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Tharmal and Biological Estimate and Characterization of Cu(II) Complexes Based on Getifloxacin

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ABSTRACT:

Some newly heterochelates synthesized by reflux of different Coumarin derivative, Getifloxacin and transition metal. 1H, 13C, 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. The compounds were subjected to antimicrobial, antioxidant and anti-tubercular activity viewing using serial broth dilution method and Minimum Inhibitory Concentration (MIC) is determined.

Key words: Getifloxacin, biological facet, Coumarin derivative

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1. INTRODUCTION

Coumarin and its derivatives represent one of the most active classes of compounds possessing a wide spectrum of biological activity [1-4]. Many of these compounds have proved to be active as antitumor [5], antibacterial [6], antifungal[7], anticoagulant[8] and antiinflammatory[9]. In addition, these compounds are used as additives to food and cosmetics[10].dispersed Fluorescent and laser[11]. Various analogues of 3- Substituted coumarins suchas 3-amino coumarins exhibit antimicrobial activity[12-13]. From the above line of reasoning we directed this paper toward synthesis of various coumarin derivatives of biological interest using 3-amino coumarin a key starting material. Coumarins are both naturally occurring as well as synthetic derivatives, and are having widespread applications as HIV protease inhibitors, anticoagulant, spasmolytic and bacteriostatic agents.[14-15] However, the most widely reported activities for coumarin derivatives are their anti-inflammatory and anti-cancer sulfonamides, and 3-bromophenyl 6acetoxymethyl-2-oxo-2H-benzopyran-3carboxylate. Some of the medicinal compounds containing coumarin nucleus are Warfarin (vitamin K antagonists), Ensaculin (antidementia agent), Umbelliferone or 7-hydroxycoumarin (find use in sunscreen creams and lotions), Psoralen (for psoriasis, eczema and vitiligo). activities. Coumarin derivatives with anticancer activities include aromatase inhibitors, carbonic anhydrase inhibitors, and steroid sulfatase inhibitors.

Coumarin in itself possess much of broad range of biological activities namely anticoagulation, antibiotic, antifungal, antpsoriasis, cytotoxic, anti-HIV, anti-inflammatory. Especially 7- hydroxycoumarin has antioxidant properties and cytostatic, antibacterial, antiviral, xanthine oxidase inhibitor, antihyperglycemic, [16] casein kinase 2 inhibitor[17] activities,vasorelaxant[18], antitubercular [19]. Recently, coumarin derivatives have been evaluated in the treatment of human immunodeficiency virus, due to their ability to inhibit human immunodeficiency virus integrase.

Levofloxacin is a broad spectrum antibiotic of the fluoroquinolone drug class,[20] and the levo isomer of its predecessor ofloxacin. Its spectrum of activity includes most strains of bacterial pathogens responsible for respiratory, urinary tract, gastrointestinal, and abdominal infections, and atypical bacterial pathogens. Levofloxacin and other fluoroquinolones are valued for their broad spectrum of activity, excellent tissue penetration, and for their availability in both oral and intravenous formulations.[21] Levofloxacin is used alone or in combination with other antibacterial drugs to treat certain bacterial infections including pneumonia,[22] urinary tract infections,[7][8] and abdominal infections. Levofloxacin is used to treat infections including: respiratory tract infections, cellulitis, urinary tract infections, prostatitis, anthrax,[23, 24] endocarditis, meningitis, pelvic inflammatory disease, traveler's diarrhea, tuberculosis and plague. Levofloxacin plays an important role in professional medical society guidelines for the treatment of pneumonia, urinary tract infections, and abdominal infections. The Infectious Disease Society of America (IDSA).

The aim of this study was to prepare the mixed ligand complexes of Cu (II) using Livofloxacin with Dicoumarol derivatives and to determine their properties. In our previous reports, we have mentioned a series of fused coumarin derivatives and its transition metal complexes.[25] In continuation of our preceding work, we describe here synthesis, characterization and spectroscopic features of new mixed ligand Cu (II) complexes along with antimicrobial, anti-oxidant and anti-tubercular activities.

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 [26]. 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. The experiments were performed in N_2 atmosphere at a heating rate of 20 °C min^{-1} in the temperature range 30-800 °C.

2.3. Preparation of ligands

4-hydroxy-2H-chromen-2-one: 4-hydroxycoumarin was synthesized as reported method [27].

2.4. Synthesis of ligands (L1-L5)

General procedure for synthesis of the ligands (L) is shown in Scheme 1. The ligands were characterized using elemental analysis, FT-IR, Mass and NMR (^{1}H & ^{13}C) spectroscopy.

2.4.1 Synthesis of 6-chloro-3-(3-(3-chlorophenyl)acryloyl)-2H-chromen-2-one: (L^1)

Yield: 86%, m.p.: 164-165 °C. FT-IR (KBr, cm^{-1}): v(C=O, α, β-unsaturated ketone) 1627, v(C=O, lactone carbonyl of coumarin). ^{1}H NMR (DMSO-d^6 400 MHz) δ: 6.88 (1H, d, CH=CH- protons), 7.74-8.03 (7H, m, aromatic protons), 8.16 (1H, d, CH=CH- protons), 8.59 (1H, s, C$_4$-H). ^{13}C NMR (DMSO-d^6 100 MHz) δ: 118.1, 119.8, 124.8, 125.5, 126.2, 126.7, 128.4, 130.1, 130.8, 133.6, 134.0, 134.7, 136.2, 142.6, (14 different types of aromatic carbons), 147.3(C-4), 152.6(C-9), 158.7(C=O, lactone carbonyl of coumarin), 182.9(C=O, α, β-unsaturated ketone). MS (ESI) m/z 344.0 [M+H]$^+$, 394.0 [M+H]$^{+4}$; Elemental analysis found (%): C, 62.63; H, 2.92; Calculated for C$_{18}$H$_{10}$ClClO$_3$ (345.18): C, 62.49; H, 2.59.

2.4.2 Synthesis of 6-chloro-3-(3-(3-hydroxyphenyl)acryloyl)-2H-chromen-2-one: (L^2)

Yield: 72%, m.p.: 167-169 °C. FT-IR (KBr, cm^{-1}): v(C=O, α, β-unsaturated ketone) 1623, v(O-H) 3426, v(C=O, lactone carbonyl of coumarin) 1740. ^{1}H NMR (DMSO-d^6 400 MHz) δ: 6.77 (1H, d, CH=CH- protons), 6.92-8.14 (7H, m, aromatic protons), 8.22 (1H, d, CH=CH- protons), 8.56 (1H, s, C$_4$-H). 9.72 (1H, s, -OH). ^{13}C NMR (DMSO-d^6 100 MHz) δ: 115.5, 117.9, 118.6, 120.2, 121.4, 124.8, 125.6, 130.2, 130.8, 134.5, 134.9, 135.4, 142.9, (13 different types of aromatic carbons), 147.2(C-4), 152.2(C-9), 158.4(C-16, carbon attach to phenolic OH), 160.5(C=O, lactone carbonyl of coumarin), 183.2(C=O, α, β-unsaturated ketone). MS (ESI) m/z 326.03 [M+H]$^+$; Elemental analysis found (%): C, 66.67; H, 3.39; Calculated for C$_{18}$H$_{11}$ClO$_4$ (326.73): C, 66.24; H, 3.21.

2.4.3 Synthesis of 6-chloro-3-(3-(4-hydroxyphenyl)acryloyl)-2H-chromen-2-one (L^3)

Yield: 74%, m.p.: 158-160 °C. FT-IR (KBr, cm^{-1}): v(C=O, α, β-unsaturated ketone) 1617, v(C=O, lactone carbonyl of coumarin) 1734, v(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, C$_4$-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 326.03 [M+H]$^+$; Elemental analysis found (%): C, 58.09; H, 2.78; Calculated for C$_{18}$H$_{11}$ClO$_4$ (326.73): C, 58.24; H, 2.99.

2.4.4 Synthesis of 6-chloro-3-(3-(4-nitrophenyl)acryloyl)-2H-chromen-2-one: (L^4)

Yield: 75%, m.p.: 158-160 °C. FT-IR (KBr, cm^{-1}): v(C=O, α, β-unsaturated ketone) 1625, v(C=O, lactone carbonyl of coumarin) 1740. ^{1}H NMR (DMSO-d^6, 400 MHz) δ: 6.86 (1H, d, CH=CH- protons), 7.10-8.05 (7H, m, Ar-H), 8.26 (1H, d, CH=CH- protons); 8.57(1H, s, C$_4$-H). ^{13}C NMR (100 MHz, DMSO-d^6) δ: 114.2, 116.5, 118.7, 124.4, 125.9, 126.5, 127.7, 129.8, 130.9, 134.2, 146.4 (11 different types of aromatic carbons), 147.6 (C-4), 148.9(C-16, carbon attach to NO$_2$), 154.6(C-9), 159.8(C=O, lactone carbonyl of coumarin), 189.5(C=O, α, β-unsaturated ketone). MS (ESI) m/z 355.20 [M+H]$^+$; Elemental analysis found (%): C, 60.77; H, 2.83; N, 3.94; Calculated for C$_{18}$H$_{10}$ClNO$_5$ (455.73): C, 60.02; H, 2.52; N, 3.63

2.4.5 Synthesis of 6-chloro-3-(3-(3-methoxyphenyl)acryloyl)-2H-chromen-2-one: (L^5)

Yield: 76%, m.p.: 168-169 °C. FTIR (KBr, cm^{-1}): v(C=O, α, β-unsaturated ketone) 1621, v(C=O, lactone carbonyl of coumarin) 1743, (C-O-C, asymmetric) 1249, (C-O-C, symmetric) 1,044, (aromatic C=C & C-H Stretching) 1607, 3014. ^{1}H NMR (DMSO-d^6, 400 MHz,) δ: 6.83(1H, d, CH=CH- protons), 7.07-8.05 (7H, m, Ar-H), 8.25 (1H, d, CH=CH- protons); 3.79 (3H, s, -OCH$_3$); 8.58(1H, s, C$_4$-H). ^{13}C NMR (100 MHz, DMSO-d^6) δ: 55.7 (C-17, OCH$_3$), δ: 114.1, 116.3, 118.5, 124.9, 125.7, 126.9, 127.1, 129.5, 130.6, 134.8, 146.8 (11 different types of aromatic carbons), 147.8(C-4), 154.4(C-9), 159.2(C=O, lactone carbonyl of coumarin), 160.4(C-16), 189.5(C=O, α, β-unsaturated ketone). MS (ESI) m/z 340.05 [M+H]$^+$; Elemental analysis found (%): C, 60.67; H, 3.85; Calculated for C$_{19}$H$_{13}$ClO$_4$ (340.76): C, 60.24; H, 3.40.

Where R = m-Cl; m-OH; p-OH; p-NO₂ and m-OCH₃

Coumarin derivatives (L)

Scheme 1 General scheme for Ligand

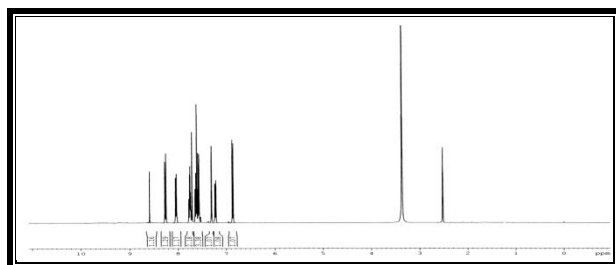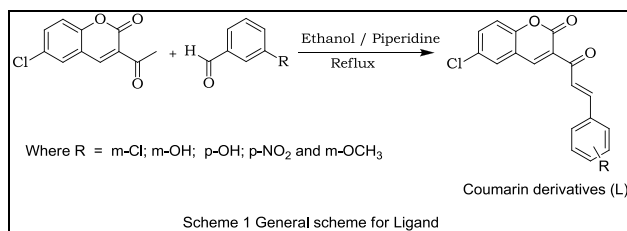Fig.1 ¹H-NMR spectrum of L4

Fig.2 IR spectrum of L4

Where R = m-Cl; m-OH; p-OH; p-NO₂ and m-OCH₃

Scheme 2 General scheme for Cu(II) complexes (C1-C5)
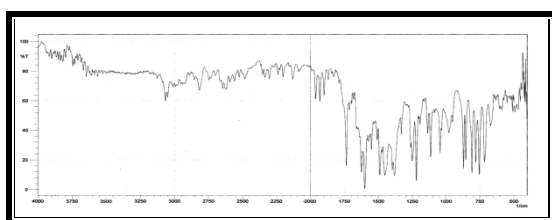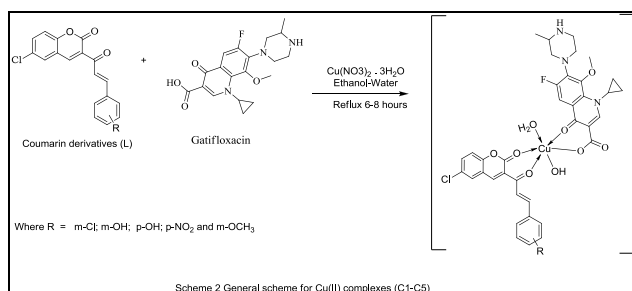
Scheme 2. General procedure for synthesis of complexe (C)

2.5 Synthesis of metal complexes

$[Cu(L)(LF)(H_2O)OH].2H_2O$ (C^1-C^5)

The Coumarin derivative (0.01 mol) was dissolved in water(25 ml) by gradually adding aqueous solution of $Cu(NO_3)_2.6H_2O$ (0.01 mol, 25 ml) and then was slowly added to an ethanolic solution of Getifloxacin (0.01 mol, 25 ml). The pH was adjusted to 4.5-6.0 with diluted NH_4OH solution. Furthermore, the mixture was heated under reflux for 5-8 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 amorphous powder was obtained by filtration and dried in air. The complexes comprise high melting points (above 300 °C) and insoluble in common organic solvents and partially soluble in DMSO. Complexes C1-C5 was prepared according to same method. The synthetic protocol of complexes is shown in scheme 2.

Table 1 Analytical and physical parameters of complexe

Comp.	Elemental analyses, % found (required)				M.p. (°C)	Yield (%)	Mol. Wt.	μ_eff (B. M.)
	C	H	N	Metal(II)				
C¹	47.29 (47.67)	3.41 (3.72)	5.13 (5.23)	5.82 (5.97)	>350	61	1092.12	1.82
C²	49.90 (49.99)	3.62 (3.79)	3.97 (4.17)	6.00 (6.19)	>350	70	1059.14	1.84
C³	48.57 (48.81)	3.33 (3.54)	3.82 (4.97)	6.12 (6.39)	>350	73	1043.23	1.87
C⁴	48.63 (48.89)	3.39 (3.64)	3.70 (4.02)	6.16 (6.45)	>350	75	1063.13	1.78
C⁵	47.29 (47.65)	3.41 (3.76)	5.13 (5.26)	5.82 (5.95)	>350	63	1092.12	1.84

2.6 Antimicrobial activity

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 and 6µg/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* anti-tubercular activity. The MICs were determined and interpreted for *M. tuberculosis* H37Rv according to the procedure of the approved micro dilution reference method of antimicrobial susceptibility testing [27]. Compounds were taken at concentrations of 100, 50, 25 and 12 µg/mL in DMSO, 1.0 ml of each concentration was used for the study. To this, 9.0 ml of Lowenstein-Jensen medium was added. A sweep from *M. tuberculosis* H37Rv strain culture was discharged with the help of nichrome wire loop with a 3 mm external diameter into a vial containing 4 ml of sterile distilled water. The vial was shaken for 5 min. Then using nichrome wire loop suspension was inoculated on the surface of each of Lowenstein-Jensen medium containing the test compounds. Further test media was incubated for four weeks at 37 °C. Readings were taken at the end of fourth week. The appearance of turbidity was considered as bacterial growth and indicates resistance to the compound. Test compounds were compared to reference drugs Isoniazid (MIC = 0.025µg/mL), Streptomycin (MIC = 6.25µg/mL) and Ethambutol (MIC = 20µg/mL). Lowenstein-Jensen medium containing standard drugs as well as DMSO was inoculated with *M. tuberculosis* H37Rv strain. The anti-tubercular activity tests were run in triplicate.

2.8 Antioxidant studies

Ferric reducing antioxidant power (FRAP) was determine using an adapted method [28]. The antioxidant potentials of the compounds were examine by their reducing power of the TPTZ-Fe(III) complex to TPTZ-Fe(II) complex for the total antioxidant capacity of tested samples, This method was employed because of its simple, fast and also results can be obtain was reproducible. Initially following solutions were prepared, A) acetate buffer, 300 mM pH 3.6 (3.1g sodium acetate trihydrate and 16 ml conc. acetic acid per L of buffer

solution), B) 10 mM 2,4,6-tripyridyl-s-triazine in 40 mM HCl, C) 20 mM $FeCl_3 \bullet 6H_2O$ in distilled water, D) 1mM of ascorbic acid dissolved in 100 mL distilled water. FRAP working solution was prepared by mixing the above (A), (B) and (C) solutions in the ratio of 10:1:1 respectively. A mixture of 40.0 µL, 0.5 mM sample solution and 1.2 mL FRAP reagent was incubated at 37 ℃ for 15 min. The working solution was necessary to use as freshly prepared. The ascorbic acid was used as a standard antioxidant compound and results were expressed with compared to ascorbic acid.

3. RESULT AND DISCUSSION

The synthesized Cu(II) complexes were characterized by elemental analysis, FTIR and mass spectra, The metal ion in their complexes were determined after mineralization. The metal content in chemical analysis was estimated by complexometrically[29], while geometry of the complexes was confirmed from electronic spectra, magnetic moment and thermal properties However, ligands and its complexes have been screened for their *in vitro* antitubercular and antimicrobial activities.

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;

$Cu(NO_3)_2 \bullet 3 H_2O + L + LF$ -----------> $[Cu(L)(LF)(H_2O)_2] \bullet x H_2O + 2HNO_3 + n H_2O$
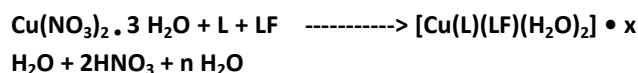
(Where L = L^1, L^2, L^3, L^4, L^5; x = 1, 0, 2, 0, 1 and n = 2, 3, 1, 3, 2)

3.2 FT-IR spectra

The coordination sites of ligand are elucidated using IR. The IR band assignments of dicoumarol derivatives and its complexes are included in Table 2. The IR data of free ligands and its metal complexes were carried out within the IR range 4000-400 cm^{-1}. The IR spectra of the dicoumarol derivatives show weak bands at ~3127-3054 cm^{-1} and ~1331-1337 cm^{-1}, corresponding to v(O–H) and v(C–OH) respectively. On complexation O–H peak has vanished, indicates deprotonation of O–H proton. The

v(C=O) of lactone rings observed at ~1647 and 1655 cm^{-1} in free ligand is shifted to lower frequencies (~12-14 cm^{-1} and 40-50 cm^{-1}) due to complex formation, and further supported by shifting of v(C–C), v(C–O), and v(C–O–C) stretch frequencies to higher values. Two bands at ~1613 and ~1565 cm^{-1} were assigned to stretching vibration of conjugate double bonding in the free ligand. The H–O–H bending mode occurring about ~1602 cm^{-1} has not been observed because of the presence of strong absorbing group like methine group (–CH=). It is difficult to resolve both these bands. A broad band at ~3424-3453 cm^{-1} observed in the complex was due to the v(O–H) characteristic peak of a coordinated water molecule. The bands at ~1732 cm^{-1} and ~1250 cm^{-1} attributed to the stretching vibrations v(C=O)$_{carboxylic}$ and v(C-O)$_{carboxylic}$ respectively, of the carboxylic moiety (–COOH) of livoofloxacin, have been shifted in the range ~1584-1603 cm^{-1} and ~1361-1382 cm^{-1} assigned as antisymmetric, v(C=O)$_{asym}$, and symmetric, v(C=O)$_{sym}$, stretching vibrations of the carboxylato group, respectively. The difference $\Delta=[v(C=O)_{asym}-v(C=O)_{sym}]$, a useful

characteristic tool for determining the coordination mode of the carboxylato ligands, reaches a value of ~204–222 cm^{-1} indicative of a monodentate coordination mode. Whereas v(C=O)p is shifted from ~1623–1644 cm^{-1} upon bonding. The overall changes of the IR spectrum suggest that livoofloxacin and ligands are coordinated to the copper via the ketone oxygen and carboxylato oxygen. These changes in the IR spectra suggest that livoofloxacin is coordinated to metal via pyridone and one carboxylate oxygen atoms. These data are further supported by v(Cu–O) which appear at ~519cm^{-1}.[30-32]
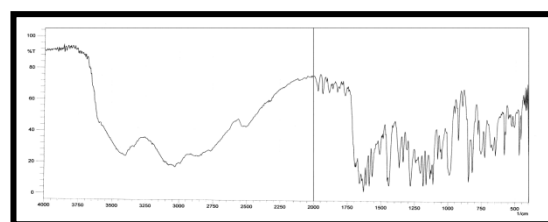

Fig.3. FT-IR spectrum of complex C4

Table 2 FT-IR data of synthesized compounds

Comp	v(O-H)^{br} cm^{-1}	v(C=O) cm^{-1}	v(C=C) cm^{-1}	v(C-C), v(C-O), v(C-O-C) cm^{-1}	v(COO)sy	v(COO)asy	v(C=O) of pyridone	v(Cu-O)^w cm^{-1}
C^1	3432	1657,1605	1587	1188,1136,1120,835,744	1366	1574	1631	510
C^2	3447	1642,1607	1594	1183,1148,1117,848745	1369	1586	1643	525
C^3	3428	1652,1611	1588	1194,1143,1131,847,755	1379	1570	1629	514
C^4	3425	1656,1608	1589	1185,1148,1127.841,749	1365	1580	1622	505
C^5	3419	1639.1013	1601	1197,1134,1135,839,752	1383	1585	1638	502

s = strong, **w** = weak, **br** = broad

3.3 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.[33].

The electronic spectral data of the complexes in DMF are shown in Table 3. The Cu(II) complexes display three prominent bands. Low intensity broad band in the region 16,930-17,880 cm^{-1} was assigned as 10 Dq band corresponding to ^{2}E$_g$→^{2}T$_{2g}$ transition [34]. In addition, there was a high intensity band in the region 22,910-27,200 cm^{-1}. This band is due to symmetry forbidden ligand → metal charge transfer transition [35]. The band above 27,200 cm^{-1} was assigned as ligand band.

Therefore distorted octahedral geometry around Cu(II) ion was suggested on the basis of electronic spectra [36]. (Fig. 4).
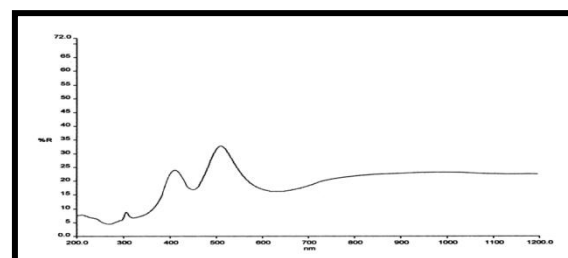

Fig.4. Electronics Spectrum of complex Cu(II)

3.4 Antioxidant studies

Antioxidant power was specifically the ability of transfer a single electron for compound and antioxidant capacity of complexes C^1-C^5 was determined by a FRAP method.

The FRAP results 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 C^1 and C^2 showed relatively high antioxidant activity while compound C^5 shows poor antioxidant power (Table 4). However, none of the compounds have been found to show excellent activity with compared to standard ascorbic acid.

Table 3 Electronic spectral data of the complexes

Compounds	Transition band observed (cm^{-1})			μ_{eff} B.M	Geometry
C^1	17,320	25,210	28,730	1.82	Octahedral
C^2	17,520	23,430	27,350	1.84	Octahedral
C^3	17,880	24,660	27,570	1.87	Octahedral
C^4	17,360	26,240	28,430	1.78	Octahedral
C^5	17,540	26,650	27,640	1.84	Octahedral

3.5 Anti-tubercular activity

The anti- tubercular activities of all the synthesized compounds were assessed against *M. tuberculosis* H37RV at 25, 50 and 100 µg/mL. The Minimum Inhibitory

Concentrations of compounds compared with Isoniazid, Streptomycin and Ethambutol, the standard drugs and are summarized in Table 4. Ligands show inhibition at concentration 100µg/mL. Complexes C^1 also exhibits activity at 50µg/mL concentration while C^2 and C^4 complexes have shown enhancement in activity with MIC of 25µg/mL. None of the tested compounds have the inhibition more than standards.

3.6 Antimicrobial bioassay

All the synthesized ligands and complexes were evaluated for their antibacterial and antifungal studies. The antibacterial and antifungal tests were carried out using the serial broth dilution method. The *in vitro* antimicrobial activities of the investigated compounds were screened against the bacterial species *SA*, *BS*, *EC*, *PA* and fungal species *CA*, *AN* and *AC*. The minimum inhibitory concentration (MIC) values of the compounds are summarized in Table 4.

A relative study for MIC values of the ligands and their complexes signify that complexes display higher antimicrobial activity than the free ligands. In present investigation, the antimicrobial activity of the ligands may be due to the heteroaromatic residues. Compounds

Table 4 Antimicrobial, Anti-tubercular and antioxidant results of compounds

Compounds	Minimal Inhibition Concentration [a] of microorganisms (µg/mL)						Antioxidant Activity [b] FRAP value (mmol/100g)	Anti-tubercular activity [a]
	Bacteria				Fungi			
	S.P.	*B.S.*	*E.C.*	*P.A.*	*C. A.*	*A. N.*		
L^1	100	100	50	100	50	100	NT	12.5
L^2	50	100	50	100	50	100	NT	25
L^3	100	100	50	100	50	100	NT	12.5
L^4	50	50	100	50	50	100	NT	25
L^5	100	50	50	100	50	100	NT	12.5
C^1	3.25	3.25	3.125	6.125	6.25	3.125	441.3457	6.25
C^2	6.25	25	12.5	12.5	12.5	25	310.3547	12.5
C^3	25	12.5	25	12.5	25	25	422.1435	12.5
C^4	6.25	25	12.5	25	12.5	12.5	311.3453	6.25
C^5	12.5	6.25	12.5	25	25	12.5	364.5262	12.5
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

containing C=N group have improved antimicrobial activity than C=C group. The growth of certain microorganisms takes place even in the absence of oxygen.The results were compared with those of the standard drug. All the metal complexes were more potent bactericides than the ligand. C1 complex was much less microbially active than the other complexes. From Table 4, it can be seen that the highest inhibition of growth occurred on C^1 complex against the microorganism, while C^2, C^4 and C^3 shows enhance activity than C^1 but less potent than C^5. There was a marked increase in the bacterial activities of the Cu(II) complex as compared with the free ligand under test, which is in agreement with antibacterial properties of a range of Cu(II) complexes evaluated against several pathogenic bacteria. The fungal strains used to demonstrate the antifungal potency of the synthesized compounds were *Candida albicans* (ATCC 66027) and *Aspergillus niger* (ATCC 64958). The results of inhibition are compared with standard antifungal drug Flucinozole (Table 4).

4. CONCLUSIONS

Here Newly the synthesised heterochelates from biological active Ligand (L) and livoofloxacin. 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 all Metal(II) complexes dispense on the basis of electronic. All Cu(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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