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Metalloantimalarials: Synthesis, X-ray crystal structure of potent antimalarial copper (II) complex of arylazo-4-hydroxy-1,2-naphthoquinone

Nikhil H. Gokhale,[a,†] K. Shirisha,[a] Subhash B. Padhye,[a,*] Simon L. Croft,[b] Howard D. Kendrick[b] and Vickie Mckee[c]

[a]*Department of Chemistry, University of Pune, Ganeshkhind Road, Pune-411007, India*
[b]*London School of Hygiene and Tropical Medicine, Keppel Street, London, WC1, UK*
[c]*Department of Chemistry, Loughbrough University, Leicestershire, LE11 3TU, UK*

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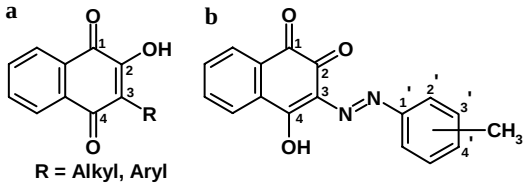
Abstract—The crystal structure of copper (II) complex of 3-arylazo-4-hydroxy-1,2-naphthoquinone is reported. The in vitro antimalarial activity of analogous compounds against *Plasmodium falciparum* 3D7 strain reveals correlation with metal redox couple, suggesting component of parasitic electron transport chain as a possible target.

The emergence of drug resistance to most clinically used antimalarial drugs by *Plasmodium* parasite is a matter of great concern and hence new effective antimalarial agents targeting novel biochemical pathways need to be evolved for treatment and control of this disease. Metalloantimalarials are a recent addition to the arsenal of therapeutic compounds for treating malarial infections especially those involving drug resistant organisms.[1,2] The redox active C-3 alkyl substituted 2-hydroxy-1,4-naphthoquinones represent an extensively investigated class of antimalarials with site-specific action on the mitochondrial electron transport chain of the parasite.[3] Metal complexation of these alkyl hydroxynahthoquinone ligands, especially with copper, has been found to be most advantageous with promising antimalarial activities against both chloroquine susceptible and resistant strains of *Plasmodium falciparum* recently.[4] The utility of copper (II) complexes in experimental therapeutics is well documented with superior efficacies encountered against experimental cancer models[5]; however, their potential as antimalarial agents has remained relatively unexplored.



Scheme 1. (a) C-3 substituted 2-hydroxy-1,4-naphthoquinone (b) 3-(arylazo)-4-hydroxy-1,2-naphthoquinone (L1H), 3-(2′-methylarylazo)-4-hydroxy-1,2-naphthoquinone (L2H), 3-(3′-methylarylazo)-4-hydroxy-1,2-naphthoquinone (L3H), 3-(4′-methylarylazo)-4-hydroxy-1,2-naphthoquinone (L4H).

We were, therefore, motivated to examine the effect of copper (II) complexation on the antimalarial activity of yet another class of naphthoquinone ligands, viz. 3-arylazo-4-hydroxy-1,2-naphthoquinones. In the present communication, we describe synthesis and characterization of Cu(II) complexes of these ligands including single crystal X-ray structure of one of the metal conjugates along with the in vitro evaluation of their antimalarial activities (Scheme 1).

The ligands, viz. (**L1H–L4H**) and their respective copper (II) complexes (**C1–C4**), were synthesized according to

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* Corresponding author. Tel.: +91 20 25601227; fax: +91 20 25691728; e-mail: sbpadhye@chem.unipune.ernet.in
† Present address: The Ohio State University, Department of Chemistry, 100 W. 18th Ave., Columbus, OH 43210, USA.

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procedures described previously.[6] The orange-colored arylazo-naphthoquinone ligands interact with copper (II) ions yielding brown copper complexes with 1:2 metal to ligand stoichiometry as judged from the compositional data (supplementary information). IR spectra of the ligands show broad hydroxyl absorption centered around 3400 cm^{-1} due to intramolecularly hydrogen bonded C-4 hydroxyl group which is lost upon copper complexation suggesting deprotonation and replacement of the hydroxyl proton by metal. The two absorptions observed for the ligands at 1680–1690 cm^{-1} and 1650–1660 cm^{-1} are typical of 1,2-quinone carbonyls, which do not undergo significant shift upon copper complexation suggesting their non-involvement in metal coordination.[7]

The reaction of **L1** with copper chloride dihydrate in methanol yielded deep brown single crystals suitable for X-ray diffraction studies upon re-crystallization from DMSO solution over several days. The ORTEP representation of this complex **C1** with numbering scheme is shown in Figure 1 along with select bond angles and interatomic distances. In **C1** complex, the ligand is coordinated to metal as a bidentate ON donor (bite angle 88.9°) via the deprotonated hydroxyl group at O (3) and azo nitrogen N (2) forming a six-membered chelate ring. The environment around the central copper atom is essentially four-coordinate with axial contacts provided by the adjacent molecules (Cu (1)–O1# 2.554 Å) yielding an overall pseudo-octahedral geometry. The axial interactions result in chains along the 'a' direction in the crystal lattice.

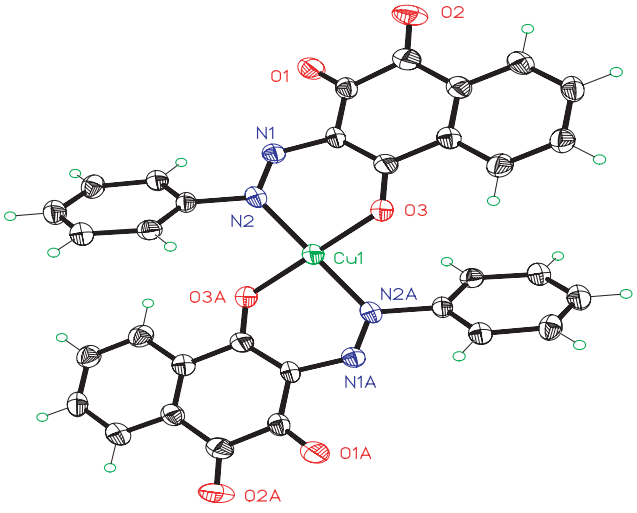

Figure 1. ORTEP representation of [Cu $(L1)_2$] **C1**. Selected bond lengths (Å) and angles (°): C (1)–O (1) 1.229(5), C (1)–C (10) 1.444(6), C (1)–C (2) 1.544(6), C (2)–O (2) 1.215(5), C (2)–C (3) 1.468(7), C (3)–C (8) 1.400(6), C (3)–C (4) 1.403(6), C (4)–C (5) 1.380(7), C (5)–C (6) 1.388(7), O (3)–Cu (1)–N (2) 88.89(13), O (1)–C (1)–C (10) 123.4(4), O (1)–C (1)–C (2) 117.7(4), C (10)–C (1)–C (2) 118.8(4), O (2)–C (2)–C (3) 124.1(5), O (2)–C (2)–C (1) 118.9(4), C (3)–C (2)–C (1) 117.0(4), C (8)–C (3)–C (4) 119.6(4), C (8)–C (3)–C (2) 1.0(4), C (4)–C (3)–C (2) 119.3(4), C (5)–C (4)–C (3) 120.0(5), O (3)#2–Cu (1)–O (3) 180.00(16), O (3)–Cu (1)–N (2)#2 91.11(13), O (3)#2–Cu (1)–N (2) 91.11(13), N (2)#2–Cu (1)–N (2) 180.00(18), O (3)–Cu (1)–O (1)#1 98.34(12), N (2)–Cu (1)–O (1)#1 94.88(13).

The two naphthoquinone ligands were found to bind the central copper atom in a trans manner resulting in equivalent average bond distances of 1.93 Å for (Cu (1)–O (3)) and 1.97 Å for (Cu (1)–N (2)), respectively. The azo bond distance exhibits a typical double bond character (N (1)–N (2) 1.27 Å), while the N (1)–C (10) bond distance of 1.37 Å confirms no electron delocalization onto the azo linkage. Thus, in the present copper complexes the ligand essentially behaves as a 1,2-naphthoquinone moiety coordinating to the metal center through nitrogen and oxygen donor atoms rather than through the 1,2-carbonyl oxygens.[8] The polycrystalline X-band EPR spectra of the copper complexes **C1–C4** measured at room temperature in DMSO solvent are axial and resolve both parallel and perpendicular features of ^{63}Cu. The g‖/A‖ quotient greater than 200 found for the complex **C3** is suggestive of the tetragonal distorted geometry around the copper center.[9]

All synthesized compounds including the ligands and their copper complexes were evaluated for their antimalarial activities against *P. falciparum* 3D7 (chloroquine susceptible) strain (Table 1). Among the ligand compound containing *ortho*-methyl substituent in the arylazo ring (**L2H**) is found to be the most potent one. All synthesized copper complexes show enhanced activities against *P. falciparum* 3D7 strain when compared with their parent ligands. The copper complex, **C3**, was found to be the most active, showing approximately 10-fold enhancement in the antimalarial activity over the parent ligand **L3H**. Interestingly this complex possesses the 'meta' substitution of the methyl group in the arylazo ring, an aspect which needs to be investigated further.

Comparison of antimalarial efficacy versus cytotoxicity against normal KB cells[4] reveals that an unsubstituted 3-arylazo-4-hydroxy-1,2-naphthoquinone almost shows no toxicity toward normal cells as compared to its methyl substituted analogs (**l2H–L4H**) nor does it show any promising antimalarial activity. The methyl substituent on the aryl ring dramatically introduces cytotoxicity. Nevertheless, it is quite evident from the cytotoxicity data (Table 1) that copper complexation provides some degree of selectivity in preferentially being toxic toward parasites besides being less toxic for the normal cells as

Table 1. Antimalarial activity and cytotoxicity of compounds **L1H–L4H** and **C1–C4**

Compound	Antimalarial activity ED_{50} (μg/ml)[a]	Cytotoxicity ED_{50} (μg/ml)[b]	S.I.[c]
L1H	>30	149.3	ND
C1	22.9	229.3	10.01
L2H	13.7	7.0	0.51
C2	12.5	9.9	0.80
L3H	>30	0.90	ND
C3	3.5	8.6	2.45
L4H	>30	1.1	ND
C4	6.3	6.6	1.04

[a] Against *P. falciparum* 3D7 strain.[4]
[b] Against KB cells[4] (ND = not determined).
[c] In vitro selectivity index (ED_{50} cytotoxicity/ED_{50}).

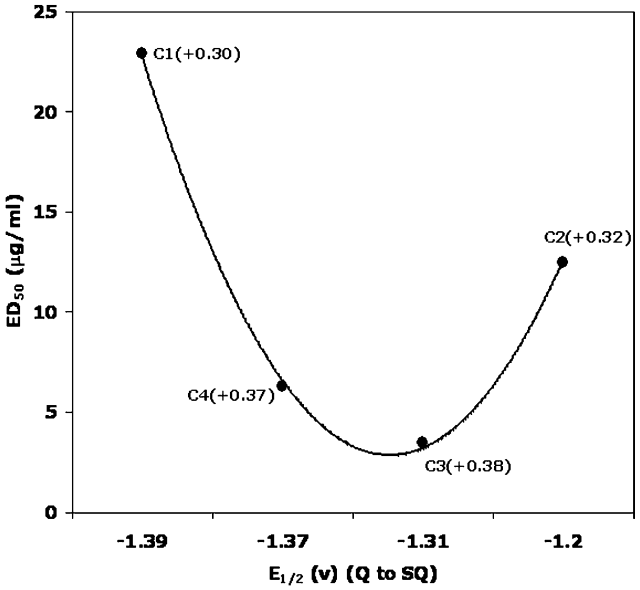

Figure 2. Plot of antimalarial ED_{50} (μg/ml) values for copper conjugates (C1–C4) of 3-arylazo-4-hydroxy-1,2-naphthoquinone against *Plasmodium falciparum* 3D7 strain versus the redox potential ($E_{1/2}$ volts against SCE) for quinone to semiquinone. The values in parentheses represent the metal redox couples.

compared to the free ligands. For example, the lead complex **C3** renders selectivity index of about 2.5 but is 10-fold less toxic than that of its corresponding free ligand (**L3H**).

A plot of (ED_{50} μg/ml) values of the antimalarial activities of present complexes versus their redox potentials for the quinone → semiquinone redox couple conversion is shown in Figure 2, along with the metal redox couples in parentheses. It is apparent that there is perhaps a small window of quinone redox potentials over which these compounds are highly active and any departures from these values result in loss of their antimalarial activity. This seems to suggest that these compounds could be targeting a specific component of the parasitic electron transport chain.

It is interesting to note that the most active **C3** copper complex (ED_{50} = 3.5 μg/ml) has the most positive metal redox potential (+0.38 V) indicating that facile reduction to the cuprous species with subsequent activation of intracellular oxygen may be one of the likely mechanisms of their antiparasitic activities.[10]

The present work thus illustrates the advantages of using copper conjugates as antimalarial agents through multiple modes of action, which may be useful additions to the second line of antimalarial agents.

Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bmcl.2005.09.061.

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