Metalloantimalarials: synthesis and characterization of a novel agent possessing activity against *Plasmodium falciparum*†

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The synthesis, characterization, and antimalarial potency of an amine-phenol complex of gallium(III), [{1,12-bis(2-hydroxy-3-methoxy-5-(quinolin-3-yl)-benzyl)-1,5,8,12-tetraazadodecane}-gallium(III)]$^+$, [Ga-3-M-5-Quadd]$^+$ (7) is described; a novel agent that targets *Plasmodium falciparum* strains.

The emergence of resistance to chloroquine (CQ), an inexpensive, effective and safe drug, poses difficulties in underdeveloped regions of the world for treatment of malaria.[1] Antimalarial treatments exemplified by mefloquine,[2–4] halofantrine,[5] atovaquone-proguanil,[6] and artemether-lumefantrine[7] retain their efficacy, but have limitations including their high cost[8] that negatively impact their access and distribution in the regions with limited economic resources.[9] Therefore, alternative antimalarials would be desired that are synthesized from inexpensive precursors *via* short synthetic routes and capable of overcoming resistance pathways.

Previously, we have shown that hexadentate Schiff-base phenols, and amine-phenol ligands containing an N_4O_2 donor core in their scaffold are capable of generating cationic, moderately hydrophobic, stable, and membrane permeant complexes with iron(III) and gallium(III). The former is a biocompatible metal and the latter represents an ideal surrogate marker for the former due to similar six coordinate ionic radii and ligand exchange kinetics.[10,11] These compounds have been shown to possess antimalarial properties.[12,13] Selected compounds in this class exemplified by the metalloantimalarial, [{1,12-bis(2-hydroxy-3-methoxybenzyl)-1,5,8,12-tetraazadodecane}-gallium(III)]$^+$; [Ga-3-Madd]$^+$ preferentially targeted CQ-resistant organisms and demonstrated a mechanism of action similar to that of CQ by inhibiting heme sequestration (hemozoin formation).[14] These cationic compounds have been postulated to inhibit hemozoin formation *via* the formation of specific drug/heme propionate salt.[15] However, CQ and other related quinolines block heme aggregation by participating in non-covalent pi-cation (aromatic moiety-iron) interactions. These non-covalent binding forces play a dominant role in molecular recognition for determining the structures of macromolecules exemplified by proteins in promoting drug–receptor interactions, enzyme-substrate binding, enzymatic transformation and substrate transportation.[16] Furthermore, these non-covalent interactions are also known to act as a stabilizing force between a given cation and the pi-face of the aromatic ring.[17] Therefore, we reasoned that incorporation of quinoline moieties at the 5-position into the aromatic ring of the organic scaffold would enhance efficacy of [Ga-3-Madd]$^+$ against CQ-sensitive (HB3) lines, while retaining its activity against CQ-resistant (Dd2) lines. Herein, we report the synthesis and characterization of a gallium(III) complex of the amine-phenol ligand **7**, and evaluate its cyotoxic potency as a metalloantimalarial in CQ-sensitive (HB3) and CQ-resistant (Dd2) lines.

For evaluation, 5-bromo-2-hydroxy-3-methoxy-benzaldehyde was reduced with LAH in anhydrous THF to yield **1**. The resulting phenol (**1**) was protected with methoxymethyl (MOM) ether by its treatment with an excess 1-chloro-1-methoxy-methane in the presence of diisopropylethylamine[18] to yield **2**. Further, **2** was treated with *n*-butyllithium in THF at −78 °C to obtain the organolithium derivative that in-turn was reacted with anhydrous zinc chloride to yield the organozinc compound through transmetallation reaction. Finally, the organozinc compound[19–21] obtained *in situ* was coupled to 3-bromo-quinoline under an inert atmosphere in the presence of $Pd(PPh)_4$ (7%) to yield **3**. Further, **3** was deprotected[22] with 10% HCl in methanol to give **4** and oxidized using ethylmagnesium bromide, *p*-formaldehyde and HMPA in THF[23] to give **5**. The resultant aldehyde was condensed with the linear tetramine, *N*,*N*′-bis(3-aminopropyl)ethylene diamine in 2 : 1 molar ratio to generate a Schiff-base phenol ligand that was reduced *in situ* with KBH_4 to yield **6**.

For a given drug to be beneficial as antimalarial, it should have ability to permeate membrane bilayers, and previous data in this class of compounds indicated that neither a metal salt itself nor an organic ligand have displayed any preferential cytotoxicity due to lack of membrane permeability.[12] In addition, previous data indicated that cationic and moderately hydrophobic compounds in this class have ability to permeate membrane bilayers due to delocalized positive charge. Towards this objective, a cationic metallodrug was synthesized. The gallium(III) complex (**7**) was obtained *via* transmetallation reaction involving gallium(III) acetylacetonate using previously published procedures.[9,14] The coordination with a donor core of **6** resulted in the formation of a pseudo-octahedral gallium(III) complex (**7**) wherein the central metal was engaged by four nitrogens forming an equatorial plane and two phenolate oxygens occupying axial positions. Like previously published metal(III) complexes of amine-phenol ligands[14] or Schiff-base ligands,[24,25] the presence of a single set of resonance signals assigned to the aromatic protons in the ^{1}H NMR spectrum, along with 21 resonance signals in proton-decoupled ^{13}C NMR spectrum suggested the existence of a symmetrical structure in solution. However, the complexity of various multiplets in the aliphatic region of the ^{1}H NMR spectrum was attributed to the chirality of the coordinated amines indicating rigidity in the structure of **7**. Thus, a chiral gallium(III) complex (**7**) was obtained from an achiral ligand (**6**).

† Electronic supplementary information (ESI) available: experimental details. See http://www.rsc.org/suppdata/cc/b4/b415771k/

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We evaluated the ability of **7** to inhibit the growth of trophozoites in intraerythrocyte culture by assessing ^{3}H-hypoxanthine incorporation.[26] Hypoxanthine incorporation is a measure of parasite growth and its inhibition correlates well with direct blood smear counts but provides a more reliable quantification. Compared with a half-maximal inhibitory concentration (IC_{50}) value >20 μM in the CQ-sensitive HB3 clone with $[Ga\text{-}3\text{-}Madd]^{+}$,[14] the incorporation of quinoline into the metalloantimalarial **7** improved the IC_{50} value to 0.6 μM (Fig. 1) thereby indicating a substantial (>33 fold) enhancement of its efficacy. In addition, potency of **7** was insignificantly altered in the CQ-resistant Dd2 clone [IC_{50}: 1.4 μM (**7**) *vs.* IC_{50}: 1.8 μM ($\{Ga\text{-}3\text{-}Madd\}^{+}$)] Fig. 2. These results suggest that cytotoxic targeting properties of this molecule lie in the spatial orientation of substituents on the peripheral part of the molecule and that rational drug design of metallotherapeutics may be feasible. Furthermore, *pfcrt*, a gene on chromosome **7** encoding a transmembrane protein PfCRT has been shown to confer chloroquine resistance on parasites.[10,27] The presence of the quinoline substituents may alter the interaction of the **7** with the *crt* transporter. Alternatively, the quinoline moiety itself may be active on the chloroquine sensitive parasite clone. Therefore, it will be interesting to evaluate whether different parasite clones with altered susceptibility to quinolines are also inhibited by the quinoline-metalloantimalarial **7**.

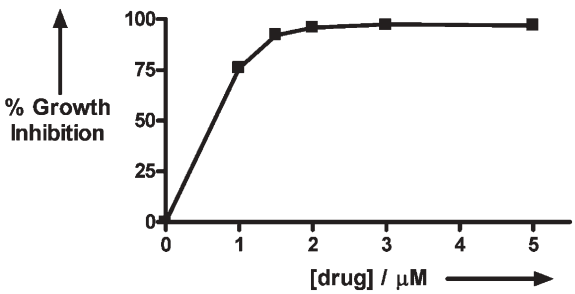


Fig. 1 Effect of **7** on growth of *P. falciparum* strains in the intraerythrocytic culture. Concentration–effect curve for chloroquine-sensitive (HB3) lines grown in the presence of various concentrations of compound, as mean values of triplicate determinations.

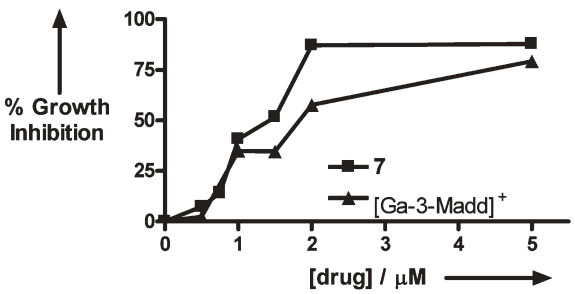
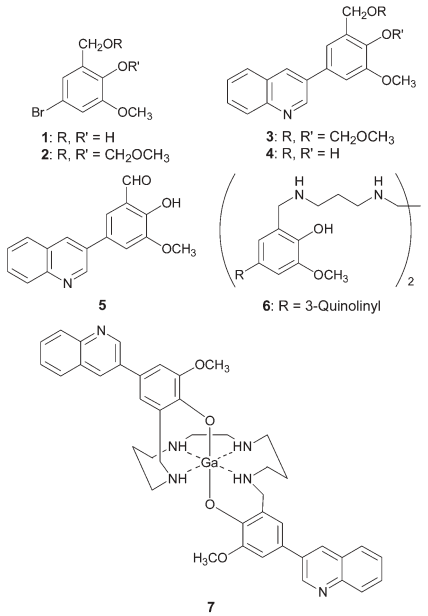


Fig. 2 Effect of **7** and $[Ga\text{-}3\text{-}Madd]^{+}$ on growth of *P. falciparum* strains in the intraerythrocytic culture. Concentration–effect curve for chloroquine-resistant (Dd2) lines grown in the presence of various concentrations of compound, as mean values of triplicate determinations.



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Notes and references

1 J. Hyde, *Microb. Infect.*, 2002, **4**, 165–174.
2 J. MacArthur, G. Stennies, A. Macheso, M. Kolczak, M. Green, D. Ali, L. Barat, P. Kazembe and T. RueBush, *Am. J. Trop. Med. Hyg.*, 2001, **6**, 679–684.
3 E. Udry, F. Bailly, M. Dusmet, P. Schnyder, R. Lemoine and J. Fitting, *Eur. Respir. J.*, 2001, **18**, 5, 890–2.
4 J. Kang, X. Chen, L. Wang and D. Rampe, *J. Pharmacol. Exp. Ther.*, 2001, **299**, 1, 290–6.
5 D. Wesche, B. Schuster, W. Wang and R. Woosley, *Clin. Pharmacol. Ther.*, 2000, **67**, 5, 521–9.
6 B. Hogh, P. Clarke, D. Camus, D. Nothdurft, D. Overbosch, M. Gunther, I. Joubert, K. Kain, D. Shaw, N. Roskell and J. Chulay, *Lancet*, 2000, **356**, 9245, 1888–94.
7 M. van Vugt, S. Looareesuwan, P. Wilairatana, R. McGready, L. Villegas, I. Gathmann, R. Mull, A. Brockman, N. White and F. Nosten, *Trans. R. Soc. Trop. Med. Hyg.*, 2000, **94**, 5, 545–8.
8 P. Winstanley, S. Ward and R. Snow, *Microb. Infect.*, 2002, **4**, 2, 157–64.
9 J. Ocheskey, V. Polyakov, S. Harpstrite, A. Oksman, D. Goldberg, D. Piwnica-Worms and V. Sharma, *J. Inorg. Biochem.*, 2002, **92**, 265–270.
10 S. Harpstrite, A. Beatty, S. Collins, A. Oksman, D. Goldberg and V. Sharma, *Inorg. Chem.*, 2003, **42**, 2294–2300.
11 V. Sharma, S. P. Wey, L. Bass, C. L. Crankshaw, M. A. Green, M. J. Welch and D. Piwnica-Worms, *J. Nucl. Med.*, 1996, **37**, 51P.
12 D. E. Goldberg, V. Sharma, A. Oksman, I. Y. Gluzman, T. E. Wellems and D. Piwnica-Worms, *J. Biol. Chem.*, 1997, **272**, 6567–6572.

13 V. Sharma and D. Piwnica-Worms, *Chem. Rev.*, 1999, **99**, 2545–2560.
14 V. Sharma, A. Beatty, D. E. Goldberg and D. Piwnica-Worms, *J. Chem. Soc., Chem. Commun.*, 1997, 2223–2224.
15 J. Ziegler, T. Schuerle, L. Pasierb, C. Kelly, A. Elamin, K. Cole and D. Wright, *Inorg. Chem.*, 2000, **39**, 16, 3731–3733.
16 D. Dougherty, *Science*, 1996, **271**, 163.
17 S. Mecozzi, A. West and D. Dougherty, *J. Am. Chem. Soc.*, 1996, **118**, 2307–2308.
18 H. Abe, S. Aoyagi and C. Kibayashi, *J. Am. Chem. Soc.*, 2000, **122**, 4583–4592.
19 G. Lessene, *Aust. J. Chem.*, 2004, **57**, 1, 107.
20 B. Kaae, P. Krogsgaard-Larsen and T. Johansen, *J. Org. Chem.*, 2004, **69**, 1401–1404.
21 E. Negishi, A. King and N. Okukado, *J. Org. Chem.*, 1977, **42**, 1821.
22 J. Auerbach and S. Weinreb, *J. Chem. Soc., Chem. Commun.*, 1974, 298.
23 G. Casiraghi, G. Casnati, M. Cornia, A. Pochini, G. Puglia, G. Sartori and R. Ungaro, *J. Chem. Soc., Perkin 1*, 1978, 318–321.
24 B. Tsang, C. Mathias and M. Green, *J. Nucl. Med.*, 1993, **34**, 1127–1131.
25 B. Tsang, C. Mathias, P. Fanwick and M. Green, *J. Med. Chem.*, 1994, **37**, 4400–4406.
26 R. E. Desjardins, R. J. Canfield, J. D. Haynes and J. D. Chulay, *Antimicrob. Agents Chemother.*, 1979, **16**, 710–718.
27 T. E. Wellems, *Science*, 2002, **298**, 124–126.