.2 (C5), 124.6, 126.7 (C8), 130.0 (C6), 138.7, 144.5 (C2), 160.9. Anal. Calcd for $C_{12}H_{12}ClNOS$: C, 56.80; H, 4.77; N 5.52. Found: C, 56.83; H, 4.76; N, 5.69.

2-[(7-Chloroquinolin-4-yl)sulfanyl]ethanol (**4**): Column chromatography DCM: EtOAc:MeOH (7:2:1). White solid, yield: 73%. The data are identical in all respect (m.p., IR, ^1H NMR) with an authentic specimen of **4**.[16]

3-[(7-Chloroquinolin-4-yl)sulfanyl]propan-1-ol (**5**): Column chromatography DCM: EtOAc:MeOH (7:2.5:0.5). Yellow solid, yield: 86%. The data are identical in all respect (m.p., IR, ^{1}H NMR) with an authentic specimen of **5**.[16]

4-(7-Chloroquinolin-4-ylthio)butan-1-ol (**6**): Column chromatography DCM: EtAc:MeOH (7:2.5:0.5). White solid; yield: 82%; m.p. 127–128 °C. IR (KBr) cm^{-1}: 3227, 2930, 1600. ^{1}H NMR (DMSO d_6, 270 MHz): δ 1.77–1.82 (2H, m, H10 or 11), 1.88–1.96 (2H, m, H10 or 11), 3.15 (2H, t, J=7.1 Hz, H9), 3.74 (2H, t, J=6.2 Hz, H12), 7.18 (1H, d, J=4.9 Hz, H3), 7.49 (1H, dd, J=2.1, 9.1 Hz, H6), 8.05 (1H, s, H8), 8.07 (1H, d, J=6.1 Hz, H5), 8.69 (1H, d, J=4.8 Hz, H2). ^{13}C NMR (DMSO-d_6 67.9 MHz): δ 24.9 (C10 or 11), 31.2 (C11 or 10), 31.9 (C9), 62.3 (C12), 116.1 (C3), 125.2 (C5), 127.4 (C8), 128.8 (C6), 135.8, 148.1, 148.3, 150.3 (C2). Anal. Calcd for $C_{13}H_{14}ClNOS$: C, 58.31; H, 5.27; N, 5.23. Found: C, 58.35; H, 5.27; N, 5.43.

General procedure for the synthesis of compounds **7–18**

A solution of the selected benzoic acid derivative (1.2 mmol) in dry DCM (15 mL) was treated with EDCI (1.5 mmol) and DMAP (0.4 mmol). The mixture was left shaking at −10 °C for 30 min. The respective intermediates **3–6** (0.65 mmol) dissolved in dry DCM (1 mL) were slowly added, and the resulting mixture was left stirring for 24 h at room temperature under a N_2 atmosphere. Next, water was added and the aqueous fraction was extracted with DCM (2 × 10 mL). The organic layer was washed with 10% sodium bicarbonate (2 × 10 mL), saturated NaCl solution (3 × 10 mL), and finally dried over Na_2SO_4, filtered, and evaporated under reduced pressure to give the crude product. The compounds were then purified by recrystallization or column chromatography. Compounds **10**–**15** have been reported earlier, and their spectral data matched with those presented in the literature.[16]

(*R, S*)-*1-(7-Chloroquinolin-4-ylthio)propan-2-yl 4-methoxybenzoate* (**7**): White solid, yield: 60% from ethanol; m.p. 144–146 °C. IR (KBr) cm^{-1}: 3040, 1730, 1200. ^{1}H NMR ($CDCl_3$, 270 MHz): δ 1.56 (3H, d, J=5.5 Hz, CH_3), 3.32 (1H, dd, J=6.7, 13.6 Hz, H9a), 3.61 (1H, dd, J=5.7, 13.6 Hz, H9b), 3.85 (3H, s, OCH_3), 5.41 (1H, m, H10), 6.86 (2H, d, J=6.9 Hz, H3′,5′), 7.55 (1H, dd, J=1.9, 8.9 Hz, H6), 7.63 (1H, d, J=5.2 Hz, H3), 7.90 (2H, d, J=6.9 Hz, H2′,6′), 8.11 (1H, d, J=8.9 Hz, H5), 8.30 (1H, d, J=1.9 Hz, H8), 8.71 (1H, d, J=5.2 Hz, H2). ^{13}C NMR ($CDCl_3$, 67.9 MHz): δ 19.5 (C11), 37.0 (C9), 55.5 (OCH_3), 68.7 (C10), 113.8 (C3′,5′), 116.6 (C3), 122.1, 125.0 (C5), 126.3 (C8), 128.7 (C6), 131.7 (C2′,6′), 138.0, 143.9, 146.9 (C2), 152.5, 163.9, 165.7 (C12). Anal. Calcd for $C_{20}H_{18}ClNO_3S$: C, 61.93; H, 4.68; N, 3.61. Found: C, 61.95; H, 4.69; N, 3.87.

(*R, S*)-*1-(7-Chloroquinolin-4-ylthio)propan-2-yl 3,4-dimethoxybenzoate* (**8**): White solid, yield: 65% from ethanol; m.p. 105 °C. IR (KBr) cm^{-1}: 3050, 1733, 1240. ^{1}H NMR ($CDCl_3$, 270 MHz): δ 1.56 (3H, d, J=6.4 Hz, H11), 3.29 (1H, dd, J=6.9, 13.6 Hz, H9a), 3.60 (1H, dd, J=5.4, 13.6 Hz, H9b), 3.89 (3H, s, OCH_3), 3.92 (3H, s, OCH_3), 5.41 (1H, m, H10), 6.84 (1H, d, J=8.4 Hz, H5′), 7.46 (1H, d, J=1.7 Hz, H2′), 7.56 (1H, dd, J=1.9, 8.4 Hz, H6), 7.55–7.59 (1H, m, H3,6’), 8.09 (1H, d, J=8.9 Hz, H5), 8.21 (1H, d, J=1.9 Hz, H8), 8.72 (1H, d, J=4.4 Hz, H2). ^{13}C NMR ($CDCl_3$, 67.9 MHz): δ 19.5 (C11), 37.0(C9), 56.1 (OCH_3), 69.0 (C10), 110.5, 112.4, 116.9 (C3), 122.3, 123.8 (C6), 125.2 (C5), 127.3, 128.3 (C8), 137.2, 145.5, 148.1 (C2), 149.0, 150.5, 153.6, 165.8 (C12). Anal. Calcd for $C_{21}H_{20}ClNO_4S$: C, 60.35; H, 4.82; N, 3.35. Found: C, 60.35; H, 4.84; N, 3.63.

(*R, S*)-*1-(7-Chloroquinolin-4-ylthio)propan-2-yl 3,4,5-trimethoxybenzoate* (**9**): White solid, yield: 67% from ethanol; m.p. 130–132 °C. IR (KBr) cm^{-1}: 3067, 1765, 1243. ^{1}H NMR ($CDCl_3$, 270 MHz): δ 1.56 (3H, d, J=6.2 Hz. H11), 3.27 (1H, dd, J=6.7, 13.58 Hz, H9a), 3.56 (1H, dd, J=5.7, 13.6 Hz, H9b), 3.85 (3H, s, 2 × OCH_3), 3.89 (3H, s, OCH_3), 5.41 (1H, m, H10), 7.20 (1H, s, H2′,6′), 7.45–7.50 (1H, m, H6,3), 8.06 (1H, d, J=1.9 Hz, H8), 8.07 (1H, d, J=8.9 Hz, H5), 8.72 (1H, br s H2). ^{13}C NMR ($CDCl_3$, 67.9 MHz): δ 19.5 (C11), 36.8 (C9), 56.4 (O CH_3), 60.9 (O CH_3), 69.5 (C10), 107.3 (C2′,6′), 117.3 (C3), 124.8, 125.1 (C5), 127.6 (C6 or 8), 128.6 (C6 or 8), 136.2, 143.0, 147.6, 149.9 (C2), 153.1, 165.6 (C12). Anal. Calcd for $C_{22}H_{22}ClNO_5S$: C, 58.99; H, 4.95; N, 3.13. Found: C, 59.02; H, 4.97; N, 3.29.

4-[(7-Chloroquinolin-4-yl)thio]butyl 2,4,5-trimethoxybenzoate (**16**): Column chromatography: DCM: EtAc (9:1). Cream solid; yield: 87%; m.p. 108–110 °C. IR (KBr) cm^{-1}: 3083, 2959, 1669, 1243. ^{1}H NMR ($CDCl_3$, 270 MHz): δ 1.84–1.90 (4H, m, H10,11), 3.04 (2H, t, J=6.5 Hz, H9), 3.72 (6H, s, 2 × OCH_3), 3.81 (3H, s, OCH_3), 4.26 (2H, t, J=7.5 Hz, H12), 6.39 (1H, s, H2′), 7.00 (1H, d, J=4.9 Hz, H3), 7.28 (1H, s, H6′), 7.32 (1H, dd, J=2.1, 9.2 Hz, H6), 7.88 (1H, d, J=9.2 Hz, H5), 7.88 (1H, d, J=2.2 Hz, H8), 8.51 (1H, d, J=4.9 Hz, H2). ^{13}C NMR ($CDCl_3$, 67.9 MHz): δ 25.2 (C10 or 11), 28.2 (C10 or 11), 30.9 (C9), 56.2 (OCH_3), 56.6 (OCH_3), 57.1 (OCH_3), 63.9 (C12), 97.8 (C2’), 110.7, 114.7 (C6′), 116.1 (C3), 125.1 (C5), 127.3 (C6), 129.0 (C8), 135.8, 142.7, 148.0, 148.1, 150.3 (C2), 153.9, 155.8, 165.9 (C13). Anal. Calcd for $C_{23}H_{24}ClNO_5S$: C, 59.80; H, 5.24; N, 3.03. Found C, 59.81; H, 5.26; N, 3.21.

4-[(7-Chloroquinolin-4-yl)thio]butyl 3,4,5-trimethoxybenzoate (**17**): Column chromatography: DCM: EtAc (8:2). White solid; yield: 85%; m.p. 92 °C. IR (KBr) cm^{-1}: 3030, 2941, 1703, 1215. ^{1}H NMR ($CDCl_3$, 270 MHz): δ 1.92–2.01 (4H, m, H10,11), 3.15 (2H, t, J=6.6 Hz, H9), 3.85 (6H, s, 2 × OCH_3), 3.88 (3H, s, OCH_3), 4.37 (2H, t, J=5.5 Hz, H12), 7.11 (1H, d, J=4.8 Hz, H3), 7.25 (2H, s, H2′,6′), 7.44 (1H, dd, J=1.9, 9.0 Hz, H6), 7.99–8.01 (2H, m, H5,8), 8.63 (1H, d, J=4.8 Hz, H2). ^{13}C NMR ($CDCl_3$, 67.9 MHz): δ 24.9 (C10), 28.1 (C11), 30.8 (C9), 56.3 (2 × OCH_3), 61.0 OCH_3), 64.3 (C12), 106.9 (C2′,6′), 116.0 (C3), 125.0 (C5), 125.1, 127.3 (C6), 128.9 (C8), 135.7, 142.4, 147.7, 148.0, 150.2 (C2), 153.0, 166.2 (C13). Anal. Calcd for $C_{23}H_{24}ClNO_5S$: C, 59.80; H, 5.24; N, 3.03. Found: C, 59.87; H, 5.23; N, 3.17.

4-[(7-Chloroquinolin-4-yl)thio]butyl 4-(trifluoromethyl)benzoate (**18**): Column chromatography: DCM: EtAc

(8:2). White solid; yield: 99%; m.p. 122–124 °C. IR (KBr) cm^{-1}: 3056, 2932, 1689, 1226. ^1H NMR ($CDCl_3$, 270 MHz): δ 1.87–2.03 (4H, m, H10,11), 3.10 (2H, t, J=6.8 Hz, H9), 4.38 (2H, t, J=5.9 Hz, H12), 7.06 (1H, d, J=4.8 Hz, H3), 7.39 (1H, dd, J=2.1, 8.9 Hz, H6), 7.62 (2H, d, J=8.3 Hz, H3′,5′), 7.95 (1H, d, J=8.9 Hz, H5), 7.97 (1H, d, J=2.1 Hz, H8), 8.05 (2H, d, J=8.1 Hz, H2′,6′), 8.60 (1H, d, J=4.8 Hz, H2). ^{13}C NMR ($CDCl_3$, 67.9 MHz): δ 24.8 (C10), 27.9 (C11), 30.7 (C9), 64.6 (C12), 116.0 (C3), 124.9 (C5), 125.3 (J=19.65 Hz), 127.1 (C8), 128.9 (C2′ or 6′), 129.9 (C2′or 6′), 133.3 (J=4.29 Hz), 134.4 (J=133.23 Hz), 135.6, 147.6, 148.0, 150.2 (C2), 165.2 (C13). Anal. Calcd for $C_{21}H_{17}ClF_3NO_2S$: C, 57.34; H, 3.90; N, 3.18. Found: C, 57.32; H, 3.93; N, 3.39.

Estimation of the ADME/Tox profile

A computational study of compounds **3**–**18** was performed to predict the ADME/Tox properties using the *SwissADME* program, a free platform available online through the site (http://www.swissadme.ch/).[17] Another free online access tool used in this study was the *pkCSM-pharmacokinetics* web tool (http://structure.bioc.cam.ac.uk/pkcsm), which is a method used for predicting and optimizing the ADME/Tox properties of small molecules.[21]

Biological evaluation

Inhibition of β-hematin formation. The assay was performed according to a previously described protocol.[25,28] Hemin chloride solution (50 µL, 4 mM) dissolved in dimethyl sulfoxide (DMSO) (5.2 mg mL^{-1}) was distributed in 96-well microplates. The compounds were dissolved in DMSO, and different concentrations (100–5 mM) were added to the test wells (50 µL). Water (50 µL) and DMSO (50 µL) were used as controls. The experiments were performed in triplicate. Acetate buffer (100 µL, 0.2 M, pH 4.4) was used to generate β-H. The plates were incubated at 37 °C for 48 h and centrifuged (4000 RPM × 15 min, IEC-CENTRA, MP4R). The supernatant was discarded, and the pellet was washed twice with DMSO (200 µL) and dissolved in NaOH (200 µL, 0.2 N). The aggregates were further solubilized with NaOH (0.1 N), and their absorbance values were recorded at 405 nm (BIORAD-550 microplate reader). The results are expressed as the percent inhibition of β-H formation.

Parasite, experimental host, and strain maintenance. The protocol for the mouse model was followed as previously described.[19,26] Male BALB/c mice weighing 18–22 g were maintained on a commercial pellet diet and handled according to local and national regulations, and the research protocols were approved by the Institute of Biomedicine Committee on Animal Research. A rodent malaria ANKA strain of *P. berghei* was used to infect the animals. Mice were infected by *ip* injection with 1×10^6 infected erythrocytes diluted in phosphate-buffered saline (PBS; 10 mM, pH 7.4, 0.1 mL). Parasitemia was monitored by microscopic examination of Giemsa-stained smears.

4-day suppressive test. The percentage of parasitemia and the survival times of the mice infected with *P. berghei* and treated were determined following a protocol previously described.[13,27] Caudal vein *i.v.* infection of BALB/c mice (18–23 g) was performed with 10^6 *P. berghei-infected* red blood cells (n=6). However, 2 h after infection, treatment began with the active compounds from the in vitro test (inhibition of β-H formation). The compounds were dissolved in DMSO (0.1 M) and subsequently diluted with a saline-Tween 20 solution (2%). Each compound (dose 25 mg kg^{-1}) was administered *ip* for 4 days. On Day 4, the parasite load was assessed by examining Giemsa-stained smears. CQ (25 mg kg^{-1}) was used as a positive control. The survival time of the mice infected with *P. berghei* and treated with saline solution was used as a base control. The results are expressed as the percentage of parasitemia, and the survival curve was based on the number of days of mouse survival after treatment with compounds over the survival of infected but not treated.

In vitro toxicity on mouse red blood cells. To evaluate the in vitro toxicological effects of the compounds, we used a model based on the lysis of red blood cells (RBCs), measuring the hemoglobin released into the supernatant fraction.[29] The hemoglobin released was measured using a spectrophotometer at 550 nm. Mouse blood was centrifuged at 800 g for 10 min and then washed three times with saline solution to obtain RBCs at a value of 100%. The synthesized compounds (1 mM) were incubated with a 2% final suspension of RBCs at 37 °C for 45 min. The release of hemoglobin by hypotonic lysis in 1% saponin by an equal number of RBCs was used as a 100% positive control, while RBCs treated with saline solution served as negative controls. The results are expressed as the concentration at which half of the RBCs were lysed ($LyticC_{50}$).

Statistical analysis

Statistical analysis was performed using GraphPad Prism version 5.3. The difference was considered significant when the p-value was $\leqslant 0.05$ (GraphPad Prism Software Inc., 1992–2004).

Declaration of conflicting interests

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