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Metalloantimalarials: Targeting of *P. falciparum* Strains with Novel Iron(III) and Gallium(III) Complexes of an Amine Phenol Ligand

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Emergence of chloroquine (CQ)-resistant *Plasmodium falciparum* strains necessitates discovery of potent and inexpensive antimalarial drugs. The high cost of new drugs negatively impacts their access and distribution in the regions of the world with scarce economic resources. While exploring structure–activity relationships, using gallium(III) as a surrogate marker for iron(III), we found cationic, and moderately hydrophobic, compounds, [{1,12-bis(2-hydroxy-3-ethyl-benzyl)-1,5,8,12-tetraazadodecane}metal(III)]$^+$ (metal = Fe(III) and Ga(III); [Fe-3-Eadd]$^+$, **3**; [Ga-3-Eadd]$^+$, **4**), that possessed antimalarial activity. Crystal structure analyses revealed octahedral geometry for these complexes. The RP-HPLC analysis, after incubation in PBS or HEPES buffer (pH 7.4) at 37 °C for 3 days, detected only parental compounds thereby providing evidence for stability under physiological conditions. Both **3** and **4** demonstrated promising half-maximum inhibitory concentration (IC_{50}) values of ~80 and 86 nM in the CQ-sensitive HB-3 line, respectively. However, both **3** and **4** were found to possess elevated IC_{50} values of 2.5 and 0.8 μM, respectively, in the CQ-resistant Dd2 line, thus displaying preferential cytotoxicity toward the CQ-sensitive HB3 line. In cultured parasites, **3** and **4** targeted hemozoin formation. Thus, these compounds acted similarly to chloroquine with regard to action and resistance, despite the lack of structural similarity to quinolines. Finally, similarity in coordination chemistry, stability, and antimalarial cytotoxicity profiles indicated that gallium(III) ion can serve as a template for iron(III) in structure elucidation of active molecules in solution.

Introduction

Malarial parasites emerging from the genus *Plasmodium* infect 300−500 million people and result in the death of 1−3 million children each year.[1,2] Recently, interdisciplinary efforts involving a wide consortium of several laboratories have resulted in the genome sequencing of *Plasmodium falciparum*[3] and the mosquito vector *Anopheles gambiae*.[4] These genome discoveries will provide an enormous amount of information about the interaction of the parasite with its host and carrier, including the genes involved in parasite recognition by the human immune system. However, translating this information into effective remedies will be an immensely challenging task due to the resistance pathways devised by the parasite. Resistance to chloroquine (CQ), an inexpensive and potent antimalarial, is becoming rampant.[5−7] New drugs exemplified by mefloquine[8−10] and halofantrine[11]

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(1) Woster, P. *Annu. Rep. Med. Chem.* **2001**, *36*, 99.

(2) Sturchler, D. *Parasitol. Today* **1989**, *5*, 39−40.

(3) Gardner, M.; Hall, N.; Fung, E.; White, O.; Berriman, M.; Hyman, R.; Cariton, J.; Pain, A.; Nelson, K.; Bowman, S.; Paulsen, I.; James, K.; Eisen, J.; Rutherford, K.; Salzberg, S.; Craig, A.; Kyes, S.; Chan, M.; Nene, V.; Shallom, S.; Suh, B.; Peterson, J.; Angiouli, S.; Pertea, M.; Allen, J.; Selengut, J.; Haft, D.; Mather, M.; Vaidya, A.; Martin, D.; Fairlamb, A.; Fraunholz, M.; Roos, D.; Ralph, S.; Mcfadden, G.; Cummings, L.; Subramanian, G.; Mungall, C.; Venter, J.; Carucci, D.; Hoffman, S.; Newbold, C.; Davis, R.; Fraser, C.; Barrell, B. *Nature* **2002**, *419*, 498−511.

(4) Holt, R.; Subramanian, G.; Halpern, A.; Sutton, G.; Charlab, R.; Nusskern, D.; Hoffman, S. *Science* **2002**, *298*, 129−149.

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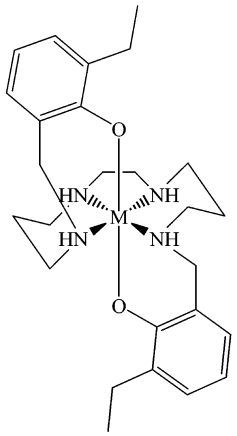
are efficacious, but their high cost negatively impacts their access and distribution in the underdeveloped regions of the world. Therefore, alternative therapeutic drugs that are synthesized from inexpensive precursors, and capable of overcoming resistance pathways, are needed.[12]

While broadening horizons of conventional medicinal chemistry restricted primarily to organic molecules, various bioinorganic complexes have been explored for their antimalarial therapeutic effects.[13−15] Given the importance of iron metabolites in malarial physiology and toxicity, various chelators such as deferoxamine[16−19] and reversed siderophores[20,21] have been explored as potential antimalarials. However, these chelators may bind other essential metals such as zinc, calcium, and magnesium, in intracellular compartments, thereby resulting in deprivation of these vital nutrients that are required for the mammalian host as well. Therefore, metallodrugs that possess an optimal balance of hydrophobicity/hydrophilicity, or relative lipophilicity, for permeation across membrane bilayers, and remain nonmetabolized in the intracellular target sites, may offer alternative remedies. Previously, we found a novel class of hexadentate amine phenol ligands that can bind efficiently various metals to generate moderately hydrophobic and membrane permeant compounds.[22] Obtained from commercially available inexpensive precursors, these compounds were found to possess antimalarial properties. Like CQ, these compounds target the vital biocrystallization process, heme sequestration (hemozoin formation).[12]

Herein, using gallium(III) as a surrogate marker for iron(III) due to similarities in their coordination chemistry, we report the chemical characterization, including crystal structures, of novel iron(III) and gallium(III) complexes of an amine phenol ligand, [{1,12-bis(2-hydroxy-3-ethyl-benzyl)-1,5,8,12-tetraazadodecane}metal(III)]$^+$, [M-3-Eadd]$^+$ [M = iron(III), **3**; gallium(III), **4**], and we evaluate their antimalarial cytotoxic profiles in the CQ-sensitive HB3 and CQ-resistant Dd2 lines. Both compounds **3** and **4** demonstrated similar half-maximum inhibitory concentration (IC_{50}) values in the CQ-sensitive HB3 line, and only 3-fold IC_{50} value differences in the CQ-resistant Dd2 line, suggesting similarities in the mechanism of action for these metal(III) complexes. Furthermore, these compounds were also found to be inhibitors of heme sequestration.



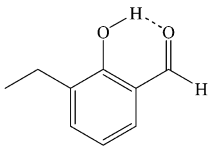
M = Fe(III) **3**; Ga(III) **4**

Results and Discussion

Due to inherent difficulties attributed to the large spectral line widths and low spatial resolution with high spin iron(III), the structural characterization of active compounds in solution poses some difficulties. Previously, gallium(III) substitution was performed for paramagnetic [Fe_2S_2] ferredoxins to characterize the structural and functional features of the ferric forms of the native iron−sulfur proteins with use of conventional NMR techniques.[23] On the basis of the rationale, both iron(III) and gallium(III) have similar charge/radius ratios and produce similar octahedral complexes, and neither high spin d^5 iron(III) nor d^{10} gallium(III) has any crystal field stabilization energy rendering similar ligand exchange kinetics. We postulate that the group 13 metal ion, gallium(III), may serve as an excellent surrogate marker of the iron(III) for structure elucidation of the active molecules in the solution. For evaluation, 3-ethyl-2-hydroxy-benzaldehyde (**1**) was obtained via selective ortho-formylation[24] of (2-ethylphenolato)magnesium(II) chloride, purified and spectroscopically characterized. The existence of resonance signals at δ 11.29 ppm, assigned to the hydroxyl proton, and at δ 196.8 ppm, assigned to the carboxylic carbon, in ^{1}H and ^{13}C NMR spectra, respectively, indicated the presence of hydrogen bonding between the hydroxyl and lone pair of the carbonyl

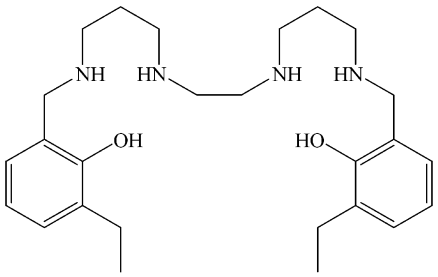
(5) Beeson, J.; Winstanley, P.; Mcfadden, G.; Brown, G. *Nat. Med. (N.Y.)* **2001**, *7*, 149−150.
(6) Hein, T.; White, N. *Lancet* **1993**, *341*, 603.
(7) Olliaro, P.; Cattani, J.; Wirth, D. *J. Am. Med. Assoc.* **1996**, *275*, 230−233.
(8) MacArthur, J.; Stennies, G.; Macheso, A.; Kolczak, M.; Green, M.; Ali, D.; Barat, L.; Kazembe, P.; RueBush, T. *Am. J. Trop. Med. Hyg.* **2001**, *6*, 679−684.
(9) Udry, E.; Bailly, F.; Dusmet, M.; Schnyder, P.; Lemoine, R.; Fitting, J. *Eur. Respir. J.* **2001**, *18*, 890−892.
(10) Kang, J.; Chen, X.; Wang, L.; Rampe, D. *J. Pharmacol. Exp. Ther.* **2001**, *299*, 290−296.
(11) Wesche, D.; Schuster, B.; Wang, W.; Woosley, R. *Clin. Pharmacol. Ther.* **2000**, *67*, 521−529.
(12) Goldberg, D. E.; Sharma, V.; Oksman, A.; Gluzman, I. Y.; Wellems, T. E.; Piwnica-Worms, D. *J. Biol. Chem.* **1997**, *272*, 6567−6572.
(13) Hubel, R.; Polborn, K.; Knizek, J.; Noth, H.; Beck, W. *Z. Anorg. Allg. Chem.* **2000**, *626*, 1701−1708.
(14) Obaleye, J.; Adeyemi, O.; Balogun, E. *Int. J. Chem.* **2001**, *11*, 101−106.
(15) Obaleve, J.; Balogun, E.; Adeyemi, O. *Biokemistri* **1999**, *9*, 23−27.
(16) Pollack, S.; Rossan, R.; Davidson, D.; Escajadillo, A. *Proc. Soc. Exp. Biol. Med.* **1987**, *184*, 162−164.
(17) Stahel, E.; Mazier, D.; Guillouzo, A.; Miltgen, F.; Landau, I.; Mellouk, S.; Beaudoin, R.; Langlois P.; Gentilini, M. *Am. J. Trop. Med. Hyg.* **1988**, *39*, 236−240.
(18) Hershko, C.; Peto, T. *J. Exp. Med.* **1988**, *168*, 375−387.
(19) Gordeuk, V.; Thuma, P.; Brittenham, G.; Biemba, G.; Zulu, S.; Simwanza, G.; Kalense, P.; M'Hango, A.; Parry, D.; Poltera, A.; Aikawa, M. *Am. J. Trop. Med. Hyg.* **1993**, *48*, 193−197.
(20) Lytton, S.; Cabantchik, Z.; Libman, J.; Shanzer, A. *Mol. Pharmacol.* **1991**, *40*, 584−590.
(21) Shanzer, A.; Libman, J.; Lytton, S. D.; Glickstein, H.; Cabantchik, Z. I. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 6585−6589.
(22) Sharma, V.; Rao, V.; Reichert, D.; Crankshaw, C.; Welch, M.; Piwnica-Worms, D. *J. Nucl. Med.* **1996**, *37*, 71P.
(23) Vo, E.; Wang, H.; Germanas, J. *J. Am. Chem. Soc.* **1997**, *119*, 1934−1940.
(24) Hofslokken, N.; Skattebol, L. *Acta Chem. Scand.* **1999**, *53*, 258−262.

for **1**. Further, compound **1** was condensed with a linear



1

tetraamine, *N*,*N*′-bis(3-aminopropyl)ethylenediamine, to obtain the Schiff-base ligand that was reduced in situ to yield H_2-3-Eadd (**2**). ^{1}H and proton-decoupled ^{13}C NMR spectra

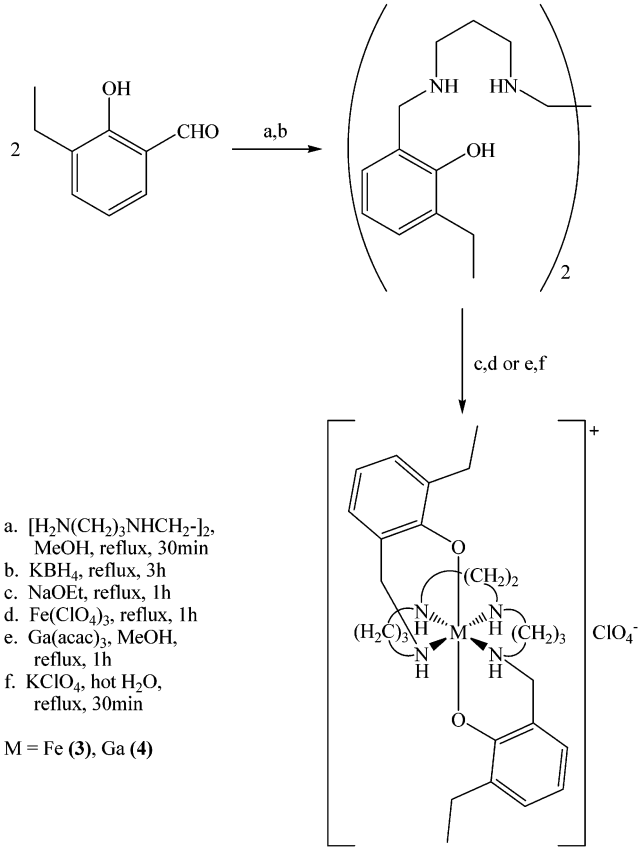


2

demonstrated **2** to be symmetrical around the central ethylene moiety of the linear tetraamine hydrocarbon backbone. Further, the presence of single set of resonance signals assigned to aromatic and benzylic protons supported the existence of a symmetrical structure in the solution. In addition, mass spectral data confirmed its formulation. A metalloantimalarial, [Fe-3-Eadd]$^+$ (**3**), was prepared through deprotonation of H_2-3-Eadd (**2**) with sodium-ethoxide, and reaction of the resultant sodium phenolate with iron(III) perchlorate, Scheme 1. The surrogate marker, [Ga-3-Eadd]$^+$ was obtained through transmetalation reaction using H_2-3-Eadd (**2**), and gallium(III)-acetylacetonate, Scheme 1. Both compounds **3** and **4** were soluble in methanol, and acetonitrile. RP-HPLC analyses, using an isocratic solvent system (MeOH/water; 25:75), demonstrated retention times of 13.3 and 11.5 min for **3** and **4**, respectively, suggesting them to be moderately hydrophobic. Furthermore, the ^{1}H NMR spectrum of **4** recorded in DMSO-d_6 demonstrated a single set of signals assigned to the aromatic protons at δ 7.05(d), 6.90(d), and 6.58(t) as well as δ 1.15(t), and δ 2.62(q) for the ethyl substituent, overall indicating that the aromatic rings remained chemically equivalent upon coordination of donor core of the ligand (**2**) with the central gallium. Although the ligand (**2**) was achiral and flexible, it yielded a rigid chiral complex **4** on coordination of the ligand to the gallium. Thus, the protons assigned to the hydrocarbon backbone appeared as a complex series of multiplets between δ 2.80 and 4.10, arising due to the chirality of the coordinated amine nitrogens. Further, proton-decoupled ^{13}C NMR of **4** recorded in DMSO-d_6 at room temperature demonstrated 13 resonance signals supporting the existence of a 2-fold symmetry for the structure of the compound in solution. These observations were found to be in accord with other metal complexes of similar ligands.[25−27]

Crystals suitable for X-ray crystallography were grown by slow evaporation of the solution of **3** dissolved in ethanol,

Scheme 1



while crystals for **4** were obtained either by slow diffusion of ether into a solution of **4** dissolved in the mixture of methanol/acetonitrile (9:1) or slow evaporation of methanolic solution of **4**. The ORTEP drawings showing the crystallographic numbering scheme are illustrated, in Figures 1 and 2. The crystal data, including refinement parameters and selected bond angles and interatomic distances, are given in Tables 1 and 2. The crystal structures demonstrated iron(III) and gallium(III) being involved in pseudo-octahedral geometry, wherein central metals were surrounded by four secondary amine nitrogen atoms of the hydrocarbon backbone in the equatorial plane and two axial phenolate oxygen atoms. Both [Fe-3-Eadd]$^+$ (**3**) and [Ga-3-Eadd]$^+$ (**4**) formed four six-membered rings and one five-membered ring upon coordination of the organic scaffold to the central core metals. Among the four six-membered rings, those arising due to participation of aliphatic hydrocarbons were found to be in a chairlike configuration, whereas those forming a boatlike configuration resulted from participation of both aliphatic and aromatic carbon atoms. The angle N2−M−N3 was found to be the narrowest in both **3** and **4** due to restrictions of the five-membered ring. The angles involving O1−M−O2, N1−M−N3, and N2−M−N4 averaged 174.5° and 175.6° for **3** and **4**, respectively. Further evaluation of the

(25) Sharma, V.; Wey, S. P.; Bass, L.; Crankshaw, C. L.; Green, M. A.; Welch, M. J.; Piwnica-Worms, D. *J. Nucl. Med.* **1996**, *37*, 51P.
(26) Sharma, V.; Beatty, A.; Goldberg, D. E.; Piwnica-Worms, D. *J. Chem. Soc., Chem. Commun.* **1997**, 2223−2224.
(27) Polyakov, V. R.; Sharma, V.; Crankshaw, C. L.; Piwnica-Worms, D. *Inorg. Chem.* **1998**, *37*, 4740−4742.

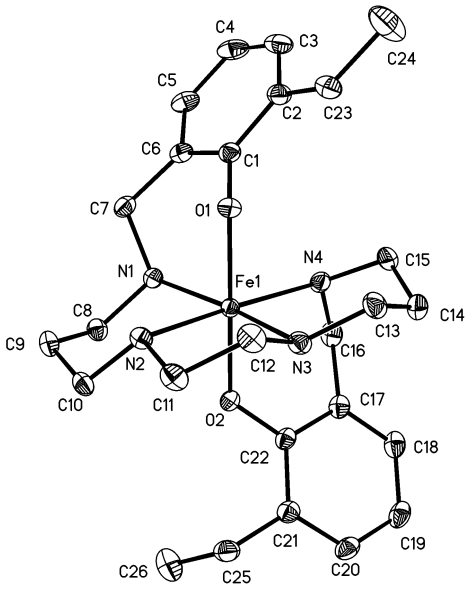


Figure 1. ORTEP drawing of $[Fe\text{-}3\text{-}E\ add]^+$ cation in $[Fe\text{-}3\text{-}E\ add]^+[ClO_4]^-$ (**3**) showing the crystallographic numbering scheme. Atoms are represented by thermal ellipsoids corresponding to 20% probability.

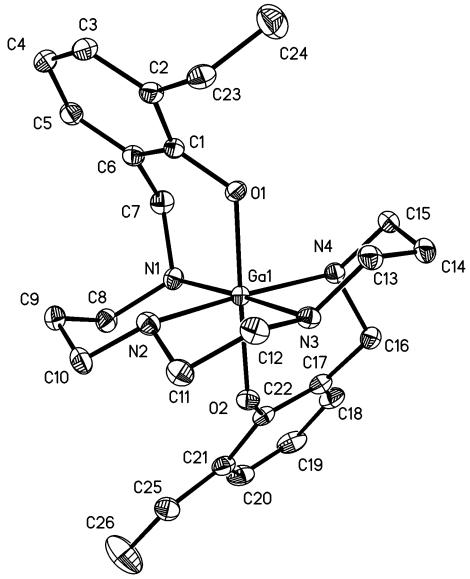


Figure 2. ORTEP drawing of $[Ga\text{-}3\text{-}E\ add]^+$ cation in $[Ga\text{-}3\text{-}E\ add]^+[ClO_4]^-$ (**4**) showing the crystallographic numbering scheme. Atoms are represented by thermal ellipsoids corresponding to 20% probability.

cis angles in the donor core around central metal cores involving O−M−N indicated slightly less distortion in **3** compared with **4**. These bond angles were found to be similar compared to other amine−phenol−iron(III)[28,29] and gallium(III) complexes.[26,30] Therefore, combined results of NMR spectral and crystal data demonstrated symmetrical contribution of two anionic phenolate oxygen atoms, and four secondary amine nitrogen atoms of the ligand (**2**) for **3** and **4** both in solution, and in the solid state. Further, mass spectral data of **3** and **4** were consistent with the proposed

Table 1. Crystal Data and Refinement Parameters for $[Fe\text{-}3\text{-}Eadd]^+[ClO4]^-$ and $[Ga\text{-}3\text{-}Eadd]^+[ClO4]^-$

	$[Fe\text{-}3\text{-}Eadd]^+[ClO4]^-$	$[Ga\text{-}3\text{-}Eadd]^+[ClO4]^-$
chemical formula	$C_{26}H_{40}ClFeN_4O_6$	$C_{26}H_{40}ClGaN_4O_6$
fw, g mol^{-1}	595.92	609.79
T (K)	100	100
wavelength (Å)	0.71073	0.71073
crystal system	monoclinic	monoclinic
space group	$P2_1/n$	$P2_1/n$
unit cell dimensions	$a = 11.1321(5)$ Å	$a = 11.1232(4)$ Å
	$b = 16.2569(7)$ Å	$b = 16.2188(7)$ Å
	$c = 15.9053(6)$ Å	$c = 15.7708(6)$ Å
	$\alpha = \gamma = 90.0°$	$\alpha = \gamma = 90.0°$
	$\beta = 103.6010°$	$\beta = 103.6110°$
V (Å^3)	2797.7(2)	2765.23(19)
d(calcd)	1.415 g cm^{-3}	1.465 g cm^{-3}
Z	4	4
GOF	1.043	1.021
final R indices $[I > 2\sigma(I)]$	0.0370	0.0308

formulation. The absence of any species in the higher mass range in the mass spectral data for **3** and **4** supported the presence of monomeric cationic compounds. Molar conductance measurements for **3** and **4** showed values of 126 and 125 Ω^{-1} mol^{-1} cm^2, respectively, consistent with a 1:1 electrolyte (monocationic metal complexation).[31]

Because these compounds were designed for their therapeutic applications, it was, therefore, essential to evaluate the stability of **3** and **4** under physiological conditions. Thus, we evaluated the stability of these compounds through RP-HPLC. Both **3** and **4** were found to be stable at pH 7.35 in PBS or HEPES (100 mM) buffer for at least 3 days at 37 °C. The existence of only parental peaks for these metallodrugs during RP-HPLC analysis suggested absence of demetalation reactions under these conditions. However, it is likely that metabolites of these compounds also elute coincidentally with the same retention times. Therefore, the peaks were collected and analyzed through mass spectroscopy. The mass spectral data of these individual HPLC fractions showed the presence of only parental compounds. Thus, the data seemed to be consistent with the postulation that these metallodrugs might permeate into the putative site of action, the digestive vacuole, as intact compounds rather than behave as a vehicle for delivery of metal(III) salts or chelators for the extraction of iron, in the digestive vacuole of the parasite.

Antimalarial Activity: Cytotoxicity against *Plasmodium falciparum* Strains. Compounds **3** and **4** were assessed for their antimalarial efficacy against *P. falciparum* trophozoites in intraerythrocytic culture (Figure 3). The ability of these compounds to induce parasite death was measured by ^{3}H-hypoxanthine incorporation assays and correlated directly with blood smear counts. Control experiments confirmed the HB3 line to be CQ-sensitive, whereas the Dd2 line was established to be CQ-resistant. The compounds were found to be most efficient at the mid-trophozoite stage. Compounds **3** and **4** demonstrated half-maximum inhibitory concentration (IC_{50}) values of 80 and 86 nM, respectively, in the CQ-sensitive HB3 line. In addition, compounds **3** and **4** were

(28) Kimura, S.; Bill, E.; Bothe, E.; Weyhermuller, T.; Wieghardt, K. *J. Am. Chem. Soc.* **2001**, *123*, 6025−6039.
(29) Yamahara, R.; Ogo, S.; Watanabe, Y.; Funabiki, T.; Jitsukawa, K.; Masuda, H.; Einaga, H. *Inorg Chim Acta* **2000**, *300*−*302*, 587−596.
(30) Wong, E.; Caravan, P.; Liu, S.; Rettig, S.; Orvig, C. *Inorg. Chem.* **1996**, *35*, 715−724.
(31) Geary, W. J. *Coord. Chem. Rev.* **1971**, *7*, 81−122.

Table 2. Selected Bond Angles (deg) and Interatomic Distances (Å) for $[Fe\text{-}3\text{-}Eadd]^{+}[ClO4]^{-}$ and $[Ga\text{-}3\text{-}Eadd]^{+}[ClO4]^{-}$

bond angles (deg)		interatomic distances (Å)	
N1−Fe−N2, 89.23(4)	N1−Ga−N2, 93.39(5)	Fe−O1, 1.9575(10)	Ga−O1, 1.9187(11)
N2−Fe−N3, 81.45(5)	N2−Ga−N3, 83.27(5)	Fe−O2, 1.9142(10)	Ga−O2, 1.9542(11)
N3−Fe−N4, 92.62(5)	N3−Ga−N4, 89.71(5)	Fe−N1, 2.1554(11)	Ga−N1, 2.1160(13)
N4−Fe−N1, 96.70(4)	N4−Ga−N1, 93.62(5)	Fe−N2, 2.1661(12)	Ga−N2, 2.0954(13)
O1−Fe−O2, 178.86(4)	O1−Ga−O2, 177.54(5)	Fe−N3, 2.1520(12)	Ga−N3, 2.1109(13)
N1−Fe−N3, 170.62(5)	N1−Ga−N3, 176.57(5)	Fe−N4, 2.1744(12)	Ga−N4, 2.1019(13)
N2−Fe−N4, 174.06(5)	N2−Ga−N4, 172.95(5)	O1−C1, 1.3384(17)	O1−C1, 1.3450(19)
O1−Fe−N1, 89.58(4)	O1−Ga−N1, 91.87(5)	O2−C22, 1.3455(16)	O2−C22, 1.3428(18)
O1−Fe−N4, 90.06(4)	O1−Ga−N4, 88.57(5)		
O2−Fe−N2, 90.70(4)	O2−Ga−N2, 88.20(5)		
O2−Fe−N3, 91.83(5)	O2−Ga−N3, 88.56(5)		
C1−O1−Fe, 121.74(9)	C1−O1−Ga, 124.37(10)		
C22−O2−Fe, 127.37(9)	C22−O2−Ga, 120.12(10)		

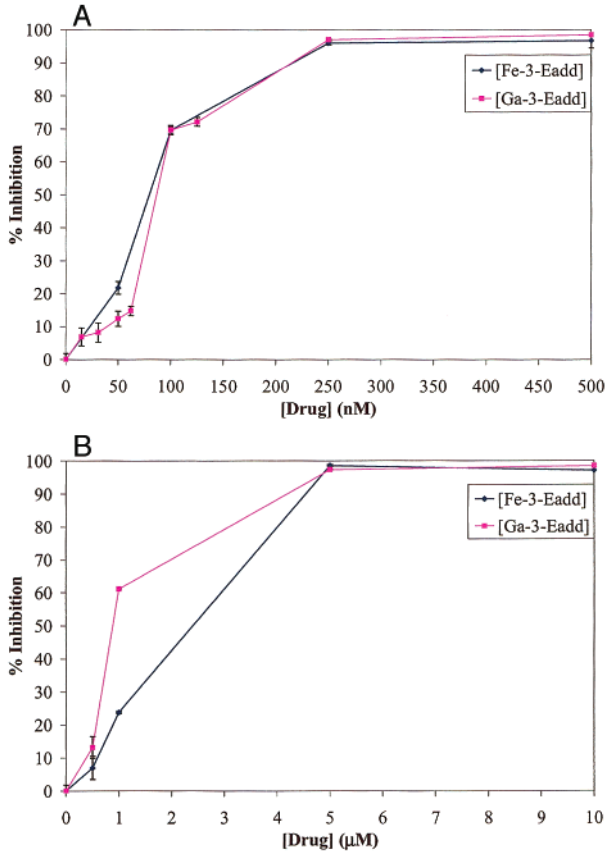


Figure 3. Effect of **3** and **4** on intraerythrocytic *P. falciparum* in culture: CQ-sensitive (HB3, A) and -resistant (Dd2, B) lines were grown in the absence or presence of various concentrations of metalloantimalarials. Growth inhibition relative to control was measured by the ^{3}H-hypoxanthine incorporation assay. Data are shown as mean values of triplicate determinations.

found to possess modest efficacy with IC_{50} values of 2.5 and 0.88 μM, respectively, in the CQ-resistant Dd2 line, thereby displaying preferential cytotoxicity toward the CQ-sensitive HB3 line. Similarity in the cytotoxicity profiles for these iron(III) and gallium(III) complexes suggested that gallium(III) ion may serve as a substitute for iron(III) ion in the biological environments. Further, these observations will facilitate the design of radiolabeled analogues of these metalloantimalarials for pharmacokinetic studies. Commercially available radioisotopes Ga-67 and Fe-59 have half-life ($t_{1/2}$) values of 78.2 h and 45 days, respectively. Therefore, Ga-67 can be substituted into the organic scaffold instead of Fe-59 for preliminary pharmacokinetic studies to avoid long-term radiochemical storage.

During its intraerythrocytic life cycle, the parasite digests hemoglobin to obtain requisite amino acids for its growth and survival. While this process produces needed amino acids, it also produces toxic free heme (ferriprotoporphyrin IX; FE(III)PPIX; FP). To balance the metabolic needs for amino acids against toxic effects of heme, the parasite has evolved a unique detoxification process that involves formation of a crystalline, insoluble, black pigment commonly known as hemozoin. The structure of this biocrystal was found to consist of dimers of the five-coordinate FP linked by reciprocating monodentate carboxylate linkages from one of FP's propionate functionalities.[32] These dimers are linked via an extensive network of hydrogen bonds contributed by the second propionic acid group of protoporphyrin IX. Inhibition of this vital sequestration process is believed to be the mechanism of action of antimalarial quinolines such as chloroquine, and it is a target for the development of new antimalarial drugs.

The mechanism of action for hemozoin-targeted antimalarial agents necessitates transport across four bilayers: the erythrocyte plasma membrane, the parasitophorous vacuolar membrane, the parasite plasma membrane, and finally the digestive vacuole membrane. Chloroquine, the classic hemozoin-targeted agent, is a moderately hydrophobic base possessing titratable protons that confer net positive charge in the acidic environments of digestive vacuole. Thus, CQ is thought to diffuse in its nonprotonated form across the vacuolar membrane and be trapped in the acidic compartment of the digestive vacuole.[33] Once in the vacuole, chloroquine prevents sequestration of toxic heme into hemozoin, by binding with heme. The ability of drugs to act as inhibitors of heme aggregation may be dependent upon two factors: (a) formation of a heme−drug complex and (b) interaction of the heme−drug complex with a heme polymer. Furthermore, hemozoin from both CQ-sensitive and -resistant strains was found to have similar affinity for the CQ−heme complex, thereby suggesting failure of CQ to access its target site in the drug-resistant strains.[34] We have demonstrated that these metal(III) complexes also target this critical heme

(32) Pagola, S.; Stephens, P.; Bohle, D.; Kosar, A.; Madsen, S. *Nature* **2000**, *404*, 307−310.
(33) Yayon, A.; Cabantchik, Z.; Ginsburg, H. *EMBO J.* **1984**, *3*, 2695−2700.

sequestration process, similarly to CQ.[12,26] In addition, selected analogues in this class of metallodrugs demonstrate modest antimalarial activity against the CQ-resistant Dd2 line.

To determine if there is a correlation between antimalarial activity of these metalloantimalarials in *Plasmodium* culture and their ability to target heme sequestration (hemozoin formation), both compounds **3** and **4** were incubated at increasing concentrations in *P. falciparum* culture (HB3 and Dd2 lines). The efficacy of a given compound to act as inhibitor was assessed from the amount of hemozoin recovered. Compounds **3** and **4** were found to be inhibitors of hemozoin formation with IC_{50} values of 160 and 150 nM in the HB3 line. However, both compounds demonstrated higher IC_{50} values, ~2 μM in the Dd2 line, consistent with observed cytotoxicity profiles, thereby displaying preferential hemozoin targeting in the CQ-sensitive HB3 line. While the inhibition of hemozoin formation in the digestive vacuole of the parasite continues to be an attractive target for antimalarial drug development,[35] the resistance remains an obstacle to be overcome. The consensus in the field indicates *pfcrt,* a gene on chromosome 7, to be the chloroquine-resistant determinant.[36,37] This gene encodes a transmembrane protein PfCRT, localized in the vacuolar membrane of the parasite. PfCRT resembles the protein that facilitates transport of organic cations and protonated amino acids.[38] The simplest hypothesis is that the wild-type PfCRT fails to recognize protonated chloroquine, thus allowing interaction with heme to inhibit its sequestration process. However, the mutant PfCRT would have high affinity for protonated chloroquine thereby mediating its efflux and allowing continuation of heme sequestration.[39] Compared to our earlier results, both **3** and **4** demonstrated enhanced potency [(IC_{50}) 80 nM (**3**) and 86 nM (**4**)] in chloroquine-sensitive strains. However, these compounds were found to be 10−30-fold less potent [IC_{50} 2.5 μM (**3**) and 0.88 μM (**4**)] in chloroquine-resistant strains. The results suggest that these metal complexes are recognized by the mutant PfCRT mediating their efflux from the digestive vacuole of the parasite, thereby resulting in a decreased concentration of available drug within the intracellular compartment. Finally, that both **3** and **4** target heme sequestration as chloroquine may help us understand the drug resistance mechanism(s) and may offer promising leads for SAR development.

Conclusions

In summary, novel iron(III) and gallium(III) complexes of an amine phenol ligand were synthesized and structurally characterized. These cationic and moderately hydrophobic compounds were found to be stable under physiological conditions for a 3 day period. Similar to chloroquine, these compounds were found to be inhibitors of hemozoin formation. Both gallium(III) and iron(III) complexes demonstrated similarity in their cytotoxicity profiles that provided supporting evidence that gallium(III) ion can serve as a template for elucidation of structure and pharmacokinetics studies of iron(III) ion in biological environments. While important factors such as optimization of selectivity and bioavailability are under investigation, these results offer a scaffold in this class of compounds for further modification to generate potential inexpensive metalloantimalarial drugs.

Experimental Section

All starting materials were purchased from Sigma-Aldrich, unless otherwise stated. The ^{1}H and ^{13}C NMR spectra were recorded on a Varian 300 MHz spectrometer and chemical shifts reported in δ (ppm) with reference to TMS. Mass spectra were obtained from the Washington University Resource for Biomedical and Bioorganic Mass Spectrometry with 3-nitrobenzyl alcohol as a matrix. The crystal data was collected on a Siemens P4 four-circle diffractometer with graphite monochromated Mo Kα radiation. HPLC analysis was performed with a Waters system 600 equipped with dual λ detector 2487 set to 280 and 240 nm. The compounds were assessed for their purity on the Xterra C-18 reversed-phase column (5 μm, 123 Å) using an eluent MeOH/water (25:75) as an isocratic system. Elemental analyses (C, H, N) were performed by Galbraith Laboratories, Knoxville, TN. Molar conductance (κ, Ω^{-1} mol^{-1} cm^2) was determined with a portable conductivity meter (Orion Research, model 120) at 25 °C in acetonitrile with a 0.33 mM solution of the metal(III) complexes.[31]

Synthesis of 3-Ethyl-2-hydroxy-benzaldehyde (1). Anhydrous triethylamine (4.8 mL, 34.6 mmol) and anhydrous magnesium chloride (1.97 g, 20.7 mmol) were added to 2-ethyl-phenol (1.52 g, 12.5 mmol) dissolved in anhydrous acetonitrile (30 mL). The contents were stirred for 1 h at room temperature, reacted with *p*-formaldehyde (2.62 g, 87.3 mmol), and heated at reflux for 3 h. After cooling to room temperature, the solvent was evaporated. The residue was acidified with 5% HCl (75 mL), and the resultant acidic mixture was extracted with ether (3 × 125 mL). The combined organic extracts were dried over anhydrous sodium sulfate and filtered, and the filtrate was evaporated to give crude product. The crude product was purified over silica (Merck silica gel, mesh 70−230, 40 Å) using an eluent mixture of hexanes/chloroform (1:4) (R_f = 0.46) to yield **1** (0.87 g, 5.8 mmol, 46%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.20 (t, 3H, methyl H), 2.70 (q, 2H, methylene H), 6.95 (t, 1H, Ar−H), 7.40 (dd, 2H, Ar−H), 9.87 (s, 1H, CHO), 11.29 (s, 1H, OH). ^{13}C NMR (75.4 MHz, $CDCl_3$) δ: 13.6 (C8), 22.2 (C9), 119.5 (C5), 120.2 (C1), 131.3 (C6), 132.7 (C3), 136.2 (C4), 159.7 (C2), 196.8 (C7).

Synthesis of 1,12-Bis(2-hydroxy-3-ethyl-benzyl)-1,5,8,12-tetraazadodecane [H_2-3-Eadd] (2). H_2-3-Eadd (**2**) was synthesized by a modification of the procedure reported in the literature.[26,40,41] Briefly, *N*,*N*′-bis(3-aminopropyl)ethylenediamine (0.77 g, 4.40 mmol) was dissolved in methanol (20 mL) and treated with dropwise addition of 3-ethoxy-2-hydroxy-benzaldehyde (1.33 g, 8.87 mmol) dissolved in methanol (20 mL). The contents were heated at 70 °C for 1 h, then treated with addition of KBH_4 (0.49

(34) Sullivan, D.; Matile, H.; Ridley, R.; Goldberg, D. *J. Biol. Chem.* **1998**, *273*, 31103−31107.
(35) Wellems, T. *Science* **2002**, *298*, 124−126.
(36) Fidock, D.; Nomura, T.; Talley, A.; Cooper, R.; Dzekunov, S.; Ferdig, M.; Ursos, L.; Sidhu, A.; Naude, B.; Deitsch, K.; Su, X.; Wootton, J.; Roepe, P.; Wellems, T. *Mol. Cell* **2000**, *6*, 861−871.
(37) Carlton, J. M. R.; Fidock, D. A.; Djimde, A.; Plowe, C. V.; Wellems, T. E. *Curr. Opin. Microbiol.* **2001**, *4*, 415−420.
(38) Warhurst, D. *Parasitol. Today* **1988**, *4*, 211−213.
(39) Warhurst, D. *N. Engl. J. Med.* **2001**, *344*, 299−301.
(40) Wong, E.; Liu, S.; Lugger, T.; Hahn, F. E.; Orvig, C. *Inorg. Chem.* **1995**, *34*, 93−101.
(41) Sharma, V.; Crankshaw, C.; Piwnica-Worms, D. *J. Med. Chem.* **1996**, *39*, 3483−3490.

g, 9.18 mmol), and heated for an additional 2 h. After cooling the mixture, excess solvent was removed through rotatory evaporation to yield a white fluffy solid. The residue was acidified to pH 0.1, and the resulting aqueous solution was washed with methylene chloride (3 × 50 mL). The pH of the aqueous layer was raised to 8.4 and the compound extracted with methylene chloride (3 × 50 mL). Organic extracts were combined, dried with Na_2SO_4, filtered, and evaporated, and the residue was dried under vacuum to yield a colorless viscous oil **2** (1.24 g, 2.80 mmol, 63.6%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.21 (t, 6H, methyl H), 1.68 (m, 4H, methylene H), 2.69 (m, 16H, methylene H), 3.93 (s, 4H, benzylic H), 4.94−5.54 (bs, 6H, NH, OH), 6.69 (t, 2H, Ar−H), 6.81 (d, 2H, Ar−H), 7.03 (d, 2H, Ar−H). ^{13}C NMR (75.4 MHz, $CDCl_3$) δ: 13.9 (C24), 22.7 (C23), 29.3 (C9), 46.9 (C10), 47.7 (C8), 48.9 (C11), 52.6 (C7), 118.4 (C4), 121.8 (C6), 125.8 (C5), 127.9 (C3), 130.9 (C2), 155.8 (C1). HRMS(FAB) Calcd for $[C_{26}H_{42}N_4O_2]$: $(M + H)^+$ 443.3380. Found: 443.3380.

Synthesis of [Fe-3-Eadd]$^+$[ClO$_4$]$^-$ (3). Sodium (0.013 g; 0.57 mmol) was taken in ethanol (2 mL) and heated at 80 °C for 15 min. The resulting sodium-ethoxide was added to H_2-3-Eadd (0.1 g; 0.23 mmol) dissolved in ethanol (5.0 mL). The contents were heated for 1 h and treated with iron(III) perchlorate (0.11 g; 0.23 mmol) dissolved in ethanol (2 mL). The reaction mixture was refluxed for 1 h. The contents were cooled and filtered, and the filtrate was evaporated at room temperature for 2 days. The hexagonal deep purple crystals were obtained on slow evaporation of ethanol solution. The resulting crystals were washed with cold methanol and ether and dried under reduced pressure to yield **3** (0.062 g; 0.06 mmol; 45.7%). κ, 126 Ω^{-1} mol^{-1} cm^2. LRMS(FAB) for $[C_{26}H_{40}N_4O_2Fe]$: $(M + H)^+$ 497.2. Anal. Calcd for $C_{26}H_{40}ClFeN_4O_6$: C, 52.40; H, 6.77; N, 9.40; O, 16.11. Found: C, 52.38; H, 7.06; N, 9.46; O, 16.07%.

Synthesis of [Ga-3-Eadd]$^+$[ClO$_4$]$^-$ (4). H_2-3-Eadd (**2**) (0.16 g, 0.33 mmol) was dissolved in methanol (5 mL) and treated with dropwise addition of gallium(III) acetylacetonate (0.12 g, 0.33 mmol) dissolved in methanol at room temperature and refluxed with stirring for 1 h. Thereafter, $KClO_4$ (46 mg; 0.33 mmol) dissolved in hot water was added and refluxed for 15 min. The contents were cooled, and slow evaporation of the solvent at room temperature over 2 days provided colorless crystals, that were separated, then recrystallized by a two-phase technique through slow diffusion of ether into the methanol/acetonitrile (9:1) solution of compound **4**, and washed with cold methanol and ether to yield crystals of **4** (0.1 g, 0.16 mmol, 49.1%). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.15 (t, 6H, methyl H), 1.61 (m, 4H, methylene H), 2.62 (q, 4H, methylene H), 2.65 (m, 2H, methylene H), 2.80−2.98 (m, 6H, methylene H), 3.14−3.30 (m, 2H, methylene H), 3.74−3.82 (m, 2H, methylene H), 3.90−4.15 (m, 4H, benzylic H), 4.54 (bs, 4H, NH), 6.58 (t, 2H, Ar−H), 6.90 (d, 2H, Ar−H), 7.05 (d, 2H, Ar−H). ^{13}C NMR (75.4 MHz, $CDCl_3$) δ: 13.9 (C24), 23.2 (C23), 23.7 (C9), 47.6 (C10), 49.0 (C8), 50.6 (C11), 52.5 (C7), 115.5 (C6), 123.7 (C4), 127.0 (C5), 128.4 (C3), 132.4 (C2), 160.2 (C1). κ, 125 Ω^{-1} mol^{-1} cm^2. HRMS(FAB) Calcd for $[C_{26}H_{40}N_4O_2Ga]^+$: 509.2398. Found: 509.2430. Anal. Calcd for $C_{26}H_{40}ClGaN_4O_6$: C, 51.21; H, 6.61; N, 9.19. Found: C, 50.03; H, 6.60; N, 9.34%.

X-ray Crystallography. Crystalline samples of **3** and **4** were placed in inert oil, mounted on a glass pin, and transferred to the cold gas stream of the diffractometer. Crystal data were collected and integrated using a Bruker Apex system, with graphite monochromated Mo Kα (λ = 0.71073 Å) radiation at 100 K. The structure was solved by direct methods using SHELXS-97 and refined using SHELXL-97 (Sheldrick, G. M., University of Göttingen). Non-hydrogen atoms were found by successive full-matrix least-squares refinement on F^2 and refined with anisotropic thermal parameters. All hydrogen atoms were located from difference Fourier maps. A riding model was used for subsequent refinements of the hydrogen atoms, and their thermal parameters were allowed to refine independently.

***Plasmodium* Culture.** *Plasmodium falciparum* lines HB3 and Dd2 were grown in intraerythrocytic culture by the method of Trager and Jensen.[42] Cultures were maintained at 2% parasitemia, 2% hematocrit using bovine AlbuMax I (Gibco) in a 3% oxygen/3% carbon dioxide atmosphere. Synchronization of developmental stage was achieved by sorbitol treatment.[43] Parasite growth inhibition and half-maximal inhibitory concentration values (IC_{50}) were determined by measuring ^{3}H-hypoxanthine incorporation.[44] Parasites were incubated with drug starting at the late ring stage, and then ^{3}H-hypoxanthine was added for 4 h at the mid-trophozoite stage before harvesting parasites (Schizont stage) and assaying for incorporated radioactivity. Compounds **3** and **4** were added in the cytotoxicity assay as dilutions of a 10 mM DMSO stock. Vehicle alone had no effect on ^{3}H-hypoxanthine incorporation.

Hemozoin Inhibition Assay. *Plasmodium* lines (HB3, Dd2) were incubated with increasing concentration of drug. The cultures were maintained at 10% parasitemia, and Triton-X100 was added to 1% final concentration. The samples were centrifuged (13000 rpm) at 4 °C for 45 min, and supernatant was discarded. The pellet was resuspended in water (1 mL) and centrifuged (13000 rpm; 4 °C) for 15 min, and supernatant was discarded. The pellet was transferred into a conical tube (15 mL) using water (1.6 mL), treated with sodium hydroxide (1 N, 184 μL) and pyridine (368 μL). The heme concentration at a given data point was assessed by the method.[45]

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Supporting Information Available: Tables of X-ray crystallographic data (atomic coordinates, interatomic distances and angles, anisotropic and isotropic displacement parameters, and hydrogen coordinates). This material is available free of charge via the Internet at http://pubs.acs.org.

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(42) Trager, W.; Jensen, J. B. *Science* **1976**, *193*, 673−675.
(43) Lambros, C.; Vanderberg, J. *J. Parasitol.* **1979**, *65*, 418−420.
(44) Desjardins, R. E.; Canfield, R. J.; Haynes, J. D.; Chulay, J. D. *Antimicrob. Agents Chemother.* **1979**, *16*, 710−718.
(45) Fuhrhop, J.; Smith, K. *Porphyrins and metalloporphyrins*; Elsevier/North-Holland Biomedical Press: Amsterdam, 1975.