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Study on the Synthesis, Thermal Behavior and Biological Evaluation of DicoumarolNi Complexes Based on Enrofloxacin

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ABSTRACT

Synthesized a series of newNi complexes by usingEnrofloxacinanddicoumarol derivatives.Physico-chemical, spectroscopic and thermal properties of the complexes have been studied on the basis of infrared spectra, mass spectra, NMR spectra, electronic spectra, elemental analyses.All the compounds were screened for their antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis* and antifungal activity against *Candida albicans*,*Aspergillusclavatus*and *Aspergillusniger*. Ferric-reducing antioxidant power (FRAP) of all complexes were measured.Also the compounds against Mycobacterium tuberculosis shows clear enhancement in the antitubercular activity uponNickel complex.

Introduction

Dicoumarol is a natural chemical substance of combined plant and fungal origin. It is a derivative of coumarin, a bitter substance made by plants that does not itself affect coagulation, but which is (classically) transformed in mouldy feeds or silages by a number of species of fungi, into active dicoumarol. Dicoumarol does affect coagulation, and was discovered in mouldy wet sweet-clover hay, as the cause of a naturally occurring bleeding disease in cattle.

Like all 4-hydroxycoumarin drugs it is a competitive inhibitor of vitamin K epoxide reductase, an enzyme that recycles vitamin K, thus causing depletion of active vitamin K in blood (1). This prevents the formation of the active form of prothrombin and several other coagulant enzymes (2). These compounds are not direct antagonists (in the pharmaceutical sense) of vitamin K itself, but rather act to deplete reduced vitamin K in tissues. For this reason, vitamin K antagonizes their effect (rather than the reverse), and this has led to the loose terminology of vitamin K antagonism (3). Administration of vitamin K is therefore the antidote for dicoumarol toxicity. The action and toxicity of the drug and the antidote effectiveness are measured with the prothrombin time (PT) blood test.

Enrofloxacin is a fluoroquinolone antibiotic sold by the Bayer Corporation under the trade name Baytril. Enrofloxacin is currently approved by the FDA for the treatment of individual pets and domestic animals in the United States. In September 2005, the FDA withdrew approval of Baytril for use in water to treat flocks of poultry, as this practice was noted to promote the evolution of fluoroquinolone-resistant strains of the bacterium Campylobacter, a human pathogen [4].

It is a bactericidal agent. The bactericidal activity of Enrofloxacin is concentration-dependent, with susceptible bacteria cell death occurring within 20–30 minutes of exposure. Enrofloxacin has demonstrated a significant post-antibiotic effect for both Gram-negative and Gram-positive bacteria and is active in both stationary and growth phases of bacterial replication.

The aim of this study was to prepare the mixed ligand complexes of Ni using Enrofloxacin with coumarin derivatives and to determine their properties. In our previous reports, we have mentioned a series of fused coumarin derivatives and its transition metal complexes.[8-9] In continuation of our preceding work, we describe here synthesis, characterization and spectroscopic features of new mixed ligand Ni complexes of Enrofloxacin with dicoumarol derivatives along with antimicrobial, anti-oxidant and anti-tubercular activities. Thermal behavior of the complexes has been investigated by using thermogravimetric(TG) analysis.

Experimental

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 [10].

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

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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.

Preparation of ligands

6-chloro-4-hydroxy-2H-chromen-2-one was synthesized as reported method[11].

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 (^1H &^{13}C) spectroscopy.

3,3'-((4-chlorophenyl)methylene)bis(6-chloro-4-hydroxy-2H-chromen-2-one) (L1)

The 6-chloro-4-hydroxy-2H-chromen-2-one (0.1296 g, 0.04 mole) was dissolved in 20 ml of ethanol and heated in water bath for 4–5 h, to obtain a clear solution. Ethanolic solution of (20 ml) 4-chlorobenzaldehyde (0.0424 g, 0.02 mole) was added to hot solution and refluxed in presence of Piperidine. The fine crystals obtained were separated out and were recrystallized from ethanol. Yield: 72%, m.p.: 211 °C. FT-IR (KBr, cm^{-1}): ν(-OH/H_2O) 3136, 3052, ν(C=O) 1665,1654 ν(C=C) 1623, 1578, ν(C-O) 1151, 1126, 1091, 815, 795, 777. ^1H NMR (DMSO-d6 400 MHz) δ: 6.36 (1H, Aliphatic), 6.97-7.75 (12H, m, Aromatic proton), 9.39, 10.37 (-OH phenolic); ^{13}C NMR (DMSO-d6 100 MHz): δ: 36.7 (C-9), 101.5 (C-3, 18), 113.4, 114.7, 116.3, 116.9, 120.5, 123.2 125.7, 128.5, 130.5, 142.3 (10C, Ar-C), 152.3(C-8a, 23a), 157.5(C-12, carbon attach to phenolic OH) 161.4(C-2, 17), 164.5(C-4, 19); ESI-MS (m/z): 428.09. Elemental analysis found (%): C, 70.09; H, 3.76; Calculated for C25H16O7 (428.09): C, 69.93; H, 3.62.

***3,3'-((4-hydroxyphenyl)methylene)bis(6-chloro-4-hydroxy-2H-chromen-2-one)* (L2)**

L2 was synthesized same as L1 by using 4-hydroxybenzaldehyde in place of 4-chlorobenzaldehyde. Yield: 65 %, m.p.: 223 °C. FT-IR (KBr, cm^{-1}): *ν*(-OH/H2O) 3172, 3046, *ν*(C-OH) 1343, 1315, *ν*(C=O)1661, 1653, *ν*(C=C)1625, 1572, ν(C-O)1162, 1127, 1083, 812, 786, 743. ^1H NMR (DMSO-d^6 400 MHz) δ: 6.39 (1H, Aliphatic), 7.09-7.86 (12H, m, Aromatic proton), 9.71, 10.78 (-OH phenolic); ^{13}C NMR (DMSO-d^6 100 MHz): δ: 35.9 (C-9), 101.2 (C-3, 1), 115.3, 116.6, 117.2 123.5, 125.5, 128.9, 130.3 137.1(8C, Ar-C), 152.7(C-8a, 23a), 156.4(C-13, carbon attach to phenolic OH) 163.0(C-2, 17), 165.7(C-4, 19); ESI-MS (*m/z*): 428.09. Elemental analysis found (%): C, 70.09; H, 3.76; Calculated for $C_{25}H_{16}O_7$: (428.09): C, 69.87; H, 3.59.

***3,3'-((4-nitrophenyl)methylene)bis(6-chloro-4-hydroxy-2H-chromen-2-one)* (L3)**

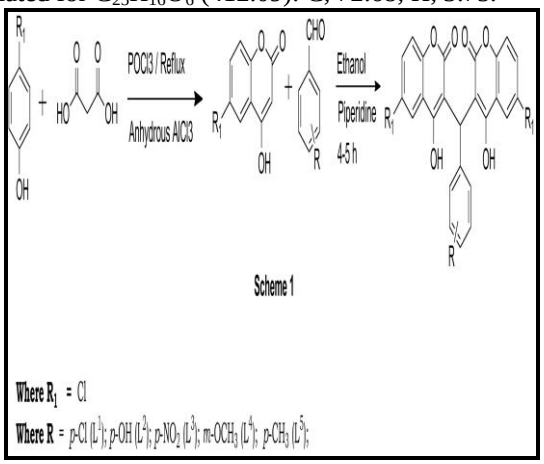
L3 was synthesized same as L1 by using 4-nitrobenzaldehyde in place of 4-hydroxy benzaldehyde. Yield: 71%, m.p.: 263 °C. FT-IR (KBr, cm^{-1}): *ν*(-OH/H2O) 3194, 3054, *ν*(C=O) 1665,1653, *ν*(C=C) 1646, 1559, *ν*(C-O) 1204, 1122, 1085, 818, 783, 748. ^1H NMR (DMSO-d^6 400 MHz) δ: 6.45 (1H, Aliphatic), 7.18-8.79 (12H, m, Aromatic proton), 10.42 (-OH phenolic); ^{13}C NMR (DMSO-d^6 100 MHz): δ: 36.2 (C-9), 102.1 (C-3, 18), 116.2, 116.9, 123.4, 125.4, 125.9, 125.9, 128.3, 128.9, 131.2, 134.4, 144.8 (11C, Ar-C), 151.6(C-8a, 23a), 163.4(C-2, 17), 165.2(C-4, 19); ESI-MS (*m/z*): 446.06, 448.05(M +H)$^+$.Elemental analysis found (%): C, 67.20; H, 3.38; Calculated for $C_{25}H_{15}ClO_6$ (446.06): C, 67.07; H, 3.27.

***3,3'-((3-methoxyphenyl)methylene)bis(6-chloro-4-hydroxy-2H-chromen-2-one)* (L4)**

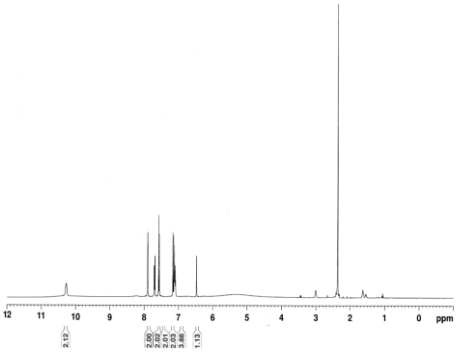
L4 was synthesized same as L1 by using 3-methoxybenzaldehyde in place of 4-nitrobenzaldehyde. Yield: 69 %, m.p.: 263 °C. FT-IR (KBr, cm^{-1}): *ν*(-OH/H2O) 3192, 3053, *ν*(C-OH) 1345, 1330, *ν*(C=O) 1664, 1652, *ν*(C=C) 1627, 1575, *ν*(C-O) 1165, 1125, 1087, 815, 785, 713. ^1H NMR (DMSO-d^6 400 MHz) δ: 6.48 (1H, Aliphatic), 7.10-8.84 (12H, m, Aromatic proton), 10.26 (-OH phenolic). ^{13}C NMR (DMSO-d^6 100 MHz): δ: 36.6 (C-9), 100.7 (C-3, 18), 115.8, 117.1, 124.4, 126.7 128.3, 128.5 130.7, 132.1, 142.6 (9C, Ar-C), 151.7(C-8a, 23a), 162.9(C-2, 17), 168.9(C-4, 19); ESI-MS (*m/z*): 446.06, 448.05(M+H)$^+$. Elemental analysis found (%): C, 67.08; H, 3.24; Calculated for $C_{25}H_{15}ClO_6$ (446.06): C, 67.20; H, 3.38.

***3,3'-(p-tolylmethylene)bis(6-chloro-4-hydroxy-2H-chromen-2-one)* (L5)**

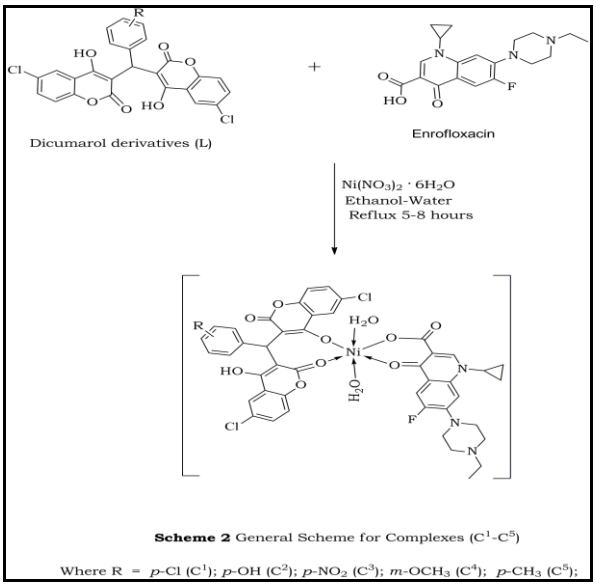
L5 was synthesized same as L1 by using 4-methylbenzaldehyde in place of 3-methoxybenzaldehyde. Yield: 65 %, m.p. 227 °C. FT-IR (KBr, cm^{-1}): *ν*(-OH/H2O) 3181, 3052, *ν*(C=O) 1660,1651, *ν*(C=C) 1645, 1563, *ν*(C-O) 1176, 1121, 1085, 820, 792, 748. ^1H NMR (DMSO-d^6 400 MHz) δ: 6.51 (1H, Aliphatic), 7.11-7.93 (13H, m, Aromatic proton), 10.35 (-OH phenolic); ^{13}C NMR (DMSO-d^6 100 MHz): δ: 36.4 (C-9), 103.4 (C-3, 18), 116.2, 117.2, 123.3 125.2, 125.9, 127.72, 128.2, 128.9, 143.8(9C, Ar-C), 152.5(C-8a, 23a), 164.7(C-2, 17), 167.5(C-4, 19); ESI-MS (*m/z*): 412.09. Elemental analysis found (%): C, 72.68; H, 3.91; Calculated for $C_{25}H_{16}O_6$ (412.09): C, 72.68; H, 3.75.



Scheme 1. Procedure for synthesis of ligands (L)



^1H-NMR spectrum of L4



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

Synthesis of metal complexes

[Ni(L)(EN)(H_2O)OH].2H_2O (C^1-C^5)

The Dicoumarol derivative (0.01 mol) was dissolved in water (25 ml) by gradually adding aqueous solution of Ni(NO3)2.6H2O(0.01 mol, 25 ml) and then was slowly added to an ethanolic solution of Enrofloxacin (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.

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.

Anti-tubercular activity

Test compounds were evaluated for *in vitro* anti-tubercular activity.

Table 1. Analytical and physical parameters of complex

Comp.	Elemental analyses, % found (required)				M.p. (°C)	Yield (%)	Mol.Wt.	μ_{eff} (B.M.)
	C	H	N	Metal(II)				
C^1	55.33 (55.47)	4.31 (4.44)	4.61 (4.79)	6.97 (7.12)	>350	74	911.77	1.85
C^2	55.35 (55.46)	4.29 (4.41)	4.58 (4.77)	6.95 (7.10)	>350	71	911.77	1.81
C^3	55.37 (55.49)	4.65 (4.81)	4.62 (4.78)	6.96 (7.11)	>350	69	910.19	1.88
C^4	57.63 (57.79)	4.38 (4.51)	4.80 (4.92)	7.27 (7.42)	>350	73	875.31	1.79
C^5	58.70 (58.83)	4.46 (4.58)	4.89 (4.94)	7.40 (7.56)	>350	79	959.31	1.86

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 [12]. 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.

Antioxidant studies

Ferric reducing antioxidant power (FRAP) was determine using an adapted method[13]. 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 mMHCl, C) 20 mM $FeCl_3$•$6H_2O$ 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 °C 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.

Result and Discussion

The synthesized Ni 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[14], 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 andantimicrobial activities.

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.

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 ~3125-3050 cm^{-1} and ~1320-330 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 1650 cm^{-1} in free ligand is shifted to lower frequencies (~11-14 cm^{-1} and 45-50 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 [15-17].Two bands at ~1619 and ~1563 cm^{-1} were assigned to stretching vibration of conjugate double bonding in the free ligand.The H–O–H bending mode occurring about ~1600 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 ~3425-3450 cm^{-1} observed in the complex was due to the *v*(O–H) characteristic peak of a coordinated water molecule. Spectra of the mixed-ligand Nicomplexes reveals that a broad band in the region ~3420-3460 cm^{-1} is due to stretching vibration of OH group. The *v*(C=O) stretching vibration band appears at ~1705 cm^{-1} in the spectra of Enrofloxacin, and the complexes show this band at ~1625 cm^{-1}; this band shifted towards lower energy, suggesting that coordination occurs through the pyridone oxygen atom. The strong absorption bands obtained at ~1623 and ~1385 cm^{-1} in Enrofloxacin are observed at ~1572-1581 and ~1343-1372 cm^{-1} for $\nu(COO)_a$ and $\nu(COO)_s$ in the complexes, respectively; in the present case the separation frequency $\Delta\nu$> 200 cm^{-1} ($\Delta\nu = \nu COO_a - \nu COO_s$) suggesting unidentate binding of the carboxylato group. In all the complexes, a new band is seen in the ~ 430-455 cm^{-1} range can be attributed to *v*(Cu-O).

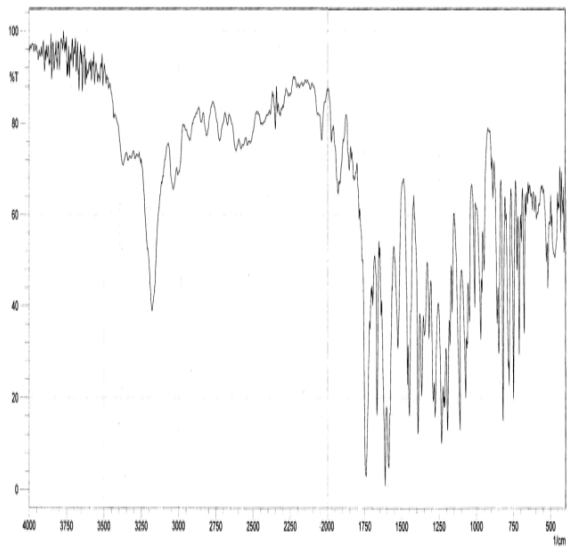


Fig 1. FT-IR spectrum of L4

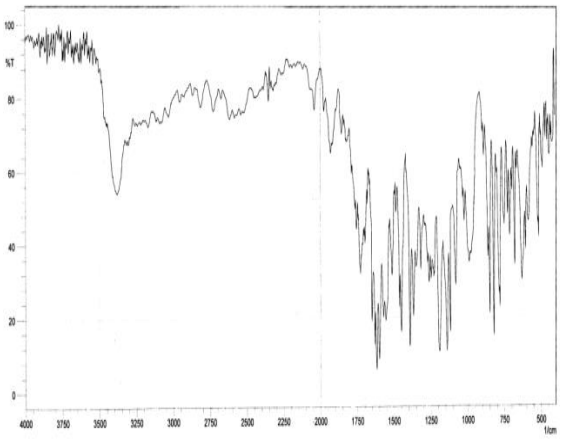


Fig 2. FT-IR spectrum of complex C4

Electronic spectra

In the electronic spectra of the complexes, the wide range bands were observed due to either the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ of C=N chromophore or charge transfer transition arising from π electron interactions between the metal and ligand, which involves either a metal to ligand or ligand to metal electron transfer [18]. Moreover, the electronic spectra of hexa coordinate Ni complexes were either D_{4h} or C_{4v} symmetry, the E_g and T_{2g} level of ^2D free ion term will split into B_{1g}, A_{1g}, B_{2g} and E_g levels, respectively under the influence of the distortion, which cause the two transitions such as $^2B_{1g} \rightarrow {}^2B_{2g}$ and $^2B_{1g} \rightarrow {}^2A_{1g}$. This promotes the distorted octahedral Ni complex which was usual in the d^9 system [19]. The electronic spectra of Ni complexes display three prominent bands. Low intensity broad band in the region 16,900-17,900 cm^{-1} was assigned as 10 Dq band corresponding to $^2E_g \rightarrow {}^2T_{2g}$ transition [20]. 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 [21]. The band above 27,100 cm^{-1} was assigned as ligand band. Therefore distorted octahedral geometry around Ni ion was suggested on the basis of electronic spectra [22], which was further discovered by its magnetic moment of 1.79-1.87 B.M. falls within the range generally observed for octahedral Ni complexes [23]. Therefore the electronic spectral data and magnetic moment data support the octahedral geometry of the all complexes.

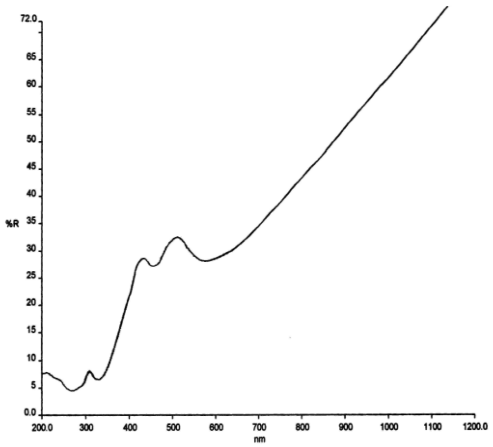


Fig 4. Electronics Spectrum of complex Ni

Table 2. FT-IR data of synthesized compounds

Comp	υ(O-H)^{br} cm^{-1}	υ(C=O) cm^{-1}	υ(C=C) cm^{-1}	υ(C-C), υ(C-O),υ(C-O-C)cm^{-1}	υ(COO)sy	υ(COO)asy	υ(C=O) of pyridone	υ(Cu-O)^{w} cm^{-1}
C^1	3437	1642,1611	1598	1191.1141,1138,856,754	1379	1588	1637	501
C^2	3433	1655,1609	1592	1182,1142,1124,855,725	1360	1575	1620	527
C^3	3427	1635,1604	1590	1182,1146,1139.840786	1374	1582	1634	511
C^4	3425	1656,1608	1589	1185,1148,1127.841,749	1365	1580	1622	505
C^5	3434	1645,1607	1584	1193,1153,1135,836,757	1378	1580	1632	503

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

Table 3. Electronic spectral data of the complexes

Compounds	Transition band observed (cm^{-1})			μ_{eff}B.M	Geometry
C^1	17,240	25,200	28,720	1.84	Octahedral
C^2	17,560	23,450	27,340	1.83	Octahedral
C^3	17,890	24,650	27,560	1.81	Octahedral
C^4	17,340	26,230	28,420	1.78	Octahedral
C^5	17,530	26,670	27,650	1.79	Octahedral

Antimicrobial studies

The antibacterial activity of synthesized compounds was tested against skin disease causing bacteria like *Streptococcus pyogenes*(ATCC12384), *Bacillus subtilis* (ATCC11774), *Escherichia coli* (ATCC25922), and *Pseudomonas aeruginosa* (ATCC25619). The ligand and its metal complexes were screened for their antibacterial activities according to the respective literature protocol[24] and the results obtained are presented in Tables 4. The results were compared with those of the standard drug. All the metal complexes were more potent bactericides than the ligand. C^1 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^3 complex against the microorganism, while C^2, C^4 and C^5 shows enhance activity than C^1 but less potent than C^3. There was a marked increase in the bacterial activities of the Ni complex as compared with the free ligand under test, which is in agreement with antibacterial properties of a range of Ni complexes evaluated against several pathogenic bacteria[25]. The fungal strains used to demonstrate the antifungal potency of the synthesized compounds were *Candida albicans* (ATCC 66027) and *Aspergillusniger* (ATCC 64958). The results of inhibition are compared with standard antifungal drug Flucinozole (Table 4). The complexes exhibit stronger activity with lower MIC value as compared to free ligand except C^1 complex whose activity is nearly same as that of the ligand. Among all the complexes, C^3 complex is found to be highly active against *A.n.* with a MIC of 3.125µg/mL. Thus activity of ligand has enhanced on complexation. However, activity exhibited by the ligand as well as the corresponding complexes is less compared to the standard antifungal drug used in the study.

Antituberculosis studies

The encouraging results from the antibacterial studies prompted us to go for preliminary screening of complexes for their in vitro antituberculosis activity. The resulted antituberculosis activity was expressed as minimal inhibition concentration (MIC). Compounds were assayed for their inhibitory activity toward *M. tuberculosis* H37Rv (MTCC200). The minimum inhibitory concentration as well as % inhibition of growth was determined for all compounds including standard drugs Table 4. Isoniazid, Rifampicin and Ethambutol were used as standard drugs for comparison purpose. From reviewing the activity data of ligands $\mathbf{L^2}$ and $\mathbf{L^4}$ were shown good activity, while $\mathbf{L^1}$, $\mathbf{L^3}$ and $\mathbf{L^5}$shows moderate activity. In conclusion, all complexes shows clear enhancement in the antitubercular activity then its free ligands. The most effective compound $\mathbf{C^3}$ and $\mathbf{C^4}$was significantly enhanced at MIC (30 µg mL^{-1}), which effected 86% inhibition of growth.

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 C^1, C^3 and C^4 showed relatively high antioxidant activity while compound C^2 and C^3 shows poor antioxidant power (Table 4).

In conclusion, the antimicrobial testing results reveal that complexes possess higher activity at lower concentration compared to parent ligand. It is known that chelation tends to make the Schiff bases more powerful and potent bacteriostatic agents[26],

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	50	100	100	100	50	100	NT	25
L^2	100	50	50	100	50	100	NT	12.5
L^3	50	50	100	50	50	100	NT	25
L^4	100	100	50	100	50	100	NT	12.5
L^5	50	100	50	100	50	100	NT	25
C^1	25	25	12.5	12.5	25	25	422.1435	12.5
C^2	6.25	12.5	12.5	25	25	12.5	364.5262	12.5
C^3	3.25	3.25	3.125	6.125	6.25	3.125	441.3457	6.25
C^4	6.25	25	12.5	12.5	25	12.5	311.3453	6.25
C^5	6.25	25	12.5	12.5	12.5	25	310.3547	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

[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

Conclusions

Here Newly the synthesised heterochelatesfrom biological active Ligand (L) and Enrofloxacin. 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 allMetal(II) complexes dispenseon the basis of electronic. All Metal(II) complexes tested by *In vitro* antimicrobial, anti-tubercular and antioxidant activity which showsfineresults 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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