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RESEARCH ARTICLE

Preparation, Structures and Antimicrobial Activity of Ni(II) Complexes on the Base of Enrofloxacin with Dicumarol Derivative

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Abstract

Objective: To synthesize Ni(II) complexes on the base of enrofloxacin with dicumarol derivative and to investigate their antioxidant and antimicrobial potentials. **Methods:** Ni(II) complexes have been synthesized by classical thermal methods by mixing aqueous metal solutions with ethanol ligands and enrofloxacin in a 1:1 molar ratio. A biologically strong ligand was prepared by refluxing a dicoumarol derivative in aldehyde and ethanol. The structures of the ligands and their nickel complexes were investigated and confirmed by elemental analysis, FT-IR, ^{1}H-NMR, ^{13}C-NMR and mass spectroscopy. The thermal behavior of newly synthesized mixed-ligand Ni(II) complexes was studied by thermogravimetric analysis. Ferric-reducing antioxidant power (FRAP) (antioxidant activity) of all complexes were measured. All the compounds were screened for their antimicrobial activity against *E. coli; P. aeruginosa; S. pyogenes; C. albicans and A. niger* using Luria Broth dilution method for MIC values. Both the ligand and its complex were screened for antioxidant and antimicrobial activity *in vitro*. **Findings:** Antioxidant and antimicrobial activities of complexes were found to be better than the parent ligands used for complex formation. MIC values for complex C^5 (-CH_3 derivative) were found to be between 40-70 μg/mL and FRAP values was 82.44 mmol/100g, indicative of its ferric reducing antioxidant power. These result show that complex C^5 (-CH_3 derivative) was found more potential as antimicrobial and antioxidant agent. **Novelty :** The coordination of metal complex with enrofloxacin (antibiotic) draws a scheme for the synthesis of new drug as well as increasing the activity of enrofloxacin that is currently in use. Current work supports that the activity of antibiotics could be increased by coordination of metal ion complex. There could be a possibility that these complexes allow to reduce the dose of drug introduced into the body.

Keywords: Dicumarol; Antimicrobial; Antioxidant; Thermal Analysis; TGA

1 Introduction

Coumarin (2H-1-Benzopyran-2-one) is an important class of natural compounds that exhibit anti-tumor, anti-oxidant, anti-inflammatory, antimicrobial, and antidiabetic activities [1,2] . Coumarins and their derivative dicumarol are effective drugs for the treatment of microbial, neuronal diseases, and AIDS [3] . Dicumarol is a natural chemical bitter substance made by plants. It has anticoagulant properties. As in the human body, several metals like Cu, Zn, Ni, Co, Fe, etc play an important role in the proper functioning of cells and their slight disruption leads to serious diseases like Alzheimer's, Wilsom's, Perkinson's, etc. [4,5].

Enrofloxacin (EN) is an antibiotic that is very effective in the treatment of bacterial infections [6,7]. It can be taken by the oral or by injection into a vein; it is effective for pneumonia, cellulitis, urinary tract infections, prostatitis, plague, and other types of infectious diarrhea. It is also effective for medication for multidrug-resistant tuberculosis. It can be used in an eye drop for a superficial bacterial infection of the eye and an ear drop may be for otitis media when there is a hole in the eardrum [8].

In the present work, we synthesized derivatives (-OH, -Cl, -NO_2,-CH_3, -H) of the dicumarol Ni metal complex with the substitute of the Enrofloxacin drug (Figures 1 and 2). Characterization of complexes was done by elemental analysis, FT-IR spectra, Electronic spectra, Magnetic moment, and Thermal analysis (Figures 3, 4, 5, 6, 7, 9 and 10, Tables 1 and 2). Antimicrobial and antioxidant activities were studied. Comparative studies were performed with pure ligand and their complex (Table 3). Antioxidant activity studies were performed on microorganisms like *E. coli, P. aeruginosa; S. pyogenes, B. subtilis, C. albicans* and *A. Niger*. Norfloxacin, Flucanazole, and Nystatin were used as standards.

2 Methodology

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 before use [9].

2.2 General procedure for the preparation of Dicumarol derivatives (L)

All reactions were monitored by thin-layer chromatography (TLC on silica gel 60 F254, 0.25 mm thickness, E. Merck, Mumbai, India on coated aluminum plates), and component detection was measured under UV light or, examined in an iodine chamber. Carbon, hydrogen, and nitrogen were estimated on an elemental analyzer Perkin Elmer, USA 2400-II CHN analyzer. Metal ion analysis was performed by dissolving the solid complexes in hot concentrated nitric acid, further diluting with distilled water, and filtering to remove precipitated organic ligands. The remaining solution was neutralized with ammonia solution and metal ions were titrated against EDTA. ^{1}H and ^{13}C NMR measurements were performed on an Advance-II 400 Bruker NMR spectrometer, SAIF, Chandigarh. Chemical shifts were determined using TMS as the internal standard and DMSO-d^6 as solvent. Infrared spectra of solids were recorded in the range 4000-400 cm^{-1} on a Nicolet Impact 400D Fourier transform infrared spectrophotometer using KBr pellets. The melting points of ligands and metal complexes were determined by the open capillary tube method. Solid-state susceptibility measurements were performed at room temperature using a Gui susceptibility balance using mercury tetrathiocyanatocobaltate (II) as a reference standard (g = 16.44 x 10^{-6} c.g.s. units).

2.3 Physical measurements

2.3.1 3,3'-((3-hydroxyphenyl) methylene)bis(6-bromo-4-hydroxy-2H-chromen-2-one): (L^1)

Yield: 70%, m.p.: 217 °C. FT-IR (KBr, cm^{-1}): ν(-OH/H_2O) 3136, 3054, ν(C=O) 1664,1657 ν(C=C) 1625, 1576, ν(C-O) 1155, 1126, 1092, 814, 797, 774. ^{1}H NMR (DMSO-d6 400 MHz) δ: 6.35 (1H, Aliphatic), 6.97-7.74 (12H, m, Aromatic proton), 9.37, 10.34 (-OH phenolic); ^{13}C NMR (DMSO-d^6 100 MHz): δ: 36.5 (C-9), 101.3 (C-3, 18), 113.2, 114.5, 116.3, 116.8, 120.3, 123.4 125.6, 128.8, 130.4, 142.4 (10C, Ar-C), 152.3(C-8a, 23a), 157.8(C-12, carbon attach to phenolic OH) 161.6(C-2, 17), 164.5(C-4, 19); ESI-MS (m/z): 471.01(M +H)$^+$. Elemental analysis found (%): C, 60.09; H, 2.76; Calculated for $C_{25}H_{16}Br_2O_7$ (470.28): C, 60.38; H, 2.82.

2.3.2 3,3'-((3-chlorophenyl methylene bis(6-bromo-4-hydroxy-2H-chromen-2-one : (L^2)

Yield: 70%, m.p.: 260 °C. FT-IR (KBr, cm^{-1}): ν(-OH/H_2O) 3191, 3055, ν(C=O) 1664,1656, ν(C=C) 1648, 1557, ν(C-O) 1205, 1124, 1083, 817, 783, 744. 1H NMR (DMSO-d^6 400 MHz) δ: 6.44 (^{1}H, Aliphatic), 7.19-8.78 (12H, m, Aromatic proton), 10.43 (-OH phenolic); ^{13}C NMR (DMSO-d^6 100 MHz): δ: 36.2 (C-9), 102.6 (C-3, 18), 116.3, 116.8, 123.4, 125.6, 125.7, 125.8, 128.2, 128.7, 131.3, 134.5, 144.7 (11C, Ar-C), 151.6(C-8a, 23a), 163.4(C-2, 17), 165.4(C-4, 19); ESI-MS (m/z): 489.98(M +H)$^+$. Elemental analysis found (%): C, 67.24; H, 3.38; Calculated for $C_{25}H_{15}Br_2O_6$ (488.75): C, 58.22; H, 2.53.

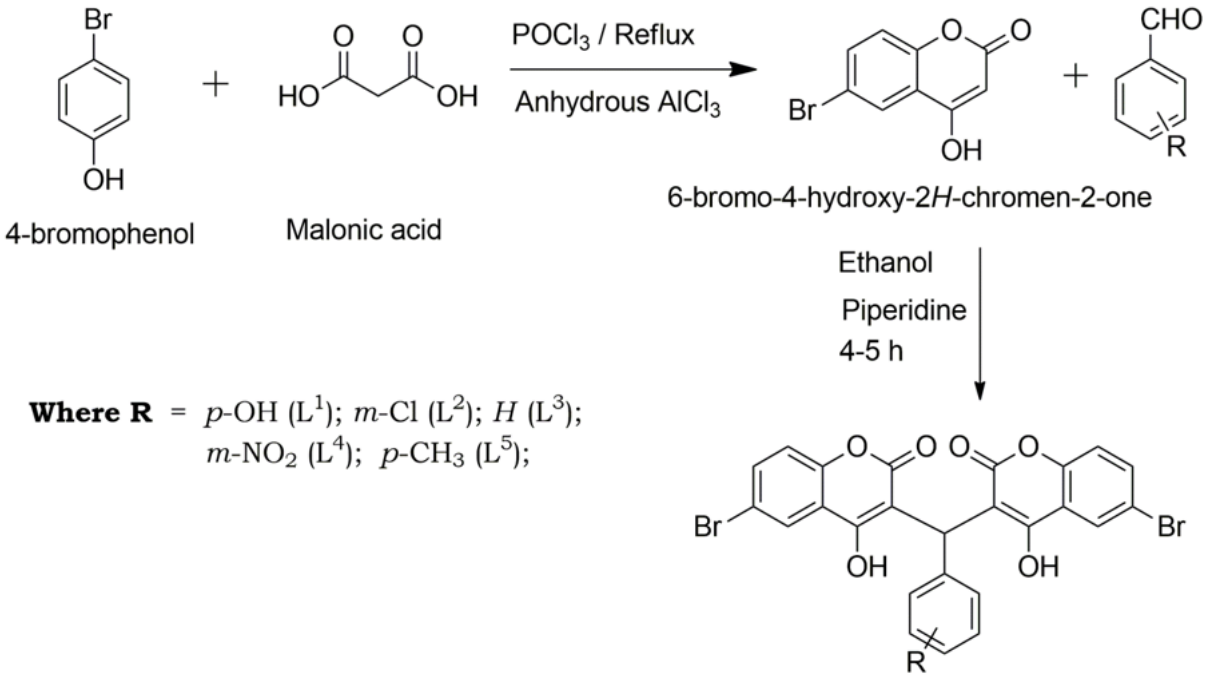

Fig 1. General scheme for dicumarol derivatives

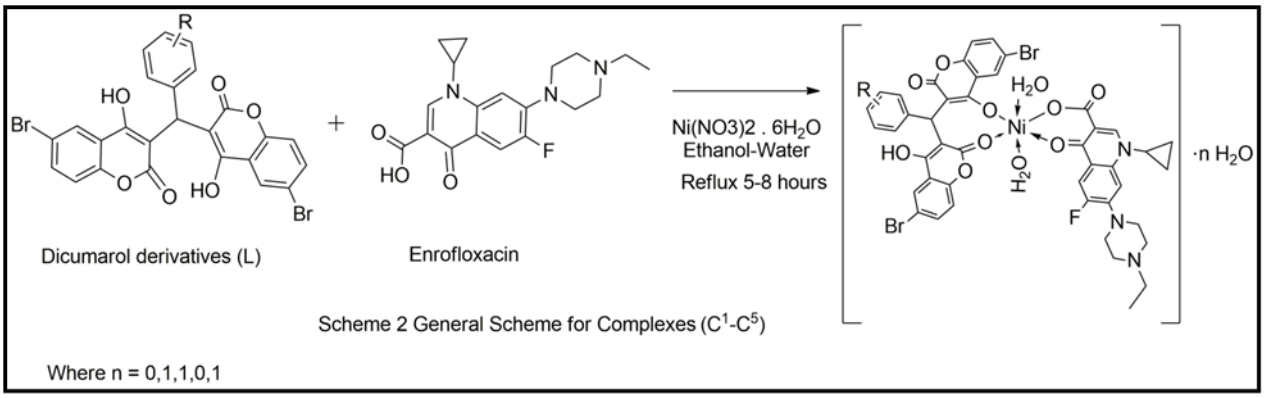

Fig 2. The synthetic protocol of complexes

2.3.3 3,3′-(phenylmethylene) bis(6-bromo-4-hydroxy-2H-chromen-2-one): (L^3)

Yield: 67 %, m.p. 225 °C. FT-IR (KBr, cm^{-1}): ν(-OH/H_2O) 3181, 3052, ν(C=O) 1662,1655, ν(C=C) 1646, 1564, ν(C-O) 1177, 1123, 1085, 823, 794, 748. ^{1}H NMR (DMSO-d^6 400 MHz) δ: 6.53 (^{1}H, Aliphatic), 7.11-7.92 (13H, m, Aromatic proton), 10.36 (-OH phenolic); ^{13}C NMR (DMSO-d^6 100 MHz): δ: 36.4 (C-9), 103.5 (C-3, 18), 116.3, 117.3, 123.5 125.4, 125.2, 127.71, 128.2, 128.4, 143.5(9C, Ar-C), 152.3(C-8a, 23a), 164.2(C-2, 17), 167.3(C-4, 19); ESI-MS (m/z): 455.02$(M+H)^+$. Elemental analysis found (%): C, 62.26; H, 2.82; Calculated for $C_{25}H_{14}Br_2O_6$ (454.26): C, 62.38; H, 2.91.

2.3.4 3,3′-((3-nitrophenyl) methylene)bis(6-bromo-4-hydroxy-2H-chromen-2-one): (L^4)

Yield: 69%, m.p.: 287 °C, FT-IR (KBr, cm^{-1}): ν(m,-OH/H_2O) 3157, 3035, ν(C=O) 1665,1651 ν(C=C) 1624, 1574, ν(C-O) 1160, 1123, 1076, 813, 782, 748. ^{1}H NMR (DMSO-d^6 400 MHz) δ: 6.41 (1H, Aliphatic), 7.18-8.26 (12H, m, Aromatic proton), 10.83 (-OH phenolic). ^{13}C NMR (DMSO-d^6 100 MHz): δ: 35.3 (C-9), 100.7 (C-3, 18), 115.8, 116.7, 119.6, 120.24, 121.3 122.3 124.55, 127.7, 133.6, 144.5, 148.3 (11C, Ar-C), 151.6(C-8a, 23a), 162.3(C-2, 17), 165.2(C-4, 19); ESI-MS (m/z): 500.00. Elemental analysis found (%): C, 57.63; H, 23.33; N, 2.04; Calculated for $C_{25}H_{13}Br_2NO_8$ (499.28): C, 57.06; H, 2.45; N, 2.65.

2.3.5 3,3'-(p-tolylmethylene bis(6-bromo-4-hydroxy-2H-chromen-2- one : (L^5)

Yield: 63 %, M.P.: 275 °C, FT-IR (KBr, cm^{-1}): ν(-OH/H_2O) 3153, 3032, ν(C-OH) 1342, 1335, ν(C=O) 1668, 1653, ν(C=C) 1628, 1575, ν(C-O) 1165, 1125, 1075, 813, 787, 744., ^{1}H NMR (DMSO-d^6 400 MHz) δ: 2.35 (3H, s, -CH_3), 6.56 (1H, Aliphatic), 7.12-7.95 (10H, m, Aromatic proton), 10.84 (-OH phenolic), ^{13}C NMR (DMSO-d^6 100 MHz): δ: 22.4 (-CH_3), 37.3 (C-9), 102.4 (C-3, 18), 116.5, 117.3, 124.6 126.35, 128.15, 128.7, 128.7, 136.4, 142.5 (9C, Ar-C), 152.2(C-8a, 23a), 162.8(C-2, 17), 165.8(C-4, 19), ESI-MS (m/z): 463.03$(M+H)^+$, Elemental analysis found (%): C, 67.45; H, 3.49; Calculated for $C_{26}H_{16}Br_2O_6$ (462.40): C, 67.55; H, 3.51.

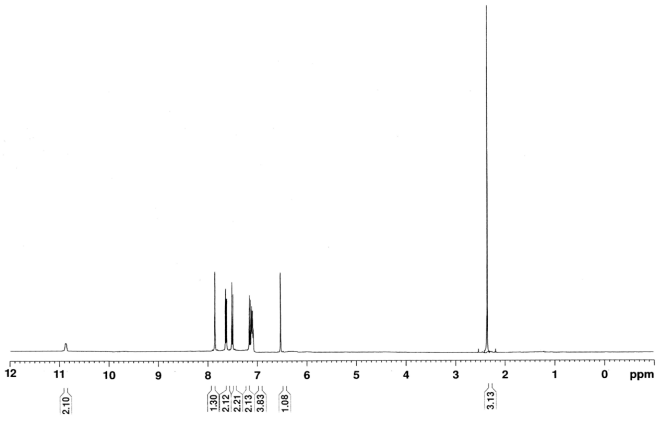

Fig 3. ^{1}H NMR Spectra of L^5

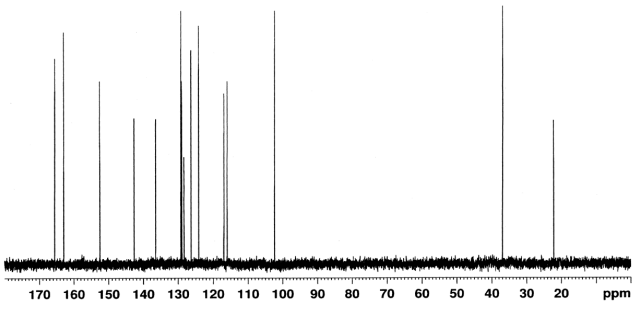

Fig 4. ^{13}C NMR Spectra of L^5

2.4 Synthesis of metal complexes: [M(L) (EN)(H2O)OH].xH2O

An aqueous solution of $Ni(NO_3)_2.6H_2O$ salt (10 mmol) was poured into an ethanol solution of ligand (L) (10 mmol) and an ethanol solution of enrofloxacin (10 mmol) was added with continuous stirring. The pH was then adjusted to 4.5-6.0 by adding dilute NH_4OH solution. The resulting solution was refluxed for 5 hours and then heated in a steam bath to evaporate to half its volume. The reaction mixture was kept overnight at room temperature. A slightly colored crystalline product was obtained. The product obtained was washed with ether and dried in a vacuum desiccator. Complexes C^2-C^5 were prepared according to the same method and their physicochemical parameters are summarized in Table 1. The synthetic protocol of complexes is shown in Figure 2 while FT-IR spectrum of C^5 is given in the Figure 7

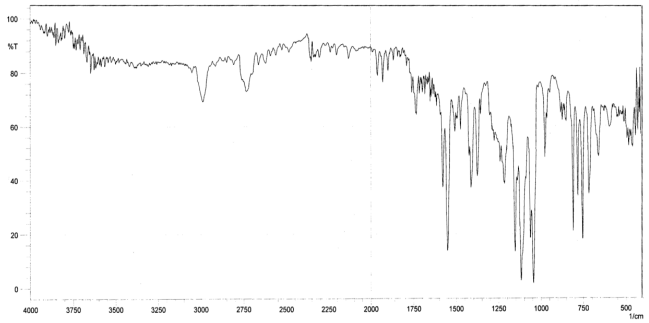

Fig 5. IR Spectra of L^5

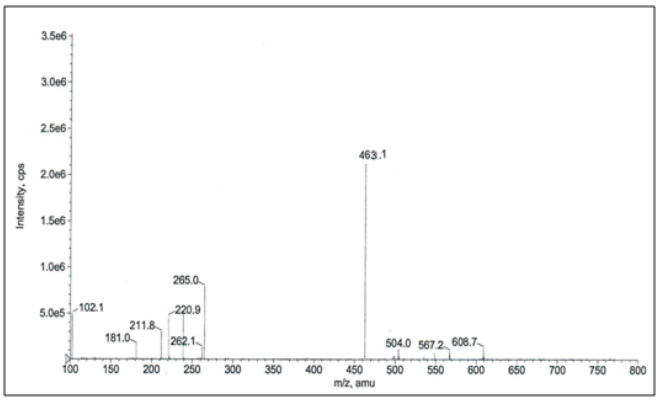

Fig 6. Mass Spectra of L^5

2.5 Antimicrobial activity

All ATCC cultures were collected from the Institute of Microbiology Technology, Bangalore. A 2% Luria broth solution was prepared in distilled water while adjusting the pH of the solution to 7.4 ± 0.2 at room temperature and autoclaved at 15 pounds' pressure for 25 minutes. Bacterial and fungal strains tested were prepared in Luria broth and incubated overnight in an orbital incubator at 37°C and 200 rpm. Sample solutions were prepared in DMSO at concentrations of 200, 150, 100, 50, 25, 12, and 6 μg/ml. Standard solutions of streptomycin (antibacterial) and nystatin (antifungal) were prepared in DMSO. A stepwise broth micro-dilution was used as a reference method. 10 μL of test compound solution was inoculated into 5 mL of Luria culture at each concentration and an additional test tube was added as a control. Each tube was inoculated with a suspension of the test standard organism and incubated at 35°C for 24 hours. At the end of the incubation period, the tubes were checked for turbidity. Turbidity in the test tubes indicated that the antibiotics contained in the media did not inhibit microbial growth at the concentrations tested. The antibacterial activity test was performed in triplicate.

2.6 Antioxidant studies

Iron reducing antioxidant capacity (FRAP) was determined using an adapted method[(10)]. The antioxidant capacity of the compounds was evaluated based on the reducing power of His-TPTZ-Fe(III) complex to His-TPTZ-Fe(II) complex relative to the total antioxidant capacity of the tested samples. This method was used due to its simplicity, rapidity, and reproducibility of the results obtained. The following solutions were initially prepared: A) Acetate buffer, 300 mM pH 3.6 (3.1 g sodium acetate trihydrate and 16 ml concentrated acetic acid per liter buffer), B) 10 mM 2,4, 6-tripyridyl-s-triazine 40 mM HCl, C) 20 mM $FeCl_3.6H_2O$ in distilled water, D) 1 mM ascorbic acid dissolved in 100 ml distilled water. A FRAP working solution was prepared by mixing the above solutions (A), (B) and (C) in a 10:1:1 ratio. A mixture of 40.0 μl of 0.5 mM sample solution and 1.2 ml of FRAP reagent was incubated at 37° C. for 15 minutes. Working solutions had to be freshly prepared. Ascorbic acid was used as a standard antioxidant compound and results were expressed relative to ascorbic acid.

3 Result and Discussion

The synthesized Ni(II) complexes were examined by elemental analysis, FTIR spectra. The metal ion in their complexes were determined after mineralization. The metal content in chemical analysis were evaluated by complexometrically[11] while the geometry of the complexes was confirmed from thermogravimetry anlysis, electronic spectra, and magnetic moment.

$$Ni(NO_3)_2.\ 3\ H_2O\ +\ L\ +\ EN \longrightarrow [Ni(L)(EN)(H_2O)OH]\ .\ x\ H_2O\ +\ 2\ HNO_3\ +\ n\ H_2O$$

(Where $L = L^1, L^2, L^3, L^4, L^5, L^6$; x = 3, 2, 2, 3, 2 and n = 0, 1, 1, 0, 1)

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 shown.

Table 1. Analytical and physical parameters of complexes

Comps.	Elemental analyses, % found (required)				M.P. (° C)	Yield (%)	Molecular weight	μeff /B.M.
	C	H	N	Ni(II)				
C^1	56.65(56.75)	4.35(4.43)	4.51(4.59)	6.27(6.42)	>350	72	934.39	1.83
C^2	56.56(56.66)	4.15(4.28)	4.42(4.57)	6.15(6.27)	>350	72	952.93	1.85
C^3	57.64(57.73)	4.38(4.51)	4.64(4.82)	4.56(4.68)	>350	70	918.49	1.82
C^4	54.95(54.95)	4.09(4.27)	5.83(5.93)	6.08(6.17)	>350	73	963.49	1.86
C^5	57.95(58.04)	4.56(4.68)	4.52(4.63)	6.28(6.36)	>350	67	932.52	1.84

3.2 FT-IR spectra

The coordination sites of ligands are elucidated using IR. The IR data of free ligands and their metal complexes were carried out within the IR range of 4000-400 cm^{-1}. The IR spectra of the dicoumarol derivatives show weak bands at ~3120-3050 cm^{-1} and ~1321-337 cm^{-1}, corresponding to ν(O–H) and ν(C–OH) respectively. On complexation O–H peak has nowhere to be found, indicating the deprotonation of the O–H proton. The ν(C=O) of lactone rings observed at ~1647 and 1659 cm^{-1} in the free ligand is shifted to lower frequencies (~12-14 cm^{-1} and 45-55 cm^{-1}) due to complex formation, and further supported by shifting of ν(C–C), ν(C–O), and ν(C–O–C) stretch frequencies to higher values. Two bands at ∼1617 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 ∼1605 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 ∼3420-3455 cm^{-1} observed in the complex was due to the ν(O–H) characteristic peak of a coordinated water molecule. The bands at ~1740 cm^{-1} and ~1254 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 ~1360-1390 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 ~205–235 cm^{-1} indicative of a monodentate coordination mode. Whereas ν(C=O)p is shifted from ~1625–1655 cm^{-1} upon bonding. The overall changes in the IR spectrum suggest that enrofloxacin and ligands are coordinated to the Ni(II) via the ketone oxygen and carboxylato oxygen. These changes in the IR spectra suggest that enrofloxacin is coordinated to the metal via pyridone and one carboxylate oxygen atom. These data are further supported by ν(Ni–O) which appears at ∼525 cm^{-1} (Figure 7)

3.3 Thermogravimetric analysis

The thermal behaviour of the complexes was studied using TGA, whereas the TG curve corresponding to the complex (C^2) is represented in Figure 9 The thermal decomposition occurs in four steps in the air are observed. According to the mass losses, the following degradation pattern might be proposed for complex $[Ni(L^2)(EN)(H_2O)OH]\bullet H_2O$ (C^2) is represented in Figure 8

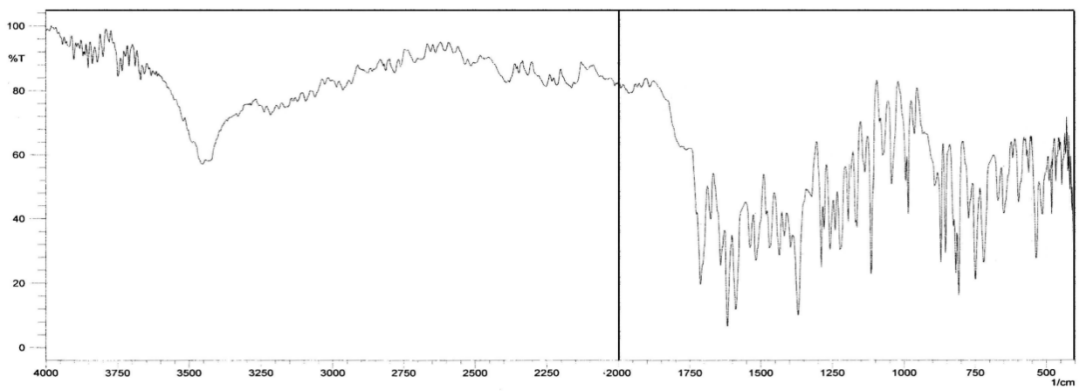

Fig 7. FT IR Spectrum of C^5

. All the compounds decompose with time respectively. Thermal decomposition started with the dehydration process and was accompanied by an endothermic effect between 70-100 °C, which was due to the loss of one lattice of water molecules in the first step. The observed mass loss was 2.63% which was nearly equal to the theoretical value of 2.25%. In the second step, exothermic decomposition between 200-240 °C corresponds to a loss of one coordinated water and one hydroxyl molecule. The observed mass loss was 4.55% which was nearly equal to the theoretical value of 4.30%. The next two steps are exothermic and endothermic, which are related to the removal of coordinated Enrofloxacin and ligand (coumarins) respectively. As temperature raise, the intermediate complexes [Ni(L^2)(EN)] (350-410 °C) and [Ni(EN)] (510-730 °C) convert to NiO residue of fragments. The observed mass loss for the third and fourth stages was 50.07% (calc. 36.84%) and 36.59% (calc. 36.07%) respectively. The final solid product of decomposition was NiO (obs. 08.55%; calc.8.25%) accompanied by the broad exothermic effect on above 750 °C.

$$[Ni(L^2)(EN)(H_2O)(OH)]\,H_2O \xrightarrow[\text{Removal of lattice water molecules}]{70\text{-}100\ ^oC} [Ni(L^2)(EN)(H_2O)(OH)] + H_2O$$

$$[Ni(L^2)(EN)(H_2O)(OH)] \xrightarrow[\text{Removal of hydroxyl and water molecules}]{140\text{-}240\ ^oC} [Ni(L^2)(EN)] + OH$$

$$[Ni(L^2)(EN)] \xrightarrow[\text{Removal of } L^2 \text{ ligand molecules}]{310\text{-}410\ ^oC} [Ni(L^2)] + EN$$

$$[Ni(L^2)] \xrightarrow[\text{Removal of EN ligand molecules}]{510\text{-}730\ ^oC} \text{Metal residue (NiO)} + L^2$$

Fig 8. Thermal degradation pattern for C^2

3.4 Electronic spectra and magnetic measurement

Electronic spectral data and magnetic susceptibility measurements provided sufficient support to determine the shape of the metal complex. The electronic spectrum of the complex was recorded in DMF solution over the scanning range of 200–1200 nm. The octahedral geometry is usually found in Ni(II) complexes[12], but the Jahn-Teller distortions in triangular and octahedral geometries are well known. The very faint, low-intensity absorption bands associated with the d–d transition of the Ni(II) complex at 465, 532 nm are octahedral transitions, indicating the octahedral geometry of the complex[13].

The absorption spectra of Ni(II)-complexes display three d-d transition bands appear at ~10,800(ν_1), ~14,600(ν_2) and ~21,180(ν_3) cm^{-1}. The transitions correspond to the $3A_{2g}$ (F) → $3T_{2g}$ (F), $3A_{2g}$ (F) →$3T_{1g}$ (F), and $3A_{2g}$ (F) →$3T_{2g}$ (P), respectively. These transitions reveal that the Ni(II)-complexes possess octahedral geometry, which was further supported by the observed magnetic moment values between 2.85-3.17 B.M.[14]. The observed magnetic moments of Ni(II)-complexes are in the range expected for a spin-free d^8-system. The electronic spectral data and magnetic moment data are summarized in Table 2, which supports the octahedral geometry of all complexes, while the electronic spectra of C^3 are given in Figure 10.

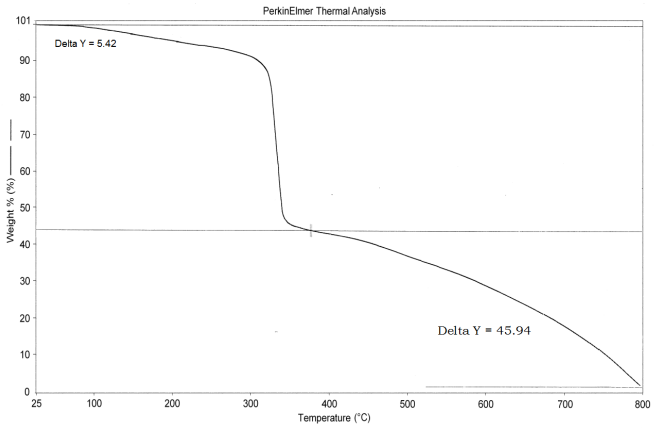

Fig 9. TGA of C^2

Table 2. Electronic spectral data and magnetic moment value of Ni(II) Octahedral complexes. (C^1-C^5)

Complexes	Transition band observed (cm^{-1})			μ_{eff} B. M.
Ni(II)-31	10801	14596	17250	2.85
Ni(II)-32	9627	13450	16732	2.97
Ni(II)-33	9991	13570	16427	2.84
Ni(II)-34	9862	13643	17758	2.89
Ni(II)-35	10053	12917	16515	2.90

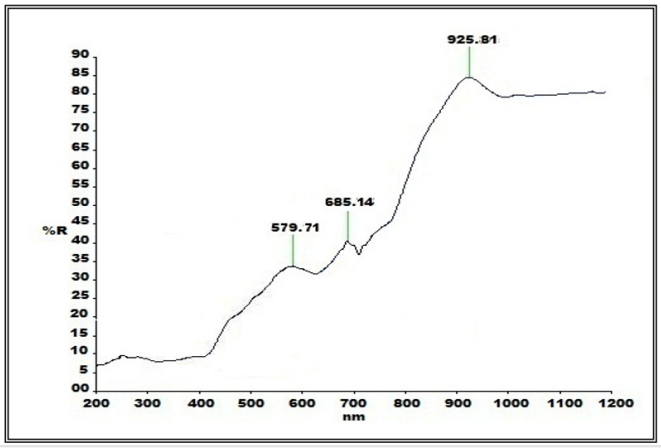

Fig 10. Electronic spectra of C^3

3.5 Antimicrobial bioassay

The MIC values of the ligands and their complexes signify that complexes display higher antimicrobial activity than the free ligand. In the present investigation, the antimicrobial activity of the ligands may be due to the heteroaromatic residues. Compounds containing the C=N group have improved antimicrobial activity than the C=C group. The growth of certain microorganisms takes place even in the absence of oxygen. Hence, compounds containing the C=C group are still capable of absorbing oxygen which is not related to the growth of microorganisms. The greater activity of the complexes can be clarified based on Overtone's concept [15] and Tweedy's chelation theory [16]. According to Overtone's concept of cell permeability, the lipid membrane surroundings in the cell permit only the lipid–soluble resources, which makes liposolubility a key part that controls the antimicrobial activity. During complexation, the polarity of the metal ion will decrease up to a certain level due to the overlapping of the ligand orbital and partial sharing of the positive charge of the Ni(II) ion with hetero atoms. Furthermore,

it enhances the delocalization of p-electrons around the entire complex ring and enhances the lipophilicity of the complexes. This increased lipophilicity enhances the permeation of the complexes into lipid membranes and blocking of the metal binding sites in the enzymes of microorganisms. These complexes also troubled the respiration process of the cell and hence block the synthesis of proteins, which restricts additional growth of the organism, and as a result microorganisms died.

Coordination, locking the polar electronegative atoms in the inner core around the metal and confining the polar residues in an external lipophilic envelope, favors diffusion through biomembranes [17]. It is also suspected that factors such as solubility, conductivity, and dipole moment may be the possible reasons for the increase in activity. Since all complexes have not shown enhanced activity as compared to parent ligands were rationalized the fact that steric and pharmacokinetic factors also play a decisive role in deciding the potency of an antimicrobial agent [18]. In review, the antimicrobial testing results reveal that complexes possess higher activity at lower concentration compared to parent ligands. All ligands show inhibition concentrations between 200 and 100 lg/mL. The low MIC value of C^5 complex indicates better activity among all complexes, while in antifungal activity C^2 complex shows poor activity from Table 3.

3.6 Antioxidant studies

The 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 the dried compound). FRAP values indicate that all the compounds have a ferric reducing antioxidant power. Complex C^3 and C^5 showed relatively high antioxidant activity while compounds C^1, C^2, and C^4 show poor antioxidant power (Table 3).

Table 3. Antimicrobial and antioxidant results of complexes

Antimicrobial Activity (Minimal Inhibition Concentration, in μg/mL)							**Antioxidant Activity**
Compounds	**Gram negative bacteria**		**Gram positive bacteria**		**Fungus**		**FRAP value (mmol/100 g)**
	E. coli	***P. aerugi-nosa***	***S. pyogenes***	***B. subtilis***	***C. albicans***	**A. niger**	
L^1	400	400	400	>600	400	200	NT
L^2	100	100	100	200	200	200	NT
L^3	100	200	100	200	200	200	NT
L^4	200	200	400	400	200	400	NT
L^5	400	200	200	600	200	200	NT
C^1	70	100	100	100	100	100	63.92
C^2	100	100	100	100	200	100	75.76
C^3	70	100	70	100	100	100	86.32
C^4	100	100	100	200	100	100	54.05
C^5	40	70	40	40	100	100	82.44
Norfloxacin	10	10	10	10	NT	NT	NT
Flucanazole	NT	NT	NT	NT	10	10	NT
Nystatin	NT	NT	NT	NT	100	100	NT

E. coli = ATCC25922; *P. aeruginosa* = ATCC25619; *S. pyogenes* = ATCC12384 ; *B. subtilis* = ATCC11774 ; *C. albicans* = ATCC 66027; *A. niger* = ATCC 64958
NT = Not tested

4 Conclusion

This study elucidated the synthesis of biological active dicumarol derivatives with enrofloxacin and Ni(II) complexes (C^1-C^5). Characterization of complexes was done by the elemental analysis, FT-IR, ^{1}H-NMR, ^{13}C-NMR, mass spectral studies, thermo-gravimetric analysis and magnetic moment data. Measurement of inhibition zone of ligand and complex confirms that the prepared complexes have enhanced antimicrobial and antioxidant activity. The complexation of ligands, with Ni(II) doubled, its activity. MIC values for complex C^5 (-CH_3 derivative) were found between 40-70 μg/mL and FRAP values were 82.44 mmol/100g indicating its ferric reducing antioxidant power; it also indicates C^5 complex for its potentcy as antimicrobial and antioxidant agent. C^5 complexi.e.-CH_3 derivative can be used for the treatment of common disease caused by these bacteria.

Further studies on the anti-tuberculosis and anticancer activity of these complexes are planned in future.

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