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In Vitro Antibacterial Studies of Ciprofloxacin-imines and Their Complexes with Cu(II),Ni(II),Co(II), and Zn(II)

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Abstract: Some new transition metal complexes of ciprofloxacin-imines derived from ciprofloxacin and *p*-substituted anilines were synthesized and characterized on the basis of physical properties, conductance measurements, elemental analysis, UV/Vis., infrared and nuclear magnetic resonance spectroscopy. These ligands as well as their metal complexes were also evaluated for their antibacterial activity against several bacterial strains, such as *Staphylococcus aureus, Bacillus subtilus, Salmonella typhae,* and *E. coli.* It was found that metal complexes are more antibacterial as compared to uncomplexed ligands. The present study was carried out in search of a target antibiotic instead of a broad spectrum one.

Key Words: Ciprofloxacin imines, coordination compounds, antibacterial activity

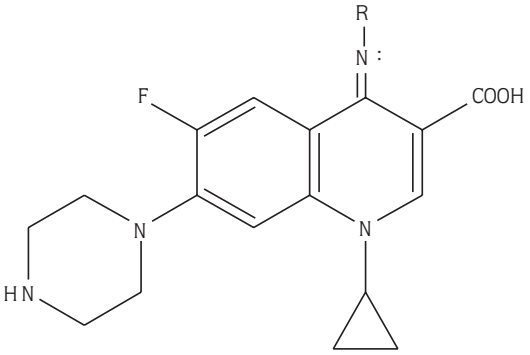
Introduction

Compounds containing an azomethine group are known as imines (Schiff bases). The chelating abilities, and analytical and biological applications of these compounds have attracted remarkable attention (1-3). Antibiotics such as Streptomycin, aspergillic acid, usnic acid, and tetracycline are known to have chelating properties. Presumably some antibiotics are delicately balanced so as to be able to compete successfully with the metal-binding agents of bacteria while not disturbing the metal processing by the host. There is evidence that at least some bacteria have developed resistance to antibiotics through the development of altered enzyme systems that can compete successfully with antibiotics. The action of the antibiotic need not be a simple competitive one. The chelating properties of antibiotics may be used in metal transport across membranes or to attach the antibiotic to a specific site from which it can interfere with the growth of bacteria (4).

Quinolones are a group of antibiotics that have been extensively used in treating several bacterial diseases (5). Quinolone compounds have been synthesized and are being used as antibacterials since the discovery of nalidixic acid in 1962 (6). They have antibacterial activity against gram-negative and gram-positive strains. The primary mechanism of their antibacterial action is inhibition of Topoisomerase II, which is accountable for coiling the long DNA molecule into the restrained space inside the bacterial cell (7).

Transition metal complexes of these antibiotics with enhanced potentiality against bacterial strains have been reported elsewhere (8-12). Ciprofloxacin (1-cyclopropyl-6-flouro-1, 4-dihydro-4oxo-7- (1-piperazinyl)-3 quinolone carboxylic acid is a synthetic fluoroquinolonone antibiotic with a broad spectrum of activity. It is active against a wide variety of aerobic gram-negative and gram-positive bacteria. The mechanism of ciprofloxacin action involves inhibition of bacterial DNA gyrase (13), which is essential for DNA replication, and it has been proposed (14,15) that metal complex intermediates are involved in this process.

In continuation of our series of investigations (16), we attempted to widen the scope of derivatization by providing more flexibility through Schiff base formation with ciprofloxacin containing keto group, -COOH and complexation with metal ions. The Schiff base structure provides for a greater choice and flexibility, and complexation with a metal ion adds to the stability and versatility of the compounds. The novel investigated compounds and their metal complexes were also evaluated for their antibacterial activity against several bacterial strains. The structures of the ciprofloxacin-imines are shown in Figure 1.



For L^1

R = C_6H_5Cl–,

For L^2

R = $C_6H_5NO_2$–

Figure 1. Structure of the ligands.

Materials and Methods

All chemicals were of analytical grade and were obtained from E. Merck. Ciprofloxacin was obtained in pure form from a local pharmaceutical company and was used without further purification. IR spectra were recorded on a Philips analytical PU 9800 FTIR. UV- visible spectra were obtained in DMSO by SPECORD-200 spectrophotometer using the software ACTUA 710. ^{1}H-NMR spectra were recorded on a Bruker 250 MHz spectrophotometer in DMSO-d_6. Conductances of the metal complexes were determined in DMF on a Hitachi YSI-32 model conductometer. Magnetic measurement was done on the solid complexes using the Gouy method. Melting points were determined by digital Gallenkamp apparatus. Antibacterial activity was determined by disk diffusion method (16).

Synthesis of ciprofloxacin imines

A methanolic solution of ciprofloxacin (0.01 mol) with *p*-nitro aniline (0.01 mol) and *p*-chloro aniline (0.01 mol) was boiled under reflux in the presence of glacial acetic acid separately for 4 h. The resulting solution was concentrated to 8 ml on a water bath and allowed to cool at 0 °C. Colored solids were filtered, washed with methanol and ethanol, and dried.

Synthesis of metal complexes

The Schiff base ligands (0.02 mol) dissolved in methanol (25 ml) were mixed with respective transition metals salts (0.01 mol) in methanol (20 ml). The reaction mixture was refluxed for 2-3 h. The resulting solutions were concentrated and cooled. On cooling colored precipitates formed, which were filtered, washed with methanol, and dried. The complexes thus obtained are listed in Table 1.

Table 1. UV/VIS and IR data of ligands and metal complexes.

Compound	IR (cm^{-1})							λmax (nm)		
	COO^- ν(C=O)	COO^- ν(C-O)	ν_a (COO^-)	ν_s (COO^-)	ν (-C=N)	ν (M-N)	ν (M-O)	Π→Π*	d-d	n→Π*
L^1	1726	1253	--	--	1626	--	--	275	--	308
L^2	1729	1263	--	--	1621	--	--	270	--	312
$Cu(L^1)_2$	--	--	1638	1470	1616	463	370	--	556	376
$Cu(L^2)_2$	--	--	1631	1483	1609	460	373	--	562	379
$Co(L^1)_2$	--	--	1628	1488	1613	466	383	--	632	389
$Co(L^2)_2$	--	--	1630	1486	1604	470	387	--	612	379
$Ni(L^1)_2$	--	--	1618	1474	1612	455	376	--	592	370
$Ni(L^2)_2$	--	--	1620	1482	1598	461	374	--	580	377
$Zn(L^1)_2$	--	--	1625	1480	1615	458	382	--	570	374
$Zn(L^2)_2$	--	--	1631	1483	1609	459	379	--	575	372

Table 2. Physico-analytical data of ligand and metal complexes.

Compound	MP (°C)	Yield (%)	Calculated (Found) (%)			
			C	H	N	M
L^1	168	54	77.43(76.80)	6.12(5.98)	16.43(13.89)	-------
L^2	173	61	74.60(70.20)	5.97(5.80)	19.73(19.60)	-------
$Cu(L^1)_2$	265	51	55.75(55.70)	4.64(4.52)	14.78(14.39)	7.12(6.98)
$Cu(L^2)_2$	260	53	56.41(56.20)	4.70(4.64)	14.95(14.45)	7.21(7.10)
$Co(L^1)_2$	320	65	56.11(55.98)	4.67(4.60)	14.87(13.98)	6.44(6.28)
$Co(L^2)_2$	313	69	56.77(56.30)	4.74(4.62)	15.05(14.98)	6.52(6.30)
$Ni(L^1)_2$	290	74	56.05(55.79)	4.67(4.57)	14.86(14.80)	6.56(6.49)
$Ni(L^2)_2$	273	73	56.72(56.25)	4.72(4.52)	15.03(14.95)	6.64(6.60)
$Zn\ (L^1)_2$	280	48	55.62(55.45)	4.63(4.53)	14.75(14.50)	7.35(7.28)
$Zn\ (L^2)_2$	285	51	56.28(56.23)	4.69(4.60)	14.92 (14.86)	7.44(7.23)

Antibacterial studies

Antibacterial activity of the complexes/ligands was investigated by a previously reported (16) method against different bacterial strains such as *Staphylococcus aureus*, *Bacillus subtilus*, *Salmonella typhae* and *E. coli.* The nutrient agar medium (Peptone, Beef extract, NaCl and Agar-Agar) and 5 mm diameter paper disks (Whatman No.1) were used. The investigated compounds, i.e. ligands and their complexes, were dissolved (30 µg) in DMF (0.01 ml). The filter paper disks were soaked in solutions of ligands as well as complexes, dried, and then placed in petri plates previously seeded with the test organisms. The plates were incubated for 24 h at 37 °C and the inhibition zone around each disk was measured. The results obtained are tabulated in Table 3.

Results and Discussion

The analytical data of the ligands and its complexes along with some physical properties are summarized in Table 1. The ligands on interaction with Cu(II), Co(II), Ni(II) and Zn(II) chlorides yield complexes corresponding to the general formula $[ML_2]$. The low molar conductance values of the complexes reveal their non-electrolytic nature (17). These complexes are more soluble in organic solvents and less soluble in inorganic solvents. Elemental analysis data (Table 1) show that the metal to ligand ratio is 1:2.

In order to study the binding mode of both the ligands to the metal in complexes, the IR spectra of the uncomplexed ligands were compared with the spectra of metal complexes. The IR spectra of the ligands show a band in the region 1725-1730 cm^{-1} and 1248-1254 cm^{-1} assignable to the COOH group (8).

Table 3. MIC (mm) values of ligand and metal complexes.

Compound	Microbial species			
	a	b	c	d
Ciprofloxacin	20	19	19	25
L^1	22	20	20	24
L^2	21	22	20	23
$Cu(L^1)_2$	22	21	22	25
$Co(L^1)_2$	22	22	23	25
$Cu(L^2)_2$	23	22	24	24
Co(L2) 2	21	21	22	22
$Ni(L^1)_2$	21	25	20	25
$Ni(L^2)_2$	25	23	21	23
Zn (L1) 2	24	24	22	23
Zn(L2) 2	23	25	22	22

a. *Staphylococcus aureus*
b. *Bacillus subtilus*
c. *Salmonella typhae*
d. *E. coli*

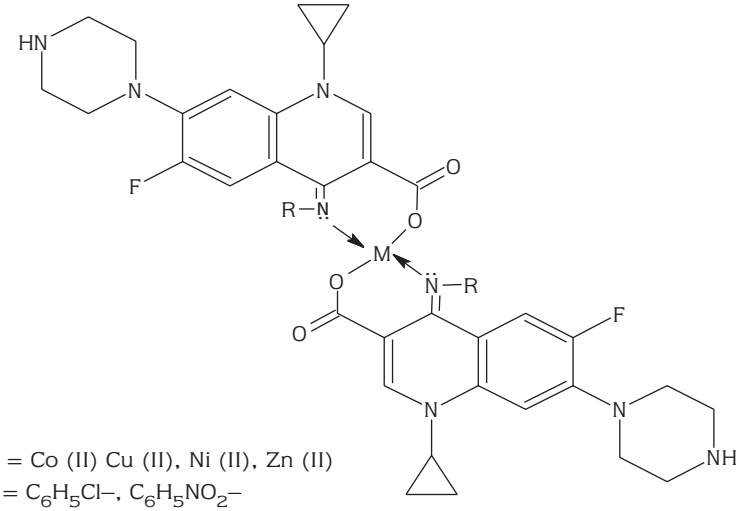
The absence of these bands in metal complexes reveals the deprotonation of the -COOH group on complexation. These ligands also exhibit their characteristics bands in the region 1650-1630 cm^{-1} assigned to azomethine linkage in the ligands (16). The IR spectra of metal complexes indicate shifting of these bands to lower frequencies (1610-1590 cm^{-1}), thus confirming the involvement of the azomethine group in bonding with metal ions. The above description of IR data supports the monoanionic bidendate nature of these investigated ligands.

The IR spectra of the metal complexes also show some new bands in the region 470-455 cm^{-1} and 390-350 cm^{-1}, which are assigned to $\bar{\nu}$ (M-N) and $\bar{\nu}$ (M-O) bands, respectively (16,18). The appearance of these bands also favors complex formation (Figure 2).

The ^{1}H-NMR spectra of the Schiff base and its complex with Zn(II) in DMSO-d_6 exhibit a multiplet at 7.1-7.4 δ assigned to C_6H_5- and at 2.7 δ, which can be attributed to the $-CH_2$ group. The peak in the downfield region at 10.8-11.3 δ in the spectra of the ligand can be assigned to the proton of the -OH group (19). Comparisons of chemical shift of the uncomplexed ligands with their Zn (II) complexes showed that all the signals are in the expected range except for that of the OH group. The absence of this signal suggested the deprotonation of the hydroxyl group and the involvement of the oxygen atom in complexation.

The electronic spectra of the metal complexes are compared with those of the ligands. Two bands appeared at 265-273 nm and 308-315 nm, which can be assigned to $\Pi \rightarrow \Pi^*$ and $n \rightarrow \Pi^*$ transition, respectively, in both ligands (20). The complexes of Cu(II), Ni(II), and Co(II) show less intense bands in the region 550-640 nm, which can be assigned to d-d transition of metal ions. The former band may be due to $^{4}A_2 \rightarrow {}^{4}T_1$ (P) for Co $(L)_2$, $^{3}A_2 \rightarrow {}^{3}T_2$ (F) for Ni $(L)_2$ and $^{2}T \rightarrow {}^{2}E$ (G) for Cu $(L)_2$ transition of tetrahedral geometry. All the complexes show an intense band at 370-390 nm, which can be assigned to $n \rightarrow \Pi^*$ transition associated with azomethine linkage. The shifting of these bands in metal complexes together with color change authenticates complex formation.

The in vitro biological screening effects of the investigated compounds were tested against 4 bacterial strains: *Staphylococcus aureus*, *Bacillus subtilus*, *Salmonella typhae*, and *E. coli*. The minimum inhibition concentration (MIC) values of the novel investigated compounds against the growth of organisms are summarized in Table 3. A comparative study of ligands and their metal complexes showed that they exhibit higher antibacterial activity than uncomplexed ligands. The results are promising compared with the previous studies (15,21,22). Such increased activity of metal chelate can be explained on the basis of the overtone concept and chelation theory. According to the overtone



M = Co (II) Cu (II), Ni (II), Zn (II)
R = C_6H_5Cl–, $C_6H_5NO_2$–

Figure 2. Proposed structure of metal complexes.

concept of cell permeability, the lipid membrane that surrounds the cell favors the passage of only lipid-soluble materials in which liposolubility is an important factor that controls the antimicrobial activity. On chelation the polarity of the metal ion will be reduced to a greater extent due to overlap of ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. Further it increases the delocalization of Π-electrons over the whole chelate ring and enhances the lipophilicity of complexes (23). This increased lipophilicity enhances the penetration of complexes into the lipid membranes and blocks the metal binding sites in enzymes of microorganisms. These complexes also disturb the respiration process of the cell and thus block the synthesis of proteins, which restricts further growth of the organism.

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