



# New silver complexes with levofloxacin: Synthesis, characterization and microbiological studies



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## ABSTRACT

Levofloxacin is a third generation fluoroquinolone antibiotic with a broad-spectrum including both Gram positive bacteria and Gram negative bacteria, as well atypical bacteria. In order to extend the spectrum of activity and to add new biological effects, several metal complexes of levofloxacin have been obtained and reported recently. The aim of our study was to obtain new silver complexes with levofloxacin with potential broad spectrum antibacterial and antifungal activity. Therefore three new silver complexes of levofloxacin with the proposed chemical structures (levofloxacin)<sub>2</sub>Ag(NO<sub>3</sub>), (levofloxacin)<sub>2</sub>Ag(NO<sub>3</sub>)(CH<sub>3</sub>OH) and (levofloxacin)Ag(C<sub>6</sub>H<sub>6</sub>O<sub>7</sub>)·3H<sub>2</sub>O were synthesized. In order to characterize the obtained complexes elemental analysis, conductivity measurement, spectroscopic, and thermal methods were used. Optimized molecular structures were determined using DFT (density functional theory) analysis. The antibacterial activity against Gram negative and Gram positive bacteria and antifungal activity against *Candida* spp of the complexes was tested by determination of minimum inhibitory concentration through microtitre broth dilution method.

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## 1. Introduction

Antibacterial fluoroquinolones are chemotherapeutic compounds commonly used in therapy to treat bacterial infections. As in the case of most classes of antibiotic compounds, the development of bacterial resistance represents a major challenge and a serious threat to therapists. Therefore, the development of metal complexes with fluoroquinolones had captivated attention of many researchers recently, taking into account the potential of broadening the activity spectrum of the ligand or the emergence of

possible new biological effects. Many studies report the excellent complexing capacity of fluoroquinolones particularly due to the chemical structure [1–3]. Among the large variety of metal cations which can be used in the production of metal complexes of the fluoroquinolones, silver appears to be adequate, mainly due to its antibacterial, antifungal and cytotoxic activity [4–6]. Thus, taking in account the biological effects of silver ions and the activity spectrum of fluoroquinolones, it makes sense to study, if there is a possible summation and potentiation of these properties in case of metal complexes which contain silver and a fluoroquinolone. Some studies have approached this line of research using as ligands first generation quinolones (nalidixic acid, norfloxacin, pefloxacin, and ciprofloxacin) or a modern fluoroquinolone (moxifloxacin) [7–16]. From a structural point of view, silver complexes with fluoroquinolones have a particular aspect, namely silver binding that may occur at the nitrogen atom of the ligand N4'-piperazine, different from most of fluoroquinolone metal complexes which have the complexing location 3-carboxyl and 4-oxo groups [3]. Levofloxacin (LEV) or (S)-9-fluoro-2,3-dihydro-3-methyl-10-(4-

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methylpiperazin-1-yl)-7-oxo-7H-pyrido [1,2,3-de]-1,4-benzoxazine-6-carboxylic acid is a third generation fluoroquinolone, being the levorotatory isomer of racemic ofloxacin (Fig. 1). As mechanism of action, LEV directly inhibits DNA synthesis by acting on two target enzymes, respectively DNA gyrase and topoisomerase IV. LEV has a broad-spectrum, acting on the Gram positive bacteria and Gram negative bacteria, and atypical bacteria, as well [17–21].

Thus far several metal complexes of LEV were synthesized and studied. Among targeted cations can be found  $Mg^{2+}$ ,  $Ca^{2+}$ ,  $Zn^{2+}$ ,  $Pd^{2+}$ ,  $Pt^{2+}$ ,  $Au^{3+}$ ,  $Cu^{2+}$ ,  $Fe^{3+}$ ,  $MoO_4^{2-}$ , and the antimicrobial activity, effect on the leukemia HL-60 and HePG-2 cell line, human colon carcinoma HCT-116 and human hepatocellular carcinoma HepG-2 tumor cell lines, and interaction with DNA were tackled in different studies [22–31]. Due to an intensive worldwide use, an increase of bacterial resistance to fluoroquinolones appeared. Thus, the need to improve the antibacterial potential of existing antimicrobial drugs is more challenging, adding the concept that metal complexes could be an alternative to conventional drugs [32]. As far as we know no reported data regarding silver complex of LEV has been described in the literature. Our study reports reliable methods for preparation of silver metal complexes of LEV which will possess the biological effects of the two constituent components. In order to determine the most probable structures of the obtained silver complexes elemental analysis, conductivity measurement, spectroscopic, thermal and density functional theory (DFT) methods were used. The antimicrobial and antifungal activity of the complexes has been evaluated.

## 2. Methods

### 2.1. Materials

LEV was purchased from Sigma, silver nitrate from SC UTCHIM SRL (România), silver citrate hydrate from Aldrich; all other chemicals used were of analytical reagent grade.

### 2.2. General

Elemental analyses of C, H and N were carried out on a Perkin Elmer PE 2400 analyzer. The metal ion content was determined by flame atomic absorption spectrometry (FAAS) using a Shimadzu AA 6300 spectrometer after destruction of the complex in a Berghof microwave digestion system. The molar conductance was determined for  $10^{-3}$  M solution of complex in dimethyl sulfoxide (DMSO) with an analyzer inoLab<sup>®</sup> pH/Cond 740. To record the FT-IR spectra we used a FT-IR Thermo Nicolet (USA) spectrometer with samples prepared as KBr pellets in the range of  $4000$ – $400$   $cm^{-1}$ , 32 scans at  $4$   $cm^{-1}$  resolution, and processed using Omnic V.6 software. Mass spectra of the obtained compounds were analysed with an Agilent 6410 Triple Quadrupole (Agilent Technologies, USA) mass

spectrometer equipped with electrospray ionization (ESI) ion source in positive ion mode. The recorded data were processed using MassHunter software. Conditions of the ionization source were: gas flow 8 L/min, 40 psi,  $300$   $^{\circ}C$ ,  $4000$  V, with a full scan on the field  $100$ – $1500$  amu data acquisition module. NMR measurements were carried out on a Varian Unity Inova DDR spectrometer (599 MHz for  $^1H$ ) with a 5 mm inverse-detection gradient probehead using dimethylsulfoxide- $d_6$  (DMSO- $d_6$ ) as solvent. Standard pulse sequences and processing routines available in Vnmrj 2.2C/Chempack 4.0 were used. The chemical shifts were referenced to the solvent signal of internal DMSO- $d_6$  (2.500 ppm). For Differential Scanning Calorimetry (DSC) analysis we used a DSC 60 Shimadzu apparatus. The weight of the samples was 3 mg. The DSC curves were recorded in the range of  $40$ – $400$   $^{\circ}C$ , with a temperature increase rate of  $10$   $^{\circ}C/min$ . UV spectra were recorded on a T70 + UV/VIS Spectrometer (PG Instruments Ltd). Electronic spectra by diffuse reflectance technique were recorded in the range  $200$ – $800$  nm with spectralon as standard, on a Jasco V650 spectrophotometer.

### 2.3. Computational study

Full geometry optimizations of the ligand and complexes (1) and (3) were carried out using the DFT method in Gaussian09 [33]. The split valence plus polarization basis set 6-31G(d) was applied for any atom that is not involved in coordination. 6-31G(d',p') basis set was used for the fluorine atoms. To give a better description of the  $Ag^+$  – ligand interactions, basis sets with additional diffuse functions were added for the piperazine nitrogen and the oxygen atoms involved in coordination (6-311G+(d,p)). The Stuttgart-Cologne small-core quasi-relativistic pseudopotential ECP28MWB (28 electrons in the core) [34,35] was used for the  $Ag^+$  ion in conjunction with its optimized valence basis set  $8s^7p^6d^2f^1g$ . DFT/B3LYP/(MWB28 for Ag) was proven to give good results for prediction of coordination polyhedral and geometry parameters of the  $Ag^+$  complexes in other [36,37].

### 2.4. Experimental procedures

In order to obtain silver metal complexes of LEV we considered several possibilities. Although in previously reported articles regarding synthesis of silver complexes with fluoroquinolones only silver nitrate was used, we tried to synthesize silver complexes starting with another silver compound in this case silver citrate. In the preliminary study we tried to adapt several methods described in the literature which used as reaction temperature  $80$ – $100$   $^{\circ}C$  [8,14–16]. These methods were not successful due to the fact that the  $Ag^+$  ion can easily generate  $Ag_2O$  (black-brown precipitate), which partially decomposed to Ag and  $O_2$  ( $Ag_2O \rightarrow 2Ag + 1/2O_2$ ) when heated. Thus, we opted for a “cold” method that does not use heat. Also for drying the obtained substance we choose to avoid as much as possible the high temperatures and direct exposure to light [38,39].

#### 2.4.1. Synthesis of complex (1)

A solution obtained from 1.38 mmol  $AgNO_3$  and 50 mL of water have been added into a mixture of 2.76 mmol of LEV and 75 mL methanol (2:1 molar ratio LEV: $AgNO_3$ ). After mixing the solutions 25 mL of concentrated ammonia was added. The entire mixture was stirred for 4 h with a magnetic stirrer, in a sealed flat-bottom flask, protected from light. A pale yellowish clear solution was obtained, which was left overnight at room temperature. The obtained solution was concentrated with a rotary evaporator at  $40$   $^{\circ}C$  under vacuum and the last 20 mL solution was slowly dried in a normal oven set at  $40$   $^{\circ}C$  for 24 h. The solid compound was kept in desiccator over anhydrous  $CaCl_2$ , protected from light.

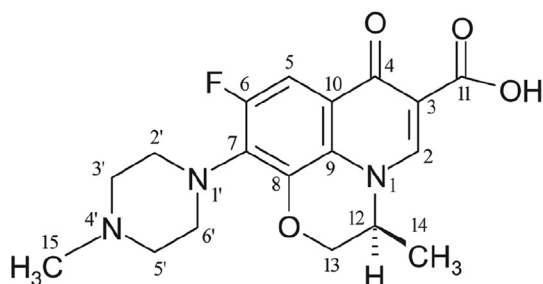


Fig. 1. The chemical structure and numbering of LEV.

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