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Crystal structure, spectroscopic, and biological study of the copper(II) complex with third-generation quinolone antibiotic *sparfloxacin*

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Abstract—The neutral mononuclear copper(II) complex with the quinolone antibacterial drug *sparfloxacin* has been prepared and characterized with IR, UV–vis, and EPR spectroscopies and X-ray crystallography. The interaction of the complex with *calf-thymus* DNA has also been investigated and the antimicrobial activity has been evaluated against three different microorganisms.
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Quinolones, a commonly used term for the quinolone-carboxylic acids or 4-quinolones, are a group of synthetic antibacterial agents containing a 4-oxo-1,4-dihydroquinoline skeleton.[1] In general, quinolones can act as antibacterial drugs that effectively inhibit DNA replication and are commonly used as treatment for many infections.[2] In comparison with first- (*nalidixic acid*, *cinoxacin*) and second- (*norfloxacin*, *enoxacin*, *ofloxacin*, and *ciprofloxacin*) generation, third-generation quinolones (*levofloxacin*, *sparfloxacin*, *gatifloxacin*, and *moxifloxacin*) show a much broader spectrum of activity providing expanded gram-negative and gram-positive activity coverage as well as expanded activity against atypical pathogens.[3–5] The interaction of transition metal ions with diverse first- and second-generation quinolones as ligands has been studied.[6] Specially, for copper(II) the crystal structures of complexes of ciprofloxacin,[7] cinoxacin,[8,9] and ofloxacin[10] have been reported as well as mixed ligands neutral mononuclear copper(II) complexes of phenanthroline with nalidixic acid,[11] cinoxacin,[12] and ciprofloxacin,[13,14] and ionic copper(II) complexes of protonated norfloxacin.[15]

Sparfloxacin, H*sf*, (Fig. 1) is a third-generation quinolone antimicrobial drug mainly used for the treatment of acute exacerbations of chronic bronchitis and community-acquired pneumonia.[3] Although electrometric studies, elemental analysis, and magnetic measurements of a parrot-green inner complex copper(II)-sparfloxacin have been reported,[16] no crystal structure of a metal complex with a third-generation quinolone has been reported yet.

The interaction of Cu(II) with the quinolone *sparfloxacin* has been studied in an attempt to examine the mode

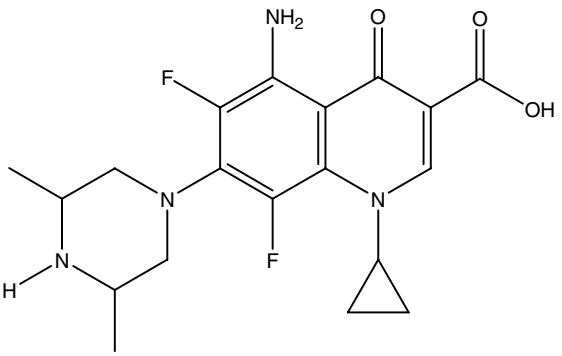

Figure 1. *Sparfloxacin* (H*sf* = 5-amino-1-cyclopropyl-7-(*cis*-3,5-dimethyl-1-piperazinyl)-6,8-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid).

Keywords: Quinolones; *Sparfloxacin*; Cu(II) complex; EPR spectroscopy; Interaction with *calf-thymus* DNA; MIC.
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of binding and possible synergetic effects. In this paper, we report the crystal structure, the solid and solution behavior of the mononuclear Cu(II) complex with the quinolone H*sf*, bis(*sparfloxacinato*)copper(II), **1**, as well as the interaction of the complex with *calf-thymus* DNA. The antimicrobial efficiency of the complex has been tested on three different microorganisms.

Complex **1** was prepared via the reaction of equimolar quantities (0.4 mmol) of H*sf* (157 mg), which was deprotonated with CH_3ONa (22 mg), and $CuCl_2{\cdot}2H_2O$ (68 mg) in CH_3OH. The reaction mixture was refluxed for 1 h. The green solution was reduced in volume and left for slow evaporation. Green crystals of **1** suitable for X-ray structure determination were deposited over a week. Yield: 220 mg, 65%. The complex is soluble in H_2O, DMSO, DMF, CH_2Cl_2, and $CHCl_3$, and is no electrolyte.

In the IR spectrum of **1**, the replacement of the valence stretching carboxylic vibration $\nu(C{=}O)_{carb}$ of H*sf* at 1716 cm^{-1} by the asymmetric, $\nu(CO_2)_{asym}$, at ~1605 cm^{-1} and the symmetric stretching ν(O–C–O) vibration, $\nu(CO_2)_{sym}$, at ~1385 cm^{-1} as well as the Δ value (~220 cm^{-1}) [$\Delta = \nu(CO_2)_{asym} - \nu(CO_2)_{sym}$] are indicative of the monodentate coordination mode of the carboxylato group.[17,18] The pyridone stretch $\nu(C{=}O)_p$ is slightly shifted from 1641 to 1635 cm^{-1} upon bonding. The overall changes in the IR spectra suggest that *sparfloxacin* is coordinated to the metal via the pyridone and one carboxylate oxygen atoms.[6]

The UV–vis spectrum of the complex has been recorded as nujol mull and in aqueous solution. The spectrum in aqueous solution is very similar with that recorded as a nujol mull. The complex exhibits one asymmetric broad d–d transition band composed with maxima at approximately ~525 nm and ~645 nm, typical for square planar geometry with CuO_4 chromophore.[19,20] An absorption band also exists as a shoulder at ~425 nm which can be assigned to the ligand-to-metal charge-transfer transition for the quinolone ligand since it is also present in the spectrum of the complex Cu(*enrofloxacin*)$_2$(H_2O) in aqueous solution.[21] The UV spectrum of the complex is practically identical with that of the quinolone ligand.

The crystal structure of **1** has been determined with X-ray crystallography.[†] An ORTEP diagram of **1** is shown in Figure 2 along with selected bond distances and angles.

The structure of the complex is centrosymmetric, the Cu(II) ion is sitting on a center of symmetry and it is

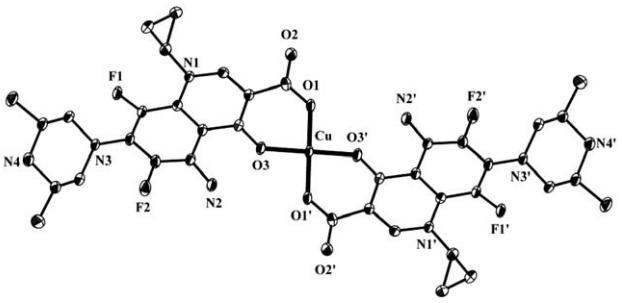

Figure 2. Partially labeled ORTEP plot of **1** with 30% thermal probability ellipsoids shown (primed atoms are related to the unprimed ones through the symmetry operation $1-x, -y, 1-z$). Selected bond distances (Å) and angles (°) of complex (**1**): Cu–O1 = 1.902(3), Cu–O3 = 1.914(2), O3–C3 = 1.292(4), O1–C1 = 1.285(5), O2–C1 = 1.230(5), Cu···O2 = 4.032(2); O1–Cu–O1′ = 180.00, O1–Cu–O3 = 92.9(1), O1–Cu–O3′ = 87.1(1), O3–Cu–O3′ = 180.00, C1–O1–Cu = 130.0(2), C3–O3–Cu = 128.1(2), O2–C1–O1 = 122.6(3), O1–C1–C2 = 119.7(3), O2–C1–C2 = 117.7(3), O3–C3–C2 = 122.0(3), O3–C3–C4 = 119.3(3).

coordinated to two bidentate *sparfloxacinato* ligands related by the inversion center. Thus, the copper ion is four-coordinate and as a result of the inversion center it displays a square planar geometry. This is one of the few cases described in which Cu(II) ion exhibits such an environment, most of them observed with the ligand *acetylacetonate* or derivatives[20] as well as in [Cu(*cinoxacin*)$_2$].[8] The coordination mode of the *sparfloxacinato* ligand in **1** is similar to that observed in other quinolone complexes.[6] The Cu–O bond lengths are similar to those of square planar copper(II) complexes with acetonato derivatives.[19] The uncoordinated carboxylato oxygen atom O(2) [Cu···O(2) = 4.032(2) Å] lies almost on the CuO_4 plane. The piperazinyl ring adopts the stable chair conformation with atoms C15 and C17 displaced at 0.57 and 0.68 Å, respectively, above and below the mean plane defined by the remaining four atoms, which form an angle of 21.3° with the quinolone ring, whereas the cyclopropyl ring forms an angle of 59.0° with the quinolone ring. Hydrogen bonding interactions between the solvate water molecules (Ow1 and Ow2), the uncoordinated carboxylato oxygen atom (O2), and the amine group of the *sparfloxacinato* ligand (N2) form a 2D polymeric network, stabilizing the lattice structure of **1**.

Figure 3 shows an X-band EPR spectrum from a powdered sample of **1** recorded at room temperature and under nonsaturating conditions. The spectrum exhibits a well-defined axial signal with $g_{\|} > g_{\perp}$ consistent with a Cu(II) monomer. Resolved hyperfine interactions due to Cu (I = 3/2) nucleus are observed at $g_{\|}$. We have fitted the spectrum within the framework of the spin Hamiltonian:

$$\boldsymbol{H} = \beta \boldsymbol{S}\boldsymbol{g}\boldsymbol{B} + \boldsymbol{I}\boldsymbol{A}\boldsymbol{S} \qquad (1)$$

where β is the Bohr magneton, $\boldsymbol{S} = 1/2$, $\boldsymbol{I} = 3/2$, and $\boldsymbol{A}$ is the tensor for the hyperfine interaction.

The fit yields $g_{\|} = 2.30 \pm 0.01$, $g_{\perp} = 2.05 \pm 0.01$, $A_{\|} = 171(\pm 3) \times 10^{-4}$ cm^{-1}, and $A_{\perp} = 4.5\ (\pm 1) \times 10^{-4}$ cm^{-1}. The lineshape of the spectrum is reproduced

[†] Crystal data of **1**. $C_{38}H_{43}{\cdot}6CuF_4N_8O_{8.8}$, $M = 892.74$, triclinic, $a = 8.867(4)$, $b = 10.418(5)$, $c = 11.260(6)$ Å, $\alpha = 97.14(2)$, $\beta = 92.01(2)$, $\gamma = 106.19(2)°$, $V = 988.5(8)$ Å^3, $T = 298$ K, space group P-1, $Z = 1$, Dc = 1.500 g/cm^3, μ(Mo-Kα) = 0.637 mm^{-1}, $F(000) = 463$. 3706 reflections measured, 3493 unique ($R_{int} = 0.0311$), which were used in all calculation. Fine $R1 = 0.0559$, $wR(F2) = 0.1583$ (all data). Full crystallographic details of **1** have been deposited at the Cambridge Crystallographic Data Center and allocated the deposition number CCDC 297689.

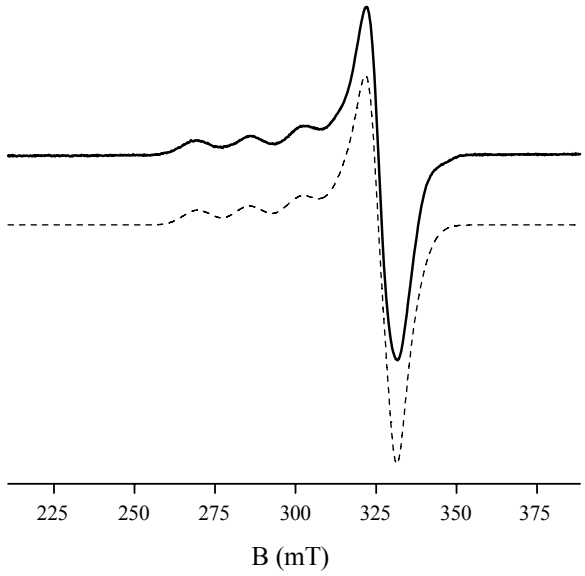

Figure 3. Experimental (solid line) and theoretical (dashed line) X-band EPR spectra from a powdered sample of **1** at room temperature. *EPR conditions:* microwave power, 0.57 mW, modulation amplitude, 0.25 mT, and microwave frequency, 9.41 GHz.

assuming Gaussian line-shape with an intrinsic line-width of 2.2 mT and a distribution of the g values (g-strain) with a width of $\sigma g_{\|} = 0.03$ and $\sigma g_{\perp} = 0.01$.

The EPR parameters are consistent with the square planar geometry of complex **1**.[19,22] Moreover, the pair of values ($g_{\|}$, $A_{\|}$) is consistent with the O_4 coordination.[8,19,23]

DNA can provide three distinctive binding sites for the quinolone complexes; namely, groove binding, binding to phosphate group, and intercalation.[24] This behavior is of great importance with regard to the relevant biological role of fluoroquinolone antibiotics in the human body.[25]

The absorption spectra (Fig. 4) of the interaction of complex **1** with *calf-thymus* DNA have been recorded for a constant DNA concentration (3.125×10^{-4} M) in different complex: *calf-thymus* DNA mixing ratios (r). The changes observed in the absorption spectra of the complex after mixing with DNA indicate that the interaction of complex **1** with DNA takes place by a direct formation of a new complex with double-helical *calf-thymus* DNA.[26]

The CD spectra of complex **1** with double-stranded DNA for different r values are shown in Figure 5 and they provide us with useful information concerning the complex-nucleotide interaction. The existence of the isosbestic point at 258 nm suggests that the conformation of the DNA-bound complex is homogeneous and independent of the r value. The CD spectra of the free ligand do not show any transitions because there is not any asymmetry or chirality in the molecules.[27]

The CD spectra of *calf-thymus* DNA in the presence of complex **1** consist of a positive band I at 279 nm and a strong negative one II at 245 nm. When r increases up

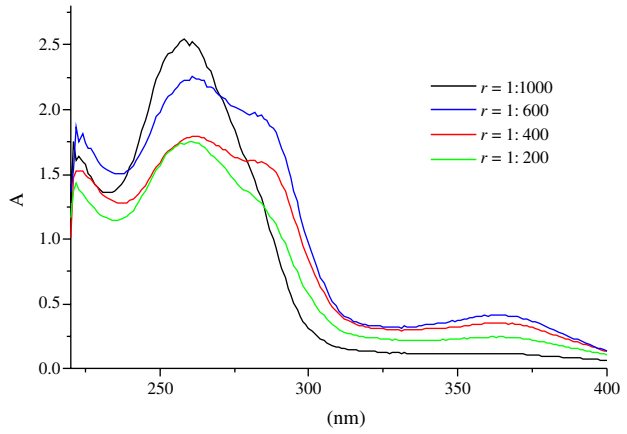

Figure 4. Absorption spectra of complex **1** in the presence of *calf-thymus* DNA for diverse r values. The spectra were recorded at 25 °C after complex **1** had been incubated with *calf-thymus* DNA for 24 h at 37 °C.

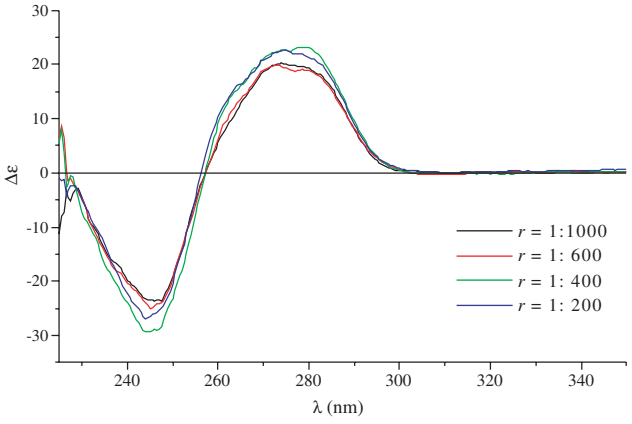

Figure 5. CD spectra of *calf-thymus* DNA in 5 mM buffer (containing 150 mM NaCl and 15 mM Tris–sodium citrate at pH 7.0) in the presence of complex **1** in different r values. The spectra were recorded at 25 °C after samples had been incubated with *calf-thymus* DNA for 24 h at 37 °C.

to 1:400, the intensity of band I at $\lambda_{max} = 279$ nm increases indicating that complex **1** is bound to *calf-thymus* DNA without being able to suggest the exact mode of binding. For r values higher than 1:600, a positive shoulder at ~264 nm appears. From all these data, we can conclude that complex **1** interacts with DNA but we cannot safely suggest the exact mode of binding.[26]

The efficiencies of the ligand and the complex have been tested against two Gram(−), *Escherichia coli* (*E. coli*), and *Pseudomonas aeruginosa* (*P. aeruginosa*), and one Gram(+), *Staphylococcus aureus* (*S. aureus*), microorganisms.[28,29] The results of these tests are presented in Table 1.

Comparing the MIC values between H*sf* and complex **1**, it is evident (Table 1) that **1** is four times more active than H*sf* against *E. coli*, while H*sf* is twice more active than **1** against *S. aureus*. Both H*sf* and **1** provide equal inhibition against *P. aeruginosa* (MIC = 0.25 μg mL^{-1}). The best inhibition provided by the two compounds is against *P. aeruginosa* (MIC = 0.25 μg mL^{-1}). This fact

Table 1. Minimum inhibitory concentration (MIC) in μg mL^{-1}

	E. coli	*P. aeruginosa*	*S. aureus*
H*sf*	8.0	0.25	0.5
Cu(*sf*)$_2$, **1**	2.0	0.25	1.0

is in accordance with the specific activity of third-generation quinolones and related compounds since third-generation quinolones are mainly used for the treatment of acute exacerbations of chronic bronchitis and community-acquired pneumonia.[3]

The synthesis and characterization of a new mononuclear copper complex with the third-generation quinolone antibacterial drug *sparfloxacin* has been realized with physicochemical and spectroscopic methods. In this complex, *sparfloxacin* is bound to copper(II) via the pyridone and one carboxylate oxygen atoms. The crystal structure of the complex has been determined with X-ray crystallography. This structure is the first reported crystal structure of a complex of a third-generation quinolone. The EPR spectrum of **1** at room temperature is consistent with the square planar geometry of the cluster and the O_4 coordination. The interaction of the complex with *calf-thymus* DNA has revealed that the complex is bound to *calf-thymus* DNA. The antimicrobial activity of the complex has been tested on three different microorganisms and the best inhibition is provided by the complex against *P. aeruginosa* (MIC = 0.25 μg mL^{-1}). Further biological experiments in order to clarify the possible mechanism of action of the complex are under investigation.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bmcl.2006.04.034.

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