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# Synthesis and biological activities of 4-*N*-(anilinyl-*n*-[oxazolyl])-7-chloroquinolines (*n* = 3′ or 4′) against *Plasmodium falciparum* in in vitro models ☆

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ABSTRACT

The synthesis (Pd-mediated coupling strategy) and characterization (NMR, IR, elemental analysis, etc.) of a short series of quinoline–oxazole hybrid compounds has been carried out. These materials are found to be moderately active against *Plasmodium falciparum* in vitro, with activities in the sub-micromolar range, and to display acceptable cytotoxicity to mononuclear leukocytes. Chemical modification strategies, with the intention to increase the biological potency of this new class of anti-malarial agents, are discussed.



Malaria infection results in over 300 million clinical cases and 1.5–2.7 million deaths worldwide per year. Over half of these cases are caused by the most virulent human malaria species, *Plasmodium falciparum*.[1] This apicomplexan protozoan alternates between asexual reproduction in humans and sexual reproduction in female mosquitoes of the genus Anopheles. Following initial infection of human hepatocytes, *P. falciparum* reproduces asexually through erythrocytic schizogony every 36–48 h.

Classical anti-malarial compounds (e.g., quinine: Fig. 1) antagonize *P. falciparum* by inducing heme accumulation in the parasite membrane, consequently disturbing cation homeostasis and resulting in parasite death.[2,3] The characteristic quinoline ring is found in both the natural product quinine[4] and the synthetic anti-malarial chloroquine (**CQ**: Fig. 1). This latter molecule is a relatively inexpensive and highly effective 4-amino-quinoline anti-malarial and was for some time the first choice for the treatment of uncomplicated falciparum malaria despite its notable side effects.[5,6] However, **CQ** is presently ineffective in the majority of areas of endemic malaria due to the development and spread of resistant *P. falciparum* isolates.[5,7–10]

Alternative anti-malarials such as mefloquine, artemisinin and their derivatives are used to treat these drug-resistant infections, but these drugs increase treatment costs as much as 10-fold. There is also an increased risk of other side effects with these novel compounds such as neurotoxicity.[3,11] Proposed solutions to the expanding drug resistance includes research into novel modes of drug action[8,12] and synthetic modifications of existing anti-malarial agents. Specifically, this latter strategy typically involves chemical alteration of side chain functional groups. The overall goal is to create novel molecular scaffolds which can evade resistance mechanisms to the original mother compound.

Oxazole derivatives, such as the 2-amino-1,3-oxazoles and the 4,5-dihydro-1,3-oxazoles (i.e., the 2-oxazolines), have long been recognized for their potent biological activity[13–15] and relatively low cost of production. To our knowledge, the application of an oxazoline derivative as a potential anti-malarial agent has not hitherto been investigated. In this report, we detail the synthesis and in vitro anti-malarial testing of a short series of quinoline–oxazole

☆ Oxazoles XXVII.

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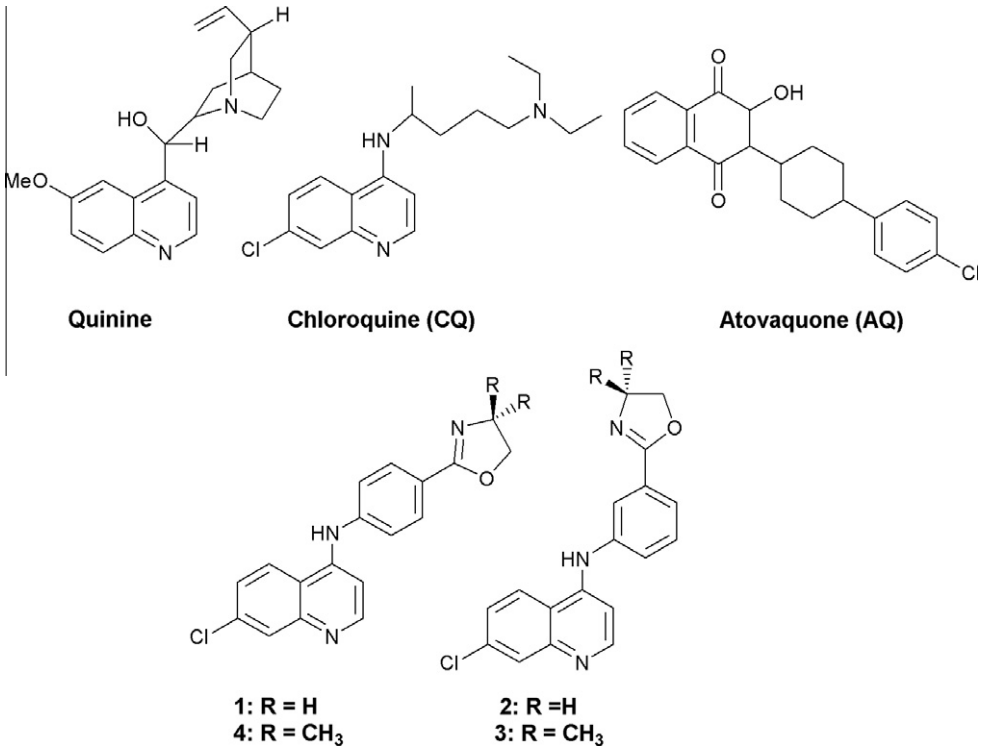
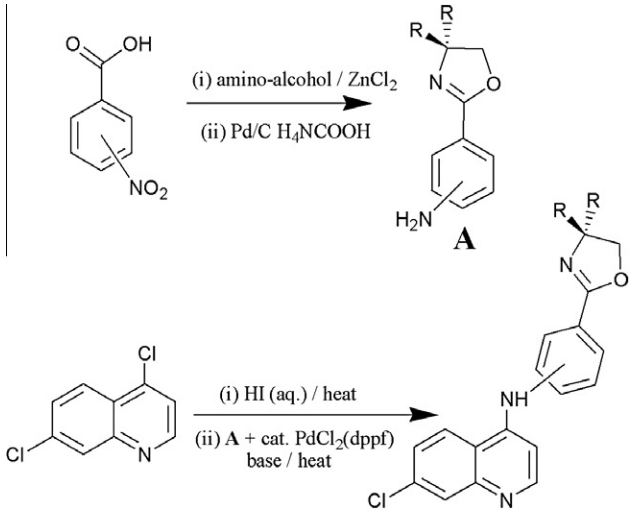



**Figure 1.** The structures of quinine, **CQ**, **AQ** and compounds **1**–**4**.




**Scheme 1.** The general reaction protocols leading to compounds **1**–**4**.

hybrids (Fig. 1: compounds **1**–**4**) and establish $IC_{50}$ data for both these molecules against **CQ** resistant (K1) and sensitive (3D7) *P. falciparum* cell lines.

Compounds **1**–**4** (Fig. 1) were synthesised (Scheme 1) by adapting a Pd-mediated cross-coupling procedure (Buchwald–Hartwig reaction)[16,17] involving 4-iodo-7-chloroquinoline[18] (via commercial 4,7-dichloroquinoline) and an appropriate aniline with a *meta*- or *para*-located 1,3-oxazole substituent.[19,20] These latter components were in turn produced from the suitably substituted 2-(nitrophenyl)-2-oxazoline[20–22] by standard reduction protocols (10% Pd/C, $HCOONH_4$ in EtOH: reflux: Scheme 1).[23,24] Purification was performed using standard flash chromatographic separation and recystallisation procedures.[25–27]

Compounds **1**–**4** were initially screened on cultures of *P. falciparum* clone 3D7A to evaluate if these materials exhibited any anti-malarial activities.[28] Qualitative estimates based on examination of blood films made from treated cultures (Supplementary data) suggested that all four compounds were active in the 1 μM range and could facilitate complete cell eradication upon extended exposures (2–7 d). Having established this aspect, precise $IC_{50}$ values were then established.[33] In addition, the general cytotoxicity profile was determined on isolated mononuclear leukocytes (MNL) using standard protocols.[34,35]

When compared to **CQ** and Atovaquone (**AQ**),[4] compounds **1**–**4** are about an order of magnitude less active against *P. falciparum* in vitro (Table 1). Of the four novel compounds, compound **3**, the *meta*-substituted derivative with further substitution on oxazoline ring position-4, is the most promising. It has been previously shown that the replacement of H by a methyl group has little effect on the donor ability of the oxazoline (i.e., the electronic nature of the heterocycle as quantified by $pK_a$ values).[36] Previous examples of aniline-derived quinoline anti-malarial agents have demonstrated superior activity for *para*-appended aromatic derivatives (e.g., pyronaridine, amodiaquine, etc.)[37] and hence our observed activity trend here is contradictory to this general observation.

Although MNL, including monocytes, macrophages and dendritic cells, are not the target of 4-aminoquinoline toxicity, these cells are easily obtained in large numbers from peripheral blood and have been used by others in simple quantitative comparisons of compound cytotoxicity.[38–40] **CQ** application to peripheral blood MNL by Winstanley et al.[40] resulted in a significant degree of cell death compared to control cells, dependent on concentration over the 1–100 μM range. This observed toxicity was consistent with 5–500 μM application of **CQ** and compounds **1**–**3** to monocytes, for a five-day incubation period (Supplementary data). Compound **4**, the lowest active novel compound, was precluded from these cytotoxicity trails. Treatment with compounds **1** and **2** resulted in gradually decreasing cell health with increased drug concentration, including the presence of cell fragments and small

**Table 1**
$IC_{50}$ data (μM) for **CQ**, Atovaquone (**AQ**) and compounds **1**–**4** tested against *P. falciparum* strains K1 (**CQ** resistant) and 3D7 (**CQ** sensitive)[a]

| Strain | $IC_{50}$ (μM) | | | | | |
|---|---|---|---|---|---|---|
| | **CQ** | **AQ** | **1** | **2** | **3** | **4** |
| K1 | NM[a] | 0.0177 (±0.0002) | 0.846 (±0.156) | 0.895 (±0.134) | 0.711 (±0.127) | >1[b] |
| 3D7 | 0.0177 (±0.0002) | NM[a] | 0.192 (±0.012) | 0.091 (±0.015) | 0.083 (± 0.026) | 0.308 (± 0.091) |

[a] NM = not measured.
[b] Value >1 not precisely determined.

macrophages without visible pseudopodia, following incubation in 500 μM (Table S1: Supplementary data). This concentration dependent pattern and the general qualitative observations of monocytes treated with compounds **1** and **2** were very similar to those cells subjected to **CQ** solutions. In the two highest concentrations of compound **3**, crystal aggregations in globular- and spike-form were clearly visible and the cells were considerably more damaged compared to application of all other compounds. A general conclusion to these observations is that compounds **1**–**3** have a general toxicity profile similar to **CQ** and are hence at least relatively non-toxic to non-target cells.

Mechanisms for drug accumulation in the food vacuole that include a 'receptor' for **CQ**, an intravacuolar receptor, free heme molecules acting as a receptor, or a carrier-mediated method, stress the importance of this 3-D drug structure and subsequent effectiveness of the compounds themselves.[4,5,37,41] Alternatively, the basicity of a compound affects anti-malarial activity in the weak base model, which proposes the difference in pH between the external medium and the food vacuole as the single determinant of **CQ** accumulation.[5] Considering the very similar expected $pK_a$ values between the novel compounds, differences in anti-malarial activity between the individual novel quinoline compounds and between these compounds and **CQ** are likely attributable to variation in the side chain structure, supportive of the receptor mechanism for drug accumulation. The in vitro results also suggest, based on these concepts, that increasing the overall basicity of derivatives having the same general structural characteristics of **3** might facilitate greater biological potency. This will be examined in later compounds by, for example, the replacement of the oxazoline by a more basic oxazole or incorporation of EDGs or more basic functionalities onto the heterocyclic ring system.[15] These modifications may result in sufficient structural changes of our materials compated to that of **CQ** with the objective of evading the **CQ**-resistance mechanism(s). It has been noted that **CQ** derivatives with shortened and lengthened amine side chains have been shown to exhibit undiminished activity against resistant isolates.[42]

In both the anti-malarial and cytotoxicity analysis, compounds **2** and **3** demonstrated slightly superior activity (in terms of $IC_{50}$ values at 48 h) and the latter an acceptable toxicity profile. Application of compound **1** resulted in the death of all detectable parasites (7 d incubation) at the three highest drug concentrations, proving to be slightly more effective at outright cell death rates than compounds **2**–**4** but all with an overall lower potency than **CQ**. Furthermore, compound **1** was minimally toxic to monocytes, compared to the other compounds (**2**, **3** and **CQ**).

These data suggest that although the general class of quinoline–oxazole hybrids appear to have promise as anti-malarial agents due to their low toxicity and ease of syntheses, considerable improvements to the general potency, most importantly increasing activity to the nanomolar level, will be necessary for these compounds to be useful drug candidates. This facet might be facilitated by the incorporation of electron withdrawing groups or other basic side chains to the generalized quinoline–oxazole structure, and such endeavors are currently a focus of our research.

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## Supplementary data

Supplementary data (Qualitative anti-parasitic properties) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.05.131.

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27. *Typical procedure* (***1***): Under an inert atmosphere, 33.5 mg (0.046 mmol) of $[Pd(dppf)Cl_2]$ and 76 mg (0.14 mmol) of dppf[16,17] were added to a solution of 0.265 g (0.917 mmol) of 7-chloro-4-iodoquinoline[18] and 0.128 g (1.14 mmol) of *t*-BuOK suspended in 8 ml of 1,4-dioxane. A sample (0.216 g: 1.14 mmol) of 2-(4′-anilinyl)-4,5-dihydro-1,3-oxazole was then added to this mixture. The contents were then heated to reflux temperature with stirring (3 h) and then cooled to room temperature; all volatile materials were then removed (rotary evaporation). The resulting products were subjected to separation by flash column chromatography ($SiO_2$: 230–400 mesh; EtOAc/$Et_2O$: 3:2 v/v as eluent) to yield *product* **1** in the form of a yellow powder (35%). Mp 212–215 °C. $R_f$

($CH_2Cl_2$/MeOH: 96:4 v/v): 0.162. Elemental analysis calculated (calcd) for $C_{18}H_{14}N_3OCl·½(H_2O)$: C, 64.97; H, 4.54; N, 12.63. Found: C, 65.06; H, 4.42; N, 13.28. $^1$H NMR ($CDCl_3$; 300 MHz: ppm vs TMS): $\delta_H$ = 4.08 (t, 2H, *J* = 9.3 Hz), 4.47 (t, 2H), 6.98 (br, 1H), 7.16 (d, 1H, *J* = 5.1 Hz), 7.29 (m, 2H), 7.47 (d, 1H, *J* = 8.1 Hz), 7.91–7.99 (m, 3H), 8.05 (s, 1H), 8.64 (d, 1H, *J* = 9.3 Hz). Selected IR (KBr: $cm^{-1}$): 3259 (m), 1646 (s), 1568 (s), 1174 (s), 1072 (s). *Physical and spectroscopic data for* ***2*****–*****4***. Compound **2** (15%): cream colored solid. Mp 190–191 °C. $R_f$ ($CH_2Cl_2$/MeOH: 96:4 v/v): 0.209. Elemental analysis calcd for $C_{18}H_{14}N_3OCl·¼(H_2O)$: C, 65.86; H, 4.45; N, 12.80. Found: C, 65.92; H, 4.48; N, 12.56. $^1$H NMR ($CDCl_3$; 300 MHz: ppm vs TMS): $\delta_H$ = 4.09 (t, 2H, *J* = 9.3 Hz), 4.47 (t, 2H), 6.81 (br, 1H), 7.01 (br, 1H), 7.40–7.50 (m, 3H), 7.78 (d, 1H, *J* = 7.2 Hz), 7.89 (s, 1H), 8.05 (s, 1H), 8.60 (br, 1H). Selected IR (KBr: $cm^{-1}$): 3285 (m), 1654 (s), 1215 (s), 1080 (s). Compound **3** (45%): yellow colored solid. Mp 109–110 °C. $R_f$ (EtOAc/$Et_2O$: 3:2 v/v): 0.372. Elemental analysis calcd for $C_{20}H_{18}N_3OCl·(H_2O)$: C, 64.96; H, 5.45; N, 11.36. Found: C, 64.78; H, 5.38; N, 11.45. $^1$H NMR ($CDCl_3$; 300 MHz: ppm vs TMS): $\delta_H$ = 1.40 (s, 6H), 2.10 (br, 1H), 4.14 (s, 2H), 6.98 (d, 1H, *J* = 5.4 Hz), 7.44–7.47 (m, 3H), 7.75 (d, 1H, *J* = 6.9 Hz), 7.87 (d, 2H, *J* = 9.0 Hz), 8.04 (d, 1H, *J* = 2.1 Hz), 8.56 (d, 1H, *J* = 5.4 Hz). Selected IR (KBr: $cm^{-1}$): 3429 (m), 1644 (s), 1569 (s), 1320 (s), 1080 (s). Compound **4** (51%): light yellow colored solid. Mp: 200–201 °C. $R_f$ (EtOAc/$Et_2O$: 3:2 v/v): 0.196. Elemental analysis calcd for $C_{20}H_{18}N_3OCl·½(H_2O)$: C, 67.41; H, 5.23; N, 11.79. Found: C, 67.48; H, 5.34; N, 11.22. $^1$H NMR ($CDCl_3$; 300 MHz: ppm vs TMS): $\delta_H$ = 1.41 (s, 6H), 4.14 (s, 2H), 7.00 (s, 1H), 7.14 (d, 2H, *J* = 5.1 Hz), 7.30 (d, 1H, *J* = 8.7 Hz), 7.47 (d, 1H, *J* = 4.9 Hz), 7.95 (m, 2H), 8.05 (s, 1H), 8.63 (d, 1H, *J* = 5.4 Hz). Selected IR (KBr: $cm^{-1}$): 3160 (m), 1648 (s), 1570 (s), 1173 (s), 1067 (s).

28. Cultures of *P. falciparum* clone 3D7A (**CQ** sensitive) were obtained (2003) from the laboratory of Prof. K. Kain (University of Toronto) and thawed from storage in $N_2(l)$ following the protocol described by Aley and colleagues.[29] Parasites were cultured in a 12-well culture plate at 5% hematocrit in endotoxin-free RPMI 1640 media supplemented with 10% heat-inactivated human serum (complete medium) as described by Trager and Jensen[30] and modified by Smith et al.[31,32] The four novel compounds and commercial CQ diphosphate salt (Sigma–Aldrich) were each dissolved in 100 μL DMSO (Sigma–Aldrich) and added to complete media to produce logarithmic concentrations of 0.5 μM, 5 μM, 50 μM, and 500 μM, plus 0.05 μM additionally for CQ trials. The drugs were applied in two-day and four-day dosages to 1 mL parasite cultures at approximately 1% total parasitaemia on day 0, consisting of a mixture of ring and mature stages. Drugs were removed from parasite cultures after the dosage period by washing them three times in complete medium. Parasitaemia was monitored for an 11-day period through microscopic examination of culture blood smears stained with Hema 3 (Fisher Scientific, Ottawa, ON). If any cultures reached 5% parasitaemia, they were diluted to 1% parasitaemia. Total parasitaemia was recorded as 0.00% when fifty 500-cell fields of view were examined with no evidence of living parasites. Each compound was tested for a total of three trials.
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34. Compounds **1**–**3** and **CQ** were each dissolved in 100 μL DMSO and added in solution to endotoxin-free RPMI 1640 supplemented with 10% fetal calf serum (HyClone, Logan, Utah), to produce logarithmic concentrations of 5 μM, 50 μM, and 500 μM. Monocytes were isolated from human volunteers following McGilvray et al.,[35] and inoculated into a 24-well culture plate. Following 45 min relaxation at 37 °C, a 1 mL volume of drug solution was added to approximately $1.25 \times 10^5$ monocytes in each well. Following 5 d incubation at 37 °C, the cells were examined using live phase-contrast microscopy to assess general health and possible failure to differentiate into macrophages. The plate was removed from incubation for a maximum time of 30 min., with re-incubation periods of 15 min., to prevent temperature-induced cell stress. Each compound was tested for a total of six trials, two for each of the three volunteers.
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