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# Synthesis of novel thiourea, thiazolidinedione and thioparabanic acid derivatives of 4-aminoquinoline as potent antimalarials

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ABSTRACT

In search of new 4-aminoquinolines which are not recognized by CQR mechanism, thiourea, thiazolidinedione and thioparabanic acid derivatives of 4-aminoquinoline were synthesized and screened for their antimalarial activities. Thiourea derivative **3** found to be the most active against CQ sensitive strain 3D7 of *Plasmodium falciparum* in an in vitro model with an $IC_{50}$ of 6.07 ng/mL and also showed an in vivo suppression of 99.27% on day 4 against CQ resistant strain N-67 of *Plasmodium yoelii*.



Malaria is a devastating disease caused by four species of genus plasmodium which afflicts more than 40% of the world population, causing an estimated mortality of 1.5–2.7 million people annually.[1] The current epidemic is fueled about the parasite *Plasmodium falciparum*, responsible for the most deadly cases of malaria and its resistance to the antimalarial drugs. Chloroquine (CQ) has historically been mainstay of malaria treatment, particularly with *P. falciparum* in pregnant women and children under the age of five.[2] It acts by binding to heme molecules released from the hemoglobin that is digested by malaria parasites as they grow within their host red blood cells. This binding interferes with the process by which heme is normally incorporated into inert crystals (β-hematin) and detoxified, thereby accumulation of toxic levels of heme leads to the death of parasite.

Chloroquine resistance (CQR) in *P. falciparum* is primarily conferred by complex point mutations in *P. falciparum* resistant transporter (PfCRT), a putative transporter involved in drug flux and proton equilibrium across the digestive vacuole membrane, which is preventing the parasite by non-accumulating lethal concentration of the chloroquine.[3,4] Thus, developing resistant strains of chloroquine and other drugs are forcing and alarming the researchers for the development of new effective antimalarial agents (see Fig. 1).

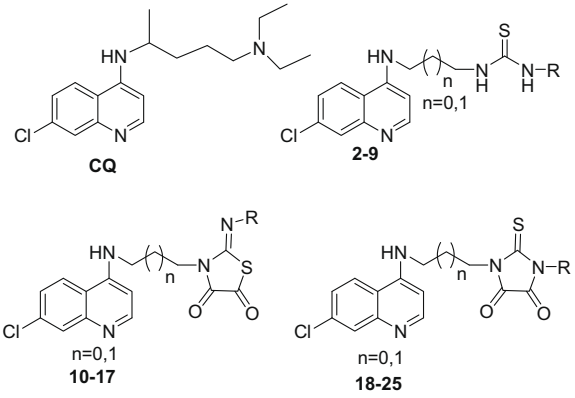


**Figure 1.** Structure of chloroquine (CQ) and synthesised compounds.

Among old and new drug targets of malaria, host heme molecule remains one of the most attractive target and 7-chloroquinoline compounds are very selective towards heme binding.[5–7] So, rather than identifying the new molecules for efficacy, 7-chloroquinolines having many advantages and efficiency are now in priority for antimalarial chemotherapy. Based on this observation, modifications of 1,4-diaminoalkyl chain of chloroquine has been done and promising results against chloroquine sensitive and resistant strains of plasmodium were obtained, due to non-recog-

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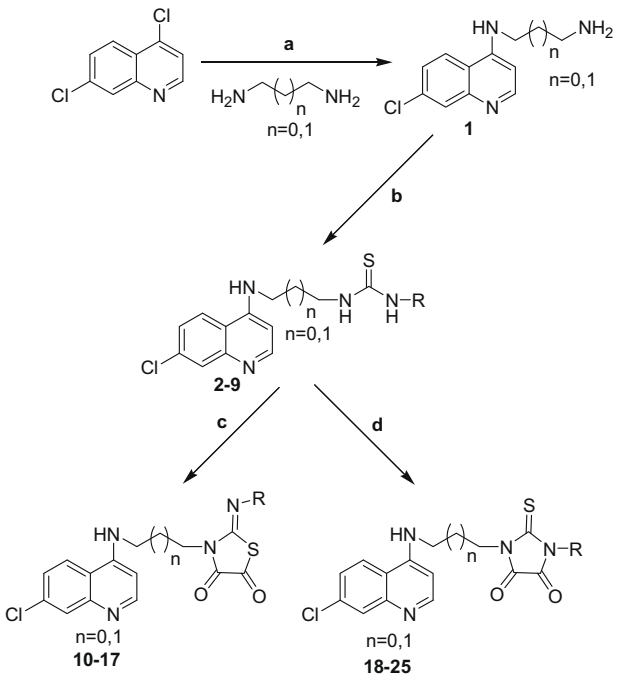
nization of these molecules by chloroquine resistant (CQR) mechanism.[8–12] In addition to 4-aminoquinolines, urea derivatives have also been identified as inhibitors of β-hematin formation,[13] while imidazolidinediones with prophylactic antimalarial activity.[14] Since our research is devoted to the synthesis of novel heterocycles as anti-infectious agents, thus considering the above points, we hypothesized and synthesized new prototypes by incorporating new entities like thiourea, thiazolidinedione and thioparabanic acid (2-thioxoimidazolidine-4,5-dione) on 4-aminoalkyl chain of 7-chloroquinoline. In this Letter, we would like to report the antimalarial activity of these new prototypes against both sensitive, resistant strains of chloroquine and also the inhibition of β-hematin formation.

Our synthesis approach toward the targeted compounds (**2**–**25**), involved less reaction time and good yielding steps with commercially available 4,7-dichloroquinoline as outlined in Scheme 1. Amination of 4,7-dichloroquinoline with α,γ-diaminoalkanes gave *N*-(7-chloro-4-quinolyl)-diaminoalkanes **1** in 80–87% yield.[8] Followed by reacting **1** with different isothiocyanate (Table 1) in acetonitrile for 30–60 min furnished respective thioureas (**2**–**9**) in 76–80% yield. Finally, to obtain the structural isomeric derivatives thiazolidinedione (**10**–**17**) and thioparabanic acid (**18**–**25**), thiourea derivatives (**2**–**9**) were cyclized with oxalyl chloride and chlorooxoethylacetate, respectively,[15] at 0 °C in DCM for 30 min yielded in the range of 72–78%. These new compounds were fully characterized by spectroscopic means and their purities were established by elemental analysis.[16]

The compounds were evaluated for their in vitro antimalarial activity against CQ sensitive 3D7 strain of *P. falciparum* BY SYBER Green I–based fluorescence (MSF) assay[17] and the compounds inhibitory activity of β-hematin formation was measured according to the described protocol.[18] Comparing the antimalarial activity, based on entities incorporated on 4-aminoalkyl chain of 7-chloroquinoline, thiourea derivatives showed good antimalarial profile of $IC_{50}$ ranging from 6.07 to 42.02 ng/mL and thiazolidinedione showed moderate activity with $IC_{50}$ in the range of 11.03–111.61 ng/mL, while thioparabanic acid derivatives showed below moderate activity of 33.08–199.31 ng/mL in comparison with chloroquine (Table 1). Among the eight thiourea derivatives (**2**–**9**), compound **3** consisting of ethyl chain and *n*-butyl group as **R** showed maximum in vitro antimalarial activity of $IC_{50}$ 6.07 ng/mL with potent inhibitory activity of $IC_{50}$ 7.11 μg/mL against β-hematin formation. On replacing ethyl with propyl chain (**7**) activity decreases to 11.82 ng/mL, though its β-hematin inhibitory activity is increased to 6.17 μg/mL. Whereas, compounds (**2**, **6**) having phenyl as **R** but with ethyl and propyl chains showed equal potency with $IC_{50}$ of 9.22, 10.01 ng/mL, respectively, although there is an increase of inhibitory activity against β-hematin formation by replacing ethyl ($IC_{50}$ = 9.86 μg/mL) with propyl chain ($IC_{50}$ = 5.67 μg/mL). While compound **9** consisting of *o*-chlorophenyl group as **R** has shown increase in antimalarial activity of $IC_{50}$ 10.16 ng/mL by replacing ethyl (**5**) with propyl chain, even though β-hematin inhibitory activity is decreased to 6.46 μg/mL from 5.56 μg/mL. Compounds with ethyl (**4**), propyl (**8**) chains and having allyl group as **R** have shown lower activity of $IC_{50}$ 26.11, 42.02 ng/mL, respectively, as compared to the other substituents of **R**.




**Scheme 1.** Reagents and conditions: (a) α,γ-diaminoalkanes, reflux, 4 h; (b) isothiocyanates, $CH_3CN$, rt, 30–60 min; (c) $(COCl)_2$, DCM, 0 °C, 30 min; (d) $ClCOCO_2Et$, DCM, 0 °C, 30 min.

Cyclization of thiourea derivatives lead to drop off in antimalarial profile of thiazolidinedione and thioparabanic acid compounds, due to decreased inhibitory activity of β-hematin formation. Compound **11** consisting of ethyl chain and *n*-butyl group as **R** shown moderate activity of $IC_{50}$ 17.44 ng/mL, while by replacing ethyl with propyl chain (**15**) showed increase in activity of $IC_{50}$ 11.03 ng/mL due to increase in inhibitory activity of β-hematin formation to 8.28 μg/mL from 9.54 μg/mL. Similarly, compound **14** having phenyl as **R** showed $IC_{50}$ of 11.05 ng/mL while its ethyl chain analogue (**10**) showed $IC_{50}$ of 29.49 ng/mL due to decrease in β-hematin formation inhibition of 9.76 μg/mL from 8.42 μg/mL. Whereas compound **13** consisting of ethyl chain and *o*-chlorophenyl group as **R** showed $IC_{50}$ of 12.11 ng/mL, while its propyl chain derivative (**17**) showed decrease in activity with $IC_{50}$ 17.48 ng/mL due to reduced β-hematin formation inhibitory activity. Compounds **12**, **16** consisting allyl group as **R** have shown poor antimalarial activity of 32.44 and 111.61 ng/mL as compared to the other substituents of **R**. Among eight thioparabanic acid derivatives (**19**–**25**), only one compound (**19**) having butyl as **R** showed moderate in vitro activity of $IC_{50}$ 33.08 ng/mL with β-hematin formation inhibition of $IC_{50}$ 9.33 μg/mL, and these derivatives showed less antimalarial activity in comparison with thiourea and thiazolidinedione derivatives (Table 1).

These compounds were also tested for their cytotoxicity against VERO cells using MTT assay.[19] Among three prototypes, thiourea and thiazolidinedione derivatives have good selectivity index in comparison with thioparabanic acid derivatives (Table 1). Compound (**9**) having an $IC_{50}$ 10.16 ng/mL showed highest selectivity index of 1113.27, while most potent compound (**3**) having an $IC_{50}$ 6.07 ng/mL showed selectivity index of 749.61, thus illustrating the good activity profile. Based on this selectivity index, compounds (**3**, **9** and **13**) were also screened in an in vivo model against chloroquine resistant N-67 strain of *Plasmodium yoelii* in swiss mice[20] at 50 mg/Kg/day for 4 days by intraperitoneal route (i.p) (Table 1). Out of three evaluated compounds, thiourea derivative (**3**) found to be the most active against chloroquine resistant strain with 99.27% suppression on day 4 and 50.50% suppression on day 10, comparable to that of standard drug chloroquine. Thus confirming that the thiourea entity on 4-aminoalkyl chain of 7-chloroquinoline is useful for generating new effective antimalarials for both chloroquine sensitive and resistant strains.

It has now become necessary to identify the new chemotherapeutic agents to overcome the parasite resistance as well as to eradicate global malaria problem. Among all synthesized molecules, thiourea derivative of 4-aminoquinoline (**3**) showed promis-

**Table 1**
Biological activity of the synthesized compounds

| Compound | *n* | **R** | In vitro antimalarial activity | SI[b] | Inhibition of β-hematin formation | In vivo % suppression | |
|---|---|---|---|---|---|---|---|
| | | | $IC_{50}$ (ng/mL)[a] | | $IC_{50}$ (μg/mL)[c] | On day 4 | On day 10[d] |
| **2** | 0 | Phenyl | 9.22 | 996.75 | 9.86 | | |
| **3** | 0 | Butyl | 6.07 | 749.61 | 7.11 | 99.27 | 50.50 |
| **4** | 0 | Allyl | 26.11 | 668.32 | 7.31 | | |
| **5** | 0 | *o*-Chlorophenyl | 20.51 | 332.06 | 5.56 | | |
| **6** | 1 | Phenyl | 10.01 | 968.11 | 5.67 | | |
| **7** | 1 | Butyl | 11.82 | 599.81 | 6.17 | | |
| **8** | 1 | Allyl | 42.02 | 581.63 | 9.23 | | |
| **9** | 1 | *o*-Chlorophenyl | 10.16 | 1113.27 | 6.46 | 16.82 | |
| **10** | 0 | Phenyl | 29.49 | 605.14 | 9.76 | | |
| **11** | 0 | Butyl | 17.44 | 480.27 | 9.54 | | |
| **12** | 0 | Allyl | 32.44 | 602.71 | 8.38 | | |
| **13** | 0 | *o*-Chlorophenyl | 12.11 | 542.58 | 8.79 | 5.45 | |
| **14** | 1 | Phenyl | 11.05 | 637.13 | 8.42 | | |
| **15** | 1 | Butyl | 11.03 | 255.76 | 8.28 | | |
| **16** | 1 | Allyl | 111.61 | 475.22 | 6.45 | | |
| **17** | 1 | *o*-Chlorophenyl | 17.48 | 433.64 | 9.72 | | |
| **18** | 0 | Phenyl | 119.10 | 118.22 | 10.71 | | |
| **19** | 0 | Butyl | 33.08 | 405.98 | 9.33 | | |
| **20** | 0 | Allyl | 104.09 | 140.64 | 9.41 | | |
| **21** | 0 | *o*-Chlorophenyl | 199.31 | 3.36 | 13.01 | | |
| **22** | 1 | Phenyl | 150.55 | 65.49 | 7.78 | | |
| **23** | 1 | Butyl | 69.70 | 27.25 | 8.65 | | |
| **24** | 1 | Allyl | 54.85 | 80.41 | 12.16 | | |
| **25** | 1 | *o*-Chlorophenyl | 39.71 | 122.38 | 9.02 | | |
| **CQ**[e] | | | 5.2 | 8983 | 4.87 | 99.05 | 73.92 |

[a] $IC_{50}$: concentration corresponding to 50% growth inhibition of chloroquine sensitive strain 3D7 of *P. falciparum*.
[b] SI = $IC_{50}$ values of toxicity against VERO cell line/$IC_{50}$ values of antimalarial activity.
[c] The 50% inhibitory concentration ($IC_{50}$) was determined using non-linear regression analysis dose–response curves.
[d] In vivo antimalarial activity against chloroquine resistant strain N-67 of *P. yoelii* in swiss mice at dose 50 mg/Kg/day by intraperitoneal route.
[e] Chloroquine at a dose of 10 mg/Kg, oral for 4 days.

ing activity against both chloroquine sensitive and resistant strains. Thus, optimization of this new prototype may be useful for the generation of effective antimalarial agents.

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16. *Spectroscopic data for* **3**: yield: 78%; mp 158–160 °C; FAB-MS: 337 (M+1); IR(KBr) 3419 (NH), 1216 (C=S) $cm^{-1}$; $^1H$ NMR (200 MHz, DMSO-$d_6$): δ (ppm) 8.38 (d, 1H, *J* = 5.41 Hz), 8.22 (d, 1H, *J* = 9.23 Hz), 7.77 (d, 1H, *J* = 2.07 Hz), 7.52 (br s, 3H), 7.44 (dd, 1H, *J* = 2.02, 8.89 Hz), 6.62 (d, 1H, *J* = 5.42 Hz), 3.71 (t, 2H, *J* = 5.48 Hz), 3.42 (t, 4H, *J* = 5.53 Hz), 1.49–1.33 (m, 2H), 1.30–1.15 (m, 2H), 0.84 (t, 3H, *J* = 7.36 Hz); $^{13}C$ NMR (50 MHz, $CDCl_3$ + $CD_3OD$): 182.05, 155.46, 155.21, 152.33, 139.67, 130.99, 129.65, 127.54, 121.53, 102.37, 49.34, 48.21, 46.61, 35.25, 24.25, 17.84; Anal. Calcd for $C_{16}H_{21}ClN_4S$: C, 57.04; H, 6.28; N, 16.63. Found: C, 57.12; H, 6.23; N, 16.57. *Compound* **11**: yield: 73%; mp 180–182 °C; FAB-MS: 391 (M+1); IR(KBr) 3426 (NH), 1773 (C=O), 1615 (C=N) $cm^{-1}$; $^1H$ NMR (200 MHz, DMSO-$d_6$): δ (ppm) 8.81 (br s, 1H), 8.56 (dd, 1H, *J* = 2.86, 6.42 Hz), 8.32–8.24 (m, 1H), 7.95 (d, 1H, *J* = 2.25 Hz), 7.62 (dd, 1H, *J* = 2.02, 9.06 Hz), 6.88 (t, 1H, *J* = 6.24 Hz), 4.08 (t, 2H, *J* = 5.56 Hz), 3.74 (t, 4H, *J* = 6.93 Hz), 1.55–1.40 (m, 2H), 1.36–1.17 (m, 2H), 0.84 (t, 3H, *J* = 7.33 Hz); $^{13}C$ NMR (50 MHz, DMSO-$d_6$): 181.57, 156.68, 154.90, 152.68, 145.71, 141.87, 135.34, 124.95, 124.27, 121.56, 115.66, 97.80, 41.62, 40.02, 39.81, 29.61, 19.75, 13.98; Anal. Calcd for $C_{18}H_{19}ClN_4O_2S$: C, 55.31; H, 4.90; N, 14.33. Found: C, 55.29; H, 4.96; N, 14.26. *Compound* **19**: yield: 75%; mp 154–156 °C; FAB-MS: 391 (M+1); IR(KBr) 3429 (NH), 1766 (C=O), 1223 (C=S) $cm^{-1}$; $^1H$ NMR (200 MHz, DMSO-$d_6$): δ (ppm) 8.47 (d, 1H, *J* = 5.39 Hz), 8.04 (d, 1H, *J* = 9.03 Hz), 7.82 (d, 1H, *J* = 1.98 Hz), 7.48 (d, 1H, *J* = 1.88 Hz), 7.44 (br s, 1H), 6.67 (d, 1H, *J* = 5.43 Hz), 4.07 (t, 2H, *J* = 5.86 Hz), 3.79 (t, 2H, *J* = 7.03 Hz), 3.63 (t, 2H, *J* = 5.98 Hz) 1.57–1.42 (m, 2H), 1.33–1.21 (m, 2H), 0.91 (t, 3H, *J* = 7.16 Hz); $^{13}C$ NMR (50 MHz, $CDCl_3$ + $CD_3OD$): 184.40, 158.04, 157.66, 149.05, 145.60, 141.89, 130.59, 127.54, 125.66, 121.56, 119.78, 101.66, 45.47, 44.08, 43.14, 32.97, 23.24, 16.76; Anal. Calcd for $C_{18}H_{19}ClN_4O_2S$: C, 55.31; H, 4.90; N, 14.33. Found: C, 55.35; H, 4.83; N, 14.31.
17. *In vitro antimalarial assay*: The compounds were dissolved in DMSO at 5 mg/mL. For the assays, fresh dilutions of all compounds in screening medium were prepared and 50 μL of highest starting concentration (500 ng/mL) was dispensed in duplicate wells in row B of 96 well tissue culture plate. The highest concentration for chloroquine was 25 ng/mL. Subsequently two fold serial dilutions were prepared up to row H (seven concentrations). Finally 50 μL of 2.5% parasitized cell suspension containing 0.5% parasitaemia was added to each well except four wells in row A which received non infected cell suspension. These wells containing non infected erythrocytes in the absence of drugs served as negative controls, while parasitized erythrocytes in the presence of CQ served as positive control. After 72 h of incubation, 100 μL of lysis buffer [20 mM tris (Ph 7.5), 5 mM EDTA, 0.008% (wt/vol) saponin, and 0.08% (vol/vol) Triton X-100] containing 1 × concentration of SYBER Green I (Invitrogen) was added to each cell (Smilkstein, M.; Sriwilaijaroen, N.; Kelly, J. X.; Wilairat, P.; Riscoe, M. *Antimicrob. Agents Chemother.* **2004**, *48*, 1803). The plates were re-incubated for 1 h at room temperature and examined for the

relative fluoroscence units (RFUs) per well using the FLUOstar, BMG lab technologies. The 50% inhibitory concentration ($IC_{50}$) was determined using non-linear regression analysis dose–response curves.

18. *Inhibition of β-hematin formation assay*: Male swiss mice, weighing 15–20 g were inoculated with $1 \times 10^5$ *P. yoelii* infected RBCs. Blood of infected animal at ~50% parasitemia was collected by cardiac puncture in 2.0% citrate buffer and centrifuged at 5000 rpm for 10 min at 4 °C. The plasma was used in assay of β-hematin formation. The assay mixture contained 100 mM sodium acetate buffer pH (5.1), 50 μL plasma, 100 μM hemin as the substrate and 1–20 μg compound/drug in a total volume of 1.0 mL. The control tube contained all reagents except compound. The reaction mixture in triplicate was incubated at 37 °C for 16 h in a rotary shaker. The reaction was stopped by centrifugation at 10,000 rpm for 10 min at 30 °C. The pellet was suspended in 100 mM Tris–HCl buffer pH (7.4) containing 2.5% SDS. The pellet obtained after centrifugation was washed thrice with distilled water (TDW) to remove free hemin attached to β-hematin. The pellet was solubilized in 50 μL of 2 N NaOH and volume was made up to 1.0 mL with TDW. Absorbance was measured at 400 nm. Pandey, A. V.; Singh, N.; Tekwani, B. L.; Puri, S. K.; Chauhan, V. S. *J.Pharm. Biomed. Anal.* **1999**, *20*, 203.
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20. The in vivo drug response was evaluated in Swiss mice infected with *P. yoelii* (N-67 strain) which is innately resistant to CQ. The mice (22 ± 2g) were inoculated with $1 \times 10^6$ parasitized RBC on day 0 and treatment was administered to a group of five mice from day 0 to 3, once daily. The aqueous suspensions of compounds were prepared with a few drops of Tween 80. The efficacy of test compounds was evaluated at 50 mg/kg/day and required daily dose was administered in 0.2 mL volume via intraperitoneal route. Parasitaemia levels were recorded from thin blood smears on days 4. The mean value determined for a group of five mice was used to calculate the percent suppression of parasitaemia with respect to the untreated control group. Mice treated with CQ served as reference controls. Puri, S. K.; Singh, N. *Expl. Parasit.* **2000**, *94*, 8.