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# Design, synthesis and antimalarial activity of a glyoxylylhydrazone library

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**Abstract**—Synthesis of a new family of quinolylhydrazone derivatives and evaluation of their activity against a chloroquine-resistant strain of *Plasmodium falciparum* are described. The best compound displayed an activity 6-fold higher than chloroquine. None of the active compounds were found to inhibit β-hematin formation in vitro in the same range as chloroquine and five among them displayed lower calculated vacuolar accumulation ratios, suggesting the implication of a different mechanism of action.


## 1. Introduction

Almost one-half of the world's population is exposed to the burden of malaria and the disease is responsible for the death of about 2 million people per year. The spread of multidrug-resistant *Plasmodium faciparum* has highlighted the urgent need to develop new antimalarial drugs, preferably those affordable to developing countries where malaria is prevalent.[1,2] Among 4-aminoquinolines, chloroquine (CQ) (Fig. 1) is believed to exert its activity by inhibiting hemozoin formation in the digestive vacuole of the parasite where it accumulates by pH gradient.[3–5] Since some members of this family are still active on CQ-resistant strains,[6] the 4-aminoquinoline structure constitutes an interesting basis for the design of novel compounds displaying increased activity.

Schiff base chemistry was selected for introducing modifications on the 4-aminoquinoline nucleus. In particular, formation of an α-oxo oxime or hydrazone linkage between a glyoxylyl derivative of 4-aminoquinoline and a collection of *O*-alkyl hydroxylamines or *N*-alkyl hydrazines was envisioned as a rapid way to generate a library of diverse 4-aminoquinoline derivatives[7] (Fig. 1).

Peptide glyoxals were found to inhibit serine and cysteine proteinases.[8] Ocain and Rich[9] have described the synthesis of α-keto amide derivatives, a novel class of aminopeptidase inhibitors. Badet and co-workers[10] have explored the ability of N-terminal glyoxylyl peptides to inhibit HIV-1 protease. Some acylhydrazones have already been described as proteinase inhibitors for

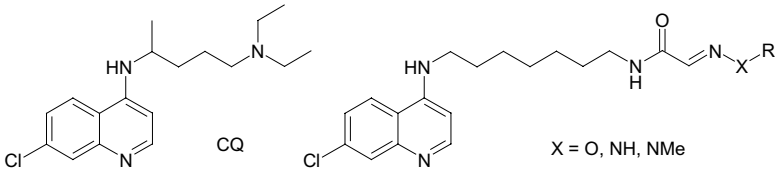


**Figure 1.** Chloroquine (CQ) and target compounds.

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antiparasitic activity against *Trypanosoma brucei*[11] and Nifurtimox, a furyl hydrazone, was commercialised for the treatment of Chagas disease. However to our knowledge, glyoxylyl chemistry was never used for the synthesis of new antimalarials.

## 2. Library synthesis

Previous work in our laboratory showed the importance of the linker between the two aromatic moieties on the activity and the localisation of antimalarials (Fig. 2).[12] Using fluorescence microscopy, experiments on the localisation of the antimalarials **1** and **3** in infected red blood cells revealed an accumulation of the drugs inside the parasite with the exception of the food vacuole, whereas in the same conditions compounds **2** and **4** concentrated into the food vacuole of the parasite.

Our previous studies conducted on quinoline derivatives with a bis-aminopropylpiperazine spacer and a diversity of aromatic and aliphatic substituents led us to compounds displaying high in vitro and in vivo antimalarial activities.[12,13]

Aliphatic spacers of between five and nine carbon atoms was successfully used in bisquinolines by Vennerstrom et al.[14] The mentioned bisquinolines displayed low nanomolar activities against both CQ-sensitive (D-6) and CQ-resistant (W-2) strains and most of them was curative against *P. berghei*.

Bearing this in mind, we decided to extend our study to aliphatic spacers and in a first step we have considered linking the quinoline moiety to the hydrazone bond by a heptamethylenic chain (Fig. 1).

The glyoxylyl derivative **7** was obtained by periodic oxidation of a tartaramide precursor **6** according to published procedures (Scheme 1).[15]

Condensation of glyoxylyl compound **7** with the selected hydroxylamines or hydrazines was performed in deep-well plates at 7 μmol scale (Scheme 2). Typically 40 μL of compound **7** (0.18 M in EtOH) were added in each well to 40 μL of hydroxylamines or hydrazines (0.18 M in DMF, without neutralisation if the compounds are sold as hydrochlorides). After 18 h at room temperature, an aliquot was taken and reaction media were evaporated. The purity of the library was assessed by RP-

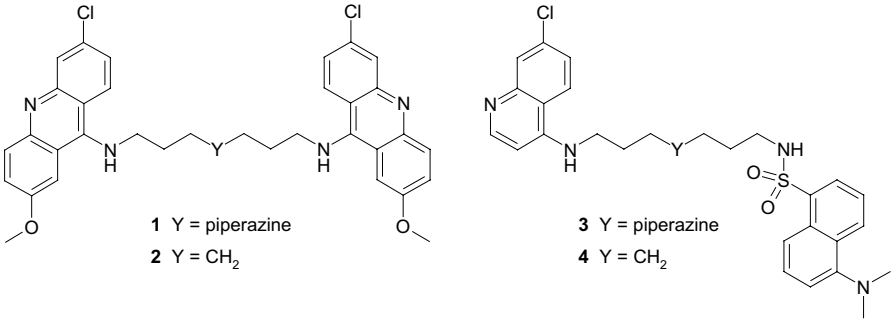
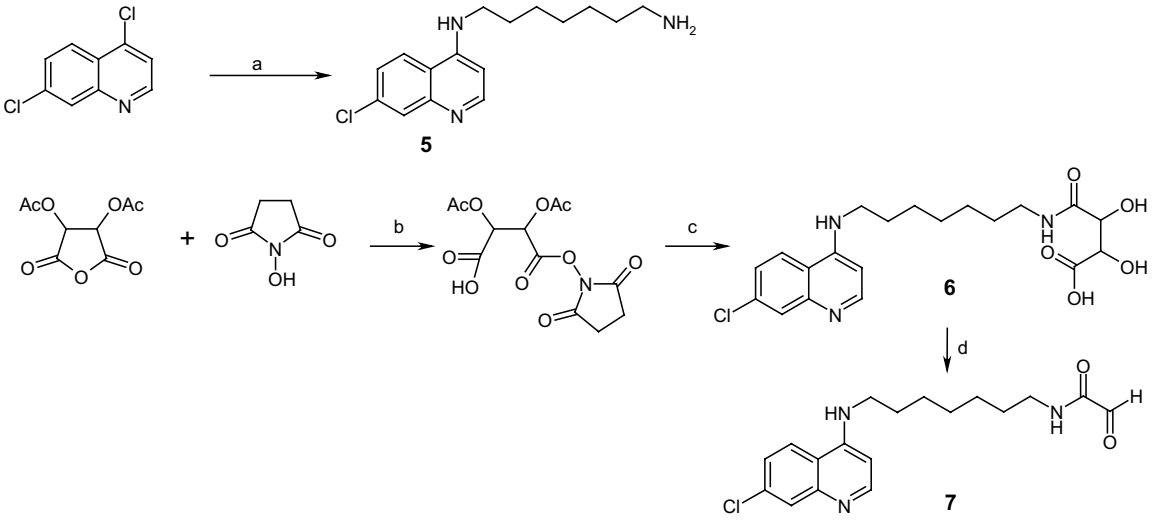
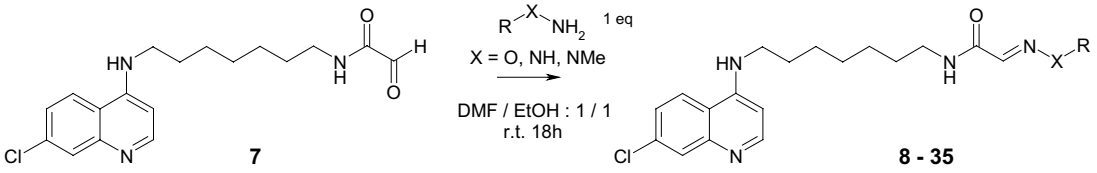



**Figure 2.** Compounds used for localisation studies.[12]




**Scheme 1.** Reagents and conditions: (a) 1,7-diaminoheptane, pentanol, 120 °C, 16 h, 80%; (b) THF, rt, overnight; (c) compound **5**, $NaHCO_3$ 1 M/THF: 1/1 then NaOH; (d) $NaIO_4$, phosphate buffer, 48% overall.




**Scheme 2.**

HPLC (>90% for oximes and 70–90% for hydrazones). All the mass spectra were consistent with the anticipated product structures.

## 3. Biological and physico-chemical assays

### 3.1. Antimalarial activity and cytotoxicity

The crude compounds were tested for their ability to inhibit parasite growth (CQ-resistant strain FcB1, $IC_{50}$ CQ = 126 nM)[16] in a rapid screening allowing us to evidence the most active of them. In order to obtain more precise values, crude compounds displaying an $IC_{50}$ below 50 nM were resynthesised at a larger scale (400 μmol) and purified to confirm the biological activity and to determine their standard $IC_{50}$ values. The results are collected in Table 1. Cytotoxicity tests ($CC_{50}$) were performed on a human diploid embryonic lung cell line (MRC-5) using the colorimetric MTT assay.[17]

### 3.2. In vitro inhibition of β-hematin formation

Compounds were tested for their ability to inhibit β-hematin formation (the synthetic equivalent of hemozoin)

**Table 1.**

| ID | X | R | $IC_{50}$ (nM)[a] (crude compds) | $IC_{50}$ (nM) (pure compds) | $CC_{50}$ (μM) | SI[c] | VAR[d] ($\times 10^4$) | Inh of β-hematin formation[e] |
|---|---|---|---|---|---|---|---|---|
| | | CQ | | 126 ± 26 | 50 | 396 | 5.4 | 55 μM[f] |
| 8 | O | Benzyl | 29%[b] | | | | 3.6 | 0 |
| 9 | O | *t*-Butyl | 14%[b] | | | | 3.6 | 0 |
| 10 | O | Methyl | 11%[b] | | | | 3.6 | 4 |
| 11 | O | H | 10%[b] | | | | 879 | 6 |
| 12 | O | Ethyl | 11%[b] | | | | 3.6 | 0 |
| 13 | O | Pentafluorobenzyl | 33%[b] | | | | 3.6 | 0 |
| 14 | O | Allyl | >500 | | | | 3.6 | 3 |
| 15 | O | 4-Nitrobenzyl | 5 | 62.3 ± 3.5 | 1.7 | 27 | 3.6 | 0 |
| 16 | NH | 2,4-Dinitrophenyl | 13 | 47.3 ± 4.9 | 11.1 | 235 | 3.6 | 25 |
| 17 | NH | Benzyl | 35 | 161 ± 3.6 | 14.6 | 91 | 3.5 | 0 |
| 18 | NH | 7-Chloroquinolin-4-yl | 7 | 19.3 ± 2.5 | 1.4 | 73 | 616 | 0 |
| 19 | NH | Ethoxyacetyl | 59%[b] | | | | 4.9 | 12 |
| 20 | NH | 2,6-Dichloro-4-(trifluoromethyl)phenyl | 16 | 88.7 ± 4.6 | 9.2 | 104 | 3.6 | 7 |
| 21 | NH | *t*-Butyl | 60 | | | | 69.8 | 0 |
| 22 | NH | 2,4-Dimethylphenyl | 86 | | | | 5.7 | 8 |
| 23 | NH | 3-Chloropyridazin-6-yl | 180 | | | | 3.6 | 0 |
| 24 | NH | 3-Chloro-4-fluorophenyl | 200 | | | | 3.7 | 0 |
| 25 | NH | 2,4-Difluorophenyl | 105 | | | | 3.8 | 0 |
| 26 | NH | 4-*t*-Butylphenyl | 32%[b] | | | | 5.7 | 1 |
| 27 | N–Me | 3-Nitropyridin-2-yl | 3%[b] | | | | 3.6 | 18 |
| 28 | NH | 2-Carbomethoxythiophen-3-yl | 13%[b] | | | | 3.9 | 11 |
| 29 | NH | Quinoxalin-2-yl | 90 | | | | 3.6 | 0 |
| 30 | NH | 1,3,4-Trimethyl-1*H*-pyrazolo[3,4-*b*]pyridin-6-yl | 30 | 63.9 ± 2.7 | 1.8 | 28 | 874 | 0 |
| 31 | NH | 7-Methoxy-1,2,4-benzotriazin-3-yl | 45 | 73.7 ± 5.3 | 2.5 | 34 | 3.6 | 28 |
| 32 | NH | 3-(2-Thienyl)-1,2,4-thiadiazol-5-yl | 8%[b] | | | | 3.6 | 19 |
| 33 | NH | 2,4-Dichlorophenyl | 0%[b] | | | | 3.7 | 12 |
| 34 | NH | 2,6-Dimethylpyrimidin-4-yl | 0%[b] | | | | 887 | 19 |
| 35 | NH | Phenyl | 0%[b] | | | | 3.8 | 0 |

[a] $IC_{50}$ values for crude compounds evaluated by rapid screening on FcB1.
[b] Inhibition of parasite growth (200 nM).
[c] Selectivity index $SI = CC_{50}/IC_{50}$.
[d] Calculated vacuolar accumulation ratios.
[e] Percentage of inhibition at 100 μM.
[f] $IC_{50}$ value obtained from duplicate experiments.

induced by 1-monooleoyl glycerol (MOG) using published procedures.[18,19]

## 4. Results and discussion

The following model of SAR for CQ has recently been proposed:[20] (i) the 4-aminoquinoline nucleus alone provides an Fe(III)PPIX complexing template but is not sufficient for inhibition of hemozoin formation; (ii) introduction of a 7-chloro group is responsible for inhibition of hemozoin formation but probably has little influence on the strength of association with Fe(III)PPIX; and (iii) the aminoalkyl side chain is a requirement for strong antimalarial activity by improving drug accumulation in the food vacuole. A further study on a family of CQ[21] derivatives showed a linear dependance of the vacuolar accumulation ratio (VAR) normalised $IC_{50}$ value on the inhibition of β-hematin formation. This study supported the proposal that both pH trapping and β-hematin inhibition are the basis of antiplasmodial activity of aminoquinolines.

Evaluation of the three parameters: inhibition of β-hematin formation, vacuolar accumulation ratio (VAR)[22] and antiplasmodial activity could allow to analyse the results.

Antimalarial activities against the FcB1 strain reach an $IC_{50}$ of 19 nM for the most potent derivative (Table 1).

In the series of oximes, compound **15** was the only one to display an activity better than CQ. Replacement of nitro substituent in **15** with an hydrogen in **8** induced a sharp loss in activity. The introduction of alkyl substituents also led to substantial loss in activity.

In the hydrazone series, the activities were more interesting (comparison between **8** and **17**, **9** and **21**) and five compounds (**16**, **18**, **20**, **30**, **31**) displayed better $IC_{50}$ than CQ. The 7-chloroquinolin-4-yl substituent provided the best result (19.3 nM).

Five compounds out of 28 provided a higher calculated VAR than CQ. Among them, two compounds (**18**, **30**) displayed a better inhibition of parasite growth than CQ, whereas the three others (compounds **11**, **21** and **34**) were found to have weaker antiplasmodial activities. Thus, an important weak-base effect (evaluated by VAR calculation) appeared not to be sufficient to provide good antimalarial activities.

None of the 28 compounds inhibited β-hematin formation in the same range as CQ. Interestingly, four compounds (**15**, **16**, **20** and **31**) displayed better antimalarial activities than CQ while they theoretically accumulated less in the food vacuole and had significantly inferior potencies as inhibitors of β-hematin formation.

Taken together, these results suggest that a mechanism of action different from that of CQ could be involved.

The cytotoxicity of compounds upon MRC-5 cells ranges from 1.4 to 14.6 μM (Table 1). All compounds displayed a selectivity index (SI) inferior to that of CQ.

## 5. Conclusion

Parallel synthesis of an α-oxo-oxime or hydrazone library provided compounds with activities superior to that of CQ on a CQ-resistant strain, especially in the hydrazone family. The synthesis of a library of analogues is on the bench, with further modulation of the linker and chloroquinolinyl moiety in order to optimise the activity and decrease the cytotoxicity.

Chloroquine and a number of quinoline-based drugs with good antimalarial activity (among which bisquinolines) inhibit β-hematin formation. They accumulate at high concentrations into the parasite's acid food vacuole deduced as their site of action.

The results obtained for compounds **15**, **16**, **20** and **31** suggest that they could have an original mechanism of action, thus, the next step would be the search for the biological target of these compounds via affinity chromatography.

## Acknowledgements

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22. Vacuolar accumulation ratios (VAR) were calculated mathematically from the equation below:

$$\mathrm{VAR} = \frac{1 + \sum_{n=1}^{4} \sum_{i=1}^{n} 10^{\mathrm{p}K_{ai} - \mathrm{pH_v}}}{1 + \sum_{n=1}^{4} \sum_{i=1}^{n} 10^{\mathrm{p}K_{ai} - \mathrm{pH_o}}}$$

where $\mathrm{pH_v}$ = pH inside the vacuole (assumed to be pH 5.0). $\mathrm{pH_o}$ = pH externally (assumed to be pH 7.4).This equation proceeds from a derivation of the Henderson–Hasselbach equation, based on predicted values of drug $\mathrm{p}K_a$ according to previous works of Hawley et al.[23] Values of $\mathrm{p}K_a$ were calculated using ACD/$\mathrm{p}K_a$ DB software from Avanced Chemistry Development Inc., Toronto, Canada.
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