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Original article

4-Aminoquinoline derived antimalarials: Synthesis, antiplasmodial activity and heme polymerization inhibition studies

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ABSTRACT

A new series of 4-aminoquinoline derivatives have been synthesized and found to be active against both susceptible and resistant strains of *Plasmodium falciparum in vitro*. Compound 1-[3-(7-chloro-quinolin-4-ylamino)-propyl]-3-cyclopropyl-thiourea (**7**) exhibited superior *in vitro* activity against resistant strains of *P. falciparum* as compared to chloroquine (CQ). All the compounds showed resistance factor between 0.59 and 4.31 as against 5.05 for CQ. Spectroscopic studies suggested that this class of compounds act on heme polymerization target.

1. Introduction

Malaria remains one of the most widespread diseases in the world, afflicting a large population living in the tropical and subtropical areas. High incidences of malaria coupled with emergence of multidrug-resistant *Plasmodium falciparum* have lent renewed impetus to the search for new antimalarial agents. Historically 4-aminoquinoline based entities (Fig. 1), particularly chloroquine (CQ), have remained the first choice in the malaria chemotherapy and the staying power of CQ may be attributed to its affordability and *Sui-generis* target [1,2]. It is generally inferred from the biochemical data that this class of compounds enter the food vacuole and inhibit the parasite growth by forming a complex with hematin thereby inhibiting the hemozoin formation [3–7]. However, over the years, extensive use of CQ has led to the emergence of drug resistant *P. falciparum*, which has to a large extent limited the number of drugs available for effective use against malaria [8]. However seminal work by Ridley et al. have demonstrated that 4-aminoquinoline derivatives with altered chain length (Fig. 2, a) are active against CQ-resistant strains of *P. falciparum*, indicating that the resistance mechanism is compound specific and not class specific [9–11]. Based on these annotations, we surmised that rational modifications at the pendant nitrogen of the CQ lateral side chain would lead to compounds with improved activity, particularly against CQ-resistant strains. Towards this objective two modifications were explored from this laboratory namely, introduction of guanidine and tetramethylguandine (Fig. 2, b), which would accentuate basicity at the lateral side chain of nitrogen [12]. Secondly, thiazolidin-4-one functionality was introduced (Fig. 2, c) that led to compounds with promising antimalarial activity [13], these results attest to a validation of the above concept and its utility in lead optimization (Fig. 2). Encouraged by these findings we thought that modification of the terminal side chain with thiourea derivatives could modulate the antimalarial activity (Fig. 2, d). It may be appropriate to mention here that thiourea group is a privileged pharmacophore found in many biologically active compounds and acts as an electrophilic warhead [14–16]. In recent years, thioureas have proven to be useful in the design of enzyme inhibitors [17] as replacement for the amide (–CO–NH–) bond in peptidomimetics [18] and as sources of self-complementary bi-directional hydrogen bonding motif in supramolecular chemistry [19]. Based on this information some research groups have introduced aryl substituted thiourea functionality on lateral side chain of 4-aminoquinoline ring system with antiplasmodial activity [20,21]. Mahajan et al. have reported a series of 7-chloroquinolinyl thiourea derivatives having thiourea in place of diethyl amino functionality and found that some of these analogs are active against CQ sensitive strains [22].

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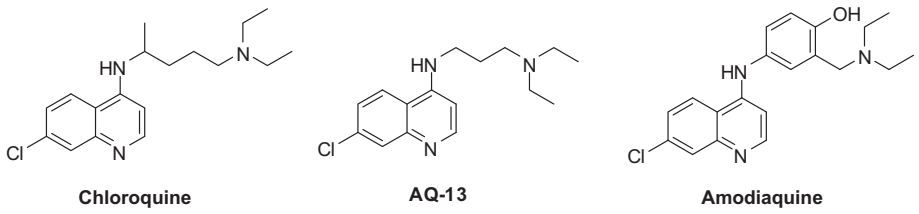


Fig. 1. Structures of 4-aminoquinoline antimalarials.

These findings have given impetus to our antimalarial drug research by further augmenting the realization that rational choice of inputs based on known 4-aminoquinoline scaffold could lead to molecules with desirable antiplasmodial activity. Therefore, we designed and synthesized new molecules to further optimize 4-aminoquinoline derived antiplasmodial agents, in which we selectively modified lateral side chain of diethyl amino functionality (Fig. 2 and Scheme 1) with a variety of heterocyclic ring substituted thiourea functionality including pyrrolidinyl, piperidyl, morpholinyl, piperazinyl and substituted piperazinyl. We have found some of them to be very promising as they inhibit parasite growth more effectively than CQ. The results are described in the present communication.

2. Chemistry

The synthesis of desired *N*-(7-chloro-quinolin-4-yl)propane-1,3-diamine (**1**) was carried out by the procedure reported earlier from this laboratory [12]. Various [3-(7-chloro-quinolin-4-ylamino)-propyl]-alkyl/aryl/heterocyclic substituted thiourea (**3**–**15**) have been synthesized in two steps. The first step comprises formation of [3-(7-chloro-quinolin-4-ylamino)-propyl]-dithiocarbamic acid methyl ester (**2**) by a one pot reaction of *N*-(7-chloro-quinolin-4-yl) propane-1,3-diamine and carbon disulfide in presence of alkali, in dimethyl sulphoxide followed by methylation with dimethyl sulfate. The product, thus obtained, was further subjected to nucleophilic substitution reaction with a variety of amines (primary, secondary and cyclic) to obtain the desired compounds (**3**–**15**) in good yields (Scheme 1). ^{1}H NMR, ^{13}C NMR, Mass and IR spectral data confirmed the chemical structures of synthesized compounds and the purity was ascertained by elemental analysis.

3. Results and discussion

All the compounds having modifications at the lateral amino group of the side chain were evaluated for their antimalarial activity against the D6-chloroquine sensitive (CQ-S) and Dd2-chloroquine resistant (CQ-R) strains of *P. falciparum* according to the procedure reported by Smilkstein et al. [23]. The IC_{50} values were calculated from experiments carried out in triplicate and the results are presented in Table 1. The in vitro activity data clearly suggest that the substituted thiourea derivatives in place of dialkyl amino moiety resulted in retention of antiplasmodial activity. The derivatives, having alkyl, aryl and hetero aryl substitution at the lateral side chain, show significant antimalarial activity. Among the thirteen compounds tested, three compounds (**7**, **12** and **13**) showed IC_{50} in the range between 23.9, 59.1 and 28.7 nM, respectively and rest of the ten compounds gave IC_{50} ranging between 103.4 and 332.9 nM, against CQ-S strain of *P. falciparum*. In the case of CQ-R strain of *P. falciparum* compounds **7**, **12** and **13** exhibited IC_{50} values 14.1, 68.4 and 50.5 nM, respectively. Remaining compounds exhibited IC_{50} values ranging between 103.7 and 778.0 nM.

The compounds obtained on replacement of SCH_3 group of **2** with alkyl amines viz. methyl amino (**3**) diethyl amino (**5**) and diisopropyl amino group (**6**) exhibited moderate antimalarial activity against both CQ-S and CQ-R strains of *P. falciparum*. In the case of cyclopropyl amino substituted compound (**7**), the activity increased as reflected in the IC_{50} value of 23.9 and 14.1 nM against CQ-S and CQ-R strains of *P. falciparum*, respectively. It is important to note that compound **7** exhibited superior activity against CQ-resistant strain as compared to CQ-S strain of parasite. Substitutions with alicyclic amino functionality namely, compound **8**–**10** led to reduction in the antimalarial activity against CQ-S and CQ-R strains of *P. falciparum.* Among all the compounds reported in the present study the piperidinyl substituted compound (**9**) was found to be better as compared to pyrrolidinyl (**8**) and morpholinyl (**10**) substituted compound, due to their lipophilic nature. In the case of 4-methyl (**12**, Log $P = 3.67$) and 4-phenyl (**13**, Log $P = 4.64$) piperazinyl substituted compound there is increased antimalarial activity 3–6 fold, respectively against CQ-S and CQ-R strains of *P. falciparum* in comparison to unsubstituted piperazinyl compound (**11**, Log $P = 2.12$). It clearly demonstrates the importance of lipophilicity for the antiplasmodial activity. It may be appropriate to mention here that resistance factor which is calculated as a ratio of IC_{50} in CQ-R vs CQ-S strains has been used as an index to assess chances of parasite developing resistance to a particular class of compounds. Accordingly, it is believed that smaller the resistance factor, the less likely is the chance of developing resistance to that class of compounds. Interestingly, all the compounds in this series showed resistance factors between 0.59 and 4.31 as compared to 5.05 for CQ. Further, the compounds reported in the present study have shown better antimalarial activity against both CQ sensitive and CQ-resistant strains compared to previously reported animalarial derived from thiureas [20–22]. Against this background the present series of compounds appear to be promising for further lead optimization to obtain compounds active against drug resistant parasites.

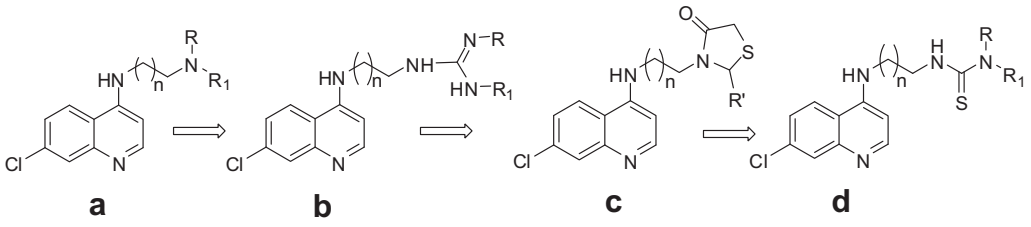
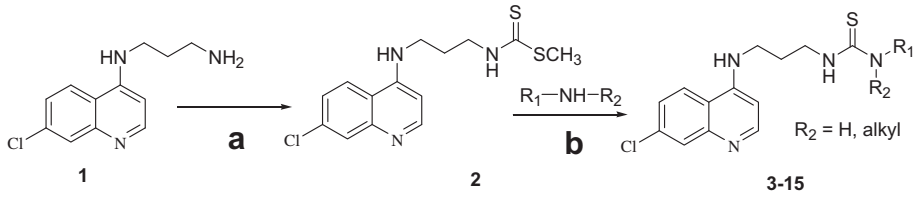
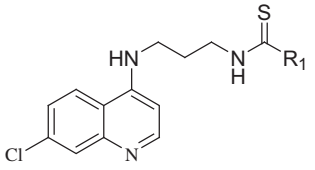


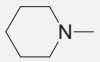
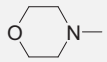
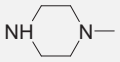
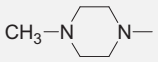
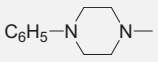
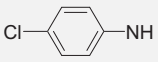
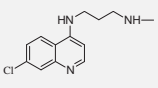
Fig. 2. 4-Aminoquinoline lead optimization from this laboratory.



Scheme 1. Synthesis of compounds **3**–**15**. Reagents and conditions: (a) CS_2 /NaOH, $(CH_3)_2SO_4$, room temperature, 4 h; (b) 80 °C for 1 h, 120–130 °C for 6–8 h.

Table 1
Biological and Biophysical data of the synthesized compounds (**3**–**15**).



Comp No	R_1	IC_{50} (nM)[a]		Resistance factor[d]	Log *P*[e]	Log K[f]	IC_{50}[g]
		D6[b]	Dd2[c]				
3	CH_3NH	177.0	212.7	1.20	2.36	4.57 ± 0.01	0.66 ± 0.03
4	$(CH_3)_2N$	139.5	ND	NA	2.74	4.87 ± 0.01	0.55 ± 0.04
5	$(C_2H_5)_2N$	103.4	125.2	1.21	3.60	5.34 ± 0.01	0.15 ± 0.01
6	$((CH_3)_2CH)_2N$	161.1	201.5	1.25	4.06	4.95 ± 0.02	0.33 ± 0.02
7		23.9	14.1	0.59	3.81	5.67 ± 0.03	0.11 ± 0.02
8		127.9	141.9	1.11	3.06	4.98 ± 0.02	0.35 ± 0.03
9		104.5	103.7	0.99	3.47	5.24 ± 0.01	0.29 ± 0.05
10		180.5	556.3	3.08	2.34	4.94 ± 0.03	0.34 ± 0.01
11		332.9	778.0	2.33	2.12	4.46 ± 0.02	0.69 ± 0.04
12		59.1	68.4	1.16	3.67	4.98 ± 0.01	0.35 ± 0.03
13		28.7	50.5	1.76	4.64	5.37 ± 0.02	0.17 ± 0.01
14		233.7	ND	NA	4.59	4.23 ± 0.04	0.48 ± 0.01
15		128.4	279.6	2.18	4.40	5.38 ± 0.01	0.27 ± 0.02
CQ		10.0	50.5	5.05	4.72	5.52 ± 0.02	0.17 ± 0.02

[a] Sigmoidal dose–response curves (variable slope) were generated using GraphPad Prism V. 4.02 (GraphPad Software Inc.).
[b] D6-chloroquine-senstive strain of *P. falciparum*.
[c] Dd2-chloroquine resistant strain of *P. falciparum*.
[d] Resistance factor calculated from dividing the IC_{50} values of Dd2 by D6.
[e] Log *P* values are calculated by ChemDraw Ultra software.
[f] 1:1 (compound: Hematin) complex formation in 40% aqueous DMSO, 20 mM HEPES buffer, pH 7.5 at 25 °C (data are expressed as means ± SD from at least three different experiments in duplicate).
[g] The IC_{50} represents the milimolar equivalents of test compounds, relative to hemin, required to inhibit beta-hematin formation by 50% (data are expressed as means ± SD from at least three different experiments in duplicate); NA – not applicable, ND – not determined.

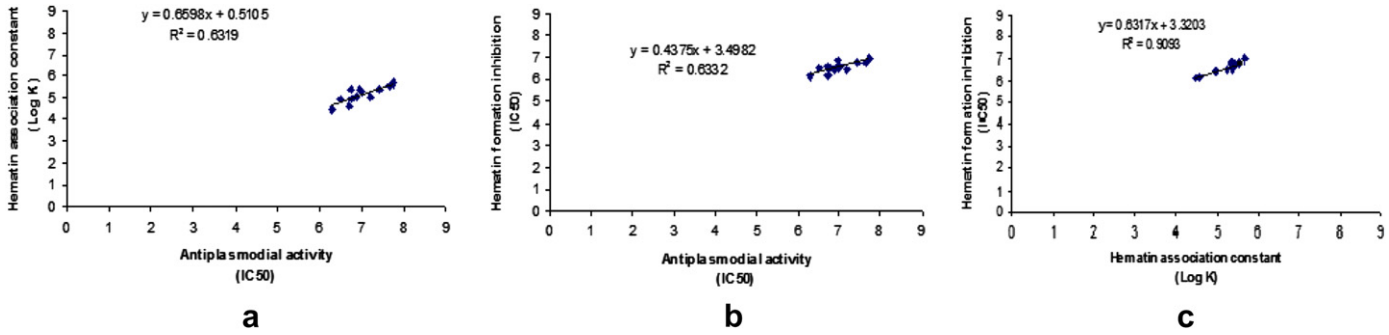


Fig. 3. Biochemical studies on synthesized 4-aminoquinoline compounds; (a) Correlation between antiplasmodial activity vs hematin association constant (b) Correlation between antiplasmodial activity vs inhibition of β-hematin formation (c) Correlation between hematin association constant vs inhibition of beta-hematin formation.

The mode of action of new series of 4-aminoquinoline derivatives (**3**–**15**) was investigated by the reported method [22–24] and the results are shown in Table 1. The data show that all the active compounds form complex with hematin and in the range Log $K = 4.24–5.67$. The piperazinyl compound (**11**) has weak lipophilic nature in comparison to CQ and shows weak binding (Log $K = 4.46$) to hematin. As expected, this compound exhibited weak antimalarial activity against CQ-S and CQ-R strains of *P. falciparum*. The piperdinyl compound (**9**) shows good binding affinity to hematin with moderate antimalarial activity against CQ-S and CQ-R strains of *P. falciparum*, due to their lipophilic character. Among all the compounds reported in the present study compound **7** having good lipophilicity shows very tight binding (Log $K = 5.67$) to hematin and it is reflected in its antiplasmodial activity. This result is concurrent with the previous results from our laboratory [12,13] as well as reported literature evidences [24–29]. Also, there is reasonable correlation between antiplasmodial activity and association constant for hematin-4-aminoquinoline binding affinity (Fig. 3a, $R^2 = 0.63$). The data suggest that the principle interaction may be hydrophobic as well as electrostatic between the 4-aminoquinoline ring and the porphyrin ring system that plays a role in hematin binding.

All the 4-aminoquinoline derived alkyl/aryl/heterocyclic substituted thiourea derivatives (**3**–**15**) inhibited the β-hematin formation in a concentration dependent manner (Table 1). Although most of the new series of 4-aminoquinoline derivatives were good inhibitors of β-hematin formation, some of them displayed moderate antimalarial activity against CQ-S and CQ-R strains of *P. falciparum*. The most potent inhibitor was compound **7** with an IC_{50} of 0.11 mM in the haemozoin inhibition assay. The IC_{50} values of β-hematin formation inhibition highlighted the importance of hydrophobic substitution. There is a moderate positive linear correlation that exists between the hematin association constant and β-hematin formation inhibition (Fig. 3b, $R^2 = 0.63$). It may suggest that this class of compounds act on heme polymerization target. It may be inferred from the above data that these compounds bind to hematin monomer or hematin μ-oxo dimer and inhibit the β-hematin formation by blocking the growing face of crystal by a capping effect [26]. Also, there is a significant positive linear correlation that exists between the hematin association constant and β-hematin formation inhibition (Fig. 3c, $R^2 = 0.91$), thereby supporting that the mechanism of action of this class of compounds is similar to that of CQ [24–29].

4. Conclusion

In summary, the synthesis of a new series of 4-aminoquinoline derivatives having substituted thiourea moiety has been described. These derivatives have exhibited promising *in vitro* antimalarial activity against the D6-chloroquine sensitive and Dd2-chloroquine resistant strains of *P. falciparum*. The most striking feature of this study is consistent antiplasmodial activity of these derivative against both CQ-S and CQ-R strains of parasite. In some cases, the activity against CQ-R strain is superior as compared to their activity against CQ-S strain of parasite. The biophysical studies have suggested that this class of compounds form association complex with hematin and thereby, inhibit the hemozoin formation. The thiourea functionality on 4-aminoquinoline-pendant amino group provided a promising lead entry for designing the heme polymerization targeted antimalarials. The present findings are in accordance with our previous findings that the basicity of the side chain nitrogen is not very essential for antiplasmodial activity of 4-aminoquinolines and provide new opportunity for the development of new antimalarials to overcome the ever-increasing problem of drug resistance.

5. Experimental protocols

5.1. General

Meting points (mp) were determined on a complab melting point apparatus and are uncorrected. IR spectra (cm^{-1}) were recorded on Perkin-Elmer 621 spectrometer using the KBr disc technique. The ^1H NMR and ^{13}C MNR spectra were recorded on a DPX-200 MHz Bruker FT-NMR spectrometer using $CDCl_3$ and DMSO-d_6 as solvent. Tetramethylsilane (δ 0.0 ppm) was used as an internal standard. Fast Atom Bombardment Mass Spectra (fab-ms) were obtained on Jeol (Japan)/SX-102 spectrometer using glycerol or *m*-nitrobenzyl alcohol as matrix. Elemental analysis was performed on a Perkin-Elmer 2400C,H,N analyzer and values were within the acceptable limits of the calculated values. The progress of the reaction was monitored on readymade TLC silica gel plates (Merck) using chloroform–methanol (9:1) as a solvent system. Iodine was used as developing agent or by spraying with Dragendorff's reagent. Chromatographic purification was performed over silica gel (100–200 mesh). All chemicals and reagents were obtained from Aldrich (USA), Lancaster (UK) or Spectrochem Pvt. Ltd (India) and were used without further purification.

5.1.1. Synthetic procedure for 3-(7-chloro-quinolin-4-ylamino)-propyl]-dithiocarbamic acid methyl ester (**2**)

To a stirred solution of N^1-(7-chloro-quinolin-4-yl)-propane-1,3-diamine (**1**) (4.75 g, 20 mmol) in dimethyl sulfoxide (10 mL) at room temperature, carbon disulphide (1.5 mL, 25 mmol) and sodium hydroxide solution (1 g, 2.5 mmol) were added dropwise over 30 min. Then the mixture was allowed to stir for another 30 min. Dimethyl sulfate (2.0 mL, 20 mmol) was added at 5–10 °C, stirring was continued for 3 h and the reaction mixture was poured

into ice water. The aqueous layer was successively washed with chloroform, the chloroform layer was washed with 5% aq $NaHCO_3$ followed by brine. The organic layer was dried over anhydrous Na_2SO_4 and solvent was removed under reduced pressure. The crude product was chromatographed over silica gel using chloroform–methanol. This compound was obtained as white solid in 60% yield. mp – 182–184 °C; R_f 0.61; IR (KBr) 2925.6 cm^{-1}; 2362.0 cm^{-1}; 1580.1 cm^{-1}; 1220.4 cm^{-1}; 1135.9 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 1.90–1.93 (m, 2H, CH_2), 1.98 (s, 3H, SCH_3), 2.17–2.19 (br s, 1H, N*H*), 2.64–2.67 (m, 2H, CH_2), 3.59–3.62 (m, 2H, CH_2), 5.87 (br s, 1H, N*H* D_2O exchangeable), 6.47–6.50 (d, J = 5.64 Hz, 1H, Ar-*H* quinoline), 7.46–7.52 (dd, J = 9.13, 2.17 Hz, 1H, Ar-*H* quinoline), 7.80–7.85 (d, J = 9.16 Hz, 1H, Ar-*H* quinoline), 8.26–8.27 (d, J = 2.13 Hz, 1H, Ar-*H* quinoline), 8.40–8.43 (d, J = 5.53 Hz, 1H, Ar-*H* quinoline); FAB-MS m/z 326 $[M+H]^+$; Anal. Calcd for $C_{14}H_{16}ClN_3S_2$; C, 51.60; H, 4.95; N, 12.89; found: C, 51.72; H, 5.02; N, 12.92.

5.1.2. General synthetic procedure for [3-(7-chloro-quinolin-4-ylamino)-propyl]-alkyl/aryl/heterocyclic substituted thiourea (**3**–**15**)

A mixture of [3-(7-chloro-quinolin-4-ylamino)-propyl]-dithiocarbamic acid methyl ester **2** (652 mg, 2.0 mmol) and corresponding amines (2.0 mmol) was heated slowly from room temp. to 80 °C over 1 h with stirring and subsequently at 120–130 °C for 6–8 h with continued stirring to drive the reaction to completion. The reaction mixture was cooled to room temp. and taken up in choloroform. The organic layer was successively washed with 5% aq $NaHCO_3$ followed by water wash and then finally with brine. The organic layer was dried over anhydrous Na_2SO_4 and solvent was removed under reduced pressure and the residue was chromatographed over silica gel using chloroform–methanol.

5.1.3. 1-[3-(7-Chloro-quinolin-4-ylamino)-propyl]-3-methyl-thiourea (**3**)

This compound was obtained as a yellowish white solid in 55% yield; mp 184–185 °C; ^1H NMR (200 MHz, DMSO-d_6 + $CDCl_3$): δ 1.81–1.93 (m, 2H, CH_2), 2.84–2.86 (m, 3H, CH_3), 3.21–3.31 (m, 4H, CH_2), 6.32–6.35 (d, J = 5.5 Hz, 1H, Ar-*H* quinoline), 7.12 (br s, 1H, N*H* D_2O exchangeable), 7.23–7.28 (dd, J = 9.0, 2.0 Hz, 1H, Ar-*H* quinoline), 7.31 (br s, 1H, N*H* D_2O exchangeable), 7.70–7.71 (d, J = 1.9 Hz, 1H, Ar-*H* quinoline), 7.80 (br s, 1H, N*H* D_2O exchangeable), 8.05–8.11 (d, J = 9.0 Hz, 1H, Ar-*H* quinoline), 8.32–8.34 (d, J = 5.4 Hz, 1H, Ar-*H* quinoline); ^{13}C NMR (DMSO-d_6 + $CDCl_3$): δ 26.63, 29.61, 37.98, 40.29, 97.40, 116.50, 122.60, 123.19, 126.36, 133.01, 147.77, 149.29, 150.44, 180.69; FAB-MS m/z 309 $[M+H]^+$; Anal. Calcd for $C_{14}H_{17}ClN_4S$; C, 54.45; H, 5.55; N, 18.14; found: C, 54.41; H, 5.58; N, 18.12.

5.1.4. 3-[3-(7-Chloro-quinolin-4-ylamino)-propyl]-1,1-dimethyl-thiourea (**4**)

This compound was obtained as a yellowish white solid in 64% yield; mp 175–176 °C; IR (KBr) 3373.7 cm^{-1}; 2923.0 cm^{-1}; 2364.0 cm^{-1}; 1579.8 cm^{-1}; 1541.5 cm^{-1}; 1457.1 cm^{-1}; 1369.3 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6 + $CDCl_3$): δ 2.00–2.04 (m, 2H, CH_2), 3.21 (s, 3H, CH_3), 3.24 (s, 3H, CH_3), 3.36–3.42 (m, 2H, CH_2), 3.77–3.80 (m, 2H, CH_2), 6.40–6.43 (d, J = 5.4 Hz, 1H, Ar-*H* quinoline), 7.19 (br s, 1H, N*H* D_2O exchangeable), 7.32–7.37 (d, J = 9.0 Hz, 1H, Ar-*H* quinoline), 7.71 (br s, 1H, N*H* D_2O exchangeable), 7.81 (s,1H, Ar-*H* quinoline), 8.09–8.14 (d, J = 9.0 Hz, 1H, Ar-*H* quinoline), 8.41–8.44 (d, J = 5.2 Hz, 1H, Ar-*H* quinoline); ^{13}C NMR (DMSO-d_6 + CDCl3): δ 26.32, 37.98, 38.26, 42.00 (2C), 97.38, 116.50, 122.37, 123.17, 126.53, 132.98, 147.91, 149.15, 150.57, 180.28; FAB-MS m/z 323 $[M+H]^+$; Anal. Calcd for $C_{15}H_{19}ClN_4S$; C, 55.80; H, 5.93; N, 17.35; found, C, 55.84; H, 5.96; N, 17.32.

5.1.5. 3-[3-(7-Chloro-quinolin-4-ylamino)-propyl]-1,1-diethyl-thiourea (**5**)

This compound was obtained as a yellowish white solid in 56% yield; mp 75–77 °C; ^1H NMR (200 MHz, DMSO-d_6 + $CDCl_3$): δ 1.09–1.15 (t, J = 6.9 Hz, 6H, 2-CH_3), 1.95–2.02 (m, 2H, CH_2), 3.22–3.42 (m, 4H, CH_2), 3.58–3.72 (m, 4H, CH_2), 6.43–6.45 (d, J = 5.5 Hz, 1H, Ar-*H* quinoline), 7.32 (br s, 1H, N*H* D_2O exchangeable), 7.36–7.40 (d, J = 8.8 Hz, 1H, Ar-*H* quinoline), 7.42 (br s, 1H, N*H* D_2O exchangeable), 7.76 (s, 1H, Ar-*H* quinoline), 8.20–8.24 (d, J = 8.8 Hz, 1H, Ar-*H* quinoline), 8.38–8.41 (d, J = 5.4 Hz, 1H, Ar-*H* quinoline); FAB-MS m/z 351 $[M+H]^+$; Anal. Calcd for $C_{17}H_{23}ClN_4S$; C, 58.19; H, 6.61; N, 15.97; found: C, 58.21; H, 6.59, N, 16.04.

5.1.6. 3-[3-(7-Chloro-quinolin-4-ylamino)-propyl]-1,1-diisopropyl-thiourea (**6**)

This compound was obtained as a yellowish white solid in 56% yield; mp 75–77 °C; ^1H NMR (200 MHz, DMSO-d_6 + $CDCl_3$): δ 1.17–1.21 (d, J = 6.7 Hz, 12H, 2-(CH_3)$_2$), 1.80–1.87 (m, 2H, CH_2), 3.13–3.34 (m, 4H, CH_2), 3.64–3.77 (m, 2H, 2-C*H*(CH$_3$)$_2$), 5.98 (br s, 1H, N*H* D_2O exchangeable), 6.67–6.70 (d, J = 6.2 Hz, 1H, Ar-*H* quinoline), 7.58–7.63 (d, J = 8.7 Hz, 1H, Ar-*H* quinoline), 7.86 (s, 1H, Ar-*H* quinoline), 8.44–8.48 (m, 2H, Ar-*H* quinoline), 8.82 (br s, 1H, N*H* D_2O exchangeable); FAB-MS m/z 379 $[M+H]^+$; Anal. Calcd for $C_{19}H_{27}ClN_4S$; C, 60.22; H, 7.18; N, 14.78; found: C, 60.19; H, 7.12; N, 14.81.

5.1.7. 1-[3-(7-Chloro-quinolin-4-ylamino)-propyl]-3-cyclopropyl-thiourea (**7**)

This compound was obtained as a pale yellowish white solid in 60% yield; mp 130–131 °C; ^1H NMR (200 MHz, DMSO-d_6 + $CDCl_3$): δ 0.56–59 (m, 2H, CH_2 cyclopropyl), 0.70–0.75 (m, 2H, CH_2 cyclopropyl), 0.80–0.95 (m, 1H, C*H* cyclopropyl), 1.96–2.02 (m, 2H, CH_2), 3.39–3.41 (m, 2H, CH_2), 3.71–3.73 (m, 2H, CH_2), 6.42–6.45 (d, J = 5.6 Hz, 1H, Ar-*H* quinoline), 7.33–7.37 (d, J = 8.6 Hz, 1H, Ar-*H* quinoline), 7.47 (br s, 1H, N*H* D_2O exchangeable), 7.72 (br s, 1H, N*H* D_2O exchangeable), 7.84 (br s, 1H, N*H* D_2O exchangeable), 7.79 (s, 1H, Ar-*H* quinoline), 8.18–8.22 (d, J = 8.8 Hz, 1H, Ar-*H* quinoline), 8.40–8.43 (d, J = 5.4 Hz, 1H, Ar-*H* quinoline); ^{13}C NMR (DMSO-d_6 + $CDCl_3$): δ 6.00 (2C), 26.75, 37.96, 40.83, 47.97, 97.45, 116.54, 122.77, 123.18, 126.34, 132.92, 147.80, 149.29, 150.47, 181.44; FAB-MS m/z 365 $[M+H]^+$; Anal. Calcd for $C_{16}H_{19}ClN_4S$; C, 57.39; H, 5.72; N, 16.73; found: C, 57.35; H, 5.69; N, 16.77.

5.1.8. Pyrrolidine-1-carbothioic acid [3-(7-chloro-quinolin-4-ylamino)-propyl]-amide (**8**)

This compound was obtained as a pale yellowish gummy matter in 65% yield; ^1H NMR (200 MHz, DMSO-d_6 + $CDCl_3$): δ 1.86–2.02 (m, 6H, CH_2), 3.26–3.29 (m, 4H, CH_2), 3.39–3.42 (m, 2H, CH_2), 3.65–3.71 (m, 2H, CH_2), 6.48–6.51 (d, J = 5.7 Hz, 1H, Ar-*H* quinoline), 7.19 (br s, 1H, N*H* D_2O exchangeable), 7.38–7.43 (dd, J = 8.9, 2.1 Hz, 1H, Ar-*H* quinoline), 7.66 (br s, 1H, N*H* D_2O exchangeable), 7.79–7.80 (d, J = 2.0 Hz, 1H, Ar-*H* quinoline), 8.22–8.27 (d, J = 9.0 Hz, 1H, 2*H* quinoline), 8.40–8.43 (d, J = 5.7 Hz, 1H, Ar-*H* quinoline); FAB-MS m/z 349 $[M+H]^+$; Anal. Calcd for $C_{17}H_{21}ClN_4S$; C, 58.52; H, 6.07; N, 16.06; found: C, 58.57; H, 6.10; N, 16.02.

5.1.9. Piperidine-1-carbothioic acid [3-(7-chloro-quinolin-4-ylamino)-propyl]-amide (**9**)

This compound was obtained as a yellowish white solid in 65% yield; mp 169–170 °C; IR (KBr) 3285.6 cm^{-1}; 2942.5 cm^{-1}; 2860.1 cm^{-1}; 1623.7 cm^{-1}; 1579.5 cm^{-1}; 1537.5 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6 + $CDCl_3$): δ 1.82–1.88 (m, 6H, CH_2), 1.94–2.01 (m, 2H, CH_2), 3.23–3.32 (m, 4H, CH_2), 3.35–3.46 (m, 4H, CH_2), 6.45 (br s, 1H, N*H* D_2O exchangeable), 6.52–6.55 (d, J = 5.8 Hz, 1H, Ar-*H* quinoline), 7.42–7.47 (d, J = 9.2 Hz, 1H, Ar-*H* quinoline), 7.58 (br s,

1H, NH), 8.08–8.09 (d, $J = 2.0$ Hz, 1H, Ar-H quinoline), 8.24–8.28 (d, $J = 8.7$ Hz, 1H, Ar-H quinoline), 8.40–8.43 (d, $J = 5.9$ Hz, 1H, Ar-H quinoline); FAB-MS m/z 363 $[M+H]^+$; Anal. Calcd for $C_{18}H_{23}ClN_4S$; C, 59.57; H, 6.39; N, 15.44; found: C, 59.62; H, 6.34; N, 15.46.

5.1.10. Morpholine-4-carbothioic acid [3-(7-chloro-quinolin-4-ylamino)-propyl]-amide (**10**)

This compound was obtained as a pale yellowish white solid in 58% yield; mp 164–165 °C; ^{1}H NMR (200 MHz, DMSO-d_6 + $CDCl_3$): δ 1.83–1.89 (m, 2H, CH_2), 1.97–2.04 (m, 2H, CH_2), 3.55–3.63 (m, 6H, CH_2), 3.72–3.83 (m, 4H, CH_2), 6.43–6.45 (d, $J = 5.5$ Hz, 1H, Ar-H quinoline), 6.71 (br s, 1H, NH), 7.34–7.39 (d, $J = 9.0$ Hz, 1H, Ar-H quinoline), 7.53 (br s, 1H, NH D_2O exchangeable), 7.79–7.80 (d, $J = 2.0$ Hz, 1H, Ar-H quinoline), 8.14–8.19 (d, $J = 9.0$ Hz, 1H, Ar-H quinoline), 8.39–8.42 (d, $J = 5.6$ Hz, 1H, Ar-H quinoline); ^{13}C NMR (DMSO-d_6 + $CDCl_3$): δ 26.53, 27.54, 36.94, 42.98, 46.74, 64.90, 65.14, 97.60, 116.28, 123.20, 123.63, 125.09, 133.50, 146.26, 149.25, 150.06, 181.06; FAB-MS m/z 365 $[M+H]^+$; Anal. Calcd for $C_{17}H_{21}ClN_4OS$; C, 55.96; H, 5.80; N, 15.35; found: C, 56.01; H, 5.84; N, 15.39.

5.1.11. Piperazine-1-carbothioic acid [3-(7-chloro-quinolin-4-ylamino)-propyl]-amide (**11**)

This compound was obtained as a pale yellowish gummy matter in 72% yield; ^{1}H NMR (200 MHz, DMSO-d_6 + $CDCl_3$): δ 1.90–1.97 (m, 2H, CH_2), 2.78–2.82 (m, 2H, CH_2), 3.21–3.34 (m, 2H, CH_2), 3.63–3.72 (m, 4H, CH_2 piperazinyl), 3.78–3.83 (m, 4H, CH_2 piperazinyl), 6.35–6.38 (d, $J = 5.7$ Hz, 1H, Ar-H quinoline), 7.26–7.31 (dd, $J = 9.2$, 2.0 Hz, 1H, Ar-H quinoline), 7.46 (br s, 1H, NH D_2O exchangeable), 7.71–7.72 (d, $J = 2.1$ Hz, 1H, Ar-H quinoline), 7.74 (br s, 1H, NH D_2O exchangeable), 7.84 (br s, 1H, NH D_2O exchangeable), 8.17–8.21 (d, $J = 9.1$ Hz, 1H, Ar-H quinoline), 8.31–8.34 (d, $J = 5.6$ Hz, 1H, Ar-H quinoline); FAB-MS m/z 364 $[M+H]^+$; Anal. Calcd for $C_{17}H_{22}ClN_5S$; C, 56.11; H, 6.09; N, 19.24; found: C, 56.14; H, 6.11; N, 19.28.

5.1.12. 4-Methyl-piperazine-1-carbothioic acid [3-(7-chloro-quinolin-4-ylamino)-propyl]-amide (**12**)

This compound was obtained as a pale yellowish gummy matter in 61% yield; ^{1}H NMR (200 MHz, DMSO-d_6 + $CDCl_3$): δ 1.95–2.02 (m, 2H, CH_2), 2.32 (s, 3H, CH_3), 2.54–2.58 (m, 4H, CH_2 piperazinyl), 3.15–3.24 (m, 2H, CH_2), 3.36–3.42 (m, 4H, CH_2 piperazinyl), 3.68–3.78 (m, 2H, CH_2), 6.41–6.44 (d, $J = 5.4$ Hz, 1H, Ar-H quinoline), 7.30 (br s, 1H, NH D_2O exchangeable), 7.33–7.37 (d, $J = 8.9$ Hz, 1H, Ar-H quinoline), 7.70 (br s, 1H, NH D_2O exchangeable), 7.76 (s, 1H, Ar-H quinoline), 8.19–8.24 (d, $J = 9.0$ Hz, 1H, Ar-H quinoline), 8.38–8.41 (d, $J = 5.3$ Hz, 1H, Ar-H quinoline); FAB-MS m/z 378 $[M+H]^+$; Anal. Calcd for $C_{18}H_{24}ClN_5S$; C, 57.20; H, 6.40; N, 18.53; found: C, 57.24; H, 6.36; N, 18.49.

5.1.13. 4-Phenyl-piperazine-1-carbothioic acid [3-(7-chloro-quinolin-4-ylamino)-propyl]-amide (**13**)

This compound was obtained as a pale yellowish white solid in 54% yield; mp 144–145 °C; ^{1}H NMR (200 MHz, DMSO-d_6 + $CDCl_3$): δ 2.00–2.06 (m, 2H, CH_2), 2.99–3.04 (m, 2H, CH_2), 3.15–3.21 (m, 2H, CH_2), 3.73–3.82 (m, 4H, CH_2 piperazinyl), 3.97–4.02 (m, 4H, CH_2 piperazinyl), 6.41–6.44 (d, $J = 5.5$ Hz, 1H, Ar-H quinoline), 6.76–6.97 (m, 3H, Ar-H), 7.19–7.23 (d, $J = 7.5$ Hz, 1H, Ar-H), 7.26–7.27 (d, $J = 2.1$ Hz, 1H, Ar-H), 7.31–7.37 (dd, $J = 8.9$, 2.1 Hz, 1H, Ar-H quinoline), 7.74 (br s, 1H, NH D_2O exchangeable), 7.77–7.78 (d, $J = 2.0$ Hz, 1H, Ar-H quinoline), 7.84 (br s, 1H, NH D_2O exchangeable), 8.16–8.20 (d, $J = 9.1$ Hz, 1H, Ar-H quinoline), 8.40–8.42 (d, $J = 5.4$ Hz, 1H, Ar-H quinoline); FAB-MS m/z 440 $[M+H]^+$; Anal. Calcd for $C_{23}H_{26}ClN_5S$; C, 62.78; H, 5.96; N, 15.92; found: C, 62.68; H, 5.94; N, 15.86.

5.1.14. 1-(4-Chloro-phenyl)–3–[3-(7-chloro-quinolin-4-ylamino)-propyl]-thiourea (**14**)

This compound was obtained as a pale yellowish white gummy matter in 56% yield; IR (neat) 3348.1 cm^{-1}; 2929.4 cm^{-1}; 2364.7 cm^{-1}; 1620.6 cm^{-1}; 1581.7 cm^{-1}; 1492.4 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6 + $CDCl_3$): δ 2.22–2.28 (m, 2H, CH_2), 2.85–2.97 (m, 2H, CH_2), 3.47–3.54 (m, 2H, CH_2), 7.07 (br s, 1H, NH), 7.12–7.16 (d, $J = 8.6$ Hz, 2H, Ar-H), 7.20–7.24 (d, $J = 8.6$ Hz, 2H, Ar-H), 7.38–7.41 (d, $J = 5.7$ Hz, 1H, Ar-H quinoline), 7.42–4.47 (d, $J = 8.9$ Hz, 1H, Ar-H quinoline), 7.51 (br s, 1H, NH), 7.55–7.56 (d, $J = 2.0$ Hz, 1H, Ar-H quinoline), 8.08 (s, 1H, Ar-H quinoline), 8.55 (br s, 1H, NH), 8.94–8.96 (d, $J = 5.6$ Hz, 1H, Ar-H quinoline); FAB-MS m/z 405 $[M+H]^+$; Anal. Calcd for $C_{19}H_{18}Cl_2N_4S$; C, 56.30; H, 4.48; N, 13.82; found: C, 56.34; H, 4.52; N, 13.91.

5.1.15. 1,3-Bis-[3-(7-chloro-quinolin-4-ylamino)-propyl]-thiourea (**15**)

This compound was obtained as a pale yellowish white solid in 66% yield; IR (KBr) 3347.5 cm^{-1}; 2922.8 cm^{-1}; 2368.2 cm^{-1}; 1653.6 cm^{-1}; 1578.9 cm^{-1}; 1450.7.4 cm^{-1}; 1367.9 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6 + $CDCl_3$): δ 1.88–1.94 (m, 4H, CH_2), 2.12–2.19 (m, 4H, CH_2), 3.33–3.42 (m, 4H, CH_2), 3.58 (br s, 2H, 2-NH D_2O exchangeable), 6.56–6.59 (d, $J = 5.4$ Hz, 2H, Ar-H quinoline), 7.24–7.29 (d, $J = 8.9$ Hz, 2H, Ar-H quinoline), 7.36–7.37 (d, $J = 1.9$ Hz, 2H, Ar-H quinoline), 7.51 (br s, 2H, 2-NH D_2O exchangeable), 8.16–8.21 (d, $J = 8.8$ Hz, 2H, Ar-H quinoline), 8.29–8.31(d, $J = 5.4$ Hz, 2H, Ar-H quinoline); ^{13}C NMR (DMSO-d_6 + $CDCl_3$): δ 25.27 (2C), 26.56 (2C), 28.27 (2C), 97.32, 97.48, 116.00, 116.46, 122.42, 123.07, 123.19, 125.95, 126.32, 126.42, 133.14, 133.42, 147.75, 149.72, 149.68, 150.33, 150.41, 151.01, 180.38; FAB-MS m/z 513 $[M+H]^+$; Anal. Calcd for $C_{25}H_{26}Cl_2N_6S$; C, 58.48; H, 5.10; N, 16.87; found: C, 58.52; H, 5.12; N, 16.92.

5.2. Biological and biophysical studies

5.2.1. Measurement of in vitro antimalarial activity

Both CQS (D6) and CQR (Dd2) *P. falciparum* maintained continuously in culture were used. Asynchronous cultures were diluted with uninfected erythrocytes and complete medium (RPMI-1640 with 0.5% Albumax II) to achieve 0.2% parasitemia and 2% hematocrit [23]. In 96-well microplates, chloroquine (positive control) or test compounds diluted in complete medium from 10 mM stock in DMSO stored at −20 °C until use. The stock solution was diluted in culture medium (0.1 nM–100 μM) immediately before use. After 72 h of incubation under standard culture conditions, plates were harvested and read by the SYBR Green I fluorescence-based method using a 96-well fluorescence plate reader (Gemini-EM, Molecular Devices), with excitation and emission wavelengths at 497 and 520 nm, respectively. The fluorescence readings were plotted against log[drug], and the IC_{50} values were obtained from curve fitting performed by non-linear regression using either Prism [30] (GraphPad) or Excel (Microsoft) software.

5.2.2. Determination of hematin-4-aminoquinoline derivatives association constant (Log K)

Association constant for hematin-4-aminoquinoline derivatives complex formation was determined by spectrometric titration procedure in aqueous DMSO at pH − 7.5 [24–26]. In this assay condition, hematin is strictly in monomeric state and interpretation of results is not complicated by the need to consider hematin disaggregation process. Association constant calculated in this technique is a good reflection of the interaction that would occur in the acidic food vacuole. The pH − 7.5 improves the stability of hematin solutions and quality of data.

5.2.3. In vitro inhibition of β-hematin polymerization

The ability of the 4-aminoquinoline derivatives to inhibit β-hematin polymerization was induced by 1-oleoyl-rac-glycerol using UV spectrophotometer and measurements were carried out at 405 nm [31]. The triplicate values obtained from the assay are expressed as percent inhibition relative to hemozoin formation in a drug free control. The 50% inhibitory concentration (IC_{50}) values for the compounds were obtained from the sigmoidal dose–response curves using non-linear regression curve fitting analyses with GraphPad Prism v.4.03 software [30]. Each IC_{50} value is the result of at least three separate experiments performed in duplicate.

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