Carbon Isosteres of the 4-Aminopyridine Substructure of Chloroquine: Effects on pK_a, Hematin Binding, Inhibition of Hemozoin Formation, and Parasite Growth

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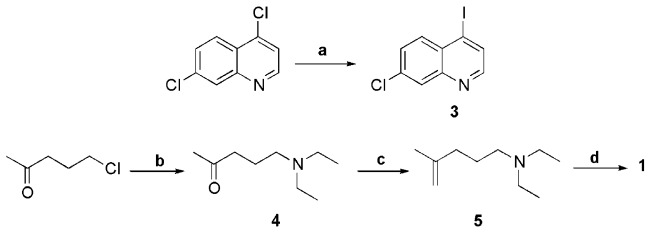
Unlike diprotic chloroquine (CQ), its two 4-aminoquinoline carbon isosteres (**1**, **2**) are monoprotic at physiological pH. Compared to CQ, hematin binding affinity of **1** decreased 6.4-fold, and there was no measurable binding for **2**. Although **1** was a weak inhibitor of hemozoin formation, neither isostere inhibited *P. falciparum* in vitro. Evidently, the CQ−hematin interaction is largely a function of its pyridine substructure, but inhibition of hemozoin formation and parasite growth depends on its 4-aminopyridine substructure.

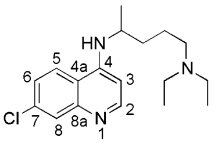
Recent data[1] continue to support the hypothesis that CQ exerts its antimalarial effect by binding to hematin,[2−4] leading to inhibition of hemozoin formation[5,6] (hematin polymerization) and death of the parasite by hematin poisoning.[7] Conversion of hematin to hemozoin, a unique parasite mechanism to detoxify hematin, is a nonenzymatic process[5] in which the hematin released from parasite digestion of hemoglobin is converted to hemozoin or malaria pigment, an insoluble ionic polymer which consists of an aggregate of reciprocally esterified hematin dimers.[8] Even though this important drug has lost much of its utility due to the spread of CQ-resistant *P. falciparum* malaria,[9] it may be possible to identify useful CQ analogues.[10] To do so efficiently, a more complete molecular understanding of the CQ−hematin binding interaction would be useful. Previous work[11−13] has established that CQ−hematin binding is largely a function of the 7-chloro-4-aminoquinoline heterocycle of CQ; the CQ diaminoalkyl side chain contributes only minimally to hematin binding. To better understand the structural specificity of this interaction, we prepared the carbon isosteres (**1**, **2**) of the 4-aminopyridine substructure of CQ and determined the effects on pK_a, hematin binding, inhibition of hemozoin formation, and parasite growth.

The carbon isostere of CQ (**1**), in which the aniline N atom is replaced with a methylene carbon, was obtained in 72% yield using a palladium-catalyzed cross-coupling reaction[14] of 7-chloro-4-iodoquinoline **3** with the borane intermediate generated in situ from diethylaminopentene **5** (Scheme 1). This one-pot coupling reaction was performed by adding 9-borabicyclo[3.3.1]nonane (9-BBN) to **5** in THF at 0 °C to generate the borane intermediate, followed by heating with iodoquinoline **3**

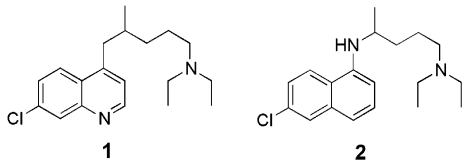
Scheme 1[a]



[a] Reagents and conditions: (a) aq HI (47%), 130 °C, 5 h, 91%; (b) Et_2NH, CH_3CN, reflux, 12 h, 68%; (c) PPh_3MeI, *n*-BuLi, Et_2O, 0 °C, 5 h, 79%; (d) (1) 9-BBN, THF, 0 °C, 6 h, (2) $PdCl_2$(dppf), 3 M NaOH (aq), **3**, reflux, 16 h, 72%.



Chloroquine (CQ)



in the presence of $PdCl_2$(dppf). 4-Iodoquinoline **3** was obtained in high yield by treatment of 4,7-dichloroquinoline with HI.[15] Vinylidine amine **5**[16] was prepared in two steps from 5-chloropentanone.[17] Heating the chloroketone with an excess of diethylamine in acetonitrile gave diethylaminoketone **4**. A subsequent Wittig olefination afforded **5** in excellent yield. The *β*-naphthylamine carbon isostere of CQ (**2**) in which the pyridine N atom is replaced with a carbon atom, was available in the Walter Reed Army Institute of Research (WRAIR) inventory and was synthesized in a three-step sequence by McCaustland et al.[17] The WRAIR sample of **2** was purified by flash column chromatography.

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Table 1. Thermodynamic Characterization of the Binding Interaction between CQ, **1**, and Hematin

compd	K_a (10^5 M^{-1})	n	ΔG (kcal/mol)	ΔH (kcal/mol)	$T\Delta S$ (kcal/mol)
CQ	5.4	0.25	-8.1 ± 0.1	-11.7 ± 0.1	-3.6
1	0.84	0.24	-7.0 ± 0.2	-8.5 ± 0.2	-1.5

Due to the poor solubility of hematin at low pH, we performed the isothermal titration calorimetry (ITC) binding experiments in 250 mM phosphate buffer at pH 6.5[3] because at this pH hematin is completely soluble. It was also necessary to add 2.5% EtOH to fully solubilize **1** and **2**. The ITC data for hematin binding of CQ and CQ isostere **1** are shown in Table 1. In aqueous solution, hematin exists almost exclusively in the form of its μ-oxo dimer,[18] and this is reflected in the observed binding stoichiometries for both CQ and CQ isostere **1** which indicates formation of a 1:2 drug:hematin μ-oxo dimer complex. As we have noted previously,[12] the stoichiometry data support a model proposed by Moreau et al.[19] in which CQ binds to hematin μ-oxo dimers in a cofacial $\pi-\pi$ sandwich-type complex. The 2.5% EtOH affected CQ:hematin binding affinty only marginally from that ($K_a = 4.0 \times 10^5$) obtained under purely aqueous conditions.[11] The binding affinity of CQ isostere **1** decreased 6.4-fold compared to CQ, and the hematin μ-oxo dimer binding interaction was, like CQ, enthalpically driven. We attribute this exothermic binding enthalpy largely to $\pi-\pi$ interactions between the compounds and hematin μ-oxo dimer, although release of perturbed water molecules from the amphiphilic surfaces[20] of hematin μ-oxo dimer and CQ and **1** could also contribute to this favorable exothermic enthalpy. There was no measurable binding interaction between CQ isostere **2** and hematin μ-oxo dimer which indicates that the pyridine N atom is essential to this binding interaction.

Although CQ isosteres **1** and **2** interact very differently with hematin μ-oxo dimer, their weak base properties differed very little; spectrophotometric titration indicated that **1** and **2** had pK_a values of 4.80 (quinoline N atom) and 4.36 (aniline N atom), respectively. The pK_a values for the side chain tertiary N atom for **1** and **2** were not measured, but it is presumed that they differ very little from that of CQ (pK_a 10.2).[21] Compared to CQ ($IC_{50} = 80$ μM), CQ isostere **1** was a weak inhibitor of hemozoin formation with an IC_{50} of 1500 μM; carbon isostere **2** had no effect on hemozoin formation at concentrations up to 2500 μM. Neither analogue inhibited parasite growth of either the NF54 or K1 strains of *P. falciparum* at concentrations up to 3000 nM; in contrast CQ has IC_{50s} of 8.5 and 150 nM against these two *P. falciparum* strains.

Electrostatic potential maps and atomic electrostatic charge densities (Table 2) for **1** and **2** were computed using ab initio quantum chemical procedures at the 6-31G** level to more fully understand the structural aspects of CQ−hematin μ-oxo dimer binding and to shed light on the underlying basis for the complete lack of binding interaction between hematin μ-oxo dimer and **2**. The contiguous three-dimensional electrostatic potential beyond the van der Waals surfaces of CQ, **1**, and **2** (Figure) reveal that, relative to CQ, the π-electron density distribution is clearly shifted toward the pyridine N atom in **1** and the aniline N atom in **2**. This is

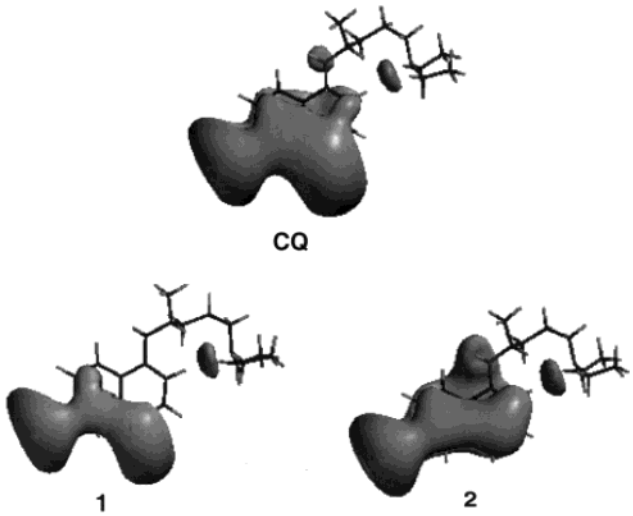

Figure 1. Three-dimensional isopotential contours of molecular electrostatic potentials at −10 kcal/mol for CQ and CQ isosteres **1** and **2**.

Table 2. Atomic Electrostatic Charge Densities of CQ, **1**, and **2** Calculated at the 6-31G** Level

atom	CQ	**1**	**2**
N1/C1	−0.76	−0.71	−0.36
C2	+0.39	+0.37	−0.11
C3	−0.61	−0.33	−0.22
C4	+0.69	+0.16	+0.32
C4a	−0.45	−0.24	−0.05
C5	−0.04	−0.1	−0.15
C6	−0.30	−0.24	−0.22
C7	+0.25	+0.18	+0.17
C8	−0.44	−0.4	−0.29
C8a	+0.76	+0.68	+0.25
N/C	−0.89	−0.14	−0.83
Cl	−0.15	−0.14	−0.16

reflected in diminished atomic electrostatic charge density values at C1 in **2** relative to N1 in **1** and CQ, and at the methylene carbon in **1** relative to the aniline N in **2** and CQ. In addition, relative to CQ and **1**, the π-electron density in **2** increased significantly at positions C2 and C8a. This suggests that increased π-electron density adjacent to the pyridine N atom in CQ can abolish the formation of a complex with hematin μ-oxo dimer. These data also provides further evidence that the tertiary nitrogen atom of CQ contributes little to hematin binding,[11−13] as both **1** and **2** possess a tertiary nitrogen atom nearly identical to that of CQ, but only **1** binds to hematin μ-oxo dimer.

Even though **1** binds to hematin μ-oxo dimer, only 6.4-fold more weakly than does CQ, **1** was a very weak inhibitor of hemozoin formation, and, like **2**, was completely inactive against *P. falciparum* in vitro. The lack of activity for **1** and **2** supports the observation of Thompson and Werbel[22] that chemical modification of the quinoline heterocycle of CQ tends to have a more pronounced impact on in vivo antimalarial activity than do modifications of the side chain. We suspect that **1** and **2**[17] may be inactive as antimalarials as a consequence of their diminished basicity that could result in reduced accumulation[23,24] in the parasite food vacuole. However this hypothesis does not fully explain the inactivity of **1**, since CQ binding to parasite-derived hematin species[2,25] is likely an even more important

contributor to the accumulation of CQ in infected erythrocytes.

In summary, this experimental and molecular modeling data suggest that enthalpy-driven CQ:hematin μ-oxo dimer binding is largely a function of the pyridine substructure in the quinoline heterocycle, not the aniline N atom. However, inhibition of both hemozoin formation and parasite growth depends on the 4-aminopyridine substructure of CQ. Apparently, the pyridine N atom of CQ creates an electron distribution which leads to an energetically favorable alignment[26,27] of the out-of-plane π-electron density in CQ and hematin μ-oxo dimer at the points of intermolecular contact.

Experimental Section

Chemistry. CQ isostere **2** was obtained from the Division of Experimental Therapeutics inventory at the Walter Reed Army Institute of Research and purified by flash chromatography. Other chemicals and reagents were obtained from Aldrich or Sigma Chemical Co's. Melting points were determined on a Mel-Temp apparatus and are uncorrected. NMR spectra were recorded using Varian XL-300 or Unity 500 spectrometers. Elemental analyses were performed by M−H−W Laboratories, Phoenix, AZ. Thin-layer chromatography was carried out on precoated Analtech silica gel coated plates.

7-Chloro-4-iodoquinoline (3). To neat HI (47% *w*/*w* in H_2O, 43 mL) was added 4,7-dichloroquinoline (5.0 g, 25.2 mmol) in small portions at room temperature. The resulting suspension was heated at 130 °C for 5 h, cooled to room temperature, poured into an ice−water mixture (60 mL), basified with 10 N NaOH, and extracted with $CHCl_3$ (3 × 50 mL). The combined organic extracts was successively washed with NH_4OAc buffer (pH 7.0), 10% $Na_2S_2O_3$, and brine, dried over $MgSO_4$, and concentrated in vacuo to give **3** (6.63 g, 91%) as an off-white solid; mp 120−122 °C (EtOH): ^{1}H NMR ($CDCl_3$) δ 7.57 (d, J = 6.8 Hz, 1H), 7.96−7.99 (m, 2H), 8.06 (d, J = 4.3 Hz, 1H), 8.45 (d, J = 5.8 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 111.27, 128.90, 129.06, 132.72, 133.11, 136.47, 148.29, 150.68.

5-Diethylamino-2-pentanone (4). To a solution of freshly distilled (94−96 °C/19 mm) 5-chloro-2-pentanone (25 g, 0.2 mol) in dry acetonitrile (100 mL) under a nitrogen atmosphere was added diethylamine (38 g, 0.5 mol), and the mixture was refluxed for 12 h. It was then cooled to room temperature and concentrated in vacuo. Distillation of the resulting dark brown liquid afforded **4** (21.3 g, 68%) as a colorless oil (bp 83−85 °C/15 mm).

5-Diethylamino-2-methylpentene (5). To a stirred suspension of methlytriphenylphosphonium iodide (7.6 g, 18.9 mmol) in dry ether (40 mL) under a nitrogen atmosphere at 0 °C was added *n*-butyllithium (2 M, 11 mL, 21.4 mmol). The resulting yellow solution was stirred for an additional 2 h at 0 °C. To this solution was added dropwise **4** (2.0 g, 12.6 mmol) in ether (10 mL). After continued stirring for 5 h at 0 °C, the reaction mixture was poured into ice-cooled water (50 mL). The ether layer was separated, washed with water (25 mL) and brine (25 mL), and then dried over $MgSO_4$. Removal of the ether in vacuo gave a yellow oil which was distilled to afford **5** (1.58 g, 79%) as a colorless oil (bp 72−74 °C/16 mm): ^{1}H NMR ($CDCl_3$) δ 1.03 (t, J = 7.3 Hz, 6H), 1.58−1.59 (m, 2H), 1.61 (s, 3H), 2.03 (t, J = 7.8 Hz, 2H), 2.41 (t, J = 5.8 Hz, 2H), 2.54 (q, J = 7.3 Hz, 4H), 4.69 (d, J = 8.3 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 11.52, 21.90, 25.04, 35.39, 46.75, 52.48, 109.40, 145.03. Anal. ($C_{10}H_{21}N$) C, H, N.

[5-(7-Chloroquinolin-4-yl)-4-methylpentyl]diethylamine (1). To a solution of **5** (0.50 g, 3.22 mmol) in THF (25 mL) under a nitrogen atmosphere was added a solution of 9-BBN (0.5 M, 7.1 mL, 4.8 mmol) in THF at 0 °C. The reaction mixture was allowed to reach room temperature and was then stirred for 6 h. To the resulting colorless solution were added $PdCl_2$(dppf) (0.08 mmol), 3 M NaOH (4.5 mL), and **3** (1.31 g, 4.51 mmol). After refluxing for 16 h, the reaction mixture was cooled to room temperature and diluted with benzene (100 mL). The organic layer was separated, washed with brine, and dried over $MgSO_4$. The solvent was removed in vacuo, and the dark-brown residue was purified by flash chromatography on silica gel with CH_2Cl_2/methanol (1:1) to yield **1** (0.74 g, 72%) as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 0.93 (d, J = 6.4 Hz, 3H), 1.01 (t, J = 6.8 Hz, 6H), 1.25−1.61 (m, 2H), 1.89−1.93 (m, 1H), 2.41 (t, J = 6.4 Hz, 2H), 2.53 (q, J = 5.8 Hz, 4H), 2.76 (dd, J = 8.2 Hz, 1H), 3.11 (dd, J = 5.8 Hz, 1H), 7.19 (d, J = 4.3 Hz, 1H), 7.48 (d, J = 6.8 Hz, 1H), 7.95 (d, J = 8.7 Hz, 1H), 8.10 (s, 1H), 8.78 (d, J = 4.3 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 11.61, 19.72, 24.76, 34.23, 35.12, 39.85, 46.97, 53.20, 122.11, 125.25, 126.41, 127.14, 129.19, 134.85, 147.64, 149.12, 150.93. Anal. ($C_{19}H_{27}ClN_2$) C, H, N.

***N*^{4}-(6-Chloronaphthalen-1-yl)- *N*^{1},*N*^{1}-diethylpentane-1,4-diamine (2).** The impure WRAIR sample was purified by flash chromatography on silica gel with CH_2Cl_2/EtOAc (1:1) to yield **2** as brown oil: ^{1}H NMR ($CDCl_3$) δ 1.01 (d, J = 6.4 Hz, 3H), 1.19 (t, J = 6.8 Hz, 6H), 1.27−1.39 (m, 2H), 1.49 (m, 2H), 2.49 (q, J = 6.4 Hz, 4H), 2.63 (t, J = 5.8 Hz, 2H), 2.76 (m, 1H), 6.56 (d, J = 6.8 Hz, 1H), 7.11 (m, 1H), 7.33 (m, 2H), 7.83 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 11.44, 20.12, 23.96, 34.63, 46.66, 48.87, 52.70, 99.11, 118.12, 121.25, 122.41, 125.54, 126.14, 129.42, 131.85, 134.64, 142.12.

Isothermal Titration Calorimetry (ITC). All ITC binding experiments were conducted in 250 mM phosphate buffer, pH 6.5, at 37 °C using a MCS titration calorimeter from Microcal Inc. (Northampton, MA). An aliquot of a 1 mM stock solution of hematin in 0.01 N NaOH was diluted up to the required concentrations (0.1−0.3 mM) in the phosphate buffer. Hematin was titrated with 0.5−5 mM solutions of **1** and **2** using a 100 μL syringe rotating at 400 rpm. Titration experiments consisted of 16 injections of 2−6 μL each, and three to four experiments were performed for each analogue. Heats of dilution and mixing were obtained by 16 injections of each analogue into the buffer medium. After having first subtracted heats of dilution and mixing from each injection heat pulse, we fitted the binding isotherms for a model of a single set of identical binding sites by nonlinear least-squares fitting of the titration data using Origin software. Association constants (K_a's), enthalpy change (ΔH), and stoichiometry (n) were obtained from binding isotherms using the Marquardt algorithm.[28]

Spectrophotometric Titration. pK_a values for **1** and **2** were determined in 10 mM acetate buffer. Solutions (2 μM) of **1** and **2** at 16 different pH values in the range of 2 to 8 were prepared. The spectra of each solution at 37 °C were obtained using a Perkin-Elimer spectrophotometer. Absorbance at 318 nm for **1** and at 328 nm for **2** vs solution pH was plotted, and sigmoid curves were obtained. The inflection points of these sigmoid curves were taken as the pK_a values for **1** and **2**.

Inhibition of Hematin Polymerization and Parasite Growth. Hematin polymerization experiments were carried out as previously described.[3] *P. falciparum* strains NF54 and K1 were cultured according to the method of Trager and Jensen[29] (1976) with minor variations as described by Dorn et al.[5] Compound inhibition of parasite growth was determined by a semiautomated microdilution assay using asynchronous stock cultures.[30]

Molecular Modeling. Conformational searches of **1** and **2** were performed by multiple rotation of the rotatable single bonds, thereby generating low energy conformers with varying population densities. This was performed using the systematic search technique with the AM1 semiempirical method[31] as implemented in SPARTAN Version 5.1 (Wavefunction, Inc.). The geometry of the most abundant minimum energy conformer was further optimized for calculation of electronic properties by using *Gaussian98* (Gaussian Inc.) at the 6-31G**/HF−SCF level of quantum chemical theory running on a Silicon Graphics Octane workstation. The 6-31G** atomic basis set has been constructed by a set of six second-order (d-type) Gaussian primitives to the spit-valence 6-31G** basis set description of each heavy non-hydrogen atom together with a single set of Gaussian p-type functions to each hydrogen and helium atom. Molecular electrostatic potential (MEP) maps

were calculated and drawn using the graphics in SPARTAN. The MEPs were sampled over the entire accessible surface of a molecule (corresponding roughly to a van der Waals contact surface), providing a measure of charge distribution from the point of view of an approaching molecule.[32]

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