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Synthesis of new 7-chloroquinolinyl thioureas and their biological investigation as potential antimalarial and anticancer agents

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Abstract—New 7-chloroquinolinyl thiourea derivatives derived from the corresponding 4,7-dichloroquinoline isothiocyanate were synthesized and evaluated for in vitro antimalarial and anticancer activity. The most active compound from the series displayed an inhibitory IC_{50} value of 1.2 μM against the D10 strain of *Plasmodium falciparum*. Lack of cytotoxicity towards HeLa cells indicates selectivity towards parasites.

Quinoline containing compounds have long been used for the treatment of malaria,[1] beginning with quinine, which is a 4,6-substituted quinoline. Systematic modification of quinine led to diverse quinoline antimalarial drugs[1] with diverse substitutions around the quinoline ring. One of the first drugs to be prepared was the potent and inexpensive chloroquine[2] (CQ), which is a 7-chloroquinoline with an amino substituent in position 4. Chloroquine's antimalarial activity appears to be linked to the parasite's haem metabolism.[3] Despite considerable therapeutic success with CQ, this drug is no longer effective due mainly to the development and spread of parasite resistance throughout endemic areas.[4–6] The spread of chloroquine-resistant *Plasmodium falciparum* strains has dashed hopes of global malaria eradication and, due to the paucity of other affordable drugs, has complicated the clinical management of malaria in endemic areas. This reason has highlighted the need to identify alternative antimalarial compounds. On the other hand, substituted quinolines possess medicinal properties for the effective control of malaria and cancer. Unfortunately the design and subsequent synthesis of new antimalarials are hindered by the fact that the mechanism of resistance is not fully understood.[7]

Recently the antitumour potential of quinolines against MCF-7 human breast cancer cells, with chloroquine being the most apoptosis-inducing agent, has been reported.[8] All differentiation-inducing quinolines caused growth suppression in MCF-7 and MCF10A cells. The mechanism of action of the differentiation-inducing quinolines has been proposed to involve strong suppression of E2F1 that inhibits growth by preventing cell cycle progression and fosters differentiation by creating a permissive environment for cell differentiation. Our ongoing efforts[9] in the direction of identifying new classes of 4-aminoquinolines with antimalarial and anticancer properties prompted us to undertake the synthesis of a variety of 7-chloroquinolinyl thioureas.

The synthetic routes towards these thioureas are simple and straight forward and commenced with commercially available 4,7-dichloroquinoline, which was transformed into the corresponding 4-quinolyl isothiocyanate **1** by refluxing with two equivalents of silver thiocyanate in anhydrous toluene for 12 h.[10] Treatment of equimolar amounts of amines/piperazines with 4-quinolyl isothiocyanate **1** in dry acetone afforded the corresponding thiourea derivatives. (Scheme 1) The new compounds

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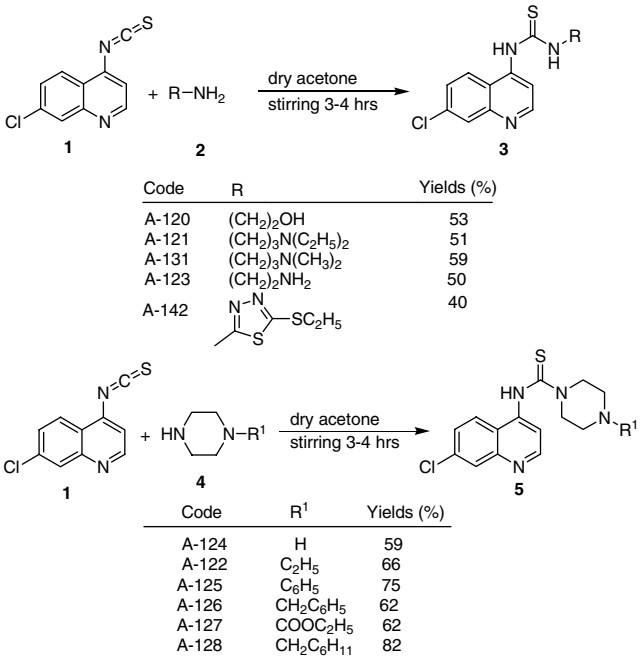

Scheme 1.

were fully characterized by spectroscopic means[11] and their purity established by elemental analysis.

CQ-sensitive (D10) strain of *P. falciparum* was maintained at 5% haematocrit in continuous culture using a modified method from Trager and Jensen.[12] The parasites were cultivated in O-positive human erythrocytes (Western Provinve Blood Transfusion Service, Groote Schuur Hospital, Cape Town, South Africa). Growth media were supplemented with Albumax II (GIBCO/Invitrogen), RPMI 1640 (Biowhittaker), 25 mM HEPES (Sigma), 1% sodium bicarbonate, hypoxanthine (44 mg/l) and gentamicin (40 mg/ml). The cultures were kept continuously at 37 °C under a gas mixture of 3% O_2, 4% CO_2 and 93% N_2. Cultures were synchronized in the ring stage with 5% D-sorbitol (Sigma) using Lambros and Vandenberg protocol.[13] The drug activities were measured using a modified parasite lactate dehydrogenase assay by Makler et al.[14] The parasites were maintained at 1% haematocrit and 2% parasitaemia for 48 h with (and without for control wells) the presence of the compound tested in 96-well microtitre plates. Upon incubation, the Malstat (Flow Inc.) reagent was used as a colourimetric indicator for parasite viability. The IC_{50} values were determined graphically using a non-linear regression analysis from GrapPad Prism (GraphPad Software Inc., 5755 Oberlin Drive, #110 San Diego, CA 92121, USA).

Cytotoxicity assays were performed on a cervical carcinoma cell line (HeLa)(CCL-2) based on the reactivity of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide), as described by Van Rensburg et al.[15] Cells were grown in EMEM supplemented with 10% foetal bovine serum in 96 multi-well plates at an initial cell concentration of 5×10^2 cells/well and incubated with the compounds for 7 after which cell survival was determined by the MTT assay. Cisplatin was used as the control anticancer drug while chloroquine was used as a control for comparison purposes.

Table 1. In vitro activity of chloroquinolinyl thioureas against HeLa cells and *Plasmodium falciparum*

Compound	D10 *P. falciparum* IC_{50} (μM)	HeLa[a] ± IC_{50} (μM)	Selectivity index[c]
CQ	0.023	9.665 ± 0.089	420.21 ± 3.86
A120	>3.5	>50	>14.28
A121	2.2	49.181 ± 0.819	22.355 ± .3727
A131	1.2	48.45 ± 1.546	40.375 ± 1.28
A123	3.3	18.012 ± 4.918	5.4581 ± 1.49
A142	>2.62	ND[b]	ND[b]
A124	>3.26	ND	ND
A122	1.8	>50	>27.77
A125	>2.61	43.64 ± 1.601	>16.72 ± 0.61
A126	>2.5	28.854 ± 2.736	>11.54 ± 1.09
A127	>2.6	40.057 ± 0.686	>15.40 ± 0.26
A128	>2.4	>50	>20.83
Cisplatin		0.803	

[a] Human adenocarcinoma of the cervix.
[b] Not determined.
[c] $\frac{IC_{50}(HeLa)}{IC_{50}(D10)}$.

The tested compounds showed moderate to good antimalarial activities against D10 (Table 1). Amongst the novel 7-chloroquinolinyl thioureas, **A121** shows the most resemblance to CQ structurally and possesses a moderate antimalarial activity of IC_{50} = 2.2 μM. **A131** differs from **A121** in having a terminal dimethylamino group and yielded better antimalarial activity (IC_{50} = 1.2 μM) but similar selectivity towards tumour (HeLa) cells. The compound, **A123**, with a shortened side chain and a terminal primary amino group displayed a slight decrease in antimalarial activity with an IC_{50} value of 3.3 μM. Similarly, the structural analogue **A122** substituted with a piperazinyl moiety also possessed comparable activity. In summary, all the synthesized compounds showed good selectivity indexes between the parasite and tumour cells. Compounds **A121**, **A131** and **A122** in particular showed similar good antiplasmodial activities against D10. Relative to chloroquine, the antitumour activity of the compounds was inferior. However the relative high concentrations necessary to inhibit 50% of tumour cell growth will not be achievable in vivo. Lack of cytotoxicity might be an indication of selectivity towards the parasite and merits further studies in a mouse model of malaria.

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11. (a) *1-(7-chloroquinolin-4-yl)-3-(3-dimethylamino) propyl thiourea* (***A131***). Yield 59%, mp 157 °C, IR: (cm^{-1}) 3500 (NH), 1223 (C═S); ^1H NMR: (400 MHz, DMSO-d_6): 1.7–1.9 (2H, m, CH_2); 2.1 (6H, s, $N(CH_3)_2$); 2.2 (2H, t, J = 6.7 Hz, N–CH_2); 3.6 (2H, t, J = 6.7 Hz, N–CH_2); 7.62–7.65 (d, 1H, J = 8.6 Hz, ArH); 7.91 (s, 1H, ArH); 8.01 (s, 1H, ArH); 8.07–8.1 (d, 1H, J = 9.2 Hz, ArH); 8.5 (br s, 1H, NH exchangeable with D_2O); 8.75 (s, 1H, ArH); 10.0 (br s, 1H, NH exchangeable with D_2O). ^{13}C NMR: (100.6 MHz, DMSO-d_6): 26.5, 39.0, 45.4, 57.5, 111, 115.5, 116.3, 122.4, 125.6, 127.2, 128.2, 135, 152.1, 176.5 (C═S). Anal. required for $C_{15}H_{19}N_4SCl$ calculated: C, 55.80; H, 5.93; N, 17.35. Found: C, 55.73; H, 5.86; N, 15.29; (b) *Ethyl-4-(7-chloroquinolin-4-ylcarbamothioyl) piprazine-1-carboxylate* (***A127***). Yield 62%, mp 205–207 °C, IR: (cm^{-1}) 3543 (NH), 1742 C═O (ester), 1239 (C═S). ^1H NMR: (400 MHz, DMSO-d_6): 1.15–1.20 (t, 3H, J = 7.0 Hz, CH_3); 3.45 (t, 4H, J = 4.8 Hz, CH_2–N–CH_2); 3.90–3.93 (br s, 4H, CH_2–N–CH_2); 4.04–4.06 (q, 2H, J = 7.0, CH_2); 6.7 (s, 1H, ArH); 7.37–7.43 (1H, dd, J = 8.8, 1.95 Hz, ArH); 7.64 (s, 1H, ArH); 8.0 (1H, s, ArH); 8.11–8.14 (d, 1H, J = 8.8 Hz, ArH); 11.2 (br s, 1H, NH exchangeable with D_2O). ^{13}C NMR: (100.6 MHz, DMSO-d_6): 14.5, 43.0, 46.9, 60.8, 114.2, 121.9, 124.5, 126.8, 128, 135.3, 147, 154.6, 155.2, 177 (C═S) 184 (C═O). Anal. required for $C_{17}H_{19}N_4O_2SCl$ calculated: C, 53.89 H, 5.05 N, 14.79. Found: C, 53.72 H, 5.12 N, 14.12. FAB MS 379 $(M+1)^+$.
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