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Synthesis of *N*1-arylidene-*N*2-quinolyl- and *N*2-acrydinylhydrazones as potent antimalarial agents active against CQ-resistant *P. falciparum* strains

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Abstract—A series of *N*1-arylidene-*N*2-quinolyl- and *N*2-acrydinylhydrazones were synthesized and tested for their antimalarial properties. These compounds showed remarkable anti-plasmodial activity in vitro especially against chloroquine-resistant strains. Their potent biological activity makes them promising lead structures for the development of new antimalarial drugs.

Malaria is a disease caused by parasitic protozoa of the genus *Plasmodium* which afflicts more than 500 million people worldwide, causing approximately 2 million deaths each year. Despite significant advances in understanding the disease and the parasite, malaria still remains one of the leading causes of morbidity and mortality, particularly in malaria-endemic regions of the world.[1,2] For decades, chloroquine (CQ, **1**, Fig. 1) provided reliable prophylaxis for travelers and therapy for those with established infection. However, the emergence in the early 1960s and subsequent spread of CQ-resistant parasites created a tremendous therapeutic void.[3] As a result, there is an urgent need for the rapid development of effective, safe, and affordable chemotherapeutics.

The exact mode of action of CQ and other 4-amino quinoline antimalarials, such as mepacrine **2** and amodiaquine **3**, remains to be elucidated,[4] but most investigators accept that a crucial step in their mechanism of action is the interference with the detoxification of free heme,[5–7] which is needed for the uninterrupted growth

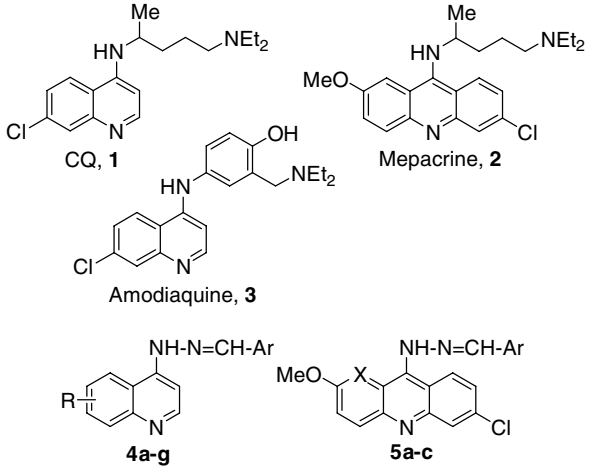

Figure 1. Reference and title compounds.

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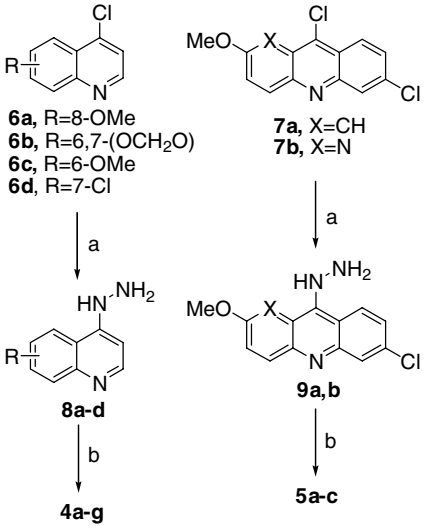
and proliferation of the parasite.[8] Although the molecular basis of CQ resistance is not fully understood, it is clear from biochemical studies that the CQ-resistant parasites accumulate fewer drugs than sensitive strains.[9,10] The fact that CQ resistance emerged after decades of widespread use suggests that CQ activity is not related to the interaction with a unique protein target.[11–13] In fact, unlike inhibitors of parasite-encoded enzymes and transporters that are currently under investigation and that may lead to the rapid appearance of resistance under drug-pressure, the parasite has difficulty in developing resistance to drugs that interfere with the heme detoxification process as it seems not to be dependent on any specific enzyme.[14] Therefore, the metabolic functions related to hemoglobin digestion and heme detoxification pathways still represent a valid target for the discovery of new antimalarial drugs.

The compounds reported here were designed to investigate novel chemical entities characterized by high activity against CQ-resistant *Plasmodium falciparum* (*Pf*) strains and by a low resistance potential due to a mechanism of action that may be similar to that of 4-aminoquinoline antimalarials. Accordingly, taking into account that CQ analogues incorporating a hydrazone or a hydrazine moiety in the side chain showed moderate antimalarial activity,[15] we developed a small series of hydrazones characterized by the presence of differently substituted quinolines (**4a**–**g**, Fig. 1) and acridines (**5a**–**c**), structurally related to CQ and mepacrine, respectively. These compounds were designed to interact with iron III FPIX (hematin), possibly generating toxic radical species capable of interfering with the crucial reducing milieu of the parasite.[16]

Compounds **4a**–**g** and **5a**–**c** were obtained starting from suitable 4-chloroquinolines **6a**–**d**,[17–20] 2,9-dichloro-6-methoxyacridine **7a**, and 7,10-dichloro-2-methoxybenzo[*b*][1,5]naphthyridine **7b**,[21] as described in Scheme 1.



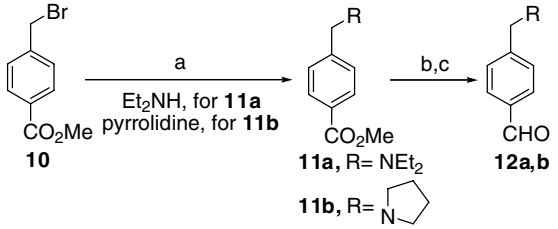
Scheme 1. Reagents and conditions: (a) $NH_2NH_2{\cdot}H_2O$, MeOH, reflux or $NH_2NH_2{\cdot}H_2O$, MW radiation, 200 W, 5 min, sealed tube; (b) ArCHO, EtOH, reflux.

These compounds were then heated under reflux for several hours in the presence of hydrazine monohydrate with methanol as the solvent.

Some of the 4-quinolylhydrazines were prepared using an alternative procedure in which microwave radiation was used as the heating source and the reaction was performed in neat hydrazine monohydrate under pressure. This latter method afforded corresponding hydrazines in 80–90% yields, generally higher than that obtained using the standard methods. In the next step of the synthesis, hydrazines **8a**–**d** and **9a**,**b** were reacted with one equivalent of the appropriate carboxaldehyde in boiling ethanol and in the presence of an equimolar amount of sodium acetate.[22] Most of carboxaldehydes used for the synthesis of final compounds were commercially available except for aldehydes **12a** and **12b** which were synthesized from the bromoester **10** as described in Scheme 2. Alkylation of diethylamine or pyrrolidine with bromide **10** was performed in refluxing acetone in the presence of potassium carbonate and of a catalytic amount of 18-crown-6. The resulting tertiary amines **11a**,**b** were reacted with lithium aluminum hydride in THF and the alcohols thus obtained were oxidized to aldehydes **12a**,**b** using manganese dioxide in refluxing dioxane.

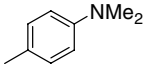
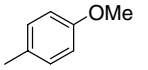
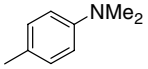
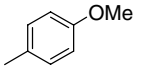
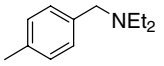
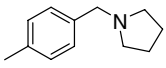
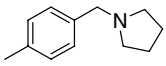
All synthesized compounds were tested in vitro against a series of *Pf* strains, namely the CQ-sensitive D10 and 3D7, and the CQ-resistant W2 and K1 strains of *Pf*. The antimalarial activity (IC_{50}, nM) was quantified as inhibition of parasite growth, measured with the production of parasite lactate dehydrogenase[23] (D10 and W2 strains) or the incorporation of [^{3}H]-hypoxanthines (3D7 and K1 strains).[24]

The results are presented in Table 1. Among the new compounds synthesized **4d**–**g** and **5a**–**c** showed an antiplasmodial activity against the CQ-sensitive D10 strain in the same range of CQ. Similarly, **4f** and **4g** displayed the same activity of CQ against CQ-sensitive 3D7 strain, while compound **5b** was 10 times more potent than CQ. Additionally, the data showed that most of the tested compounds were highly active against CQ-resistant strains, two analogues (**5b** and **5c**) being more active against W2 CQ-resistant than D10 CQ-sensitive strains. In the quinoline series (**4a**–**g**), the 8-OMe and 6,7-methylendioxy substituents gave rise to compounds generally less active than the corresponding 6-OMe and 7-Cl derivatives. These structure–activity relationships



Scheme 2. Reagents and condition: (a) K_2CO_3, 18-crown-6, acetone, reflux; (b) $LiAlH_4$, THF; (c) MnO_2, dioxane.

Table 1. Anti-plasmodial activity of compounds **4a–g** and **5a–c**

Compound	R	Ar	X	Ionic form[a]	%[b] at pH 7.2	% [b] at pH 5.5	D10[c] IC_{50}[e] (nM)	W2[d] IC_{50}[e] (nM)	3D7[c] IC_{50}[e] (nM)	K1[d] IC_{50}[e] (nM)
4a	8-OMe		—	P	3.50	64.54	519	254	5703	NT[f]
				N	96.48	35.46				
				A	0.02	—				
4b	8-OMe		—	DP	—	2.08	337	356	NT	NT
				P	3.74	64.02				
				N	96.26	33.90				
4c	6,7-(OCH_2O)		—	P	75.97	99.37	210	163	155	467
				N	24.03	0.63				
				A	—	—				
4d	6-OMe		—	DP	0.05	3.20	83	104	NT	NT
				P	67.07	95.86				
				N	32.88	0.94				
4e	6-OMe		—	P	60.77	98.73	99	128	NT	NT
				N	39.22	1.27				
				A	0.01	—				
4f	6-OMe		—	DP	59.11	98.64	39.2	79.0	11.0	55.1
				P	40.68	1.36				
				N	0.21	—				
4g	7-Cl		—	DP	4.00	67.63	28.8	58.3	19.1	16.4
				P	95.41	32.37				
				N	0.59	—				
5a	—		CH	DP	16.00	90.52	97	137	112	NT
				P	83.46	9.48				
				N	0.54	—				
5b	—		CH	DP	0.05	12.64	62.6	30.8	1.0	4.5
				P/ZW	16.90	79.59				
				N	83.00	7.77				
				A	0.05	—				
5c	—		N	DP	—	1.03	72	26.9	283	198
				P/ZW	24.87	93.29				
				ZW	74.35	5.68				
				A	0.78	—				
CQ	—	—	—	DP	12.12	87.37	22.5	280	10.1	258
				P	87.83	12.63				
				N	0.05	—				

[a] DP, di-protonated form; P, protonated form; ZW, zwitterionic form; N, neutral form; A, anionic form.
[b] ACD/pK_a DB verson 9.00 software (Advanced Chemistry Development Inc., Toronto, Canada).
[c] CQ-sensitive clone.
[d] CQ-resistant clone.
[e] IC_{50}s are the mean of at least three determinations. Standard errors were all within 10% of the mean.
[f] NT, not tested.

(SARs) can be explained by taking into account the steric hindrance of the substituent at C-8 of the quinoline ring (**4a**,**b** vs **4d–f**) with respect to the capability of the quinoline nitrogen to coordinate hematin.

Potency is also influenced by the arylidene substituent at the hydrazine *N*1. Electron-donating groups, such as the weakly basic dimethylamino (**4b**) (still not protonated at vacuole pH of 5.5, Table 1), do not improve antimalarial activity versus the unsubstituted aromatic ring (**4a**). On the contrary, a significant increase of potency against CQ-sensitive and CQ-resistant strains can be observed when the aromatic ring presents a diethylaminomethyl group, already protonated at pH 7.2 (**4f** vs **4d**). The extra basic group was introduced to mimic the distal nitrogen of CQ and amodiaquine, critical for drug sequestration into the acidic food vacuole of *Pf*.[6] SARs were also exploited by varying the bi-cyclic system of **4** introducing an acridine group (**5a–c**). The mepacrine tri-cyclic system, combined with the hydrazone moiety, was used to investigate the effects of the 7-chloro substituent of **4g** and of the 6-methoxy group of **4f**. The acridine analogues **5a–c** were synthesized and tested. The different activity against the panel of CQ-resistant strains seems to be dependent upon the ionization state of the molecule (Table 1). In fact, the introduction of an extra basic nitrogen (**4f** and **4g**), resulting into a di-protonated state at the *Pf* vacuole pH 5.5, increased activity on CQ-resistant strains. However, when the extra basic nitrogen was introduced in the acridine series (**5a**), the resulting compound did not show antimalarial activity comparable to the di-protonated analogues of the quinoline series (**4f** and **4g** vs **5a**). Interestingly, when the pyrrolidinylmethylphenyl moiety of **5a** was replaced with an imidazole ring, the resulting compound **5b** showed an increased potency profile against all tested *Pf* strains, becoming particularly active against CQ-resistant strains. Imidazoles are known to be excellent heme

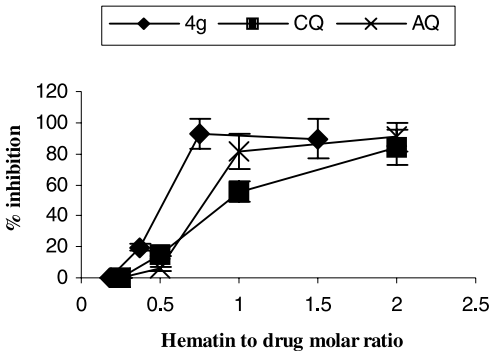


Figure 2. β-Hematin inhibitory activity assay of compounds **4g**, CQ, and **3** (AQ).

ligands; moreover, as evidenced by pK_a calculations and confirmed by computational studies (data not shown), the imidazole ring drives the formation of an intramolecular zwitterion due to the exchange of a proton from the imidazole nitrogen to the hydrazine nitrogen. Contrastingly, introduction of an extra nitrogen atom at position 5 of the acridine nucleus gave an analogue (**5c**) that retained the activity against D10 and W2 strains, being poorly active on 3D7 and K1 strains.

Compound **4g** was screened for inhibition of β-hematin (hemozoin) formation by using the BHIA (β-hematin inhibitory activity) assay (hemin in dimethylsulfoxide-acetate buffer at pH 5.0, 37 °C, 18 h).[25] Compound **4g** showed a dose-dependent inhibition in the BHIA assay ($IC_{50} = 0.53 \pm 0.09$) and became more potent than CQ ($IC_{50} = 0.91 \pm 0.23$) and amodiaquine ($IC_{50} = 0.79 \pm 0.01$) in inhibiting hemozoin formation (Fig. 2), suggesting that its antimalarial activity was related to the inhibition of the heme detoxification process.

Compounds **5b** and **5c**, bearing an imidazole ring known to have heme binding properties (e.g., azoles antifungal), are among the most promising analogues of the series. Further studies are in progress to understand the mechanism of action of these compounds and in particular to know whether the activity profile of **5b** and **5c** could be related to improved heme binding. Cytotoxicity on murine fibrosarcoma cells WEHI, clone 13, was assayed on the most active compounds **4g** and **5b** using the MTT test.[23] While **5b** showed an ED_{50} of 5.1 μM similar to that one of CQ ($ED_{50} = 9.7$ μM), compound **4g** ($ED_{50} = 19.4$ μM) proved to be much less toxic than **5b** and CQ.

To summarize, a series of novel and highly active antimalarial agents were synthesized. In particular, compounds **4f–g** and **5b–c** showed remarkable antimalarial activity especially against CQ-resistant *Pf* strains and they represent promising lead structures for the development of new antimalarial drugs. Inhibition of the heme detoxification process seems to be the basis of their mechanism of action. Notably, the synthesis of these compounds involves few steps with commercially available products and has low production costs. Stability of **4g** (over 2 h) in acidic conditions was proven by NMR techniques.[26] Further pharmacological characterization and in vivo studies to evaluate bioavailability are currently in progress.

Acknowledgments

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22. In a typical procedure: a mixture of the appropriate carboxaldehyde (1 equiv) and hydrazine derivative (1 equiv) was heated under reflux in ethanol for 2–3 h. After cooling and diluting with H_2O, the respective hydrazones precipitated from the reaction mixture. They were filtered and washed with ice-cold ethanol. The purification was carried out by recrystallization and/or by flash column chromatography (70–90% yields). The structures assigned to the synthesized compounds were in good agreement with their analytical and spectral data (elemental analyses, MS, ^{1}H NMR).

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26. Samples of **4g** in D_2O-DCl (pH 4.0) were monitored at 30′ intervals for 4 h by NMR and HPLC-MS.