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Antioxidant and antimicrobial screening of Ciprofloxacin derivative and its complexes

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ABSTRACT

The research article presents the synthesis of ciprofloxacin derivative with Benzil dihydrazone and its metal complexes with Co (II), Ni(II), Cu(II) salts. The molecular formula proposed for ligand is $(C_{48}H_{46}F_2N_{10}O_4)$ and for complexes $[M(C_{48}H_{46}F_2N_{10}O_4)X_2]$ where $M = Co(II), Ni(II), Cu(II)$ and $X = Cl$. 1H NMR, IR and UV-Vis, EPR spectra were recorded to confirm the formation of ligand (Ciprofloxacin derivative) and metal complexes. The proposed geometry of complexes was octahedral which is further confirmed by electronic spectra and magnetic moment analysis. The compounds were screened for their antimicrobial, antioxidant activity using disc diffusion method and DPPH Scavenger activity method. Ni (II) complex has shown significant antibacterial activity with maximum zone of inhibition against *E. coli* while Co(II) and Ni(II) complexes are most effective against *B. Cereus*. Antimicrobial activity of the ligand has been enhanced on complexation with Ni(II) and Co(II) ions. Antioxidant activity of complexes was compared with ascorbic acid and ciprofloxacin itself and it was observed that Cu (II) complex has shown remarkable antioxidant activity at variable concentrations.

Keywords: Antioxidant activity, zone of inhibition, E. Coli, metal complexes, DPPH

INTRODUCTION

Quinolones represent a wide spectrum of synthetic antibacterial agents, by inhibiting two crucial bacterial enzymes, DNA gyrase and topoisomerase, respectively [1–3]. Ciprofloxacin is the most common therapeutic agent among all the quinolones belonging to this class, which is currently used in clinical practice for treating mycobacterial infections [4].

Complexation of the quinolone antibiotics with metals is known to enhance biological activities by decreasing bacterial resistance to the drug perhaps due to higher liposolubilities leading to greater intracellular accumulations [5–7]. The interaction of Ciprofloxacin and metal ions have been widely studied especially due to its interesting biological and chemical applications. It is well known that metal ions present in complexes accelerate the drug action and the efficacy of the organic therapeutic agents [8].

Recent studies indicate an important role of metal ions in the mechanism of action of these drugs; it was suggested that the intercalation of the quinolone complexes to a metal is an important step in the antibacterial processes [9]. Transition metal complexes derived from hydrazones and derivatives also have been extensively studied and remarkable antimicrobial activity for these complexes has been reported.

The pharmacological efficiencies of metal complexes depend on the nature of the metal ions and the ligands [10]. There is an increasing requirement for the discovery of new compounds having antimicrobial and antioxidant activities to decrease the bacterial resistance to drugs.

In the present article synthesis of, Co (II), Ni (II) Cu (II), and Zn(II) complexes derived from ciprofloxacin and benzyl dihydrazone, their characterization and biological screening is discussed.

MATERIALS AND METHODS

2.1 Material: All reagents were commercially available and used without further purification. Solvents were distilled from appropriate drying agents immediately prior to use. Ciprofloxacin, hydrazine hydrate and Benzil were purchased from sigma Aldrich and used as received. The metal salts were purchased from S. D. Fine and Merck India. DPPH and ascorbic acid were purchased from merck India. Bacterial strains were obtained from Microbial type culture collection, IMTECH, Chandigarh.

2.2 Methods: The microanalyses of C, H and N were carried out at Sophisticated Analytical Instrument Facility. The metal contents were determined by standard EDTA methods. Electronic spectra (DMSO) were recorded on PerkinElmer LAMBDA 25 spectrophotometer in the range of 200nm-900nm. The magnetic susceptibility measurements of the complexes were carried out by Gouy balance at room temperature. The IR spectra were recorded on Thermo Scientific Nicoletis50 FT-IR Spectrometer in the range 4000-400 cm^{-1} using ATR. EPR spectra were recorded at JEOL-Japan at room temperature and liquid nitrogen temperature both. Melting points were determined by using digital melting point apparatus.

2.3 Biological assay

2.3.1 Antibacterial activity

All synthesized macrocyclic complexes were tested for in vitro antibacterial activity against bacterial strains E. Coli (gram-ve) and B. Cereus(gram +ve) using spot-on-lawn on Muller Hinton Agar(7.6g of Mueller hinton agar in 200 ml water) seeded with each of test pathogens. The organisms were isolated in nutrient agar medium and selectively cultured at 98.4°F for 24 hrs. Discs were impregnated with equal volume (100 μl) of each metal complex dissolved in DMSO at a concentration of 1mg/ml. The clinical strains were also tested for their sensitivity against the standard antibiotics, ciprofloxacin by the disc diffusion method. The negative solvent control of DMSO was also maintained throughout the experiment. The zone of inhibition was measured in mm after 24 hours incubation by using a zone reader.

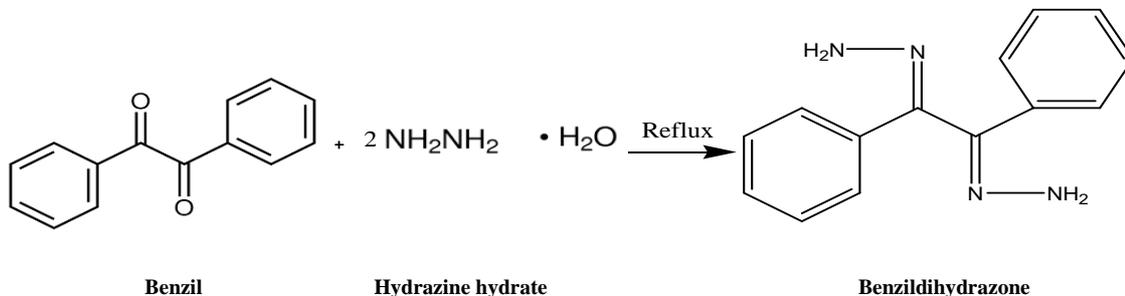
2.3.2 Antioxidant activity

Antioxidant activity was monitored for all the synthesised complexes, ciprofloxacin & standard antioxidant ascorbic acid using 1%DPPH radical scavenging method. Solutions of various concentrations 25, 50, 75 and 100 $\mu\text{g/ml}$ in DMF. 1ml of each complex was added to a 4 ml of methanolic solutions of 0.004 % (w/v) of DPPH in a test tube and mixed well. The mixtures then incubated for 30 minutes at room temperature. After that absorbance was measured at 517nm by using methanol as a blank or reference. Ascorbic acid was use as a standard antioxidant for comparison.

3. SYNTHESIS

3.1 Synthesis of Benzil Dihydrazone

Synthesis of benzildihydrazone as a reported method by taking benzil solution in ethylene glycol and mixed well with hydrazine hydrate in the ratio of 1:2. The resulting yellow solution was refluxed for 4-5 hrs with continuous stirring using magnetic stirrer. A white precipitate is obtained after 8 hours that was filtered off and washed with water and diethylether and then dried in the air (yield-70%). Recrystallize from warm methanol.



Scheme -1 Synthesis of benzil dihydrazone from Benzil and hydrazine hydrate

3.2 Synthesis of metal complexes derived from Ciprofloxacin and Benzil Dihydrazone.

3.2.1 Conventional Method

All the complexes were synthesized by template method i.e. by condensation of Ciprofloxacin and benzyl Dihydrazone in the presence of the respective divalent metal salt. To Ciprofloxacin (10 mmol) solution in

methanol(50ml) heating at 40⁰C respective divalent chromium, Ferrous cobalt, nickel, copper, zinc (5.0 mmol) dissolved in the minimum quantity of methanol (~20ml) was added. The resulting solution was refluxed for 0.5h and 2 ml of glacial acetic acid was added. Subsequently, Benzil Dihydrazone (5.0 mmol), in DMSO, was added to the refluxing mixture and refluxing was continued for 4 hrs. Temperature was maintain between 35-40⁰C The mixture was cooled to room temperature overnight. Precipitate was filtered off through G-4 crucible, washed several time by ethanol and dried under vacuum desiccators. On cooling the precipitate of metal complexes of different colour were obtained. Precipitates were collected and weighed.

3.2.2 Microwave Synthesis

Ciprofloxacin and metal salts were mixed in a beaker by taking methanol as a solvent, then the reaction mixture was irradiated by the microwave oven for 5 minutes. After 5 minutes 2 ml of glacial acetic acid was added and then add benzildihydrazone solution in DMSO(10 mmol) and irradiated again for 2 or 3 minutes. Reaction was completed in 7-8 minutes. Higher yield is obtained in this process then conventional method.

3.2.3 Chemistry

The analytical data of divalent and trivalent transition metal complexes derived from ciprofloxacin and benzil dihydrazone and the complexes formulated as $[M(C_{48}H_{46}F_2N_{10}O_4)]Cl_2$ where M=Co(II) , Ni(II) , Cu(II) and Zn(II) and benzil dihydrazone and ciprofloxacin complex without metal ions formulated as $(C_{48}H_{46}F_2N_{10}O_4)$

All the metal complexes are coloured solids. All the synthesized complexes are soluble in distilled water, DMSO & DMF and partially soluble in ethanol & Methanol while completely insoluble in acetone.

Melting points of ligand and complexes are as follows:

Co($C_{48}H_{46}F_2N_{10}O_4$)Cl₂: 256⁰C Ni($C_{48}H_{46}F_2N_{10}O_4$)Cl₂:240⁰C;
Cu($C_{48}H_{46}F_2N_{10}O_4$)Cl₂:236⁰C; Zn($C_{48}H_{46}F_2N_{10}O_4$)Cl₂ : 256.4⁰C;
Ligand: 168⁰C.

RESULTS AND DISCUSSION

4.1 Analysis of physical properties;

Physical state Colour Molecular weight of all synthesized species is given in table 1

Table:-1 Physical properties and analytical data of divalent and trivalent transition metal complexes derived from ciprofloxacin and benzil dihydrazone calculated (found)

Complex	Colour	Molecular Weight found	% of C	% of H	% of N	% of O	% of M	% of Cl	% of F
Co($C_{48}H_{46}F_2N_{10}O_4$)Cl ₂	blue	994.77	57.95 (57.2)	4.66 (4.23)	14.1 (14.02)	6.43 (6.4)	5.92 (5.7)	7.12 (7.0)	3.8 (3.7)
Ni($C_{48}H_{46}F_2N_{10}O_4$)Cl ₂	yellowish	994.53	57.96 (57.6)	4.66 (4.42)	14.08 (13.96)	6.43 (6.5)	5.90 (5.8)	7.12 (7.0)	3.8 (3.7)
Cu($C_{48}H_{46}F_2N_{10}O_4$)Cl ₂	greenish	999.39	57.68 (57.1)	4.63 (4.17)	14.01 (14)	6.40 (6.35)	6.3 (6.3)	7.09 (7.0)	3.8 (3.7)
Zn($C_{48}H_{46}F_2N_{10}O_4$)Cl ₂	white	1001.2	57.58 (57.2)	4.63 (4.25)	14.01 (13.96)	6.40 (6.3)	6.3 (6.25)	7.09 (7.0)	3.8 (3.7)
($C_{48}H_{46}F_2N_{10}O_4$)	white	864.9	66.65 (66.29)	5.36 (5.17)	16.2 (15.9)	7.4 (7.0)	-	-	-

The complexes have been characterised with the help of physical properties such as colour of complexes , solubility , melting point , elemental analysis and various physicochemical techniques such as infrared ,¹H-NMR electronic spectra , UV ,and molecular weight determination. On the basis of these studies , a six coordinate geometry was proposed for all the metal complexes.

4.2 IR Analysis

In the infrared spectra of ligand and complexes absorption peaks at ~3220 cm⁻¹ and ~3250 cm⁻¹ corresponding to $\nu(NH_2)$, were absent. In ciprofloxacin a prominent peak was observed between 3500-3450 cm⁻¹, which is assigned to stretching vibration of -OH group of acid but it was absent in the spectra of ligand and complexes and indicates the condensation of acid groups of ciprofloxacin and amino groups of benzildihydrazone. A lower shift of $\nu(C=O)$ stretching vibration from ~1715 cm⁻¹ to ~1690-1695 cm⁻¹ in the complexes was observed due to coordination with metal ions[14]. The band at ~ 1400-1450cm⁻¹ in ciprofloxacin was assign to $\nu(C=O)$ vibration of carbonyl group has been shifted to lower side in metal complexes and appears at 1390-1399cm⁻¹ and gives indication about the

coordination of metal with carbonyl oxygen[15]. The peak at 1650 to 1600 cm^{-1} may be assigned to quinolones. A strong absorption peak between 1050 and 1000 cm^{-1} was assigned to C-F group.

4.3 $^1\text{H-NMR}$ studies

Following NMR peaks were obtained for synthesized ligand.

Aromatic rings multiplet $\delta = 7.2-7.6$,

-NH group of benzyl dihydrazone at -9.5 (broad)

-NH group of ciprofloxacin at -13.6 (broad)

The signal for -NH proton of benzil dihydrazone has been shifted to downfield region due to coordination with metal through -NH group of BDH.

4.4 Magnetic measurements and electronic spectral studies

The magnetic and electronic investigation is very important to propose a geometry specially in the case of metal complexes.

Nickel complexes

The magnetic moment of nickel complex at room temperature is 2.92 B.M. The solution spectra of Ni(II) complex exhibit a well discernable band with a shoulder on the low energy side. The other two bands generally observed in the region at $\sim 16,600 \text{ cm}^{-1}$ (ν_2), and $\sim 28,350 \text{ cm}^{-1}$ (ν_3), may be assigned to $^3\text{A}_2 \rightarrow ^3\text{T}_{1g}$ (F) (ν_2), and $3\text{A}_{2g} \rightarrow ^3\text{T}_{1g}$ (P) (ν_3), respectively. The first two bands result from the splitting of one band, ν_1 , at $\sim 9,900$ and $11,750 \text{ cm}^{-1}$, which can be assigned to $^3\text{B}_{1g} \rightarrow ^3\text{E}_g$ and $^3\text{B}_{1g} \rightarrow ^3\text{B}_{2g}$, assuming the effective symmetry to be D_{4h} (component of 3T_{2g} in O_h symmetry). The intense higher energy band at $\sim 34,300 \text{ cm}^{-1}$ may be due to a $\pi-\pi^*$ transition of the (C=N) group [16].

Copper complexes

The magnetic moments of copper complexes was observed in the range of 1.74 B.M. The absorption spectra of the copper complexes exhibit bands in the region $\sim 17,500 \text{ cm}^{-1}$ with a shoulder on the low energy side at $\sim 14,950 \text{ cm}^{-1}$, and show that these complexes are distorted octahedral. Assuming tetragonal distortion in the molecule, the d-orbital energy level sequence for these complexes may be: $x^2 - y^2 > z^2 > xy > xz > yz$ and the shoulder can be assigned to: $z^2 \rightarrow x^2 - y^2$ ($^2\text{B}_{1g} \rightarrow ^2\text{B}_{2g}$) and the broad band contains both the $xy \rightarrow x^2 - y^2$ ($^2\text{B}_{1g} \rightarrow ^2\text{E}_g$) and $xz, yz \rightarrow x^2 - y^2$ ($^2\text{B}_{1g} \rightarrow ^2\text{A}_{2g}$) transitions. The band separation of the spectra of the complexes is of the order 2550 cm^{-1} , which is consistent with proposed geometry of the complexes. Therefore, it may be concluded that all the complex of Cu(II) metals of this series is distorted octahedral[17].

Cobalt complexes

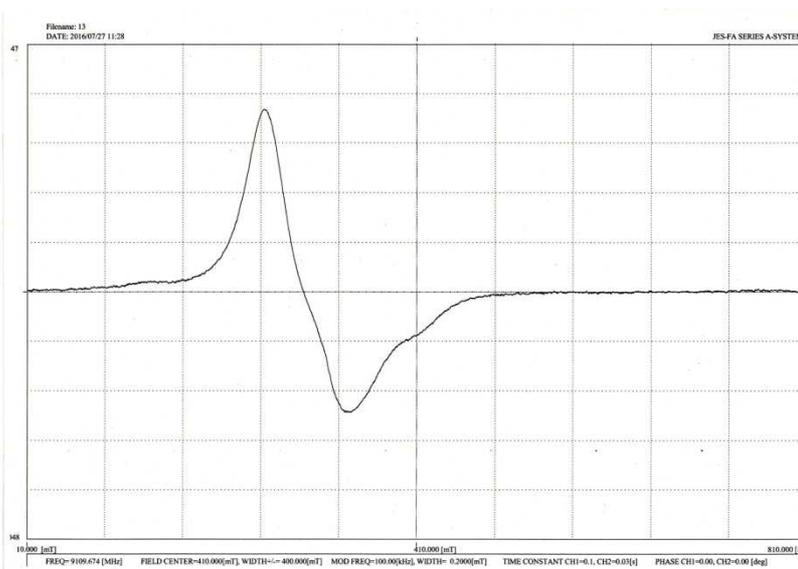
The magnetic moment was measured at room temperature and lie in the range 4.91-4.94 B.M., which corresponds to three unpaired electrons. The solution spectra of cobalt (II) complexes exhibit absorption in the regions $\sim 8100-9000$ (ν_1), $\sim 12500-15750$ (ν_2) and $\sim 18700-20250 \text{ cm}^{-1}$ (ν_3), respectively. The spectra resemble to those which are reported earlier as octahedral. Thus assuming the effective symmetry to be D_{4h} the various bands can be assigned to: $^4\text{T}_{1g} \rightarrow ^4\text{T}_{2g}$ (F), (ν_1), $^4\text{T}_{1g} \rightarrow ^4\text{A}_{2g}$ (F), (ν_2), $^4\text{T}_{1g} \rightarrow ^4\text{T}_{1g}$ (P), (ν_3), respectively. It appears that the symmetry of these complexes is not idealized O_h , but is D_{4h} . The assignment of the first spin-allowed band seems plausible since the first band appears approximately at half the energy of the visible band[18].

3.4 EPR Analysis

EPR analysis is very informative to understand the metal ion environment in complexes. X-band spectra of all three copper complexes 7, 8, and 9 were recorded in DMSO at room temperature (300K) (Fig 1) and LNT(77K).

It shows magnetic equivalence of Cu nuclei in the powder sample and possibly the exchange interactions are not enough for hyperfine splitting of lines.

g_{\parallel} & g_{\perp} values of complexes 7 were calculated and found to be 2.27 and 2.09 respectively which follows the pattern $g_{\parallel} > g_{\perp} > 2.002$ and indicates the presence of unpaired electron of copper predominantly in the dx^2-y^2 orbital [49]. The relation of g values shows considerable amount of covalent characters of M-N bond of complexes in the ligand environment. The G values were also calculated and found to be around 3.2 which suggests the covalent characters of complexes.



Based on the all characterization methods the proposed structure of ligand and its metal complexes derived from benzil dihydrazone and ciprofloxacin is shown in figure (1&2) as follows

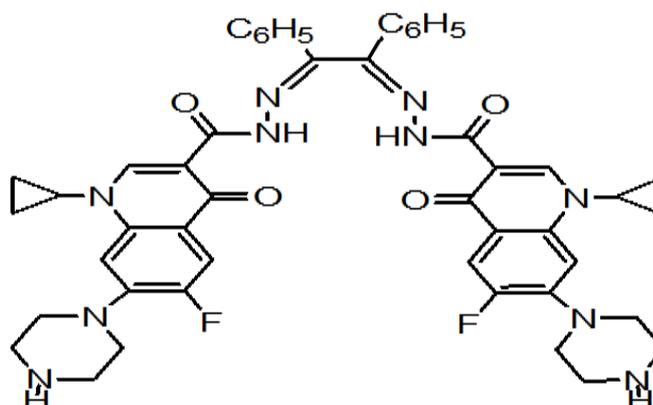


Fig. -1 Ligand Derived from Ciprofloxacin and BenzilDihydrazone

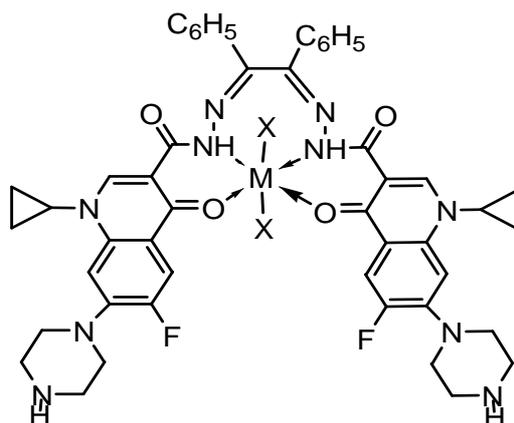


Fig. -2 Complexes derived from Ciprofloxacin and benzil dihydrazone in the presence of metal salts where M = Co(II), Ni(II), Cu(II), Zn(II), X = Cl⁻

4.5 Antibacterial activity

The synthesised metal complexes were tested against E.coli and B.cereus for their in vitro antibacterial activity.

Inhibition % calculated by this formula

$$\text{Scavenging activity (\%)} = \frac{A_c - A_s}{A_c} \times 100$$

Where A_c = absorbance of control and A_s = absorbance of sample

Graphical representation of antioxidant activity

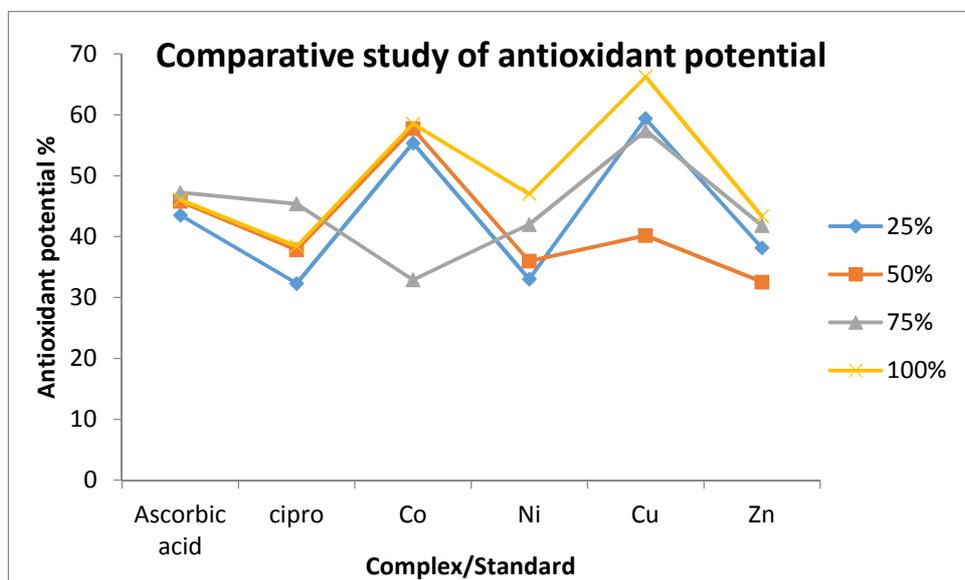


Fig. -4 Comparative study of Antioxidant activity of different metal complexes, Ciprofloxacin and Ascorbic acid at different concentrations

From figure 4 it was observed that Cu(II) complex is showing maximum antioxidant activity than other complexes and even greater than the standard antioxidant, Which supports the relevance of the present work.

CONCLUSION

The derivative of Ciprofloxacin with benzyl dihydrazone and its metal complexes were successfully synthesized and characterized.

It was observed that the microwave method is the better method for the preparation of metal complexes in terms of yield and its ecofriendly nature.

Antibacterial activity of synthesised metal complexes better than standard antibiotic Ciprofloxacin. Ni complexes have given outstanding antibacterial activity against E.coli.

Cu complex has given significant antioxidant activity than all other complexes at all concentrations.

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