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Metal binding and antibacterial activity of ciprofloxacin complexes

ZAHID H. CHOHAN[1], CLAUDIU T. SUPURAN[2], & ANDREA SCOZZAFAVA[2]

[1]*Department of Chemistry, Bahauddin Zakariya University, Multan, Pakistan, and* [2]*University of Florence, Dipartimento di Chimica, Laboratorio di Chimica Bioinorganica, Polo Scientifico, Sesto Fiorentino, Firenze, Italy*

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Abstract

Four novel cobalt(II), copper(II), nickel(II) and zinc(II) complexes of the fluoroquinolone antibiotic ciprofloxacin have been prepared. The compounds were characterized by IR, UV-Visible, molar conductivity and elemental analyses. In all of the complexes, the drug ligand, ciprofloxacin (CFL) was coordinated through two carbonyl oxygen atoms. Octahedral and square-planar geometries have been proposed for the cobalt(II), nickel(II) and zinc(II), and copper(II) complexes, respectively. *In vitro* tests of susceptibility to these metal complexes showed stronger activity than that of ciprofloxacin against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Bacillus dysenteriae*.

Keywords: *Ciprofloxacin, metal complexes, antibacterial activity*

Introduction

Ciprofloxacin (CFL) is a synthetic, broad-spectrum fluoroquinolone antibacterial agent for oral administration [1]. It is active against a wide variety of aerobic gram-negative and gram-positive bacteria. The binding of small molecules of antibacterial agents to DNA is a well-established mechanism of antibacterial action [1–5] and factors that determine their affinity and selectivity to the binding are important in designing and development of new diagnostic and chemotherapeutic agents [6–10]. Transition metals are essential elements widely distributed in biological systems, such as cells and body fluids and, many of their complexes bind to DNA through various modes [11,12]. Some metal ions are also known to cleave DNA in the presence of different ligands [13–15]. Fluroquinolones are an important class of antibacterial compounds which possess a fluorine atom at position-6 and a piperazine ring at position-7 of the quinolinone-carboxylic acid (Figure 1). These drugs are known to possess an appreciable antibacterial activity [16]. The increased affinity of these drugs for DNA is accounted for by their intercalation with the purine/pyrimidine bases of the nucleic acid [16,17].

There is not much literature available on the metal complexes of ciprofloxacin and their interaction with DNA, although the urgent problem of antibiotic resistance prompted much research in the design of novel antibacterial agents [1–5]. Novel methodologies are required to overcome this issue. After some research, it came to light that antibacterial drugs become more effective against bacteria upon chelation/coordination with the transition metal ions [1]. Transition metals are present in very low concentrations *in vivo*, and their ligand environment can be considerably altered. This change in balance between the metal ion and drug ligand may have profound effects upon the activity of a drug against potentially susceptible bacteria. This formation may increase the bioavailability of either the metal ion or the drug ligand or both [1].

In order to investigate the effect of metal ions upon antibacterial activity we have previously reported [18–27] several studies regarding the preparation and antibacterial activity of such metal complexes. Here we report the preparation and biological activity of

Correspondence: C. T. Supuran, University of Florence, Dipartimento di Chimica, Laboratorio di Chimica Bioinorganica, Polo Scientifico, Sesto Fiorentino, Firenze, Italy. Fax: 39-055-45733835. E-mail: claudiu.supuran@unifi.it

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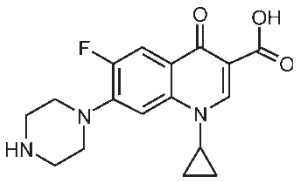

Figure 1. Structure of ciprofloxacin.

some transition metal(II) complexes of ciprofloxacin and probe the mechanism of ciprofloxacin activity against various bacterial strains. We found that the solubility of these metal(II) complexes of ciprofloxacin is better than the uncomplexed ligand both in water and ethanol. On the basis of studies of their IR, UV-Visible, molar conductivity and elemental analyses, two reasonable structures for these new complexes have been proposed.

The antibacterial activity of ciprofloxacin and its metal complexes was investigated *in vitro* against hospital isolates of gram-positive (such as *S. aureus* (ATCC 6538)) and gram-negative organisms, such as *E. coli* (ATCC 8739), *K. pneumoniae* (ATCC 5836) and *B. dysenteriae* (ATCC 7306). It is worth-mentioning that the activity of all metal complexes, particularly that of Zn(II)–CFL complex specifically against gram-negative organisms (*E. coli*, *K. pneumoniae* and *B. dysenteriae*) was stronger than that of ciprofloxacin itself.

Materials and methods

Ciprofloxacin was obtained from Pharmagen Beximco Ltd, Pakistan. Solvents used were analar grade. All metal(II) salts were used as chlorides. IR spectra were recorded on a Philips PU 9800 spectrophotometer. UV-Visible spectra were obtained in methanol on a Hitachi U-2000 double-beam spectrophotometer. C, H and N analyses were carried out by Butterworth Laboratories Ltd (U.K). Conductances of the metal complexes were determined in methanol on a Hitachi YSI-32 model conductometer. Magnetic measurements were done on solid complexes using the Gouys' method. Melting points were recorded on a Gallenkamp apparatus and are uncorrected.

Preparation of metal(II) complexes

To a warm magnetically stirred solution of ciprofloxacin sodium salt (1.0 g, 3.0 mmol) in 0.1 M HCl (10 mL) was added a solution of metal(II) chloride (1.5 mmol) in water (20 mL). The mixture was refluxed for 1 h and then cooled at room temperature. On cooling and after addition of methanol (10 mL), the mixture was left at room temperature for 24 h. The solid product which precipitated was filtered off, washed repeatedly with ethanol, then with ether and dried.

Biological activity

All the synthesized ligands and their respective metal(II) chelates were screened *in vitro* for their antibacterial activity against *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 8739) (incubation period at 37° C for 24 h), *Klebsiella pneumoniae* (ATCC 5836) and *Bacillus dysenteriae* (ATCC 7306) (incubation period at 37°C for 24 h) using agar well diffusion method [28,29]. Two to eight hours old bacterial inoculums containing approximately 10^4–10^6 colony forming units (CFU)/ml were used in these experiments. These were then melted, cooled (55°C), poured into nutrient agar plate and allowed to solidify. Wells were dug in the media with the help of a sterile metallic borer with centers at least 24 mm. Different concentrations (100 µl) of the test sample (1 mg/ml in DMSO) were introduced in respective wells. Other wells supplemented with DMSO and reference antibacterial drugs served as negative and positive controls, respectively. The plates were incubated immediately at 37°C for 20 h. Activity was determined by measuring the diameter of zones showing complete inhibition (mm).

Table I. Physical and spectral data of the metal complexes of ciprofloxacin.

Complex	M.P (°C)	Yield (%)	B.M (μ_{eff})	Calc (Found)%		
				C	H	N
1. Co(II)–CFL [825.8] $C_{34}H_{38}CoCl_2F_2N_6O_8$	208–210	63	4.1	49.4 (49.8)	4.6 (4.2)	10.2 (10.0)
2. Cu(II)-CFL [794.4] $C_{34}H_{34}CuCl_2F_2N_6O_6$	202–204	60	1.7	51.4 (51.2)	4.3 (4.0)	10.6 (10.9)
3. Ni(II)–CFL [825.6] $C_{34}H_{38}NiCl_2F_2N_6O_8$	218–220	65	3.2	49.4 (49.9)	4.6 (4.3)	10.2 (10.5)
4. Zn(II)–CFL [832.3] $C_{34}H_{38}ZnCl_2F_2N_6O_8$	210–212	62	Dia	49.0 (49.4)	4.6 (4.3)	10.1 (10.5)

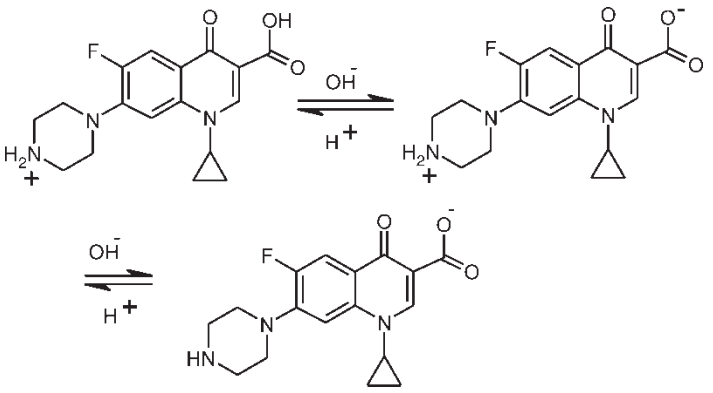

Figure 2. The zwitterionic structure of the ciprofloxacin molecule.

Results and discussion

Chemistry

The interaction of metal ions with ciprofloxacin resulted in the formation of complexes with the formula $[M(CFL)_2Cl_2]2H_2O$ where M = cobalt(II), nickel(II) or zinc(II) and, $[M(CFL)_2]Cl_2$ where M = copper(II) corresponding to the analytical data (Table I) (Figure 3). The molar conductances in 0.001 *M* in methanol are in the range 120–135 $\Omega^{-1}\,cm^2\,mol^{-1}$ indicating their electrolytic nature [30]. The complexes decomposed rather than melted above 200°C. All the complexes were stable in air and moisture and their solubility was much better than ciprofloxacin itself, both in water and methanol. The molecule of ciprofloxacin has a zwitterionic structure (Figure 2) having a good solubility in acidic or basic solvents, while its solubility in water, methanol, ethanol or other commonly used organic solvent is poor. However, the metal complexes are fairly soluble in water, methanol or ethanol.

IR spectra. The infrared spectral data and their assignments are given in Table II comparing mainly IR frequencies of the metal complexes with that of the ciprofloxacin and the following conclusion can be drawn:

1. There are two very strong absorption bands in the spectrum of the drug ligand (**L**), at 1725 and 1630 cm^{-1} assigned [31] to ν COOH and ν C=O stretching vibrations.
2. On comparison of these IR frequencies, the band at 1725 cm^{-1} and 1630 cm^{-1} completely vanished in the spectra of the metal complexes. Instead, the strong absorption bands positioned at 1580–1585 and 1350–1355 cm^{-1} indicating that ν COOH group emerged as two absorption bands $\nu_{asymmet}$ COO and ν_{symmet} COO and its coordination with the metal atoms. Similarly, the band at 1630 cm^{-1} due to the C?=O moiety in the spectrum of the drug ligand disappeared and instead a new band at 20–30 cm^{-1}lower frequency (~1600 cm^{-1}) appeared indicating [32] involvement of the carbonyl group in coordination. On the basis of three changes we propose that the drug ligand, ciprofloxacin is acting bidentately. Further conclusive evidence [33,34] observed was the

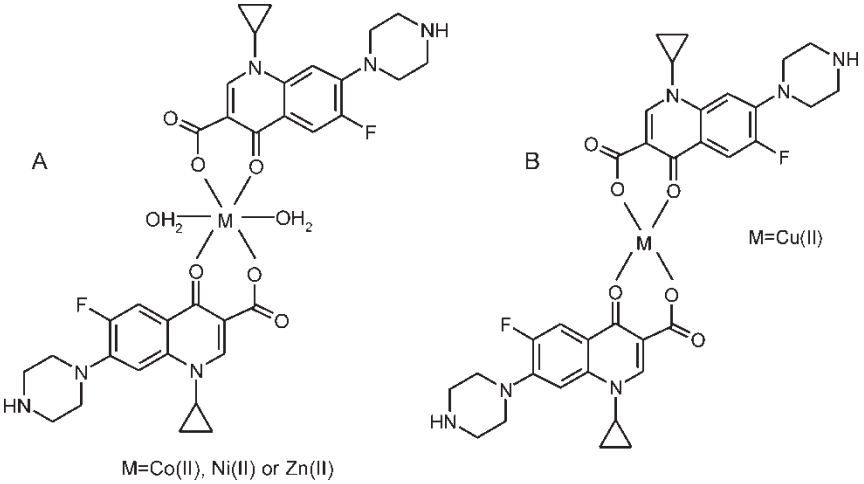

Figure 3. Proposed structure of the metal(II)–CFL complexes.

Table II. Selected IR and UV-visible spectral data of the ligand and its complexes

Complex No.	IR (cm^{-1})	λ_{max} (cm^{-1})
L	1630, 1725, 1135	–
1	1580, 1350, 1600, 550	7315, 17280, 29650
2	1585, 1355, 1610, 615	15290, 19380, 30325
3	1580, 1350, 1615, 585	10615, 15465, 26140, 30245
4	1580, 1350, 1600, 590	29215

appearance of a new band at 550–615 cm^{-1} assigned to metal-oxygen (M-O) in the spectra of the metal complexes and not observable in the spectrum of the drug ligand.

UV-visible spectra and magnetic moment. The Co(II) complex exhibited well-resolved, low-energy bands at 7,315 cm^{-1} and 17,280 cm^{-1} and a strong high-energy band at 20,355 cm^{-1} assigned [35] to the transitions $^4T_{1g}(F) \rightarrow ^4T_{2g}(F)$, $^4T_{1g}(F) \rightarrow ^4A_{2g}(F)$ and $^4T_{1g}(F) \rightarrow ^4T_{2g}(P)$ for a high-spin octahedral geometry [30,31]. A high intensity band at 29,650 cm^{-1} was assigned to metal to ligand charge transfer (Table II). The magnetic susceptibility measurements (4.1 B.M) for the solid Co(II) complex is also indicative [36] of three unpaired electrons per Co(II) ion suggesting [32] consistency with their octahedral environment (Figure 3A).

The electronic spectra of the Cu(II) complex (Table II) showed two low-energy weak bands at 15,290 cm^{-1} and 19,380 cm^{-1} and a strong high-energy band at 30,325 cm^{-1}. The low-energy bands in this position typically are expected for a square-planar configuration [37] and may be assigned to $^2B_{1g} \rightarrow ^2A_{1g}$ and $^2B_{1g} \rightarrow ^2E_g$ transitions, respectively. The strong high-energy band, in turn, is assigned to metal → ligand charge transfer. Also, the magnetic moment values (1.7 B.M) (Table I) for the Cu(II) complex was found to be consistent with the proposed square-planar structure of the Cu(II) complex (Figure 3B). The electronic spectra of the Ni(II) complex showed d–d bands in the region at 10,615, 15,465 and 26,140 cm^{-1}. These are assigned [38] to the transitions $^3A_{2g}(F) \rightarrow ^3T_{2g}(F)$, $^3A_{2g}(F) \rightarrow ^3T_{1g}(F)$ and $^3A_{2g}(F) \rightarrow ^3T_{2g}(P)$, respectively, consistent with their well-defined octahedral configuration. The band at 30,245 cm^{-1} was assigned to metal → ligand charge transfer. The magnetic measurements (3.2 B.M) showed two unpaired electrons per Ni(II) ion suggesting [39] also an octahedral geometry for the Ni(II) complex (Figure 3A). The electronic spectra of the Zn(II) complexes exhibited only a high-intensity band at 29,215 cm^{-1} which is assigned [38] to a ligand-metal charge transfer.

Table III. Antibacterial activity of the metal complexes

Ligand/complexes	Microbial species (a)	(b)	(c)	(d)
Ciprofloxacin (L)	12	18	15	22
Co(II)–CFL	14	20	18	24
Cu(II)–CFL	15	24	19	26
Ni(II)–CFL	13	20	17	25
Zn(II)–CFL	16	22	20	28

Microbial species: (a) = *Staphylococcus aureus*, (b) = *Escherichia coli*, (c) = *Klebsiella pneumoniae*, (d) = *Bacillus dysenteriae*.
Significant 14–20, Moderate 7–13, Weak < 7.

Biological activity

All the newly synthesized complexes (**1**–**4**) coordinated with the metals *i.e.* Co(II), Cu(II), Ni(II) and Zn(II) were screened for their antibacterial effect against *S. aureus*, *E. coli*, *P. aeruginosa*, *K. pneumoniae* and *B. dysenteriae*. The metal(II) complexes exhibited a marked enhancement in ciprofloxacin activity against all the test bacterial strains compared to the parent antibiotic, ciprofloxacin (**L**) (Table III).

It has been suggested that those ligands having nitrogen and oxygen donor systems might inhibit enzyme activity, since the enzymes which require these groups for their activity appear to be especially more susceptible to deactivation by the metal ions upon chelation. Chelation/coordination reduces the polarity [40,41] of the metal ion mainly because of the partial sharing of its positive charge with these donor groups and possibly the π-electron delocalization [42,43] within the whole chelate ring system. This process of chelation thus increases the lipophilic nature of the central metal atom, which in turn, favors its permeation through the lipoid layer of the membrane [42,43] thus causing the metal complex to cross the membrane of the microorganism cell wall more effectively thus increasing the activity of the drug.

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