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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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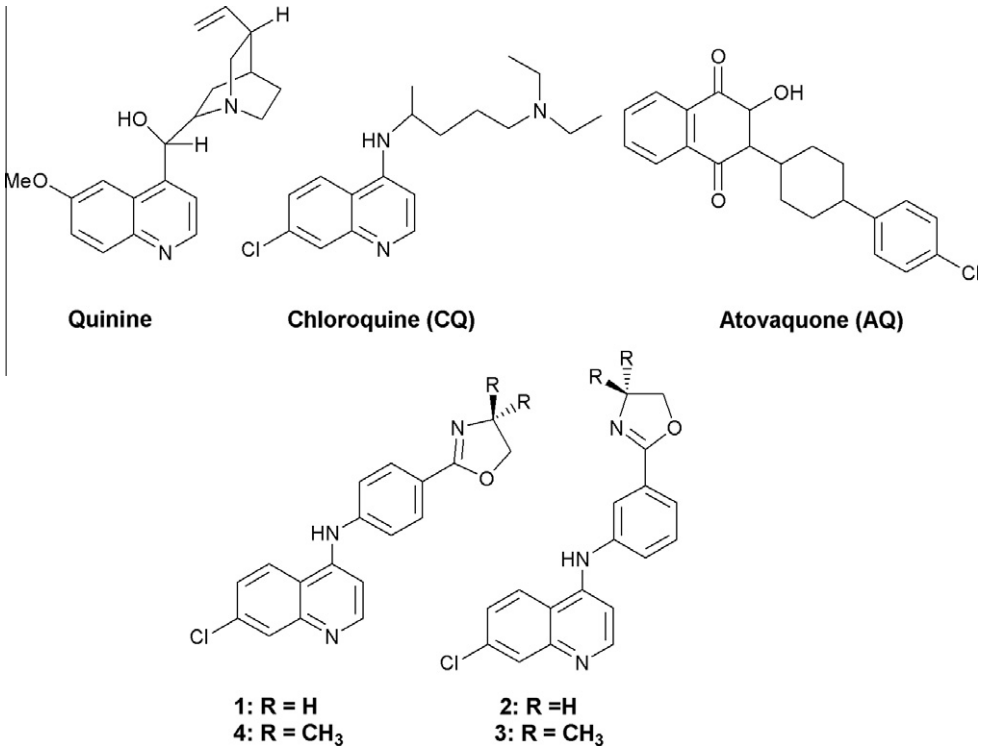
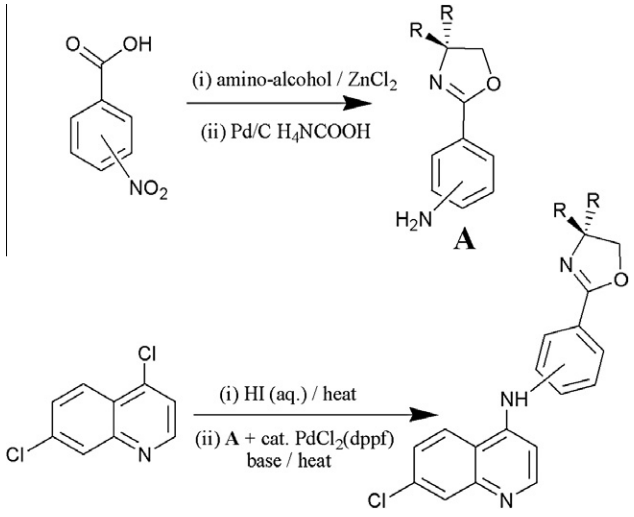


Figure 1. The structures of quinine, **CQ**, **AQ** and compounds **1**–**4**.



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