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# Synthesis and in Vitro and in Vivo Pharmacological Evaluation of New 4-Aminoquinoline-Based Compounds

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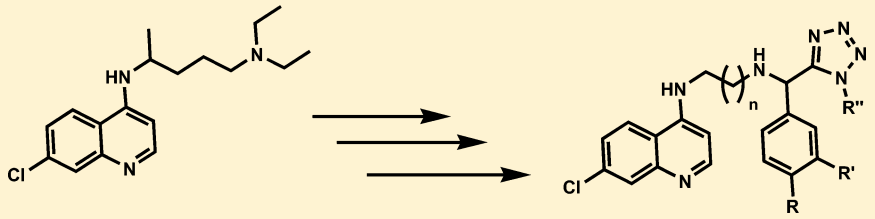
Supporting Information




**ABSTRACT:** A new class of 4-aminoquinolines was synthesized and evaluated in vitro for antiplasmodial activity against both the chloroquine-sensitive (3D7) and -resistant (K1 and W2) strains. The most active compounds **3c**−**3e** had acceptable cytotoxicity but showed strong inhibition toward a panel of cytochrome P450 enzymes in vitro. Pharmacokinetic studies on **3d** and **3e** in mice showed that they had moderate half-life (4−6 h) and low oral bioavailability. The front runner compound **3d** exhibited moderate inhibition of the malaria parasite on *P. berghei* infected mice following oral administration (5 mg/kg), achieving reduction of parasitemia population by 47% on day 7.

**KEYWORDS:** *Aminoquinolines, antiplasmodial activity, pharmacokinetics, plasma protein binding*

Chloroquine (**1**) (Figure 1) exerts its activity against the erythrocytic forms of all plasmodial species and is believed to function by inhibiting formation of hemozoin in the *Plasmodium falciparum* digestive vacuole (DV).[1] The attractive features of chloroquine (CQ) are its mild side-effects and affordability.[2]

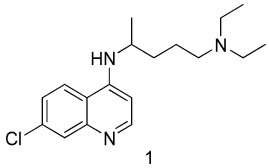



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**Figure 1.** Chloroquine.

However, the clinical use of this drug is limited by the widespread development of resistant-strains. The Roll Back Malaria (RBM) consortium devised the following strategies in mitigating the emergence and spread of antimalarial drug resistance; (i) use of combination therapies, (ii) use of parasite-resistance reversers, (iii) drug-repositioning, and (iv) rationally designing analogues of the currently administered drugs and using them in such a way that cross-resistance is circumvented.[3,4] With regards to the last point, various studies have shown that lengthening or shortening the chloroquine alkyl side-chain leads to compounds that are effective against drug-resistant strains of *P. falciparum*.[5] However, Roepe et al.[6] recently concluded that these observations only hold for quinoline derivatives that contain a diethyl substituent on the terminal nitrogen. Although, there is still some debate over the effects of modifying the length of the alkyl side-chain, there is a consensus that such modifications do not improve the

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metabolic stability, especially the N-dealkylation of the terminal tertiary amino group that normally occurs during metabolism of CQ and amodiaquine.[2,7] This N-dealkylation reduces lipid solubility of these drugs and their derivatives, an effect that is suggested to result in an increase in the potential for cross-resistance with CQ.[8] As a direct consequence of this observation, O'Neill et al.[9] and Guy et al.[10] showed that the use of bulkier substituents (such as piperidyl, pyrrolidinyl, and morpholinyl rings) attached to the terminal amino group increased the in vivo efficacy and simultaneously decreased the potential for cross-resistance, presumably by circumventing metabolic N-dealkylation.

Inspired by the work of O'Neill et al.[9] and Guy et al.,[10] we have rationally designed our target compounds largely around circumventing metabolic N-dealkylation by incorporating bulkier substituents such as aromatic and tetrazole rings while varying the length of the alkyl side-chain (Figure 2). The incorporation of the tetrazole ring is based on the rationale that was highlighted previously.[11−13]

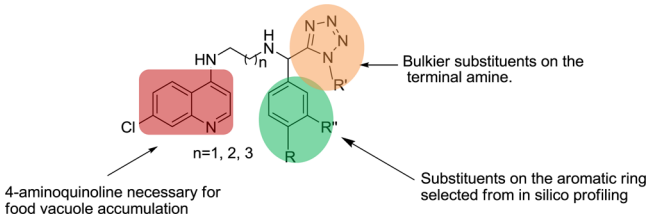
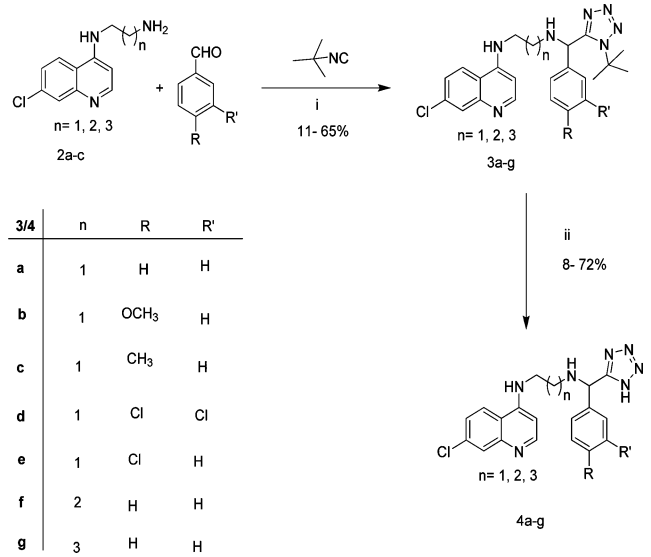



**Figure 2.** Rationale for design of 4-aminoquinoline-based target compounds.

Thus, in this letter, we describe the synthesis and pharmacological evaluation of new 4-aminoquinolines. Briefly, the synthesis commenced with the preparation of key quinoline diamines (**2a−c**) following known literature procedures (Scheme 1).[14]

These diamines (**2a−c**) were then treated under multicomponent reaction (MCR) conditions with various aromatic aldehydes in the presence of *tert*-butyl isocyanide and trimethylsilane azide ($TMSN_3$) using a previously described procedure to give compounds **3a−g**.[11−13] Subsequent, cleavage of the *tert*-butyl group gave rise to compounds **4a−g** in low to moderate yields after purification by column chromatography. All the synthesized target compounds were fully characterized by analytical and spectroscopic techniques with their HPLC purity greater than 95% (Supporting Information).

**Scheme 1**[a]




| 3/4 | n | R | R' |
|---|---|---|---|
| a | 1 | H | H |
| b | 1 | $OCH_3$ | H |
| c | 1 | $CH_3$ | H |
| d | 1 | Cl | Cl |
| e | 1 | Cl | H |
| f | 2 | H | H |
| g | 3 | H | H |

[a]Reagents and conditions: (i) $TMSN_3$, MeOH, 40 °C, 24 h; (ii) neat 32% $HCl_{(aq)}$, 120 °C, 4 to 8 h.

The protocols followed for antiplasmodial evaluation of the target compounds were the same as those described previously.[11] Furthermore, because of the poor activity of **4a−g** in the K1 strain compared to **3a−g**, we did not find it necessary to do further testing, against the 3d7 and W2 strains. Compounds **3c−3g** were all more efficacious than CQ on all the tested *P. falciparum* strains; more specifically, against the chloroquine-resistant K1 strain, 6 (**3a** and **3c−3g**) out of the 14 compounds showed greater activity than chloroquine (CQ), with compound **3e** ($IC_{50}$ = 1.0 nM) exhibiting a 36-fold greater activity (Table 1).

**Table 1. In Vitro Antiplasmodial Activity and Solubility of Target Compounds**

| | $IC_{50}$ (μM) | | | | solubility[b] (μM) | |
|---|---|---|---|---|---|---|
| entry | 3D7 | K1 | W2 | RI[a] | pH 2.0 | pH 7.4 |
| **3a** | 0.005 | 0.038 | 0.069 | 7.6 | >200 | 111 |
| **3b** | 3.821 | 0.155 | 0.046 | 0.041 | nd | nd |
| **3c** | 0.001 | 0.002 | 0.0311 | 2 | 127 | 39.6 |
| **3d** | 0.0004 | 0.008 | 0.0305 | 20 | 194 | 25 |
| **3e** | 0.002 | 0.001 | 0.0255 | 0.5 | >200 | 6.88 |
| **3f** | 0.002 | 0.027 | 0.039 | 13.5 | nd | nd |
| **3g** | 0.002 | 0.008 | 0.020 | 4 | nd | nd |
| **4a** | nd | 14.9[c] | nd[d] | | nd | nd |
| **4b** | nd | 13.2[c] | nd | | nd | nd |
| **4c** | nd | 17.4[c] | nd | | nd | nd |
| **4d** | nd | 4.39[c] | nd | | 191 | 70.1 |
| **4e** | nd | 2.38[c] | nd | | >200 | 164 |
| **4f** | nd | 14.26[c] | nd | | nd | nd |
| **4g** | nd | 6.849[c] | nd | | nd | nd |
| CQ | 0.0052 | 0.036 (0.220[c]) | 0.059 | 6.9 | >200[e] | >200[e] |

[a]Resistance index $IC_{50}$ (KI)/$IC_{50}$ (3D7). [b]Kinetic solubility. [c]Antiplasmodial testing done at the Swiss Tropical and Public Health Institute (STPH). [d]nd: not determined. [e]Chloroquine tested as a diphosphate salt.

Similarly, against another CQ-resistant W2 strain, the majority of the tested compounds were more active than CQ, with the exception of **3a** that had comparable activity to CQ. In terms of structure−activity relationships, the compound with the shortest ethylene spacer, **3a**, was less efficacious than the compounds with longer ethylene spacers, **3f** and **3g**. Moreover, compounds **3a**, **3c**, and **3g**, with resistance index (RI) values of 7.6, 2, and 4, respectively, show a low-level cross-resistance potential with CQ. At this juncture, it is worth noting that compounds **3b** and **3e** have RI values that are lower than 1, an indication of a reduced likelihood to develop cross-resistance with CQ. On the basis of in vitro antiplasmodial activity, the in vitro ADME characterization of the selected compounds, **3c−e**, was assessed. The kinetic solubility of these selected compounds was generally poorer than that of CQ diphosphate at both pH regions (Table 1). In terms of cytotoxicity, compounds **3c−3e** exhibited acceptable cytotoxicity against the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Chinese Hamster Ovarian (CHO) cell-lines, as indicated by their $IC_{50}$ values (Table 2).

**Table 2. Cytochrome P450 Inhibition and Cytotoxicity Evaluation of Compounds 3c−e**

| | CYP450 $IC_{50}$ | | | | | |
|---|---|---|---|---|---|---|
| entry | 2C8 | 2C9 | 2C19 | 2D6 | 3A4 | cytotoxicity[b] ($IC_{50}$) ($\mu$M) |
| **3c**[a] | 3.98 | 10.3 | >20 | 9.88 | 0.17 | 185.1 |
| **3d** | nd | nd | nd | nd | nd | 10.5 |
| **3e**[a] | 12.7 | 8.91 | 12.4 | 4.0 | 0.08 | 97.8 |
| emetine | | | | | | 0.129 |

[a] $IC_{50}$ was >20 $\mu$M for CYP1A2, 2A6, 2B6 and 2E1. [b] Chinese Hamster ovarian cell-lines.

Cytochrome P450 (CYP450)-mediated drug−drug interaction potential of compounds **3c** and **3e** was assessed by determining their inhibition potentials against a panel of CYP450s in human liver microsomes. Both these compounds showed moderate inhibition of P450 2C8, 2C9, 2C19, and 2D6 and relatively strong inhibition of CYP3A4. Since CQ is not a known inhibitor of CYP3A4,[15] in silico docking studies were undertaken in an attempt to elucidate the molecular basis of this inhibition. The interpretation of results was based on the proximity of the ligands to the heme and the associated binding energies. The lowest energy conformations of both compounds and CQ were docked onto a crystal structure of CYP3A4 (PDB code 2V0M) using Glide software (www.schrodinger.com).[16] One preferred ligand orientation was identified for each of **3c**, **3e**, and CQ. Compounds **3c** and **3e** adopt a conformation where their aminoquinoline rings are aligned parallel and closer to the heme, while for CQ the ring is aligned almost perpendicular to the heme (Figure 3). In addition, the binding affinities of the two molecules are higher than those of CQ, and their binding energies are comparable to that of ketoconazole, a strong CYP3A4 inhibitor (see Supporting Information). Thus, these findings suggest that the derivatives orient differently in the active site of the enzyme and that their interactions with the active site of the enzyme and near residues (e.g., in addition to hydrophobic interactions, **3c** forms a hydrogen bond with SER119) lead to more stable bound conformations, which increase their propensity for inhibition.

The metabolic stability of compounds **3d** and **3e** was assessed in human and mouse liver microsomes. Both compounds were rapidly metabolized in both species ($E_H$ > 0.8, see Supporting Information). LC−MS/MS was used to tentatively identify the metabolites of these compounds in microsomal and hepatic incubations (Supporting Information). The major metabolites were shown to result from N-oxidation of the quinoline ring and hydroxylation at the *tert*-butyl group. Details of the metabolite identification are presented in the Supporting Information.

The in vivo pharmacokinetics (PK) of compounds **3d** and **3e** were evaluated in healthy male C57/BL6 mice following oral (at 40 and 20 mg/kg doses) and intravenous (at 5 and 2.5 mg/kg doses) routes, respectively (Table 3). Both compounds displayed a relatively moderate systemic plasma clearance and high volume of distribution with elimination half-lives ranging from 1.5 to 6 h, an indication that these compounds might be slightly protein bound.

The plasma protein binding affinity studies revealed that these compounds were moderately bound to the plasma protein (see Supporting Information). In addition, the oral bioavailability of both compounds ranged from 16 to 31%.

Compound **3d** was selected for in vivo antimalarial efficacy evaluation against *Plasmodium berghei* ANKA infected male C57/BL6 mice. This compound was administered orally at four different concentrations (20, 10, 5, and 1 mg/kg dose; each dose group consists of 5 mice) once a day for four days. Blood smears were collected on days 3, 5, and 7, and the percentage parasitemia determined using light microscopy. On day 7, compound **3d** reduced the parasitemia load by up to 47% even though this activity was found to plateau above 5 mg/kg (Table 4). This plateau, which is also noticeable in the in vivo PK data, possibly indicates solubility- or dissolution-limited absorption at higher doses.

In conclusion, we have successfully synthesized a series of new 4-aminoquinoline derivatives of CQ that showed greater antiplasmodial activity than the reference drug. Moreover, the front-runner compounds **3c−3e** also exhibited acceptably cytotoxicity, albeit with strong inhibition toward a panel of CYP3A4 enzymes in vitro. The in silico CYP3A4 docking studies implicated the aminoquinoline ring's orientation relative to the heme as the major cause of this inhibition. These compounds also displayed a relatively low oral bioavailability in mice. Compound **3d** exhibited 47% reduction in parasitemia load on day 7.

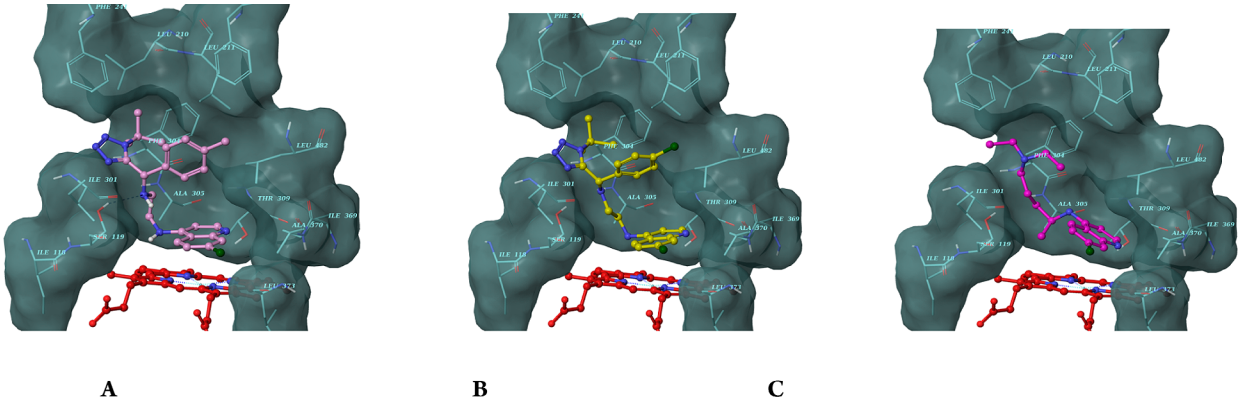


**Figure 3.** Lowest binding energy conformations for each of (A) **3c** (pink), (B) **3e** (yellow), and (C) chloroquine (magenta) showing the molecular surface of selected binding site residues at a radius of 3 Å and heme (red).


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**Table 3. Pharmacokinetic Parameters of Compounds 3d and 3e Following Intravenous and Oral Administration on Male C57/BL6 Mice[a]**

| parameters | 3d | | | | 3e | | | |
|---|---|---|---|---|---|---|---|---|
| | iv[b] | | oral[b] | | iv[b] | | oral[b] | |
| nominal dose (mg/kg) | 5.0 | 2.5 | 40 | 20 | 5.0 | 2.5 | 40 | 20 |
| apparent $t_{1/2}$ (h) | 2.3 | 1.9 | 3.9 | 6.0 | 2.5 | 1.6 | 4.0 | 3.6 |
| plasma $CL_{total}$ (mL/min/kg) | 44.8 | 48.9 | | | 51.0 | 51.2 | | |
| Vd (L/kg) | 8.9 | 7.9 | | | 10.3 | 7.0 | | |
| Vss (L/kg) | 9.1 | 8.7 | | | 12.6 | 6.5 | | |
| $C_{max}$ ($\mu$M) | | | 0.79 | 0.54 | | | 2.81 | 0.94 |
| $T_{max}$ (h) | | | 1.4 | 1.4 | | | 1.0 | 0.8 |
| $AUC_{0-\infty}$ (min· $\mu$mol/L) | 222 | 104 | 287 | 256 | 221 | 107 | 541 | 222 |
| bioavailability (%) | | | 16.2 | 30.8 | | | 30.6 | 25.9 |

[a]Empty cells indicate that the value was not measured or was not relevant. [b]Values are means of five animals.

**Table 4. Antimalarial Efficacy Using a Single Dose of Compound 3d in the *P.berghei* Mouse Model on Day 7**

| compound | dose (mg/kg) | % reduction of parasitemia |
|---|---|---|
| **3d** | 1 × 20 | 44.5 |
| | 1 × 10 | 41.4 |
| | 1 × 5 | 46.9 |
| | 1 × 1 | 22.7 |
| positive control (chloroquine) | 1 × 15 | 90.6 |
| negative control (placebo) | blank formulation solution | 0.0 |

## ■ ASSOCIATED CONTENT

### Ⓢ Supporting Information

Details regarding compound characterization and biological assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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All authors have given approval to the final version of the manuscript.


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### Notes

The authors declare no competing financial interest.

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