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Synthesis, characterization and antibacterial studies of a copper(II) lomefloxacin ternary complex

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ABSTRACT

Solution behavior of lomefloxacin (lmx) complexes with copper(II) in the presence and absence of 1,10-phenanthroline (phen) was studied in aqueous solution, by potentiometry. The results obtained showed that under physiological conditions (micromolar concentration range and pH 7.4) only copper(II):lmx:phen ternary complexes are stable. Hence, a novel copper(II) ternary complex of lomefloxacin with the nitrogen donor heterocyclic ligand phen was synthesized and characterized by means of UV–visible and IR spectroscopy, elemental analysis and X-ray crystallography. In the synthesized complex (**1**), $[Cu(lmx)(phen)(NO_3)]\cdot 5H_2O$, lmx acts as a bidentate ligand coordinating the metal cation, in its anionic form, through the carbonyl and carboxyl oxygens and phen coordinates through two N-atoms forming the equatorial plane of a distorted square-pyramidal geometry. The fifth ligand of the penta-coordinated Cu(II) center is occupied axially by an oxygen atom from the nitrate ion. Minimum inhibitory concentration (MIC) determinations of the complex and comparison with free lomefloxacin in various *E. coli* strains indicated that the Cu-complex is an antimicrobial which is as efficient as the free antibiotic but strongly suggest that the cell intake route of both species is different. Moreover, spectrophotometric stability studies suggest that the solution of the complex synthesized is considerably more photostable than the free fluoroquinolone supporting, therefore, the complex's suitability as a candidate for further biological testing in fluoroquinolone-resistant microorganisms with possible reduced side-effects.

1. Introduction

Quinolones are a group of synthetic antibiotics widely used in human and veterinary medicine, mainly due to their broad spectrum of activity and safety profile, allied to good oral absorption and bioavailability [1,2]. Quinolones act intracellularly and their mechanism of action relies on the inhibition of cell division by stabilizing the reversible cleavage complexes formed by DNA and DNA gyrase/topoisomerase IV, the enzymes responsible for DNA replication [3,4].

Due to the limited activity of the first quinolones (*e.g.* nalidixic acid), strategic structural changes to the basic nucleus were introduced to broaden their antibacterial spectrum of activity namely, the introduction of a fluorine atom at position 6 of the basic quinolone ring, giving rise to the fluoroquinolones, and a cyclic amino group at C-7 [5]. Lomefloxacin (lmx) is a second generation fluoroquinolone characterized by a double fluorination, one at position C-6 and the other at position C-8 (Fig. 1a). Although the antibacterial potency of this compound is not greatly improved over most first generation fluoroquinolones, it exhibits an extended half-life and significantly improved oral absorption [6]. Lomefloxacin can be dosed once daily in the treatment of systemic infections such as respiratory and urinary tracts, gynecological, ophthalmological and soft tissue infections. The fluorination at C-6 gives lmx a broader spectrum of action, being highly active against a wide variety of bacteria (Gram negative and Gram positive) whereas the fluorination at carbon 8 is responsible for the potency improvements observed, being directly related to increasing affinity towards the enzyme target DNA gyrase [5]. Introduction of halogen atoms (fluorine or chlorine) at the C-8 position of the quinolone nucleus results, however, in compounds with an enhanced tendency to induce photosensitivity, both in humans and in animal models [7–9]. Phototoxicity is one of the clearest examples of the effect of structure on the biological activity of quinolone agents [10]. *In vitro* irradiation experiments have suggested that exposure to UV radiation leads to de-fluorination and the formation of highly reactive species/intermediates [9,11–18]. In fact, in a number of the newer quinolones, the C-8 halogens have been abandoned in favor of a methoxy group. This substituent also provides improved *in vitro* potency over the unsubstituted analog but without the phototoxicity issues of the halogens [19]. Such is the case of moxifloxacin and gatifloxacin that have been reported to be free of phototoxicity [20,21].

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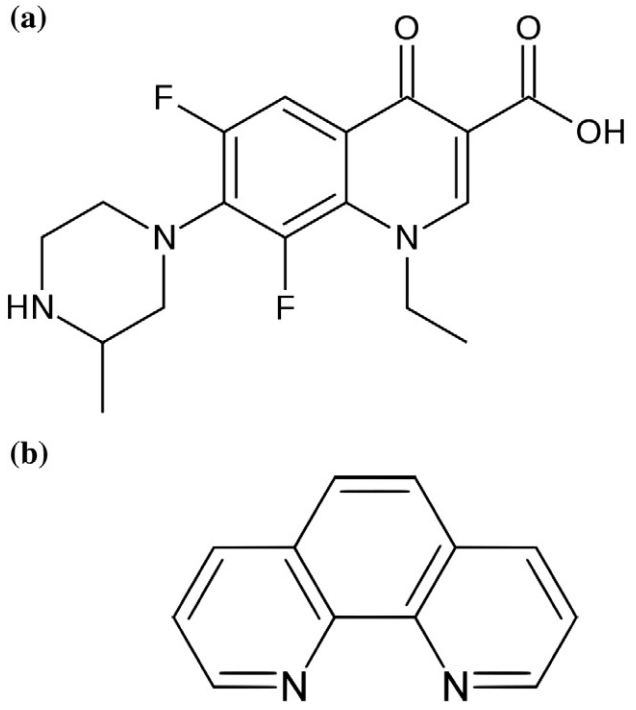

Fig. 1. (a) Structure of lomefloxacin, (RS)-1-ethyl-6,8-difluoro-7-(3-methylpiperazin-1-yl)-4-oxo-quinolone-3-carboxylic acid ($C_{17}H_{19}F_2N_3O_3$, MW 351.35 g/mol) and (b) 1,10-phenanthroline.

Unfortunately, fluoroquinolone overuse and misuse have led to the emergence of bacterial resistance to this class of antibiotics, which has disseminated rapidly in clinical and environmental settings, compromising their effectiveness as antimicrobials [22]. Microbial resistance to antibiotics sparked the need for the design of new and modified drugs. In this context, the concept of metal coordination to fluoroquinolones has gained interest as a strategy to enhance activity, reduce adverse effects and counteract resistance. Numerous studies regarding the interaction between quinolones and metal cations have been reported and reviewed in the literature [23–38]. In particular, the study of quinolone-copper-(1,10)-phenanthroline complexes has become an increasingly important field since they seem to exhibit high affinity towards DNA as well as nuclease activity towards plasmid, genomic and internucleosomal DNA [30,33,39,40].

Despite the popularity that metal cation-fluoroquinolone coordination compounds has experienced in recent years, aqueous speciation studies of these binary and ternary systems are scarce [30,33,41]. Also, to date, little work has been reported in the synthesis of coordination compounds of lomefloxacin [42] and, to the best of our knowledge, no crystal structures of ternary metal complexes of lmx with phen have been published.

In this work we report the solution behavior of this fluoroquinolone with Cu^{2+} in the presence and absence of phen. The values obtained for the stability constants of the binary and ternary divalent metal ion complexes are very high and clearly show that the ternary complexes are more stable than their binary counterparts, suggesting stabilization due to an intra-molecular interaction between the ligands. The speciation diagrams indicate that only the copper(II) ternary species are stable at physiological concentrations and pH. Hence, the synthesis, characterization and single-crystal X-ray diffraction structure of a novel copper(II) complex of lomefloxacin and N-donor heterocyclic ligand phen was undertaken and is herein described. Data on the photostability, antibacterial activity of this compound and intake route are also reported.

2. Experimental

2.1. Materials

Lomefloxacin ((RS)-1-ethyl-6, 8-difluoro-7-(3-methylpiperazin-1-yl)-4-oxo-quinolone-3-carboxylic acid) was purchased from Sigma-Aldrich and stored at −20 °C. 1,10-Phenantroline and all other general chemicals were purchased from Merck (analytical grade) and used without further purification. Solutions for potentiometric titrations were prepared in deionized water (conductivity less than 0.1 μS cm^{-1}).

2.2. Culture media and bacterial strains

Iso-Sensitest broth was obtained from Oxoid (Basingstoke, UK). All drug solutions were prepared in aqueous 10×10^{-3} M HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffer solution pH 7.4 (ionic strength 0.10 M adjusted with sodium chloride).

Antimicrobial susceptibility was assessed for the Clinical and Laboratory Standards Institute (CLSI) reference strain, *Escherichia* (*E.*) *coli* ATCC 25922 and for *E. coli* JF568, a parental strain derived from *E. coli* K12 [43,44] and *E. coli* JF701 [45] and JF703 [43,44], strains derived from the former but devoid of major porins OmpC and OmpF, respectively. Additionally, two other strains were used, *E. coli* W3110 [46] tested as control for W3110 ΔFΔC, that is devoid of both porins OmpC and OmpF (see Table 2 for a more detailed genotypic and phenotypic description).

2.3. Physical measurements

Infrared spectra, measured in the 4000–400 cm^{-1} range, were recorded on a Perkin-Elmer Spectrum BX using KBr pellets. Absorption spectra were recorded in a Shimadzu 3600 UV–Vis-NIR spectrophotometer equipped with a peltier, in 1 cm quartz cuvettes with a slit width of 2 nm. Fluorescence spectroscopy was performed at 37.0 (±0.1) °C in a VARIAN Cary Elipse spectrofluorimeter, using 1 cm quartz cuvettes also with a slit width of 5 nm.

2.4. Potentiometric pH titrations

All potentiometric measurements were carried out with a Crison 2002 pH meter and a Crison 2031 burette controlled by a microcomputer. The electrode assembly consisted of an Orion 900029 double-junction AgCl/Ag reference electrode, and a Russell SWL07 glass electrode as indicator. System calibration was performed by the Gran method in terms of hydrogen ion concentration [47], by titrating solutions of strong acid with strong base. A calibration was performed before each run to determine stability constants which also provided a check to the electrode behavior. All titrations were carried out under argon atmosphere in a thermostat-controlled double walled glass cell; the temperature was set at 25.0 (±0.1) °C and the ionic strength was adjusted to 0.10 M with sodium chloride.

2.4.1. Potentiometric determination of stability constants

Stock solutions of lmx and phen (1.0×10^{-2} M) were prepared in deionized water ($I = 0.1$ M NaCl). The concentration of fluoroquinolone was measured by checking the compliance of the absorbance of the isosbestic points with the Beer–Lambert law. Aqueous copper(II) nitrate trihydrate solution (1.0×10^{-2} M) was standardized with standard solution of EDTA 0.1 M (Titriplex).

For the determination of the acid dissociation constants, an aqueous solution (1×10^{-3} M) of the protonated ligand was titrated with NaOH (ca. 0.02 M; $I = 0.1$ M NaCl; 25 °C) under argon. For the determination of the association constants between lmx and phen, an aqueous solution of HCl ($1–2 \times 10^{-3}$ M; $I = 0.1$ M NaCl; 25 °C), in the presence of both ligands ($1–2 \times 10^{-3}$ M) was titrated with ~0.06 M NaOH, under an argon atmosphere. The stability constants of the binary and ternary