Structure of a novel antimalarial gallium(III) complex with selective activity against chloroquine-resistant *Plasmodium falciparum*†

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The crystal structure of an amine phenol complex of gallium(III), [{1,12-bis(2-hydroxy-3-methoxybenzyl)-1,5,8,12-tetraazadodecane}gallium(III)] [Ga(madd)]$^+$, a potent new antimalarial which selectively targets chloroquine-resistant organisms is reported; the compound is shown to directly inhibit heme polymerization.

Chloroquine, the mainstay of treatment and prophylaxis of malaria, disrupts polymerization of heme (ferriprotoporphyrin IX) released during catabolism of host hemoglobin within the causative organism, *Plasmodium falciparum*.[1–3] Given the importance attributed to iron metabolites in parasite toxicity, a variety of metal chelating agents such as deferoxamine and reversed siderophores have been explored as potential antimalarial chemotherapeutics.[4–6] We recently identified a class of Schiff-base and reduced amine phenol metal(III) complexes with N_4O_2 donor cores having potency as novel antimalarials.[7] We report herein the chemical characterization including crystal structure and direct demonstration of inhibition of heme polymerization by one distinctive complex, [{1,12-bis-(2-hydroxy-3-methoxybenzyl)-1,5,8,12-tetraazadodecane}gallium(III)] {[Ga(madd)]$^+$ **1**}. While the action of chloroquine is now circumvented by widely disseminated resistant organisms, **1** paradoxically possesses antimalarial activity only against chloroquine-resistant *P. falciparum* in whole cell assays. Thus, **1** may provide a lead for further development of analogous iron(III) antimalarials and, in addition, the crystal coordinates provide a structural template for exploration of the molecular determinants of chloroquine resistance in *P. falciparum*.

Large spectral linewidths and the accompanying poor resolution produced by high spin iron(III) pose difficulties for spectroscopic characterization of iron(III) complexes in solution through NMR studies. Thus, we have chosen diamagnetic gallium(III) as a template for iron(III), since both ions have the same charge and nearly identical six-coordinate ionic radii which typically result in similar coordination chemistry.[8] Furthermore, neither high spin d^5 iron(III) nor d^{10} gallium(III) possess any crystal field stabilization energy and therefore often result in similar ligand exchange kinetics.[9] For analysis, the desired amine phenol ligand, H_2madd **2**, was obtained through *in situ* potassium tetrahydroborate reduction of the condensation product derived from appropriate stoichiometric amounts of bis(*N*,*N*′-aminopropyl)ethylenediamine and 3-methoxysalicylaldehyde.[10,11] [Ga(madd)]$^+$ **1** was obtained by dissolving **2** in methanol and treating with base and the hydrated salt of gallium(III) perchlorate.† The ^{1}H NMR spectrum of **1** recorded in $(CD_3)_2SO$ demonstrated overlapping signals for the hydrocarbon backbone consistent with complex formation and a single set of signals for the aromatic protons consistent with chemically equivalent aromatic environments. The proton-decoupled ^{13}C NMR spectrum recorded under similar conditions also revealed 12 resonances out of a possible 24 further indicating the maintenance of a high degree of symmetry around the ethylene moiety upon coordination of the metal to ligand **2**.

Definitive evidence that Ga^{III} was coordinated simultaneously and symmetrically with the N_4O_2 donor core was provided by X-ray crystallography.‡ Crystals suitable for X-ray structure determination were grown through slow evaporation of methanol over a period of 2–3 d. An ORTEP drawing of **1** is shown in Fig. 1 along with selected bond angles and interatomic distances. The central Ga^{III} is six-coordinate involving two phenolate oxygens and four amine nitrogens providing overall octahedral geometry. Furthermore, the structure reveals formation of four six-rings and one five-membered ring upon coordination of the metal. The smallest angle, N(2)–Ga–N(3), is attributable to restrictions by the five-membered chelate ring involving N(2), C(11), C(12), N(3) and Ga. The *trans* angles involving N(1)–Ga–N(3), N(2)–Ga–N(4) and O(1)–Ga–O(2) averaged 175.4°, whereas the *cis* angles involving O–Ga–N averaged 90.0°, providing minimally distorted octahedral

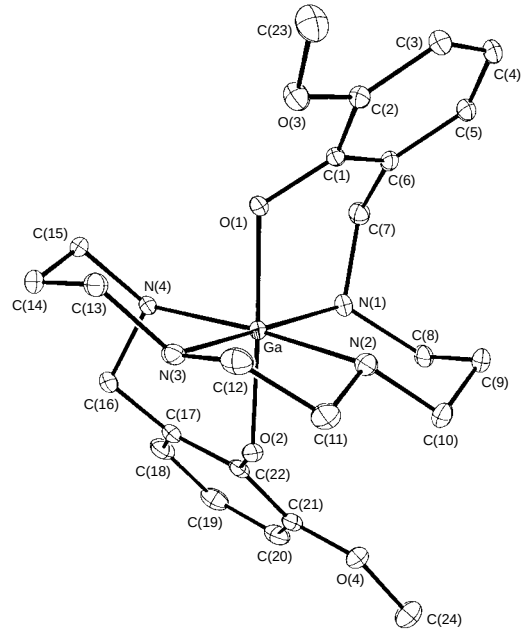

Fig. 1 ORTEP drawing of the [Ga(madd)]$^+$ cation (**1**) in [Ga(madd)]ClO_4 showing the crystallographic numbering scheme. Atoms are represented by thermal ellipsoids corresponding to 20% probability. Selected bond angles (°) and interatomic distances (Å) for **1**: N(1)–Ga–N(2) 92.15(11), N(2)–Ga–N(3) 83.22(11), N(3)–Ga–N(4) 90.62(11), N(4)–Ga–N(1) 94.08(10), O(1)–Ga–O(2) 177.99(8), N(1)–Ga–N(3) 174.87(10), N(2)–Ga–N(4) 173.43(10), O(1)–Ga–N(1) 90.29(10), O(1)–Ga–N(4), 88.59(10), O(2)–Ga–N(2) 87.87(10), O(2)–Ga–N(3) 90.87(10), C(1)–O(1)–Ga 122.4(2), C(22)–O(2)–Ga 119.1(2); Ga–O(1) 1.911(2), Ga–O(2) 1.950(2), Ga–N(1) 2.130(3), Ga–N(2) 2.103(3), Ga–N(3) 2.109(3), Ga–N(4) 2.105(3), O(1)–C(1) 1.345(4), O(2)–C(22) 1.340(4).

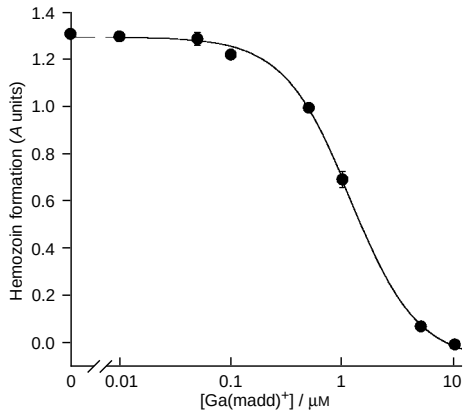

Fig. 2 Inhibition of heme polymerization by $[Ga(madd)]^+$ **1**. Heme polymerization was assayed by the preformed hemozoin nucleation reaction[7] in the absence or presence of various concentrations of **1**. Data are shown as the mean ±SEM (when larger than symbol) of six determinations.

geometry around the central metallic core. Angles and distances of atoms directly participating in the coordination sphere of **1** are similar to those recently reported for the gallium(III) complexes of a 5-bromo substituted amine phenol[12] and an analogous substituted Schiff-base phenol.[13]

Chloroquine concentrates within the parasitic digestive vacuole[14] an acidic organelle wherein the host's hemoglobin is digested as a source of amino acids, releasing monomeric heme.[15] Toxic to parasites,[2,16,17] heme is polymerized by the organisms into a crystalline matrix known as hemozoin, a black insoluble pigment which constitutes heme units coordinated through iron–carboxylate linkages.[1] Chloroquine inhibits heme polymerization, resulting in accumulation of the toxic monomeric heme moiety.[2,3] Resistance appears to be mediated by an acquired ability of the parasite to reduce uptake[18] or pump chloroquine out of the digestive vacuole.[19,20] Because hemozoin formation is unique to the malaria organism, inhibition of heme polymerization has remained an attractive target for drug development, providing an agent can be designed to bypass the organism's chloroquine-evading mechanisms.

Among dozens of analogues, including the above 5-bromo complex[21] and several Schiff-base analogues,[7] **1** was found to be unique in possessing selective antimalarial activity against chloroquine-resistant Dd2 clones of *P. falciparum*. To further determine the potency of **1** to inhibit additional chloroquine-resistant lines of *P. falciparum*, growth of trophozoites in intra-erythrocyte culture was assayed by the hypoxanthine incorporation method.[7,22] Compared to a half-maximal inhibitory concentration (IC_{50}) value $>>20$ μM in the chloroquine-sensitive HB3 line, the IC_{50} values for **1** were 0.5 and 0.6 μM in the chloroquine-resistant FCR-3 and Indo-1 lines, respectively, and further supported a general collateral selectivity of **1** for chloroquine-resistant *P. falciparum*.

However, the potency of **1** to directly inhibit heme polymerization *in vitro* was found to be similar to other agents in this class (Fig. 2). For example, to simulate the parasitic digestive vacuole environment,[16] heme was incubated along with preformed hemozoin nucleates under acidic conditions in the absence or presence of various concentrations of **1**.[2,7]§ Complex **1** was found to be a potent inhibitor of heme polymerization (IC_{50} = 1.2 ± 0.08 μM; mean ±SEM; n = 6), two- to three-fold more potent than chloroquine under identical conditions.[7] Thus, while **1** is not susceptible to the chloroquine resistance mechanism(s) in whole cell assays, it inhibits the same final molecular target as chloroquine *in vitro*. Careful evaluation of **1** revealed that the angles Ga–O(1)–C(1) and Ga–O(2)–C(22) which incorporate the aromatic rings averaged 120.8°, *ca*. 2.5° more acute than the reported 5-bromo complex, thereby suggesting that the unique targeting properties of **1** likely reside in the spatial orientation of the peripheral regions of the aromatic moieties, including the methoxy functionalities, rather than the central metal core.

The gallium(III) complex, $[Ga(madd)]^+$ **1**, is the first amine phenol metal(III) complex to show antimalarial activity selectively against chloroquine-resistant organisms. While important considerations regarding optimization of selectivity and bioavailability are under active investigation, **1** represents a significant step forward in the development of a novel antimalarial.

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Footnotes and References

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† Details of synthesis and characterization of compounds **1** and **2**; structure determination, refinement methods and tables of X-ray crystallographic data for compound **1** (atomic coordinates, interatomic distances and angles, anisotropic and isotropic displacement parameters, and hydrogen coordinates) as well as the procedure for the heme polymerization inhibition assay are included as supporting material available upon request from the authors.

‡ Colorless crystals of $[Ga(madd)]ClO_4$ (formula, $C_{24}H_{36}ClGaN_4O_8$; M = 613.74) belong to the monoclinic space group, $P2_1/n$, with a = 10.8630(8), b = 17.141(5), c = 15.181(2) Å, β = 107.711(7)°, U = 2692.8(9) Å^3, D_c = 1.514 g cm^{-3}, Z = 4 and GOF = 1.165. Data were collected at 173 K using a Mo-Kα radiation source (0.710 73 Å) and the structure resolved and refined to R_1 [$I > 2\sigma(I)$] = 0.0409. CCDC 182/625.

§ Control experiments established that **1** was stable to acidic hydrolysis: ^{1}H NMR spectra were superimposible before and after incubation in water for 72 h (37 °C, pH 4.5).

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