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A novel mixed-ligand antimycobacterial dimeric copper complex of ciprofloxacin and phenanthroline

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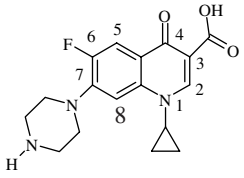
Abstract—A novel mixed-ligand Cu(II) complex of ciprofloxacin (cfH) and phenanthroline, is found to crystallize as a dimeric moiety containing monocationic and dicationic species. Two such dimeric moieties are found in the same unit cell leading to a dicationic cluster. The higher negative redox potential for this cluster dampens its antimycobacterial activity against *M. smegmatis*.

1. Introduction

Quinolones represent a large family of synthetic antibacterial agents, which inhibit two crucial bacterial enzymes, viz. DNA gyrase and topoisomerase, respectively, and hence possess a broad spectrum antibacterial activity.[1–3] Ciprofloxacin (**1**) is the most common therapeutic agent belonging to this class, which is currently used in clinical practice for treating mycobacterial infections.[4]



Ciprofloxacin (cfH,**1**)

Complexation with metals is known to enhance biological activities of the quinolone antibiotics due perhaps to resulting higher liposolubilities leading to greater intracellular accumulations.[5–7] The modes of metal coordination with quinolone ligands, however, are found to be influenced by number of factors, such as the pH of the reaction mixture,[8] anionic part of the metal salt used[9] and ancillary ligands.[10] The mixed-ligand metal complexes of quinolones are especially found to exhibit enhanced biological activities.[11] For example, the mixed-ligand copper complex of nalidixic acid and 1,10-phenanthroline is found to be more potent inhibitor of *E. coli* than the free quinolone ligand or the cupric–phenanthroline complex.[12] This motivated us to study the effect of ternary copper complexation on the antimycobacterial activity of ciprofloxacin (**1**).

In the present communication, we describe the synthesis and structural characterization of a mixed-ligand complex of **1** with the nitrogen donor ligand 1,10-phenanthroline (phen), having monocationic as well as dicationic units along with the single crystal X-ray structure, cyclic voltammetry and examination of its antimycobacterial activity against *Mycobacterium smegmatis*.

2. Experimental

Ciprofloxacin was the product of Dr. Reddy's Laboratories Ltd. (India) while $Cu(BF_4)_2{\cdot}6H_2O$ was obtained

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from Aldrich Chemicals (USA) and these were used without further purification. Analytical grade solvents were employed during all syntheses, which were distilled prior to their use. The instrumental details of all measurements are described earlier.[13]

3. Synthesis of [Cu(cfH)(phen)Cl][Cu(cfH)(phen)-(OH$_2$)](BF$_4$)$_2$Cl·8H$_2$O (2)

To a mixture of $Cu(BF_4)_2 \cdot 6H_2O$ (0.24 g, 1 mmol) and 1,10-phenanthroline (0.18 g, 1 mmol) in acetonitrile solvent (5 mL), an aqueous solution of **1** (0.36 g, 1 mmol) was added with stirring and pH of the reaction mixture was adjusted at 7–8 with a few drops of 0.1 M NaOH. The resulting green solution was concentrated on a rotavapor and kept in a desiccator for slow evaporation. The precipitated complex was filtered, washed with acetonitrile and dried in vacuum. The green crystals suitable for X-ray studies were grown after 48 h by slow evaporation. Anal. Calcd for $C_{58}H_{70}B_2Cu_2Cl_2F_{10}N_{10}O_{15}$: C, 44.75; H, 4.53; N, 8.99; Cu, 8.16. Found: C, 44.70; H, 4.47; N, 8.93; Cu 8.09%. IR: (Nujol, cm^{-1}): 1617 ν (C=O$_{keto}$); 1061 ν(B–F).

4. X-ray crystallography of 2

Crystal data and numerical parameters for the data collection and refinement are summarized in Table 1. Data were measured at 200 K on a pale green crystal of **2** with dimensions $0.18 \times 0.09 \times 0.07$ mm at 200 K, using a Stoe IPDS area detector diffractometer, equipped with an Oxford Crysostream low-temperature attachment. The structure was solved by direct method and refined against F^2 (all data, anisotropic for H atoms), using the SHELXTL software suite.[14]

Table 1. Crystal data and structure refinement summary for [Cu(cfH)(phen)Cl][Cu(cfH)(phen)(H$_2$O)](BF$_4$)$_2$Cl·8H$_2$O (**2**)

Empirical formula	$C_{58}H_{70}B_2Cl_2Cu_2F_{10}N_{10}O_{15}$
Formula weight	1556.84
Temperature (K)	200(2)
Wavelength (Å)	0.71073
Crystal system	Triclinic
Space group	P-1
a (Å)	12.6513(11)
b (Å)	13.2916(12)
c (Å)	21.9219(19)
α (°)	75.420(7)
β (°)	88.438(7)
γ (°)	64.793(6)
Volume (Å^3)	3214.4(5)
Z	2
D_{calc} (Mg/m^3)	1.609
μ (Mo-Kα) (mm^{-1})	0.849
F (000)	1600
Crystal dimensions (mm)	$0.18 \times 0.09 \times 0.07$
2θ range for data collection (°)	3.52 to 59.08°
Reflections collected	27018
Unique data	16301
R_{int}	0.0324
Completeness to $2\theta = 50.00°$	92.9%
Refinement method	Full-matrix least-squares on F^2
Parameters, restraints	935, 12
Goodness-of-fit on F^2	1.027
R indices (all data) (R_1, wR_2)	0.0762, 0.1882
R indices [$I > 2\sigma(I)$] (R_1, wR_2)	0.0630, 0.1757
Largest diff. peak and hole (e.Å-3)	0.982 and −1.264

Crystallographic data (excluding structure factors) for the structure in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC 226914. Copies of the data can be obtained, free of charge, on application to CCDC: http://www.ccdc.cam.ac.uk/perl/catreq/catreq.cgi, e-mail: data_request@ccdc.cam.ac.uk, or fax: +44-1223-336033.

Table 2. Selected bond lengths [Å] and angles [°] for **2**

Cu(1)–O(1)	1.9440(17)	Cu(2)–O(31)	1.9316(17)
Cu(1)–O(2)	1.9402(17)	Cu(2)–O(32)	1.9272(17)
Cu(1)–N(4)	2.0060(18)	Cu(2)–N(34)	2.0129(19)
Cu(1)–N(5)	2.0151(19)	Cu(2)–N(35)	2.0025(19)
Cu(1)–Cl(1)	2.5667(8)	Cu(2)–O(34)	2.303(2)
N(1)–C(1)	1.342(3)	N(31)–C(31)	1.341(3)
C(1)–C(2)	1.380(3)	C(31)–C(32)	1.380(3)
C(2)–C(3)	1.426(3)	C(32)–C(33)	1.428(3)
C(3)–O(1)	1.273(3)	C(33)–O(31)	1.275(2)
C(2)–C(10)	1.497(3)	C(32)–C(40)	1.493(3)
C(10)–O(2)	1.268(3)	C(40)–O(32)	1.266(3)
C(10)–O(3)	1.254(3)	C(40)–O(33)	1.254(3)
O(1)–Cu(1)–O(2)	92.51(7)	O(31)–Cu(2)–O(32)	93.19(7)
O(1)–Cu(1)–N(4)	91.96(7)	O(31)–Cu(2)–N(34)	92.46(8)
O(1)–Cu(1)–N(5)	165.39(8)	O(31)–Cu(2)–N(35)	170.39(9)
O(2)–Cu(1)–N(4)	163.77(8)	O(32)–Cu(2)–N(34)	164.48(8)
O(2)–Cu(1)–N(5)	90.02(7)	O(32)–Cu(2)–N(35)	90.21(7)
N(4)–Cu(1)–N(5)	81.89(8)	N(34)–Cu(2)–N(35)	82.07(8)
O(1)–Cu(1)–Cl(1)	91.77(6)	O(31)–Cu(2)–O(34)	90.81(9)
O(2)–Cu(1)–Cl(1)	97.17(6)	O(32)–Cu(2)–O(34)	93.43(9)
N(4)–Cu(1)–Cl(1)	98.28(6)	N(34)–Cu(2)–O(34)	100.94(9)
N(5)–Cu(1)–Cl(1)	102.20(7)	N(35)–Cu(2)–O(34)	97.96(9)

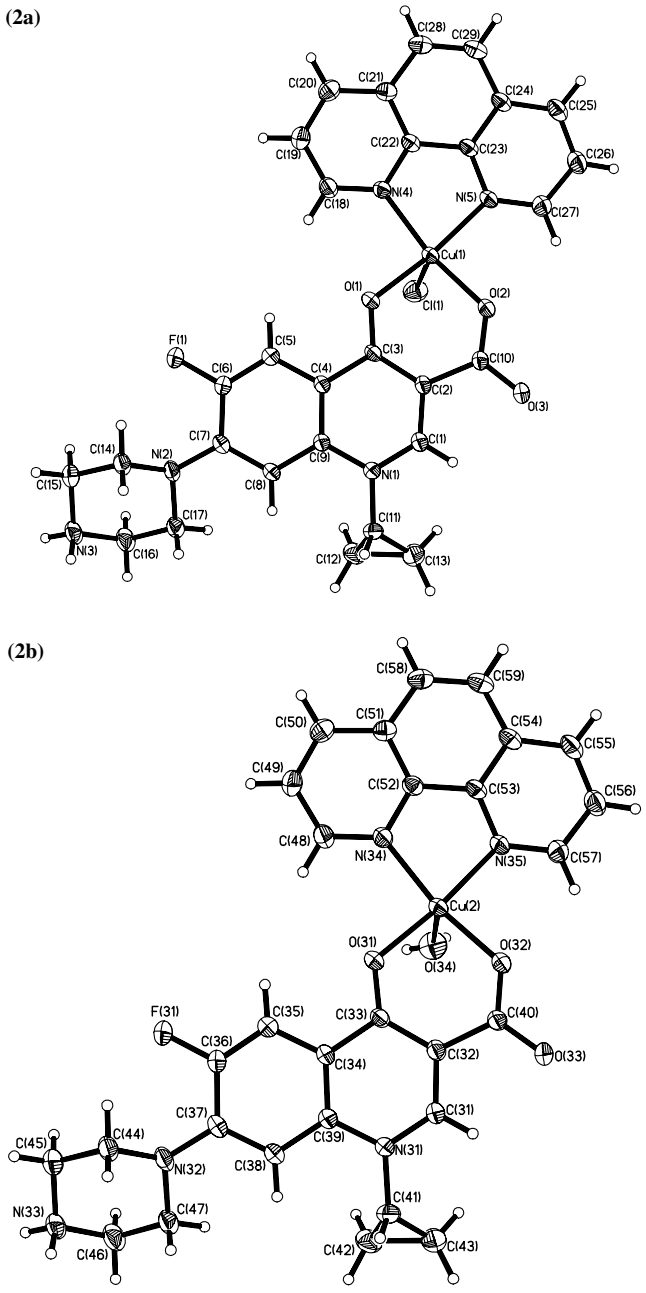

Figure 1. Structures of the monocationic unit $[Cu(cfH)(phen)Cl]^+$ (**2a**) and of the dicationic unit $[Cu(cfH)(phen)(H_2O)]^{2+}$ (**2b**).

5. Antimycobacterial activity against *M. smegmatis*

The antimycobacterial activities of the parent ligand **1** and its copper compound **2** were determined against *M. smegmatis* according to standard protocols by agar plate disc assay method. The plates were maintained at 37 °C for 24 h after which the diameters of the inhibition zones were measured.[15]

6. X-ray structural data

The structure of the copper complex **2** is shown in Figure 2. Crystal data and structure refinement parameters of compound **2** are presented in Table 1 while selected bond lengths and bond angles are listed in Table 2. In this crystal structure there are two types of cationic species, viz. $[Cu(cfH)(phen)Cl]^+$ and $[Cu(cfH)(phen)(OH_2)]^{2+}$, respectively, which are contributing to the structural properties of the compound. These are accompanied by two tetrafluoroborate and once chloride counter anions. The molecular structure is stabilized in the crystal lattice by eight uncoordinated water molecules through extensive hydrogen bonding network. The most remarkable feature of this molecule is the dimeric association of $[Cu(cfH)(phen)]^{2+}$ units through C-4 carboxyls and two such dimeric molecules are found to be present in the unit cell. Each dimeric complex consists of a five coordinated motif having a distorted square–pyramidal geometry where the differences lie in the groups occupying the apical positions. In the monocation complex, $[Cu(cfH)(phen)Cl]^+$, the apical position is occupied by the chloride anion (Fig. 1a) whereas in the dicationic complex viz. $[Cu(cfH)(phen)(OH_2)]^{2+}$, the apical position is occupied by a neutral water molecule (Fig. 1b). In the former compound, the Cu–O distances are 1.9440(17) (pyridone) and 1.9407(17) Å, (carboxylate), whereas in the latter case these distances are found 1.9316(17) (pyridone) and 1.9272(17) Å (carboxylate), respectively.[16] Both the distances are slightly longer than those found in other ciprofloxacin metal complexes probably as a result of weak dimeric associations.[17,18] The Cu(1)···Cu(1′) and Cu(1)·O(1′) (apical) distances in the dimeric, monocationic copper complex (Fig. 2) are 4.2031(7) and 3.288(2) A, respectively, while those in the accompanying dicationic copper species are 4.0946(7) and 3.263 (2) Å indicating that in the former the parallelogram consisting of Cu(1)–(O1)–Cu(1′) is slightly distorted. The extensive intermolecular hydrogen bonding network between the protonated piperazinic nitrogen and oxygens of the water as well as carboxylate groups can be seen from the packing diagram of this crystal (Fig. 3).

7. Spectroscopy and magnetism

The IR spectrum of **1** shows the carboxylate stretch at 1704 cm^{-1} while the pyridine carbonyl can be seen at 1624 cm^{-1}. Upon copper complexation the former is split into a doublet at 1572 and 1317 cm^{-1} indicating deprotonation of the carboxylic group and its participation in the metal coordination,[19] while the latter is shifted slightly to lower wavenumber 1617 cm^{-1}.[17] The strong band due to out-of-plane vibration of the hydrogen atoms of the heterocyclic phenanthroline ring in **1** is observed at 733 cm^{-1} while the hydrogens on the carboxylic ring are seen at 858 cm^{-1}, respectively.[20] The compound exhibits a broad band around 3500–3380 cm^{-1} indicative of the presence of lattice water molecules[21] and a strong absorption for the metal complexes at 1065 cm^{-1} confirms the presence of the tetrafluoroborate anions.[22]

The electronic spectrum of the copper complex exhibits intra-ligand absorptions at 272, 375, and 334 nm,

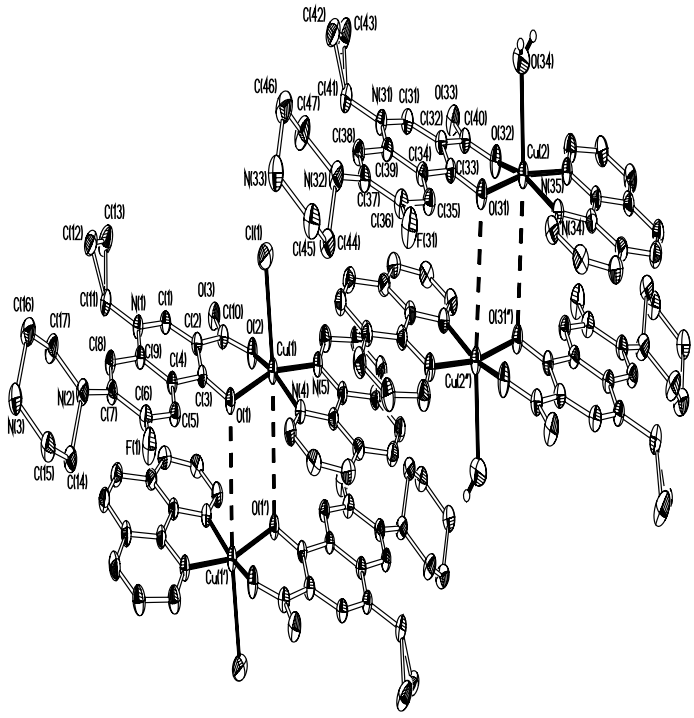

Figure 2. The two weakly-bound dimers in the structure of $[Cu(cfH)(phen)Cl][Cu(cfH)(phen)(H_2O)](BF_4)_2Cl{\cdot}8(H_2O)$ (**2**).

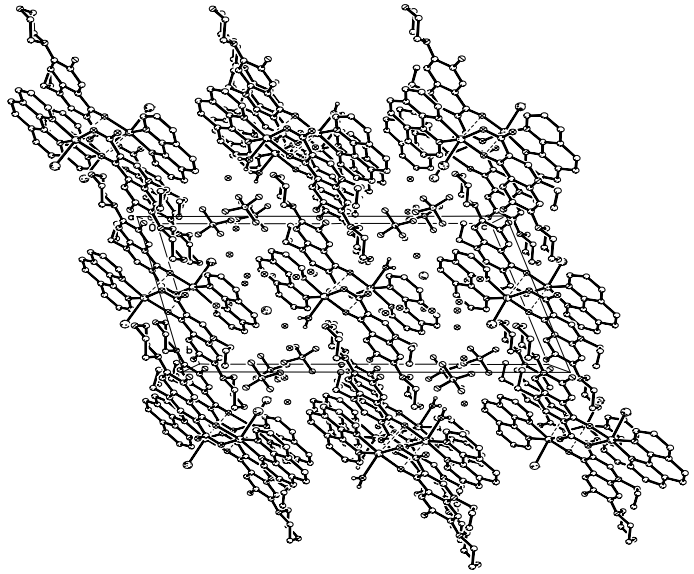

Figure 3. Packing diagram of $[Cu(cf)(phen)Cl][Cu(cf)(phen)(H_2O)](BF_4)_2Cl{\cdot}8(H_2O)$, viewed down the *a*-axis. The *pseudo*-translational symmetry along the *c*-axis, relating the very weakly-bound dimers of $[Cu(cfH)(phen)Cl]^+$ complexes and of $[Cu(cfH)(phen)(H_2O)]^{2+}$ complexes is clearly apparent.

respectively,[23,24] while the metal-based d–d transition can be located at 658 nm, which is characteristic of the square–pyramidal copper(II) compounds. Similar observations have been noted by Mendoza-Diaz et al. in case of five-coordinate copper complexes of analogous quinolone ligands such as nalidixic acid and cinoxacin.[6,25]

The lowered magnetic moment of 1.68 BM observed for compound **2** at 300 K presumably results from the weak antiferromagnetic exchanges within the dimeric units as observed for analogous compounds.[26]

The EPR parameters, viz. g_x, g_y, and g_z calculated for this compound are 2.12, 2.06, and 1.99, respectively, which are in agreement with the square pyramidal geometry observed for this compound.[27] The reduced g_o value (2.0053) observed is indicative of the weak dimeric associations observed in the present copper compound.

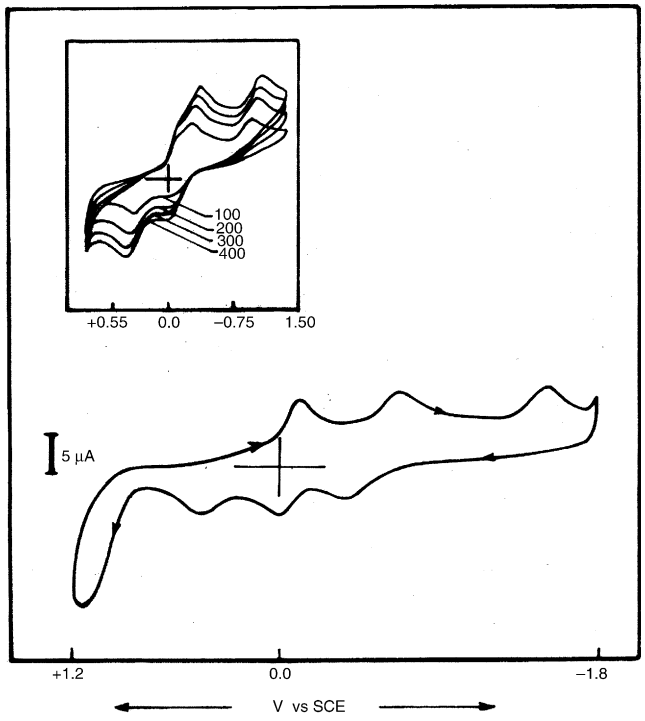

Figure 4. Cyclic voltammograms of **2** in DMSO solvent at 10^{-3} M concentration; inset showing the scan rate dependence of redox couple $Cu^{2+/+}$.

8. Cyclic voltammetry

The cyclic voltammogram for the copper compound recorded in the range of +1.2 to −1.80 V in DMSO solvent is shown in Figure 4. The redox behavior of antiferromagnetically coupled binuclear Cu(II) complexes involving bridging groups generally consists of either one step, two electron transfer[28] or two step one electron transfers.[29] The latter redox process is operative in the present case and the compound shows two successive redox peaks, one having half-wave redox potential of +0.20 V corresponding to $Cu^{2+}Cu^{2+}/Cu^{2+}Cu^{+}$ conversion while the other having potential of −0.28 V representing subsequent reduction to generate [Cu^{+}/Cu^{+}] species. The irreversible reduction wave seen at higher negative potentials (~1.55 V) is due to the reduction of the phenanthroline moiety.[30]

9. Antimycobacterial activity

The antimycobacterial activity for the parent ligand and its mixed-ligand copper complex was determined against *M. smegmatis*, which is a fast growing mycobacteria commonly used in the antimycobacterial assay by several workers.[31] The log of inhibition zone diameters observed for the ligand and their metal conjugates are depicted in Figure 5 along with those observed for analogous compounds. Contrary to the synergistic enhancement of the antimycobacterial activity observed for the copper conjugates of many antitubercular drugs including isoniazid,[32] very little enhancement is observed for the present compound. It may be envisaged that the

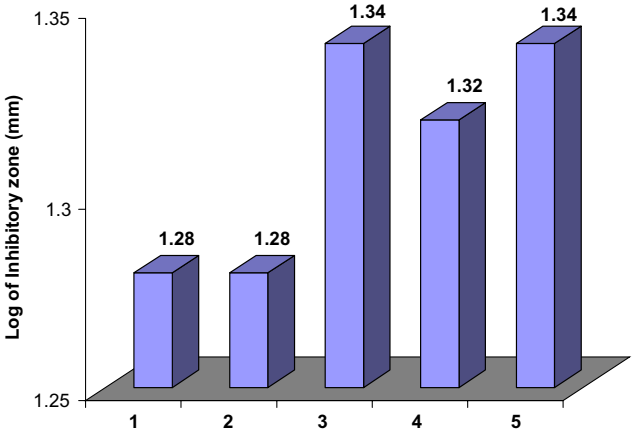

Figure 5. Antimycobacterial activities of **1** (ciprofloxacin); **2** (present complex); **3** ([Cu(cfh)(phen)Cl]; **4**([Cu(cfh)(bipy)Cl]; and **5** ([Cu(cfh)-(dafone)Cl].

dimeric nature of the present compound antagonizes the generation of fully reduced cuprous species due to its higher negative potential (−0.28 V). The presence of such cuprous moieties have been shown to result to activate intracellular oxygen producing oxidative stress, which has been shown to be detrimental for the mycobacterial species.[33] The present work thus indicates that nitrogen adducts of metal-quinolate tend to lower the antimycobacterial activities of the resulting compounds probably through stabilization of cupric species and hence may not be the appropriate strategy for designing highly active antimycobacterial compounds.

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