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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 activity. Thiourea derivative **3** found to be the most active against CQ sensitive strain 3D7 of *P. falciparum* in an *in vitro* model with an IC_{50} of 6.07 ng/mL and also showed *in vivo* suppression of 99.27% on day 4 against CQ resistant strain N-67 of *P. 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 & alarming the researchers for the development of new effective antimalarial agents.

Keywords: Antimalarial; *P. falciparum*; 4-Aminoquinoline; Thiourea; Thiazolidinedione; Thioparabanic acid.

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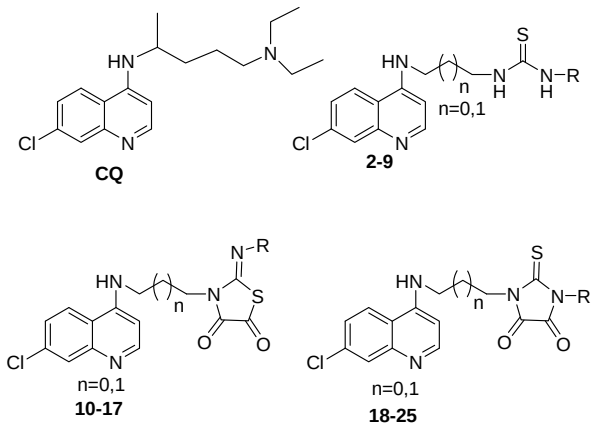
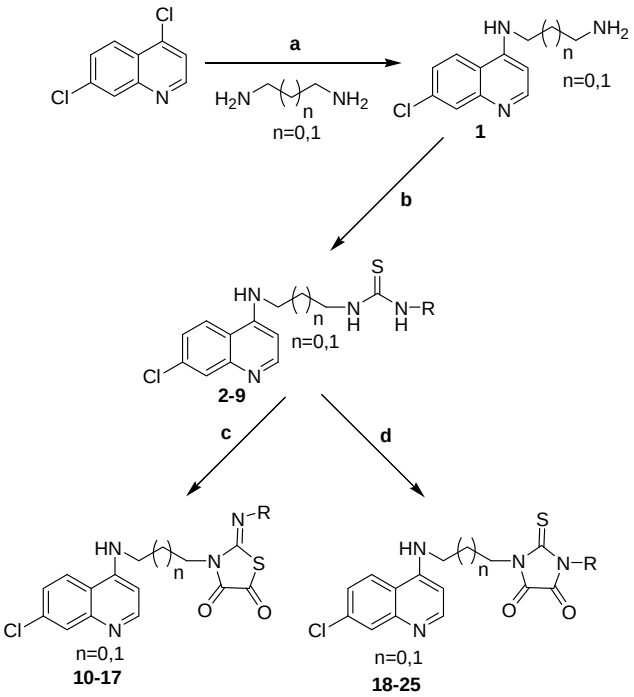


Figure 1. Structure of Chloroquine (CQ) and synthesised compounds

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-recognization of these molecules by chloroquine resistant (CQR) mechanism.[8-12] Keeping this in view we hypothesized and synthesized new prototypes by incorporating new entities like thiourea, thiazolidinedione and thioparabanic acid on 4-aminoalkyl chain of 7-chloroquinoline. In this

manuscript, 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.



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

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 minutes 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 cyclised with oxalyl chloride and chlorooxoethylacetate respectively,[13] at 0 ºC in DCM for 30 minutes with the yield in the range of 72-78%. These new compounds were fully characterized by spectroscopic means and their purity is established by elemental analysis.[14]

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.[15] The compounds were dissolved in DMSO at 5mg/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 4 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 hours of incubation, 100 µl of lysis buffer [20 mM tris (Ph 7.5), 5mM EDTA, 0.008% (wt/vol) saponin, and 0.08% (vol/vol) Triton X – 100] containing 1 x concentration of SYBER Green I (Invitrogen) was added to each cell. The plates were re-incubated for one hour 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.

The compounds inhibitory activity of β-hematin formation was measured according to the described protocol.[16] Male swiss mice, weighing 15-20 gm were inoculated with 1 X 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 16h 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 2N NaOH and volume was made up to 1.0 ml with TDW. Absorbance was measured at 400 nm.

Table 1. Biological activity of the synthesized compounds

	In vitro	Inhibition of	In vivo

Compound	n	**R**	antimalarial activity IC_{50} (ng/mL)[a]	SI[b]	β-hematin formation IC_{50} (μg/mL)[c]	% suppression on day 4[d]
2	0	Phenyl	9.22	996.75	9.86	
3	0	Butyl	6.07	749.61	7.11	99.27
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			5.2	8983	4.87	99.9

[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 antimlarial 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.

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). The most active compound is of thiourea derivative (**3**) consisting ethyl chain, n-butyl group as **R** showed IC_{50} of 6.07 ng/mL and β-hematin formation inhibition of IC_{50} 7.11 μg/mL. 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,** ethyl and propyl chains showed equal potency with IC_{50} of 9.22, 10.01 ng/mL respectively. While compound (**9**) has shown increase in antimalarial activity of IC_{50} 10.16 ng/mL by replacing ethyl (**5**) with propyl chain

and *o*-chlorophenyl group as **R** even though β-hematin inhibitory activity is decreased to 6.46 from 5.56 μg/mL. Compounds having allyl group as **R,** ethyl (**4**) and propyl (**8**) chains have shown below moderate activity of IC_{50} 26.11, 42.02 ng/mL respectively.

Cyclisation 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} 12.11 ng/mL due to increase of inhibitory activity of β-hematin formation to 8.28 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. Whereas compound (**13**) having 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 of 17.48 ng/mL. All the thioparabanic acid derivatives showed less activity in comparison with thiourea and thiazolidinedione due to poor inhibitory activity toward β-hematin formation (Table 1).

These compounds were also tested for their

cytotoxicity against VERO cells using MTT assay.[17] 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 *P.yoelii* in swiss mice[18] at 50mg/Kg/day for 4 days by intraperitoneal route (ip) (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. 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 become necessary to identify the new 4-aminoquinolines which are not identified by CQR mechanism to overcome the parasite resistance as well as to eradicate global malaria problem. Among all synthesized molecules, thiourea derivative of 4-aminoquinoline **3** showed promising 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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14. 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}; ^{1}H 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.

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17. Ctotoxicity of the compounds was determined against VERO cell lines (C-1008; Monkey kidney fibroblast cells) using MTT assay. A total of $1x10^4$ cells/well were incubated with varying concentrations of compound for 72 hours. The highest concentration of compound was 100 µg/ml. The 50% inhibitory concentration (IC_{50}) was determined using non-linear regression analysis dose-response curves and represented the concentration of compound required to kill 50% of the fibroblast cells. Mosmann T. *J. Immunol. Methods.* **1983**, *65*, 55.
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