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BIOLOGICAL ACTIVITY OF NEWLY SYNTHESIZED M(II) HETEROCHELATES OF COUMARIN DERIVATIVE AND ENROFLOXACIN

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Abstract
Some novel heterochelates synthesized by reflux of 6-bromo-3-(3-(4-chlorophenyl)acryloyl)-2H-chromen-2-one, enrofloxacin and various transition metal. ^{1}H, ^{13}C, IR and ESI Mass confirm the formation of ligand. The heterochelates were characterized on the basis of different spectroscopic techniques like IR studies and elemental analysis while the geometry of complexes was octahedral which is confirmed by electronic spectra and thermogravimetric analysis. The compounds were subjected to antimicrobial, antioxidant and anti-tubercular activity viewing using serial broth dilution method and Minimum Inhibitory Concentration (MIC) is determined. Mn(II) complex has shown significant antifungal activity with an MIC of 6.25µg/mL while Cu(II) complex is perceptible for antibacterial activity at the same concentration. Anti-TB activity of the ligand has superior on complexation with Ni(II) and Co(II) ions. While Ni(II) complex shows finer antioxidant activity than other complexes.

Keywords : Heterochelates, enrofloxacin, biological aspect

1. Introduction
Coumarins comprise a really giant category of compounds found throughout the kingdom Plantae [I]. They're found at high levels in some essential oils, notably cinnamon bark oil (7,000 ppm), cassia leaf oil (up to eighty seven,300 ppm) and lavender oil. Coumarin is additionally found in fruits (e.g. bilberry, cloudberry), tea and different foods like chicory [II]. Most coumarins occur in higher plants, with the richest sources being the rue family and carrot family. Though distributed throughout all elements of the plant, the coumarins occur at the best levels within the fruits, followed by the roots, stems and leaves. Environmental conditions and seasonal changes will influence the incidence in numerous elements of the plant. Recently six new minor coumarins are isolated from the fruits and also the stem bark of genus Calophyllum dispar (Clusiaceae). The genus Calophyllum, that includes two hundred species, is cosmopolitan within the tropical rain forest wherever many species square measure employed in people drugs [III]. Though most of the natural coumarins alive are isolated from the upper plants, some members are discovered in microorganisms. Some vital coumarin members are isolated from

microorganism sources e.g. antibiotic and coumermycin from actinomycete, and aflatoxins from Aspergillus species [IV].
Quinolones (quinolonecarboxylic acids or 4-quinolones) are antibacterial drugs and are commonly used as treatment for many infections [V] since they can effectively inhibit DNA replication. Enrofloxacin (=Herx, Fig. 1) is a typical second-generation quinolone antimicrobial drug presenting a broad spectrum of activity against a wide range of Gram-negative and Gram-positive bacteria, including those resistant to β-lactam antibiotics and sulfonamides [VI]. Enrofloxacin is the first fluoroquinolone developed for veterinary application and is usually used for the treatment of some urinary tract, respiratory tract and skin infectious diseases in pets and livestock [VII].
It is well known that metal ions present in complexes accelerate the drug action and the efficacy of the organic therapeutic agents [VIII]. The pharmacological efficiencies of metal complexes depend on the nature of the metal ions and the ligands [IX]. It is declared in the literature that different ligands and different complexes synthesized from same ligands with different metal ions possess different biological properties [X]. So, there is an increasing requirement for the discovery of new compounds having antimicrobial, antioxidant and anti tubercular activities. The newly prepared compounds may be more effective than known others in terms of their biological activities and possibly display their efficiencies with a distinct mechanism from those of well known, Also we describes the synthesis of Cu(II), Ni(II), Co(II) and Mn(II) complexes from 6-bromocoumarin and enrofloxacin as ligand. For characterization of the compounds, following spectroscopic and analytical techniques were employed: IR, NMR, TGA and elemental analyses.

2. Experimental

2.1 Materials

All reagents were of analytical reagent (AR) grade purchased commercially from Spectro chem. Ltd., Mumbai-India and used without further purification. Solvents employed were distilled, purified and dried by standard procedures prior to use [XI]. Clioquinol was purchased from Agro Chemical Division, Atul Ltd., Valsad-India. The metal nitrates used were in hydrated form.

2.2 Physical measurements

All reactions were monitored by thin-layer chromatography (TLC on alluminium plates coated with silica gel 60 F_{254}, 0.25 mm thickness, E. Merck, Mumbai-India) and detection of the components were measured under UV light or explore in Iodine chamber. Carbon, hydrogen and nitrogen were estimated by elemental analyzer PerkinElmer, USA 2400-II CHN analyzer. Metal ion analyses was carry out by the dissolution of solid complex in hot concentrated nitric acid, further diluting with distilled water and filtered to remove the precipitated organic ligands. Remaining solution was neutralized with ammonia solution and the metal ions were titrated against EDTA. ^{1}H and ^{13}C NMR measurements were carried out on Advance-II 400 Bruker NMR spectrometer, SAIF, Chandigarh. The chemical shifts were measured with respect to TMS which used as internal standard and DMSO-d_6 used as solvent. Infrared spectra of solids were recorded in the region 4000-400 cm^{-1} on a Nicolet Impact 400D Fourier-Transform Infrared Spectrophotometer using KBr pellets. Melting point of the ligands and metal complexes were measured by open capillary tube method. Thermal decomposition (TG) analysis was obtained by a model Diamond TGA, PerkinElmer, U.S.A.

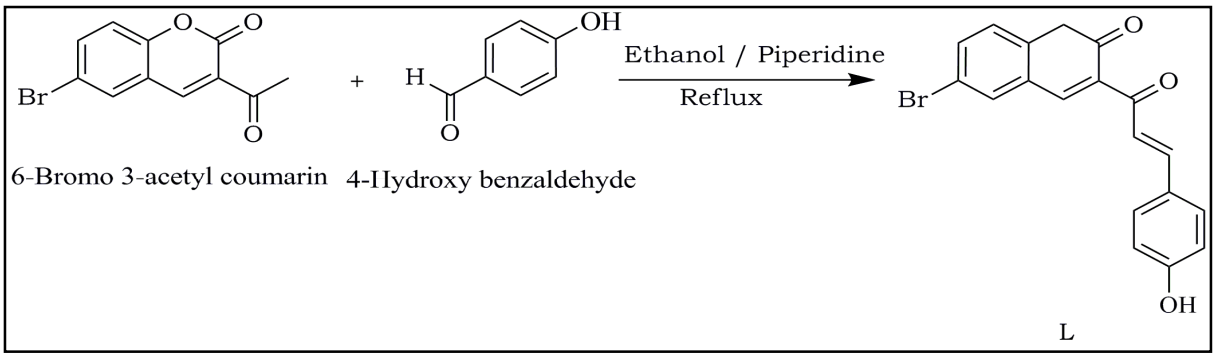
2.3 Synthesis of 6-bromo 3-acetyl coumarin

6-Bromo 3-acetyl coumarin was prepared according to the reported method [XII]. A mixture of 6-bromo salicylaldehyde (12.2 g, 0.1mol), ethyl acetoacetate (13.0 g, 0.1mol) and 3 to 4 drop

piperidine were stirred for 10 min. at room temperature in a 100 mL round bottom flask. After 10 min. it was heated for 30 min in water bath. A yellow solid obtained was taken out and washed with cold ether. It was recrystallized from chloroform-hexane. Yield: 92%; M.p.119.5 °C.

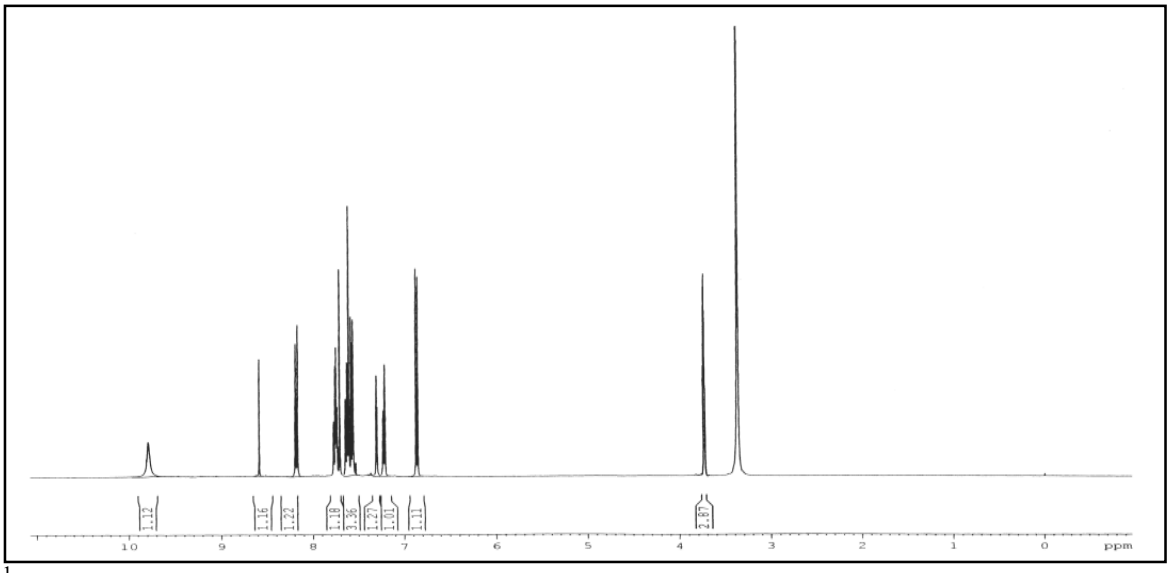
2.4 Synthesis of (E)-3-(3-(4-hydroxyphenyl)acryloyl)-2H-chromen-2-one (L)

The neutral bidentate ligands were synthesized using Claisen-Schmidt condensation [XIII]. General procedure for synthesis of the ligands (L) is shown in Scheme 1. The ligands were characterized using elemental analysis.

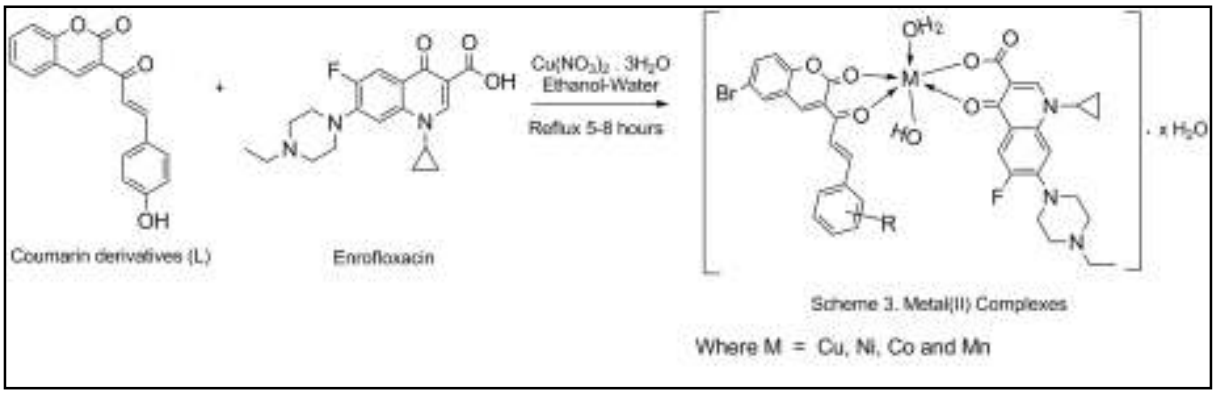


Scheme 1. Procedure for synthesis of ligands (L)

In a 100 ml round bottom flask 6-bromo 3-acetyl coumarin (0.01 mol, 1.88 g) and 4-Hydroxy benzaldehyde (0.015 mol) were taken in 15 mL of pyridine. Catalytic amount of piperidine (1.0 mL) was added and the reaction mixture was stirred for 10 min at room temperature. After clear solution obtained, the reaction mixture was refluxed on oil bath. Completion of reaction was checked by TLC using mobile phase ethyl acetate : hexane (7:3). After the completion of reaction, subsequently it was allowed to room temperature. Afterwards it was pour into ice-cold water and adjust the pH 4-5 using diluted HCl. A solid product separated out was filtered off, later on washed with cold ethanol and dried in air. It was recrystallized from ethanol. Yield: 74%, m.p.: 158-160 °C. FT-IR (KBr, cm− 1): ν(C=O, α, β-unsaturated ketone) 1617, ν(C=O, lactone carbonyl of coumarin) 1734, ν(O-H) 3426. ^{1}H NMR (DMSO-d^{6} 400 MHz) δ: 6.88 (1H, d, CH=CH- protons), 7.22 (2H, d, CH=CH- protons), 7.56 (2H, d, CH=CH- protons), 7.48-8.11 (3H, m, three aromatic protons), 8.20 (1H, d, CH=CH- protons), 8.58 (1H, s, C4-H), 9.76 (1H, s, -OH). ^{13}C NMR (DMSO-d^{6} 100 MHz) δ: 114.9, 115.8, 118.1, 119.5, 124.2, 125.5, 127.3, 130.9, 134.7, 134.4, 142.6 (11 different types of aromatic carbons), 147.7(C-4), 152.8(C-9), 157.9(C-17, carbon attach to phenolic -OH), 159.2 (C=O, lactone carbonyl of coumarin), 183.4 (C=O, α, β-unsaturated ketone). MS (ESI) m/z 370.0 $[M^{+H}]^{+}$, 372.0 $[M^{+H}]^{+2}$; Elemental analysis found (%): C, 58.09; H, 2.78; Calculated for $C_{18}H_{11}BrO_4$ (371.18): C, 58.24; H, 2.99.



^{1}H-NMR spectrum of L



Scheme 2. General procedure for synthesis of complex(C)

2.5 Synthesis of metal complexes

[Cu(L)(AN)(H_2O)OH] (C^{I})

An aqueous solution of $Cu(NO_3)_2 \cdot 3H_2O$ salt (10 mmol) was added into ethanolic solution of ligand (L) (10 mmol) and subsequently an ethanolic solution of ciprofloxacin (10 mmol) was added with continuous stirring. Then the pH was adjusted in between 4.5-6.0 by addition of diluted NH_4OH solution. The resulting solution was refluxed for 5 h and then heated over a steam bath to evaporate up to half of the volume. The reaction mixture was kept overnight at room temperature. A fine coloured crystalline product was obtained. The obtained product was washed with ether and dried over vacuum desiccators.

Complexes Ni(II), Co(II) and Mn(II) was prepared according to same method and their physicochemical parameters are summarized in Table 1. The synthetic protocol of complexes is shown in Scheme 2.

Table 1 Analytical and physical parameters of complex

Comp.	Elemental analyses, % found (required)				M.p.	Yield	Mol.	μ_{eff}
	C	H	N	Metal(II)	(°C)	(%)	Wt.	(B.M.)
Cu(II)	53.64(53.77)	4.26(4.41)	5.07(5.19)	7.67(7.72)	>350	71	828.14	1.83
Ni(II)	53.98(54.11)	4.29(4.38)	5.10(5.23)	7.13(7.21)	>300	70	823.28	3.15
Co(II)	53.96(54.05)	4.28(4.37)	5.10(5.25)	7.16(7.26)	>350	68	823.52	3.80
Mn(II)	54.23(54.35)	4.30(3.42)	5.13(5.25)	6.70(6.82)	>350	75	819.53	5.89

2.6 Antimicrobial activity

The synthesized ligand and corresponding metal(II) complexes were screened *in vitro* for their antibacterial activity against two Gram($^{+}$ve) *Streptococcus pyogenes*, *Bacillus subtilis* and two Gram($^{-}$ve) *Escherichia coli*, *Pseudomonas aeruginosa*, where antifungal against *Candida albicans* and *Aspergillus niger* using the broth dilution method [XIV]. All the ATCC culture was collected from institute of microbial technology, Bangalore. 2% Luria broth solution was prepared in distilled water while, pH of the solution was adjusted to 7.4±0.2 at room temperature and sterilized by autoclaving at 15 lb pressure for 25 min. The tested bacterial and fungal strains were prepared in the luria broth and incubated at 37 °C and 200 rpm in an orbital incubator for overnight. Sample solutions were prepared in DMSO for concentration 200, 150, 100, 50, 25, 12.5, 6.25 and 3.125, µg/mL. The standard drug solution of Streptomycin (antibacterial drug) and Nystatin (antifungal drug) were prepared in DMSO. Serial broth micro dilution was adopted as a reference method. 10 µl solution of test compound was inoculated in 5 mL luria broth for each concentration respectively and additionally one test tubes was kept as control. Each of the test tubes was inoculated with a suspension of standard microorganism to be tested and incubated at 35 °C for 24 h. At the end of the incubation period, the tubes were examined for the turbidity. Turbidity in the test tubes indicated that microorganism growth has not inhibited by the antibiotic contained in the medium at the test concentration. The antimicrobial activity tests were run in triplicate.

2.7 Anti-tubercular activity

Test compounds were evaluated for *in vitro* antimycobacterial activity. The MICs were determined and interpreted for *M. tuberculosis* H37Rv according to the procedure of the approved microdilution reference method of antimicrobial susceptibility testing [XV]. Compounds were taken at concentrations of 100, 50, 25, 12.5 6.25 and 3.125 µg/mL in 2% DMF. *M. tuberculosis* H37Rv strain was used in Middle brook 7H-9 broth which was inoculated with standard as well as test compounds and incubated at 37 °C for 4 weeks. The bottles were inspected for growth twice a week for a period of 3 weeks. Readings were taken at the end of fourth week. The appearance of turbidity was considered as bacterial growth and indicates resistance to the compound. The growth was confirmed by making a smear from each bottle and performing a ZN stain. Test compounds were compared to reference drugs Ethambutol (MIC=3.25µg/mL) and the antimicrobial and anti-tubercular activity tests were run in triplicate.

2.8 Antioxidant studies

Ferric reducing antioxidant power (FRAP) was measured by a modified method of Benzie and Strain [XVI]. The antioxidant potentials of the compounds were estimated as their power to reduce the TPTZ-Fe(III) complex to TPTZ-Fe(II) complex (FRAP assay), which is simple, fast, and reproducible. FRAP working solution was prepared by mixing a 25.0 mL, 10 mM TPTZ solution in 40 mM HCl, 20 mM $FeCl_3.6H_2O$ and 25 mL, 0.3 M acetate buffer at pH 3.6. A mixture of 40.0 mL, 0.5 mM sample solution and 1.2 mL FRAP reagent was incubated at 37 °C

for 15 min. Absorbance of intensive blue colour [Fe(II)-TPTZ] complex was measured at 593 nm. The ascorbic acid was used as a standard antioxidant compound. The results are expressed as ascorbic equivalent (mmol/100 g of dried compound). All the tests were run in triplicate and are expressed as the mean and standard deviation (SD)

3. Result and Discussion

The synthesized complexes were characterized by elemental analysis and FTIR. The metal ion in their complexes was determined after mineralization. The metal content in chemical analysis was estimated by complexometrically [XVII]. However, ligand and its complexes have been screened for their *in vitro* antimicrobial antioxidant and anti-tubercular activities, while Geometry of the complexes was confirmed from electronic spectra and TGA.

3.1 Elemental analysis

The analytical and physiochemical data of the complexes are summarized in Table 1. The experimental data were in very good agreement with the calculated ones. The complexes were colored, insoluble in water and commonly organic solvents while soluble in DMSO as well as stable in air. The structure of the complexes is assumed according to the chemical reaction as shown below;

$$M(NO_3)_2.\ 3H_2O + L + AN \longrightarrow M(L)(AN)(H_2O)OH] + 2\ HNO_3 + 3H_2O$$

(Where M = Cu(II), Ni(II), Co(II) and Mn(II) ;

3.2 FT-IR spectra

A comparison of the IR spectra of the complexes with those of the free ligands reveals interesting features relating to the metal-ligand interactions. IR spectroscopy has been used in order to confirm the deprotonation and binding mode of enrofloxacin. The bands at ~1732 cm^{-1} and ~1253 cm^{-1} attributed to the stretching vibrations $\nu(C=O)_{carboxylic}$ and $\nu\ (C\text{-}O)_{carboxylic}$ respectively, of the carboxylic moiety (–COOH) of enrofloxacin, have been shifted in the range ~1580-1600 cm^{-1} and ~1370-1385 cm^{-1} assigned as antisymmetric, $\nu(C=O)_{asym}$, and symmetric, $\nu(C=O)_{sym}$, stretching vibrations of the carboxylato group, respectively. The difference $\Delta = [\nu(C=O)_{asym} - \nu(C=O)_{sym}]$, a useful characteristic tool for determining the coordination mode of the carboxylato ligands, reaches a value of ~220–240 cm^{-1} indicative of a monodentate coordination mode [XVIII,XIX]. Whereas $\nu(C=O)p$ is shifted from ~1620–1645 cm^{-1} upon bonding. The overall changes of the IR spectrum suggest that enrofloxacin and ligands are coordinated to the copper via the ketone oxygen and carboxylato oxygen [XX]. These changes in the IR spectra suggest that enrofloxacin is coordinated to metal via pyridone and one carboxylate oxygen atoms [XXI]. These data are further supported by ν (Cu–O) which appear at ~515 cm^{-1} [XXII]. The IR spectra of the coumarin derivatives shows ~1612 and ~1745 cm^{-1} bands corresponding to α, β-unsaturated ketone and lactone carbonyl ketone respectively, on complexation these peaks shifted to a lower frequency ~1600 and ~1730 cm^{-1} due to complex formation.

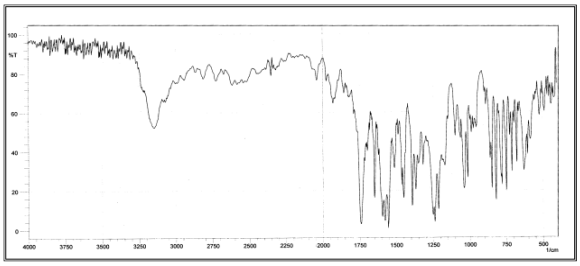


Fig. 1. FT-IR spectrum of L

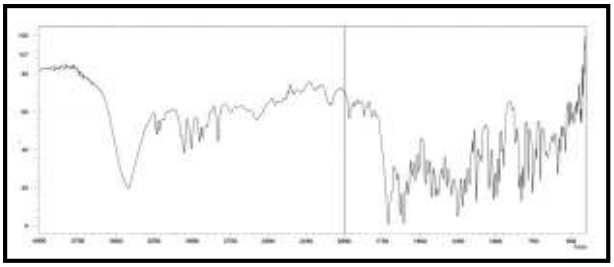


Fig.2. FT-IR spectrum of complex Co(II)

Table 2 FT-IR data of synthesized compounds

Comp.	ν (O–H)^{br} cm^{-1}	ν (COO)sy	ν (COO)asy	α, β-unsaturated ν (C=O) ^{s} cm^{-1}	lactone carbonyl ν (C=O) ^{s} cm^{-1}	ν (C=O) of pyridone	ν (M–O)^{w} cm^{-1}
C^1	3432	1375	1596	1603	1725	1620	512
C^2	3438	1374	1582	1612	1731	1622	531
C^3	3423	1378	1585	1608	1729	1632	523
C^4	3441	1373	1589	1604	1718	1626	534

^{s} = strong, ^{w} = weak, ^{br} = broad

3.3 Thermal studies of Cu(II) complexes

Thermal behaviour of the complexes was studied using TG whereas TG curves corresponding to the complex Cu(II) is characterized in Fig. 3. The thermal decomposition data for all complexes are given in Table 3. The thermal decomposition arise in four steps in air are observed. According to the mass losses, the following degradation pattern might be proposed for complex [Cu(L)(EN)(H_2O)OH] is represented in Scheme 3. All the compounds decompose with time respectively. Thermal decomposition started by dehydration process In the first step, weight loss occur at 80-270 °C corresponds to loss of one coordinated water and one hydroxyl molecule. The observed mass loss was 4.35% which was nearly equal to theoretical value 4.30%. Next two steps were related to removal of coordinated Enrofloxacin as well as ligand (coumarins) respectively. As temperature raise, the intermediate complexes [Cu(L)(EN)] (370-565 °C) and [Cu(CF)] (>650 °C) convert to CuO residue of fragments. The observed mass loss for third and fourth stage was 35.90% (calc. 35.95%) and 45.85% (calc. 45.97%) respectively. and remaining weight is in good agreement with copper oxide(CuO).

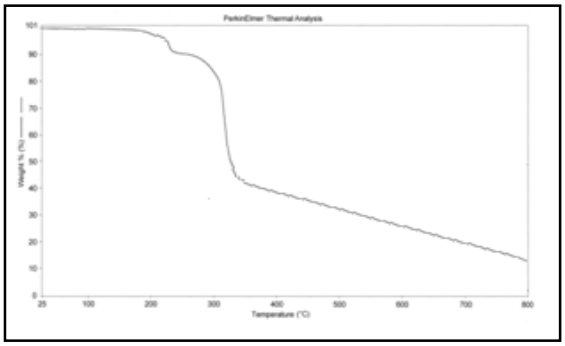


Fig.3. TGA of complex Cu(II)

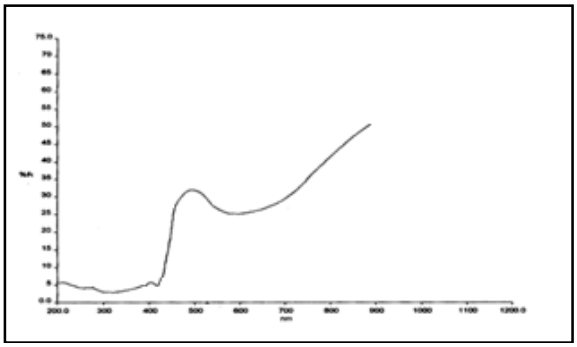
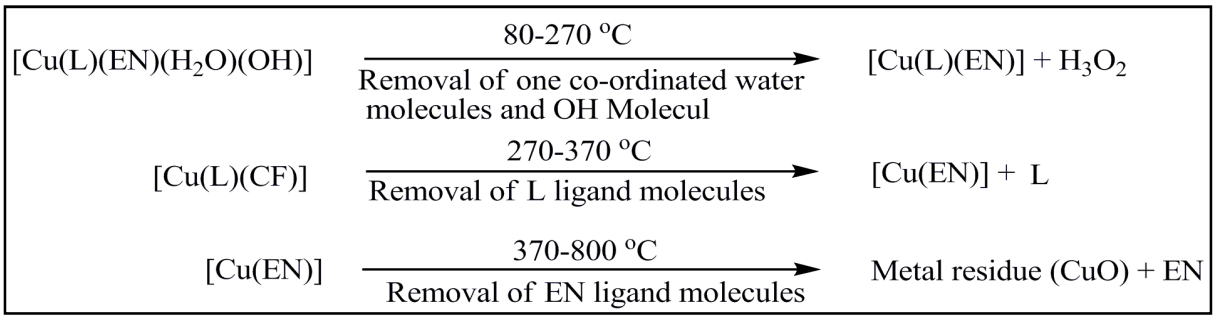


Fig.4. Electronics Spectrum of complex Cu(II)

Table 3 Thermoanalytical results (TG and DTG) of metal complexes

Complexes	TG range/ °C	DTG_{max}/ °C	Mass loss% obs.	Assignment
Cu(II)	80-370	246	4.30	Loss of one –OH & H_2O molecules
		356	35.95	Removal of L ligand
	370-800	562	45.97	Removal of Ciprofloxacin ligand
		>650	13.96	Leaving CuO residue
Ni(II)	70-380	222	4.25	Loss of one –OH & H_2O molecules
		305	35.50	Removal of L ligand
	380-800	546	45.35	Removal of Ciprofloxacin ligand
		>650	13.87	Leaving NiO residure
Co(II)	90-380	248	4.25	Loss of one –OH & H_2O molecules
		357	35.50	Removal of L ligand
	380-800	550	45.35	Removal of Ciprofloxacin ligand
		>650	13.82	Leaving CoO residure
Mn(II)	60-380	230	4.27	Loss of one –OH & H_2O molecules
		355	35.66	Removal of L ligand
	380-800	570	45.34	Removal of Ciprofloxacin ligand
		>650	13.78	Leaving MnO residure



scheme 3. Thermal fragmentation of Cu(II)

3.4 Electronic spectra

The Cu(II), Ni(II), Co(II), and Mn(II) complexes show magnetic moments of 1.82. 3.14, 3.86 and 5.93 B.M. respectively which is characteristic of mononuclear, Cu(II) (d^9, 1 unpaired electron) octahedral, Ni(II) (d^8, 2 unpaired electrons), Co(II) (d^7, 3 unpaired electrons), and Mn(II) (d^5, 5 unpaired electrons) complexes.[XXIII].

The electronic spectral data of the complexes in DMF are shown in Table 4. The Cu(II) complexes display three prominent bands. Low intensity broad band in the region 16,910-17,890 cm^{-1} was assigned as 10 Dq band corresponding to $^2E_g \rightarrow ^2T_{2g}$ transition [XXIV]. In addition, there was a high intensity band in the region 22,900-27,100 cm^{-1}. This band is due to symmetry forbidden ligand → metal charge transfer transition [XXV]. The band above 27,100 cm^{-1} was assigned as ligand band. Therefore distorted octahedral geometry around Cu(II) ion was suggested on the basis of electronic spectra [XXVI]. The electronic spectrum of the Ni(II) complex exhibits three bands at 10050, 14925 and 23529 cm^{-1}, attributable to $^3A_{2g}$ (F) → $^3T_{2g}$ (F) (ν1), $^3A_{2g}$(F) → $^3T_{1g}$(F) (ν2) and $^3A_{2g}$(F) → $^3T_{1g}$(P) (ν3) transitions, respectively, for an octahedral Ni(II) complex. The electronic spectrum of the Co(II) complex shows two bands at 15748, 19230 cm^{-1} which are assigned to $^4T_{1g}$ → $^4A_{2g}$(F) (ν2) and $^4T_{1g}$(F) → $^4T_{1g}$ (P) (ν3) transitions, respectively, as expected for an octahedral Co(II) complex[XXVII]. Mn^{+2} complexes show two bands in the region 18000-20000 cm^{-1} and a weak band in the region 23600-24350 cm^{-1} for octahedral geometry. (Fig. 4).

Table 4 Electronic spectral data of the complexes

Compounds	Transition band observed (cm^{-1})		μ_{eff} B.M	Geometry
Cu(II)	17,250	25,220	1.82	Octahedral
Ni(II)	10,150	14,925	3.14	Octahedral
Co(II)	15,755	19,300	3.86	Octahedral
Mn(II)	19,315	23,945	5.93	Octahedral

3.5 Antimicrobial bioassay

The ligand and its metal complexes were screened for their antibacterial and antifungal activities according to the respective literature protocol [XXVIII] and the results obtained are presented in Table 5. The results were compared with those of the standard drug. All the metal complexes were more potent bactericides and fungicides than the ligand. Co(II) and Mn(II) complexes have much less bacterial activity than the Cu(II) and Ni(II) complex while Mn(II) complex shows superior antifungal activity compare to other complexes. From Table 5, it can be seen that the highest antibacterial activity of Cu(II) complex against the bacterium *E. C* (3.125 µg/mL).On the other hand, Mn(II) complex showed the best activity towards fungi against *A. niger* (3.125 µg/mL). There was a marked increase in the bacterial and fungi activities of the Cu(II) and Mn(II) complexes respectively, as compared with the free ligand and other complexes under test, which is in agreement with the antifungal and antibacterial properties of a range of Cu(II) and Mn(II) complexes evaluated against several pathogenic fungi and bacteria.[XXIX] For many years it was believed that a trace of Cu(II) destroys the microbe, however, a more recent mechanism is that activated oxygen in the surface of metal Cu kills the microbe because Cu(II) activity is weak. This enhancement of metal complexes in the activity can be explained on the basis of chelation theory [XXX]. Chelation reduces the polarity of the metal atom mainly because of partial sharing of its positive charge with the donor groups and possible π electron delocalization within the whole chelate ring. Such a chelation also enhances the lipophililic character of the central metal atom, which subsequently favors its permeation through the lipid layers of cell membrane and the blocking of the metal binding sites on enzymes of microorganism. The variation in the effectiveness of different compound against different

organisms depends either on the impermeability of the cell of the microbes or differences in the ribosomes of microbial cells.

3.6 Anti-tubercular activity

The Metal(II) complexes were tested for antitubercular activity in order to check the impact of coumarin moiety to with compare activity of Ethambutol (Table 5). The anti-mycobacterial activities of all the synthesized compounds are assessed against *M. tuberculosis* H37RV at 3.125, 6.25, 12.5, 25, 50 and 100 µg/mL. The Minimum Inhibitory Concentrations of compounds compared with ethambutol as the standard anti-TB drugs and are summarized in Table 5. Lignad show inhibition at concentration 25 µg/mL. Ni(II) and Co(II) complexes also exhibit activity at same concentration while Cu(II) and Mn(II) complexes have shown enhancement in activity with MIC of 25µg/mL. None of the tested compounds have the inhibition more than standards.

3.7 Antioxidant studies

A capacity to transfer a single electron i.e. the antioxidant power of all compounds was determined by a FRAP assay. The FRAP value was expressed as an equivalent of standard antioxidant ascorbic acid (mmol/100 g of dried compound). FRAP values indicate that all the compounds have a ferric reducing antioxidant power. The compounds Cu(II) and Ni(II) showed relatively high antioxidant activity while compound Co(II) and Mn(II) shows poor antioxidant power (Table 5).

Table 5 Antimicrobial, Anti-tubercular and antioxidant results of compounds

Compounds	Minimal Inhibition Concentration [a] of microorganisms (µg/mL)						Antioxidant Activity [b]	Anti-tubercular activity [a]
	Bacteria				Fungi		FRAP value (mmol/100g)	
	S.P.	*B.S.*	*E.C.*	*P.A.*	*C. A.*	*A. N.*		
L[1]	50	100	100	100	50	100	NT	25
Cu(II)	3.25	6.125	3.125	3.25	6.25	12.5	420.1346	25
Ni(II)	6.25	12.5	12.5	25	25	12.5	435.7634	6.25
Co(II)	12.5	25	12.5	6.25	12.5	25	362.7241	12.5
Mn(II)	12.5	25	25	12.5	25	3.125	309.784	25
Streptomycin	0.025	0.025	0.020	0.020	NT	NT	NT	NT
Ethambutol	NT	NT	NT	NT	NT	NT	NT	3.25
Flucanazole	NT	NT	NT	NT	0.05	0.05	NT	NT
Ascorbic acid	NT	NT	NT	NT	NT	NT	500	NT

[a] Average value of triplicate results

[b] FRAP results expressed in mM of ascorbic acid per 100 g of sample i.e. mmol/100 g

NT = Not Tested

4. Conclusions

Here new heterochelates synthesised from biological active ligand (L) and ciprofloxacin are discussed. The structures of the ligand were investigated and confirmed by the elemental analysis, FT-IR, ^{1}H-NMR, ^{13}C-NMR and mass spectral studies. Octahedral geometry were established in all metal(II) complexes on the basis of electronic spedtra and TG analysis. All metal(II) complexes tested by in vitro antimicrobial, anti-tubercular and antioxidant activity

which shows fine results with an enhancement of activity on complexation with metal ions. This enhancement in the activity may be due to increased lipophilicity of the complexes. In review, the antimicrobial testing results reveal that complexes possess higher activity compared to parent ligand.

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