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Investigation into novel thiophene- and furan-based 4-amino-7-chloroquinolines afforded antimalarials that cure mice

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ABSTRACT

We herein report the design and synthesis of a novel series of thiophene- and furan-based aminoquinoline derivatives which were found to be potent antimalarials and inhibitors of β-hematin polymerization. Tested compounds were 3–71 times more potent in vitro than CQ against chloroquine-resistant (CQR) W2 strain with benzonitrile **30** being as active as mefloquine (MFQ), and almost all synthesized aminoquinolines (22/27) were more potent than MFQ against multidrug-resistant (MDR) strain C235. In vivo experiments revealed that compound **28** showed clearance with recrudescence at 40 mg/kg/day, while 5/5 mice survived in Thompson test at 160 mg/kg/day.

1. Introduction

Malaria is a devastating tropical disease—caused by five Plasmodium species: *P. falciparum*, *P. ovale*, *P. vivax*, *P. malariae*, and *P. knowlesi*—resulting in over 580,000 deaths in 2013 alone.[1] The increasing number of multidrug resistant *P. falciparum* (*P.f.*) strains to available drugs, and in particular to chloroquine (**1**, CQ, Fig. 1),[2] which has been the standard antimalarial chemotherapeutic for many years,[3] seriously complicates the prevention and treatment of malaria.

Clinical symptoms of malaria occur during the intra-erythrocytic asexual phases,[4] therefore the majority of antimalarial drugs target this stage of the lifecycle. During the blood stage, *P.f.* infests red blood cells (RBC) and within their food vacuole (FV) digests hemoglobin, releasing small peptides (transported to the cytoplasm) and ferriprotoporphyrin-IX (Fp(III)-IX) (obtained upon oxidation of the initially formed ferroprotoporphyrin-IX (Fp(II)-IX)). One of the defenses that protect it against the toxicity of Fp(III)-IX involves the sequestering of the toxic heme byproduct into insoluble hemozoin. Aminoquinoline-based antimalarials, such as CQ, interfere with hemozoin formation. In addition, it has been suggested that 4-amino-7-chloroquinoline-(ACQ)-based drugs may also act as inhibitors of oxidative[5] and glutathione-mediated[6] heme degradation. It appears that the heme detoxification pathway is not directly involved in CQ resistance; it has been reported that CQ resistance is related to mutations in drug transporters (PfCRT, Pgh1, and PfMRP) that affect drug accumulation in the parasite by reducing drug uptake, or increasing drug efflux, or both.[7,8] Recent research to develop novel ACQ-based compounds that bypass CQ resistance is resulting in the synthesis of potentially promising new antimalarial agents, including 2′,2′-dimethyldiamines,[9] ACQ heteroaryl derivatives,[10] 4-aminoquinoline/clotrimazole-based hybrids,[11] aminoquinoline-pyrimidine hybrids,[12] aminoquinoline-tetrazole combinations,[13] and quinoline–pyrimidine hybrids.[14] A related series of aminoquinolines with tertiary amino side chains containing furan and thiophene moieties were

Abbreviations: ART, artemisinin; ACQ, 4-amino-7-chloroquinoline; CQ, chloroquine; MFQ, mefloquine; MLM, HLM, mouse and human liver microsomes, respectively.

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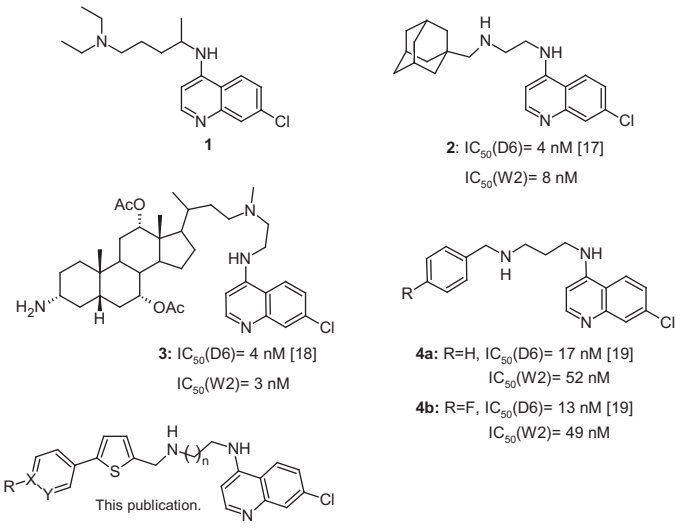

Figure 1. Aminoquinoline antimalarials.

recently reported with excellent in vitro potencies against CQR W2 and CQS 3D7 *P.f.* strains.[15,16]

Our own research in the field of antimalarial ACQs afforded adamantane-[17] and steroidal-derived compounds,[18] **2** and **3**, respectively, as well as simple aminoquinolines bound to a single aromatic ring **4a** and **4b**,[19] Figure 1.

Herein, we report on the synthesis and the activity of a new generation of aminoquinoline antimalarials with thiophene or furan ring linking aminoquinoline and aromatic moiety.

2. Results and discussion

2.1. Rationale

In our previous paper we reported on simple aminoquinoline molecules significantly inhibiting CQR W2 and CQS D6 *P.f.* strains in vitro.[19] One of them, **4a**, was metabolically stable (HLM, MLM, $t_{½} \geqslant 60$ min) and recently we decided to further examine its activity against two additional *P.f.* strains: CQS 3D7 and CQR Dd2. In vitro activity against CQS 3D7 strain was very close to that of CQS D6 strain (IC_{50} = 23 nM vs IC_{50} = 17 nM, respectively), and the activity of the same compound **4a** against CQR Dd2 strain was close to that of CQR W2 strain (IC_{50} = 36 nM vs IC_{50} = 52 nM, respectively). We then submitted compound **4a** to in vivo screening against *P. berghei* at concentrations of 160 and 80 mg/kg/day in Thompson test, resulting with 1/5 mice cured at 160 mg/kg/day (vide infra).

The driving force of hematin:CQ complex formation appears to be a π–π stacking[20,21] consequently, our strategy was to design an aminoquinoline antimalarial with an additional π-system attached to the aminoquinoline to exploit interference with hemozoin formation from hematin.[22,23] We explore the influence of introducing a thiophene (and furan) linkage between the requisite aminoquinoline and aryl group on antimalarial activity.

2.2. Synthesis

The synthesis of the target compounds **15**–**32** utilized a simple protocol in which starting thiophene **5** and furan **6** derivatives and respective arylboronic acids were submitted to Suzuki–Miyaura reaction conditions affording corresponding intermediates **7**–**14** in good to excellent yields (57–92%, Scheme 1).[24–26] Subsequent reductive amination using *N*-(7-chloroquinolin-4-yl)ethane-1,2-diamine (ACQ2) or *N*-(7-chloroquinolin-4-yl)propan-1,3-diamine (ACQ3) gave the target compounds **15**–**32** (Scheme 1), and **41**, **42**, **43** (Fig. 2) in up to 88% yield. The exception was the yield of 2-chloropyrimidin **34** (36%), an intermediate to 2-aminopyrimidines **35** and **36** (Scheme 2). Compounds **37**–**40** (Fig. 2) were obtained in an analogous manner starting from appropriate 5-(pyridinyl)thiophene-2-carbaldehydes (for the preparation of compounds **37**–**43** please consult the Supplementary material).

2.3. In vitro antiplasmodial activity

All 27 synthesized aminoquinoline compounds were analyzed in vitro for their antiplasmodial activity against three *P. falciparum* strains: D6 (CQ susceptible (CQS) strain), W2 (CQ resistant (CQR) strain), and TM91C235 (multidrug resistant (MDR) strain), and for their toxicity against rat macrophage cell line RAW 264.7 (Table 1). In brief, the assay relies on the incorporation of radiolabeled hypoxanthine by the parasites and inhibition of isotope incorporation is attributed to activity of known or candidate antimalarial drugs.

Beside relatively weakly active derivatives **41** and **42**, tested compounds were several-fold (3-71) more potent in vitro than CQ against CQR W2 strain (12 times on average, Table 1), with benzonitrile **30** being as active as mefloquine (MFQ). However, the more important finding was that 10/27 compounds were >2 times more potent against CQS D6 strain than CQ, and that almost all (22/27) synthesized aminoquinolines were more potent than MFQ against MDR strain C235.

Detailed SAR analysis of the in vitro activity against CQS D6 strain revealed that the activity of our new antiplasmodials possessing an ethylene spacer between the aminoquinoline moiety and thiophene ring does not appreciably depend on the substitution pattern on benzene ring, Table 1, compounds **15**, **17**, **19**, **21**, **23**, **27**, and pyrimidine **34**, pyridines **37**, **39**, and furan derivative **31** (IC_{50}(D6) = 2–9 nM). A similar conclusion can be drawn for CQR W2 strain and for MDR C235 strain, as well as for respective IC_{90} activities thereof (see Supplementary material). In the series with the propylene spacer the most active compound across all strains is **30** with SI ⩾400 (Table 1). The in vitro activity of the other antiplasmodials with C3 spacer is dependent on aromatic ring substitution pattern. However, it is evident that the introduction of the methyl group on the basic nitrogen, and in α-, and α′-positions thereof sharply attenuates the potency of the candidate antiplasmodials (**41**, **42**, **43** vs **28**). In addition, comparison of thiophene and furan analogs **15**, **16** vs **31**, **32** indicated that besides similar in vitro activity of the two pairs, thiophenes exhibited higher activity-to-toxicity ratio (selectivity index, Table 1), and thiophene **16** appeared metabolically considerably stable ($t_{½\,HLM}$ = 45 min). We conclude that both aminoquinoline-thiophene hybrids appear less active against CQR W2 strain than against respective CQS strains D6 and 3D7 than the thiophenes evaluated previously.[15,16] In addition, we found that 7 of our thiophene compounds (**15**, **21**, **23**, **27**, **29**, **30**, **40**) were 15–40 times more active than MFQ against MDR C235 strain.

The toxicity of the tested antiplasmodials was assessed in vitro using rat macrophage cell line RAW264.7. Several compounds were also tested for toxicity against human liver carcinoma cell line (HepG2) and peripheral blood mononuclear cells (PBMC), Table 1. In general, the assays for cytotoxicity estimation revealed that all compounds are well tolerated by the three cell lines, possessing $IC_{50} \geqslant 1000$ nM. In addition, high selectivity indices (SIs) were calculated for the most promising compounds **27**, **28**, and **30** (SI = 265–2138). This clearly indicates that our new aminoquinolines are quite interesting new antimalarials which exert low toxicity as compared to their respective in vitro activities.