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DNA binding, photo-induced DNA cleavage and cytotoxicity studies of lomefloxacin and its transition metal complexes

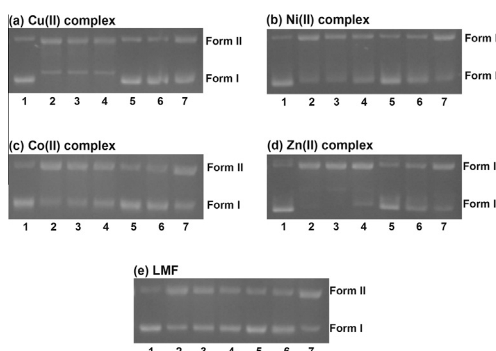
Mohamed A. Ragheb, Mohamed A. Eldesouki, Mervat S. Mohamed *

Department of Chemistry, Biochemistry Speciality, Faculty of Science, Cairo University, Egypt

HIGHLIGHTS

- DNA binding and cleavage activities of lomefloxacin and its complexes were studied.
- DNA binding studies suggest partial/moderate intercalative mode of binding.
- These compounds can efficiently photocleave pBR322 DNA via singlet oxygen pathway.
- Antimicrobial and antitumor activities of the compounds were also investigated.

GRAPHICAL ABSTRACT



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ABSTRACT

This work was focused on a study of the DNA binding and cleavage properties of lomefloxacin (LMF) and its ternary transition metal complexes with glycine. The nature of the binding interactions between compounds and calf thymus DNA (CT-DNA) was studied by electronic absorption spectra, fluorescence spectra and thermal denaturation experiments. The obtained results revealed that LMF and its complexes could interact with CT-DNA *via* partial/moderate intercalative mode. Furthermore, the DNA cleavage activities of the compounds were investigated by gel electrophoresis. Mechanistic studies of DNA cleavage suggest that singlet oxygen ($^1O_2$) is likely to be the cleaving agent *via* an oxidative pathway, except for Cu(II) complex which proceeds *via* both oxidative and hydrolytic pathways. Antimicrobial and antitumor activities of the compounds were also studied against some kinds of bacteria, fungi and human cell lines.

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Introduction

Fluoroquinolones constitute an important group of synthetic broad-spectrum antimicrobial compounds that being clinically used for nearly five decades, and derived from basic structure of nalidixic acid having substituents at N-1, C-5, C-7; position 8 and a fluorine atom at position 6. Lomefloxacin (1-ethyl-6,8-difluoro-1,4-dihydro-7-(3-methylpiperazin-1-yl)-4-oxoquinoline-3-carboxylic acid) (Fig. 1) is one of the second generation drugs of the fluoroquinolone antibiotics [1]. It has a broad spectrum of activity against Gram-negative and Gram-positive microorganisms [2], and used for treating a large number of infectious diseases. It is used for the treatment of bacterial exacerbation of chronic bronchitis and uncomplicated and complicated urinary tract infections [3]. Fluoroquinolones interfere with bacterial cell replication, transcription and DNA repair by inhibiting the enzymatic action of type II topoisomerases such as DNA gyrase or topoisomerase IV which are important to these processes [4–7].

* Corresponding author. Tel.: +20 2 101 3461447; fax: +20 2 5727213.
E-mail addresses: mervat_sayed@yahoo.com, mervat@sci.cu.edu.eg (M.S. Mohamed).

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Transition metal complexes are a subject of enormous research interest due to their interactions with nucleic acids. Transition metal complexes interact with DNA *via* different binding modes such as covalent, intercalation, groove or electrostatic binding. Binding studies of small molecules to DNA are very important due to their utility as DNA structural probes, DNA foot printing and sequence-specific cleavage agents and potential antitumor drugs [8–10]. Moreover, considerable attention has been focused on the biological properties of quinolones and their metal complexes, such as their interaction with DNA, antibacterial activity tests on diverse microorganisms, cytotoxicity and potential anticancer activity [11–15].

In this work, the binding properties of lomefloxacin and its previously prepared transition metal complexes [16] with double-stranded calf thymus DNA were investigated using UV–Vis absorption measurements, fluorescence quenching experiments and thermal denaturation studies to elucidate their binding and pharmacological potential. Furthermore, DNA cleavage activities of these compounds with pBR322 DNA have been carried out, in addition *in vitro* antiproliferative activities of these molecules against various human cancer cell lines and different microorganisms were experimentally explored.

Experimental

Materials and instruments

Calf thymus DNA and lomefloxacin were obtained from Sigma (Sigma Chemical Co., St. Louis, MO, USA). Supercoiled plasmid DNA pBR322 was purchased from SibEnzyme Ltd. (Russia). The ternary transition metal complexes of lomefloxacin complexes with glycine, namely $[Cu(LMF)(Gly)(H_2O)_2]Cl{\cdot}2H_2O$, $[Ni(LMF)(Gly)(H_2O)_2]Cl{\cdot}H_2O$, $[Co(LMF)(Gly)(H_2O)_2]{\cdot}Cl$ and $[Zn(LMF)(Gly)(H_2O)_2]{\cdot}Cl$ were prepared according to the literature procedures [16]. The other reagents were of analytical-reagent grade and were used without further purification unless otherwise noted. Human tumor cell lines were obtained frozen in liquid nitrogen (−180 °C) from the American Type Culture Collection. The tumor cell lines were maintained in the National Cancer Institute, Cairo, Egypt, by serial sub-culturing. Tris–HCl/NaCl buffer solution (5 mM Tris–HCl, 50 mM NaCl, pH = 7.1), used for binding and cleavage studies, was prepared using deionized triple distilled water. A solution of CT-DNA in Tris–HCl/NaCl buffer gave a ratio of UV absorbance at 260 and 280 nm (A_{260}/A_{280}) of about 1.8–1.9:1 at 260 and 280 nm, indicating that the DNA was sufficiently free from protein contamination [17]. The DNA concentration per nucleotide was determined by absorption spectra using the molar absorption coefficient of 6600 M^{-1} cm^{-1} at 260 nm [18]. Stock solution of DNA was stored at −20 °C. Concentrated stock solutions of drug and its metal complexes (1.0×10^{-3} M) were prepared by dissolving an appropriate amount of them in 50 ml of deionized doubly distilled water and diluted suitably with Tris–HCl/NaCl buffer to the required concentrations for all the experiments.

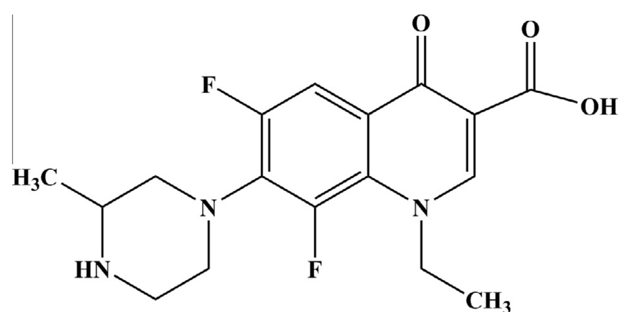

Fig. 1. Structure of lomefloxacin.

UV–Vis spectra were recorded on a Shimadzu UV-3101 PC NIR spectrophotometer (Japan). Fluorescence measurements were carried out using a Jasco FP-6200 fluorescence spectrometer (Japan Spectroscopic Company, Tokyo) with a slit width 5 nm for the excitation and emission beams. Thermal denaturation experiments were performed on TB-85-thermobath Shimadzu model UV spectrophotometer equipped with a thermostatic cell holder.

DNA binding experiments

Electronic absorption spectroscopy

Absorption titration experiments were performed by varying the concentration of CT-DNA (0, 40, 80, 120, 160, 200, 300 and 400 μM) while keeping the drug and its metal complexes concentration constant (40 μM). The reference solution was the corresponding buffer solution. Upon measuring the absorption spectra, an equal amount of CT-DNA solution was added to both the compound solution and the reference solution to eliminate the absorbance of DNA itself. The solutions were allowed to incubate for 10 min at room temperature before the absorption spectra were recorded. Each sample solution was scanned in the range of 200–400 nm. The titration processes were repeated until no change in the spectra, indicating binding saturation had been achieved. The absorption data were analyzed for an evaluation of the intrinsic binding constant (K_b) of the complexes with CT-DNA [19].

Fluorescence quenching experiments

DNA fluorescence quenching study in the presence of ethidium bromide (EB) was done by mixing the solutions of DNA (30 μM) and EB (10 μM) with variable concentrations of LMF and its metal complexes ranging from 0 to 200 μM in Tris–HCl/NaCl buffer. The excitation wavelength was kept at 500 nm, scan speed = 125 nm/min and the emission spectra were recorded in the wavelength range of 520–700 nm [20].

Thermal denaturation experiments

Melting studies were carried out by monitoring the absorbance of CT-DNA (80 μM) at 260 nm with varying temperature in the absence and presence of the LMF and its complexes (20 μM).The solution containing the compound and CT-DNA in Tris–HCl/NaCl buffer solution was stirred continuously and the temperature was elevated gradually from 20 °C to 95 °C with reading absorbance every 5 °C. The melting temperature, which is defined as the temperature at which 50% of double stranded DNA becomes single stranded, was determined from the midpoint of the melting curves. ΔT_m values were calculated by subtracting T_m of free DNA from T_m of DNA interacting with the compound [21].

DNA cleavage experiments

The cleavage experiments of supercoiled pBR322 DNA (0.3 μg) by LMF and its metal complexes in Tris–HCl/NaCl buffer were carried out using agarose gel electrophoresis. Two groups of samples were prepared; one group of them was irradiated at room temperature with an UV lamp (365 nm, 4 W) for 30 min, and the other one was incubated at 37 °C for 24 h without irradiation. Then, a loading buffer (0.01% bromophenol blue, 0.25 M EDTA, 50% glycerol) was added, and electrophoresis was carried out at 70–75 V for 2 h in TBE buffer (89 mM Tris–HCl, 89 mM boric acid, 2 mM EDTA) using 1% agarose gel containing 1.0 μg/mL ethidium bromide. Other DNA cleavage experiments were monitored in presence of different kinds of reducing agents using agarose gel electrophoresis. Photocleavage mechanistic investigation of pBR322 DNA was carried out in the presence of standard radical scavengers and

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