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# Synthesis, crystal structure and characterization of three novel copper complexes of Levofloxacin. Study of their DNA binding properties and biological activities

Anastasia Galani [a], Eleni K. Efthimiadou [b,*], George Mitrikas [b], Yiannis Sanakis [b], Vassilis Psycharis [b], Catherine Raptopoulou [b], George Kordas [b], Alexandra Karaliota [a,*]

[a] Department of Inorganic Chemistry, Faculty of Chemistry, National University of Athens, Panepistimioupoli Zographou, GR-15701 Athens, Greece
[b] Institute of Advanced Materials, Physicochemical Processes, Nanotechnology and Microsystems, Department of Materials Science, NCSR 'Demokritos', GR-15310 Aghia Paraskevi, Attikis, Greece

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ABSTRACT

Three novel copper(II) complexes of the antibacterial drug Levofloxacin (LVX), in the presence or absence of heterocyclic ligands such as 1,10-phenanthroline and 2,2-bipyridine, have been synthesized and fully characterized. The interaction of copper(II) with the deprotonated ligand LVX reveals that LVX coordinated to copper atom through the pyridone and one carboxylato oxygen. In case of the mixed ligand complexes the Cu atom is coordinated with two O atoms from LVX ligand and with two N atoms from the hetero-ligands. According to our results the formation of dimers is a typical characteristic for almost all of them. The presence of dimers in the crystal structure is also evident in the EPR spectra and this method can be used as a probe for the existence of such species. A significant characteristic of the structure is the intra- and inter-dimer π–π stacking interaction which governs the packing of complexes in the structure. The synthesized compounds were biologically evaluated. All the complexes show an increased biological activity in comparison with the free ligand.



## 1. Introduction

It is known that the biological activity of many drugs including quinolone molecules depends on their coordination chemistry with different metal ions [1,2]. The suggested mechanism of quinolones' action is based on inhibition of two bacterial enzymes: DNA gyrase (topoisomerase II) and topoisomerase IV. DNA topoisomerase II plays a crucial role in DNA replication and DNA breakage leading to cell death [3].

The first analogue of the quinolone family is nalidixic acid [4]. In order to optimize the quinolones effectiveness, chemical modification on nalidixics' acid structure presents a spectrum of new, more potent quinolone antibacterial agents [5]. LVX (LVX), a new quinolone representative, is the more potent isomer (S-isomer) of ofloxacin racemic mixture and present an exert spectrum antibacterial activity against gram-positive and gram-negative bacteria as well as against atypical organisms [6]. Many studies support that the S-ofloxacin analogue, LVX, is the most potent in contrast to the R-isomer, ofloxacin. In a series of literature studies is mentioned that the binding affinity of S-ofloxacin to double stranded calf-thymus DNA (CT-DNA) is almost five times more potent than other known isomers [7]. As far as LVX's antibacterial activity is concerned, is fourfold more active than ofloxacin's against a variety of micro organisms. It is worth mentioning that LVX is 64-fold more active than ciprofloxacin against *Staphylococcus aureus* (methicillin resistant strain) and eightfold against *Acinetobacter* spp, due to the fact that inhibit the 90% of *Streptococci* proliferation. In contrast with the above mentioned results, LVX is twofold less active than ciprofloxacin against *Enterobacteriaceae* and *Pseudomonas aeruginosa* [8] (see Scheme 1).

Copper is the most studied metal among all transition metal ions. It exhibits considerable biochemical action either binding to albumins and others proteins or binding to ligands forming complexes that interact with biomolecules such as nucleic acids. The

*Abbreviations:* CT, calf thymus; FBS, fetal bovine serum; PBS, phosphate buffer saline; EPR, electron paramagnetic resonance; MIC, minimum inhibitory concentration; IR, infrared; Phen, 1,10-phenanthroline; Bipy, 2,2-bipyridine; *E. coli*, *Escherichia coli*; LB, luria broth medium; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5 – diphenyltetrazoilum bromide; *P. aeruginosa*, *Pseudomonas aeruginosa*; *S. aureus*, *Staphylococcus Aureus*; UV–Vis, UV–Visible; Tm, DNA melting temperature.

* Corresponding authors. Tel.: +30 210 6503336.
*E-mail address:* elefth@chem.demokritos.gr (E.K. Efthimiadou).

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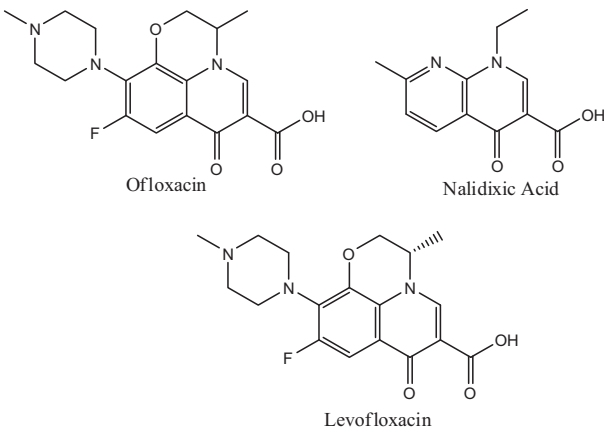


**Scheme 1.** Structure of Ofloxacin, Nalidixic Acid and Levofloxacin.

main interest in copper complexes is arising from their potential use as antiviral, antimicrobial anti-inflammatory, antitumor and as enzyme inhibitors. For instance numerous are the copper complexes of NSAIDS that exert enhanced anti-inflammatory and anti-ulcerogenic activity in conjunction with reduced gastrointestinal toxicity compared to the free ligand. Many are the studies which have focused on the chemotherapeutic properties of copper complexes as well as in their antiviral and antibacterial activity [9–12].

Today, few articles have been reported on the coordination properties of LVX in contrast with ofloxacin [13]. Macias and *co*-workers have presented a copper complex of ofloxacin with a slightly distorted square base pyramid [14]. On the other hand, Zivec et al. refers a full study about a mixed valence copper ofloxacin complex and its biological properties [15]. Furthermore a recent report described a comparative study of a ternary mixed copper complex of LVX with phenanthroline [16] and bipyridyl amine (bpa) [17]. Based on literature, quinolone complexes interact with DNA in three different ways: (1) groove binding, (2) binding to phosphate groups and (3) intercalation. These binding modes define the cytotoxic effect. In a comparative study, of the ternary copper complexes of ofloxacin and LVX with phenanthroline, it was found that the second complex has better antiproliferative activity than the ofloxacin one [13]. This data prompted us to further study both chemistry and biological activity of a single as well as of two mixed copper complexes of LVX one with bipyridine and one with phenanthroline. Aiming at comparing on the one hand the structure of copper LVX complexes with other quinolone complexes and on the other hand their biological activity we synthesized and fully characterized LVX copper complexes in the presence or absence of heterocyclic ligands. According to literature, copper quinolone complexes with phenanthroline and bipyridine present a synergetic effect in their antibacterial and cytotoxic activity [18,19].

Herein, we report the synthesis and characterization of two novel copper complexes of LVX one single in a ratio 1:2 and one mixed by using heterocyclic ligand bipyridine. Copper LVX complex with phenanthroline is synthesized in an analogues way with the bipyridine one, aiming at comparing the synergetic phenomenon in their biological activity. For the characterization of these compounds the following spectroscopic and analytical techniques were employed: Elemental analyses, thermo gravimetric (TGA) analysis, IR, EPR, UV–Vis spectroscopy, and X-ray crystallography as well. We also evaluate their antimicrobial activity, against three different microorganisms *E. coli*, *S. aureus* and *Ps. aeroginosa* through MIC determination. Calf thymus DNA-binding properties of the complexes have been investigated using absorption titration and DNA melting temperature techniques. We also evaluate the copper complexes stability in simulated body fluids (10% FBS in 10 mM PBS) and their anticancer activity against MCF-7 breast cancer cell line.

## 2. Experimental

### 2.1. Materials and methods

All chemicals used in syntheses were of analytical grade. Copper chloride dihydrate and LVX obtained from Sigma were used without further purification. Methanol obtained from Merck was used as received. 2,2-bipyridine and 1,10-phenanthroline were purchased from Sigma–Aldrich. IR spectra were recorded using a KBr pellet on a Perkin Elmer 880 IR spectrophotometer. UV–Vis spectra were recorded using a Cary 3E spectrophotometer. The TGA was determined, using a Mettler Star$^e$ SW9.30 differential thermal analysis apparatus operating at a heating rate of 10 °C per minute in the range 25–800°C in $N_2$. DNA stock solutions were prepared by dilution of CT-DNA to buffer (containing 150 mM NaCl and 15 mM tris-sodium citrate at pH 7.0) followed by exhaustive stirring at 4 °C for three days and kept there for no longer than a week. The nucleotide concentrations were determined by their absorption at 260 nm using $\varepsilon = 6600\ M^{-1}\ cm^{-1}$ [18]. CW EPR measurements at X-band were carried out on a Bruker ESP 380E spectrometer equipped with a rectangular ER 4102ST cavity or on an upgraded Bruker ER-200D spectrometer equipped with dual mode cavity that allows for running EPR spectra with the microwave magnetic field $\mathbf{B_1}$ oscillating either perpendicular or parallel to the external magnetic field $\mathbf{B_0}$. Cooling of the sample was performed with a liquid-nitrogen flow system ($T$ = 120 K). The CW EPR spectra were simulated with the EasySpin package [20] or with the SpinCount package (Prof. M. P. Hendrich, Carnegie Mellon University).

### 2.2. Synthesis of complexes

#### 2.2.1. Synthesis of {[$Cu_2$(LVX)$_4$]∗1.6 MeOH∗1.2 $H_2O$}n (**1**)

A solution of $CuCl_2 \times 2H_2O$ (48.0 mg, 0.28 mmol) in water (5 ml) was added to a previously prepared solution of LVX (100.0 mg, 0.28 mmol) in water (5 ml). The pH was adjusted to ~8 using diluted $NH_3$ (2 M). The resulting solution was stirred under reflux for at least three hours. A fine amorphous product of green color was obtained, filtered off and washed with cold water and dried in vacuum desiccator over silica gel. Yield: 43%, (M.W: 857,15) Recrystallization of the amorphous product from methanol and slow evaporation at 4 °C afforded X-ray quality crystals after ten days (**1**).

Selected bands: IR (KBr) $\upsilon_{max}$ 3421 (OH), $\upsilon_{asym}$ 1617 (C=O), $\upsilon_{sym}$ 1365(COO), $\upsilon$ 1586 (COO), $\upsilon$ 520 (Cu–O). Elemental *Anal.* Calc. for $C_{37.6}H_{46.8}CuF_2N_6O_{10.8}$ (**1**): C, 52.69; H, 5.50; N, 9.81. Found: C, 51.40; H, 5.08; N, 9.88% (Average of 3).

#### 2.2.2. Synthesis of {[$Cu_2$(LVX)$_2$(bipy)$_2Cl_2$]∗10.84$H_2O$} (**2**)

A solution of $CuCl_2 \times 2H_2O$ (23.9 mg, 0.14 mmol) in methanol (5 ml) was added to a solution of 2,2-bipyridine (bipy) (21.9 mg, 0.14 mmol) in methanol (5 ml), followed by the addition of a previously prepared solution of LVX (50.0 mg, 0.14 mmol) in methanol (5 ml) in the presence of $CH_3ONa$. The pH was adjusted to ~8 using diluted $CH_3ONa$ solution (0.1 M). The resulting solution was stirred for half hour while heating, followed by concentration to its half volume. A fine amorphous product of green color was obtained, filtered off and washed with cold methanol and dried in vacuum desiccator over silica gel. Yield: 65%, (M.W: 1426.37) Recrystallization of the above amorphous product provides green crystals after few weeks suitable for X-ray crystallography (**2**).

Selected bands: IR (KBr) $\upsilon_{max}$ 3421 (OH), $\upsilon_{asym}$ 1614 (C=O), $\upsilon_{sym}$ 1385(COO), $\upsilon$ 1579 (COO), $\upsilon$ 515 (Cu–O), $\upsilon$ 554 (Cu–N). Elemental