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RESEARCH ARTICLE

Biological and Fluorescence Activity of Newly Synthesized Ni(II) Heterochelates

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ABSTRACT

The present work staunch from our interest by the synthesis, characterization and biological evaluation of Ni(II) complexes, which have been synthesized by mixing an aqueous solution of $Ni(NO_3)_2$ in 1 : 1 molar ratios with ethanolic bidentate ligands (Bromocoumarin derivatives) and ciprofloxacin. Spectral studies confirm ligands to be mono functional bidentate and octahedral environment around metal ions. Thermal behaviour of newly synthesized mixed ligand Ni (II) complexes were investigated by means of electronic spectra and magnetic measurements. Characterization of the ligands has been carried out by elemental analysis, melting point determinations, mass spectra, ^{1}H NMR, ^{13}C NMR, and FT-IR, while structure of metal complexes were investigated and confirmed by FT-IR and FAB-mass spectral studies. Both the ligands as well as its complexes have been screened for their *in vitro* antimicrobial and fluorescence activities.

KEYWORDS

Ni(II) Complexes, Antimicrobial, Fluorescence

INTRODUCTION

Coumarins and chromones are well known pigments and have been studied widely for their luminescent properties and applications, i.e. laser dyes, optical brighteners[1]. In comparison, benzocoumarins and benzochromones are less studied for their fluorescence data and photosensitive properties. Recently, the application of benzocoumarins as non-linear optical devices and fluorescent whiteners[2] have been reported. Fluorescence emission of a bromomethyl derivative of benzocoumarin was employed for HPLC–fluorometric analysis of marine organisms. Two bioactive benzocoumarins have been isolated from Vismia guianensis and a benzochromone has been isolated from the roots of Sophora exigua.

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5,6-Benzocoumarin-5-uracil is used for a bio-medical treatment[3]. Benzochromone and benzocoumarin derivatives are under focus because of their interaction with HIV reverse transcriptase also. All of these observations hint that there is a cause of biological functions of benzocoumarins, benzochromones in nature. Liu et al. reports the antioxidative effect of 4-methylcoumarin and 7-hydroxy-4-methylcoumarin at photosensitized peroxidation of human low-density lipoprotein[4]. Fluoroquinolones represent an important group of chemotherapeutic compounds, which exhibit high antibacterial activities. An efficient representative of this group, ciprofloxacin (1-cyclopropyl-6-fluoro-4-oxo-7-(1-piperazinyl)-1,4-dihydroquinoline-3-carboxylic acid)[5] is widely used in clinical practice as a broad spectrum antimicrobial agent[6]. Quinolones comprise a group of well-known antibacterial

agents and the first members being in clinical practice over 40 years[7]. They can act as antibacterial drugs that effectively inhibit DNA replication and are commonly used in treatment of many infections[8]. Studies on the biological properties of quinolone-metal complexes have been focused on the interaction with DNA, antibacterial activity tests on diverse microorganisms, cytotoxicity and potential antitumor activity.

In earlier times, series of coumarin derivatives and their Cu(II) complexes were synthesized[9,10]. In order to have further investigation, the coordination abilities and complexation behaviors of coumarin based ligands, we extended the study to the synthesis of new bromocoumarin derivatives and their Ni(II) complexes. Present work describes FAB mass, antimicobacterial and fluorescence activity of new Ni(II) complexes.

MATERIALS AND METHODS

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. Clioquinol was purchased from Agro Chemical Division, Atul Ltd., Valsad-India. The metal nitrates used were in hydrated form.

Physical Measurements

All reactions were monitored by thin-layer chromatography (TLC on aluminum 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. The FAB mass spectrum of the complex was recorded at SAIF, CDRI, Lucknow with JEOL SX-102/DA-6000 mass spectrometer.

Melting point of the ligands and metal complexes were measured by open capillary tube method. Solid state magnetic susceptibility measurements were carried out at room temperature using a Gouy's magnetic susceptibility balance with mercury tetrathiocyanato cobaltate (II) being used as a reference standard ($g = 16.44\times10^{-6}$ c.g.s. units). Molar susceptibility was corrected using Pascal's constant[11].

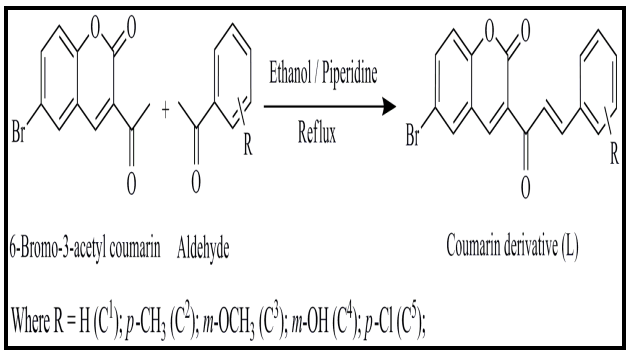
The electronic spectra were collected using LAMBDA 19 UV/Vis/NIR spectrophotometer in the region 200-1200 nm. The fluorescence behaviors of ligands and their Cu(II) complexes were studied using a Shimadzu RF-1501 Fluorescence Spectrophotometer with Xe arc lamp as the light source at room temperature. The slit width for excitation and emission was 10 nm, and the scan speed was 1200 nm/min.

Synthesis of 3-acetyl coumarin

3-acetyl coumarin was prepared according to the reported method[12]. 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.

Synthesis of ligands (A^1-A^5)

The neutral bidentate ligands were synthesized using Claisen-Schmidt condensation[13]. General procedure for synthesis of the ligands (A) is shown in Scheme 1.



Scheme 1: General procedure for synthesis of ligands (L)

Synthesis of 6-bromo-3-cinnamoyl-2H-chromen-2-one (A^1)

In a 100 ml round bottom flask 6-bromo 3-acetyl coumarin (0.01 mol, 1.88 g) and 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 72%, m.p. 163-165 °C. FTIR (KBr, cm^{-1}): *ν*(C=O, *α, β*-unsaturated ketone) 1615, *ν*(C=O, lactone carbonyl of coumarin) 1738. ^{1}H NMR (DMSO-d6, 400 MHz): δ=6.84 (1H, d, *J* = 16, CH=CH- protons), 7.19–8.04 (8H, m, eight aromatic protons), 8.24 (1H, d, *J* = 16, CH=CH- protons), 8.58 (1H, s, C4-H). ^{13}C NMR (100 MHz, DMSO-d6): 118.2, 119.7, 124.4, 125.2, 126.9, 128.7, 129.6, 130.6, 134.9, 135.4, 135.8, 142.9 (12 different types of aromatic carbons), 147.9(C-4), 152.7(C-9), 159.8 (C=O, lactone carbonyl of coumarin), 183.9 (C=O, *α, β*-unsaturated ketone). MS (ESI) *m/z* 355.0 $[M+H]^+$, 357.0 $[M+H]^{+2}$; Anal. Calculated for $C_{18}H_{11}BrO_3$ (355.18): C 60.72 (60.87); H 2.98 (3.12)

Synthesis of 6-bromo-3-(3-p-tolylacryloyl)-2H-chromen-2-one (A^2)

A^2 was synthesized by same method used for A^1 by using 4-methyl benzaldehyde instead of benzaldehyde. Yield 73%, m.p. 165-167 °C FTIR (KBr, cm^{-1}): *ν*(C=O, *α, β*-unsaturated ketone), 1618, *ν*(C=O, lactone carbonyl of coumarin) 1742. ^{1}HNMR (DMSO-d_6, 400 MHz) δ=6.87 (1H, d, *J* = 16, CH=CH- protons), 7.15-8.09 (7H, m, Ar-H), 8.22 (1H, d, *J* = 16, CH=CH- protons); 2.28 (3H, s, -CH_3); 8.55 (1H, s, C4-H). ^{13}C NMR (100 MHz, DMSO-d6): δ 21.2 (C-18, CH3), 118.5, 119.5, 124.7, 125.2, 128.4, 128.7, 130.1, 132.9, 134.4, 134.8, 137.6, 142.5 (12 different types of aromatic carbons), 147.6(C-4), 152.8(C-9), 159.6 (C=O, lactone carbonyl of coumarin), 183.4 (C=O, *α, β*-unsaturated ketone). MS (ESI) *m/z* 368.0 $[M+H]^+$, 370.0 $[M+H]^{+2}$; Anal. Calculated for $C_{19}H_{13}BrO_3$ (369.21): C 61.67 (61.81); H, 3.36 (3.55)

Synthesis of 6-bromo-3-(3-(3-methoxyphenyl)acryloyl)-2H-chromen-2-one (A^3)

A^4 was synthesized by same method used for A^1 by using 3-methoxybenzaldehyde instead of benzaldehyde. Yield 76%, m.p. 168-169 °C FTIR (KBr, cm^{-1}): *ν*(C=O, *α, β*-unsaturated ketone) 1621, *ν*(C=O, lactone carbonyl of coumarin) 1743. ^{1}HNMR (DMSO-d_6, 400 MHz,) δ=6.83(1H, d, *J* = 16, CH=CH- protons), 7.07-8.05 (7H, m, Ar-H), 8.25 (1H, d, *J* = 16, CH=CH- protons); 3.79 (3H, s, -OCH_3); 8.58(1H, s, C4-H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 55.7 (C-17, OCH3), 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), 148.7(C-16, carbon attach to phenolic NO_2), 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* 384.0 $[M+H]^+$, 386.0$[M+H]^{+2}$; Anal. Calculated for $C_{19}H_{13}BrO_4$ (385.21): C 59.08 (59.24), H 3.24 (3.40).

Synthesis of 6-bromo-3-(3-(3-hydroxyphenyl)acryloyl)-2H- chromen-2-one (A^4)

A^5 was synthesized by same method used for A^1 by using 3-hydroxy benzaldehyde instead of benzaldehyde. Yield: 72%, m.p.: 167-169 °C. FT-IR (KBr, cm^{-1}): ν(C=O, α, β-unsaturated ketone) 1623, ν(O-H) 3426, ν(C=O, lactone carbonyl of coumarin) 1740. 1H NMR (DMSO-d6 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, C4-H). 9.72 (1H, s, -OH). 13C 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 370.0 $[M^{+H}]^+$, 372 $[M^{+H}]^{+2}$; Elemental analysis found (%): C, 58.08; H, 2.82; Calculated for $C_{18}H_{11}BrO_4$ (371.18): C, 58.24; H, 2.99.

Synthesis of 6-bromo-3-(3-(4-chlorophenyl)acryloyl)-2H-chromen-2-one (A^5)

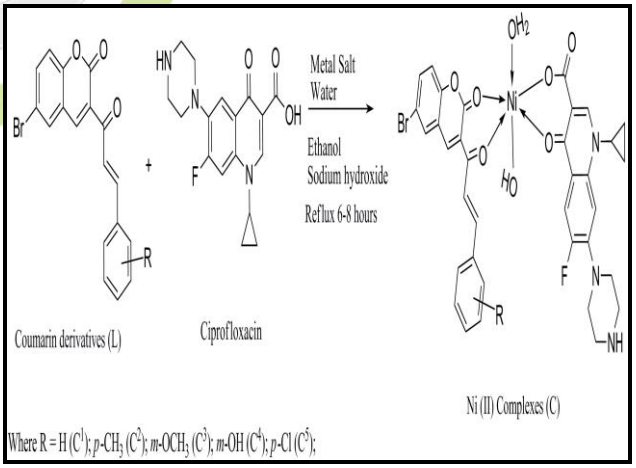
A^6 was synthesized by same method used for A^1 by using 4-chloro benzaldehyde instead of benzaldehyde. Yield: 76%, m.p.: 164-165 °C. FT-IR (KBr, cm^{-1}): ν(C=O, α, β-unsaturated ketone) 1621, ν(C=O, lactone carbonyl of coumarin). 1H NMR (DMSO-d6 400 MHz) δ: 6.81 (1H, d, CH=CH- protons), 7.34 (2H, d, CH=CH- protons), 7.58 (2H, d, CH=CH- protons), 7.78–8.08 (3H, m, three aromatic protons), 8.12 (1H, d, CH=CH- protons), 8.59 (1H, s, C4-H). 13C NMR (DMSO-d6 100 MHz) δ: 118.1 119.4, 124.4, 125.1, 128.3, 129.4, 130.8, 133.3, 133.8, 134.2, 134.7, 143.2 (12 different types of aromatic carbons), 146.8(C-4), 152.3(C-9), 159.7(C=O, lactone carbonyl of coumarin), 184.2(C=O, α, β-unsaturated ketone). MS (ESI) m/z 390.0 $[M^{+H}]^+$, 392.0 $[M^{+H}]^{+2}$, 394.0 $[M^{+H}]^{+4}$; Elemental analysis found (%): C, 55.27; H, 2.41; Calculated for $C_{18}H_{10}BrClO_3$ (389.63): C, 55.49; H, 2.59.*2.5*

Synthesis of Metal Complexes

$[Ni(A^1)(CF)(H_2O)_2]$ (C^1)

An aqueous solution of $Ni(NO_3)_2 \cdot 3H_2O$ salt (10 mmol) was added into ethanolic solution of ligand (A^1) (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 C^2-C^5 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, while FT-IR spectrum of C^2 is given in Fig.1.



Scheme 2: General procedure for synthesis of complexes (C)

Antimicrobial activity

Peptone (5 g), sodium chloride (5 g), beef extract (1.5 g) were suspended in 1000mL distilled water. The solution was boiled to dissolve all the ingredients completely. The pH of the solution at 25°C was adjusted to 7.4±0.2

and sterilized by autoclaving at 15 lb pressure (121°C) for 15 min. One day prior to the test, bacterial and fungal strains were madei n the sterile nutrient broth and incubated at 37°C overnight. Sample solutions were prepared by dissolving 1mg of sample in 10mL of 2% DMSO to give the concentration 100µg/mL. The standard solutions of Flucinozole (antifungal drug) were prepared in 2% DMSO to give concentration of 100µg/mL. Serial broth microdilution was adopted as a reference method. Serial dilutions of test compounds were made in broth, after which a standardized microorganism suspension was added (10 test tubes). Quantities of test compounds were serially diluted to attain the final concentrations of 100, 50, 25, 12.5, 6.25, 3.125, 1.6, 0.8, 0.4 and 0.2µg/mL. One of the test tubes was kept as control. Each of the 10 test tubes was inoculated with a suspension of microorganism to be tested and incubated at 35°C for 18 h. At the end of the incubation period, the tubes were visually examined for the turbidity. Cloudiness in the test tubes indicated that microorganism growth has not inhibited by the antibiotic contained in the medium at the test concentration.

RESULT AND DISCUSSION

The synthesized Ni(II) complexes were characterized by elemental analysis, FTIR and mass (ESI-MS & FAB) 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 and magnetic moment. However, ligands and its complexes have been screened for their *in vitro* antimicrobial and fluorescence 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 analysis of the FT-IR spectra of both ligands and complex provided information on the coordination mode between the ligands and the metal ion IR Spectra. The IR spectral data are summarized in Table 2. The infrared spectra of fluoroquinolones are quite complex due to the presence of the numerous functional groups in the molecules, therefore their interpretation is based on the most typical vibrations[15] being the most important region in the IR spectra of fluoroquinolones between ~1800 and ~1300 cm^{-1}[16]. Spectra of the mixed-ligand N(II) complexes reveals that a broad band in the region ~3420-3460 cm^{-1} is due to stretching vibration of OH group. The ν(C=O) stretching vibration band appears at ~1708 cm^{-1} in the spectra of ciprofloxacin, and the complexes show this band at ~1628 cm^{-1}; this band shifted towards lower energy, suggesting that coordination occurs through the pyridone oxygen atom[17]. The strong absorption bands obtained at ~1625 and ~1380 cm^{-1} in ciprofloxacin are observed at ~1570-1580 and ~1345-1375 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} ($\Delta\nu = \nu COO_a - \nu COOs$), suggesting unidentate binding of the carboxylato group[18]. 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 ~1735 cm^{-1} due to complex formation. In all the complexes, a new band is seen in the ~ 501-517 cm^{-1} region, which can be attributed to υ(Ni-O)[19].

Electronic Spectra and Magnetic Measurement

Ni(II) complexes-d^8 system are known to exhibit complicated equilibrium between coordination numbers six (octahedral) and four (square planar/tetrahedral). Their electronic spectra are typically characterized by the existence of complicated equilibria involving these different structural types[21].

Table 1: Analytical and physical parameters of complexes

Compounds	Elemental analyses, % found (required)				m.p. (^oC)	Yield (%)	Molecular weight	μ_{eff}/B.M.
	C	H	N	Ni(II)				
C^1	53.95 (54.11)	4.01 (4.23)	5.39 (5.56)	7.53 (8.71)	>350	73	779.23	3.06
C^2	54.51 (54.69)	4.19 (4.33)	5.30 (5.45)	7.40 (7.51)	>300	75	793.26	3.10
C^3	53.43 (53.55)	4.11 (4.23)	5.19 (5.34)	7.25 (7.37)	>350	62	809.26	2.98
C^4	52.86 (52.95)	3.93 (4.02)	5.28 (5.47)	7.38 (7.47)	>300	67	795.23	2.88
C^5	51.66 (51.78)	3.72 (4.86)	5.16 (5.29)	7.21 (7.34)	>300	62	813.68	3.01

Table 2: FT-IR data of synthesized compounds (C^1-C^5)

Sample No.	υ(O–H) cm^{-1} (br)	$\upsilon(COO)_s$	$\upsilon(COO)_a$	Δν	*α, β*-unsaturated c(C=O) cm^{-1} (s)	lactone carbonyl υ(C=O) cm^{-1} (s)	υ(C=O) of pyridine cm^{-1}	υ(Ni–O) cm^{-1} (w)
C^1	3435	1371	1590	219	1605	1722	1619	502
C^2	3432	1373	1586	213	1609	1732	1620	504
C^3	3428	1375	1582	207	1606	1730	1630	509
C^4	3445	1372	1587	215	1608	1725	1624	512
C^5	3447	1376	1585	211	1612	1737	1622	510

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

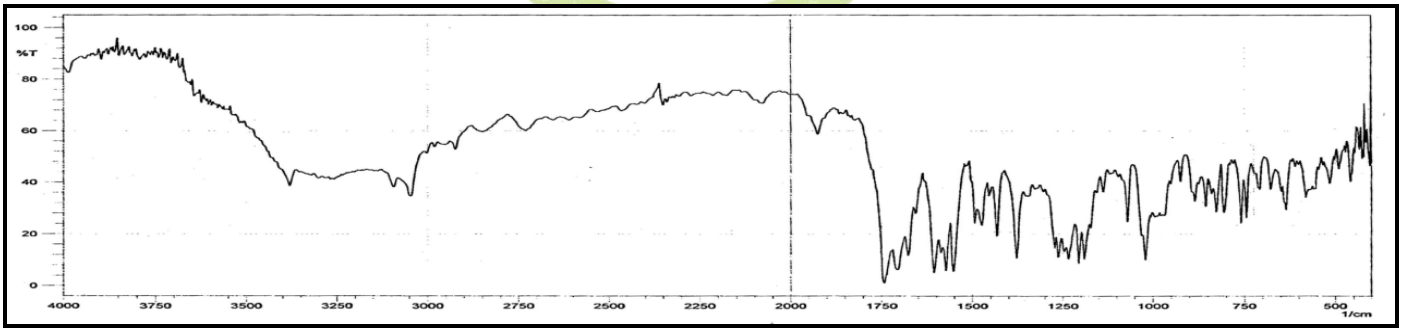


Figure 1: IR Spectra of ligand A^2

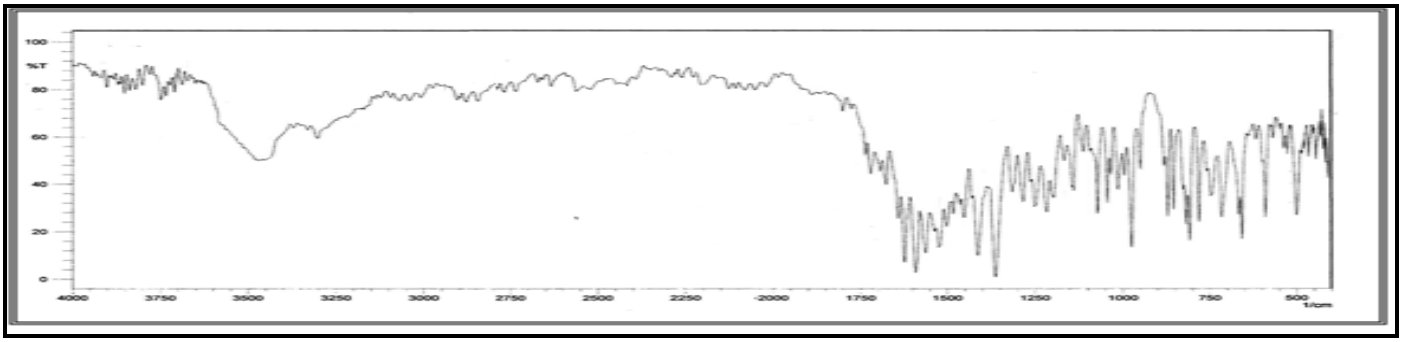


Figure 2: IR spectra of C^2

For octahedral or distorted-octahedral Ni(II)-complexes, the crystal field theory allows three d-d transitions, i.e. $^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$, $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$ and $^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$[22]. The first two bands appear over the near-IR range ~10000 and ~15000 cm^{-1} corresponding to transitions $^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$ and $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$ respectively. In the visible region of the spectrum only one band corresponding to $^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$ transition appears at ~22,000 cm^{-1}. Besides this, two spin forbidden transitions ($^3A_{2g}(F) \rightarrow {}^1E_g(D)$ and $^3A_{2g}(F) \rightarrow {}^1T_{2g}(D)$) are often observed in the case of octahedral Ni(II)-complexes, the position of these bands can be either close to the lowest energy spin allowed transition to the $^3T_{2g}$ state or close to ^3T_1g[23]. The magnetic moment value of 3.00 B.M. generally observed for octahedral environment around hexacoordinated Ni(II)-complexes[24].

The absorption spectra of Ni(II)-complexes display three d-d transition bands with D_4h symmetry appear at ~10,700(ν_1), ~14,500(ν_2) and ~21,140(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 2.82-3.15 B.M.[25]. The observed magnetic moments of Ni(II)-complexes are in the range expected for spin-free d^8-systems. The electronic spectral data and magnetic moment of Ni(II)-complexes are summarized in Table 3.

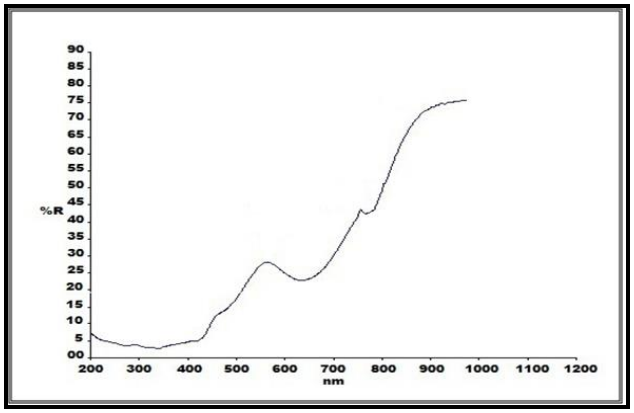


Figure 3: Electronic spectra of C^3

Table 3: Electronic spectral data of the complexes

Complexes	Transition band observed (cm^{-1})			μ_{eff} B. M.
C^1	9623	12970	17815	3.06
C^2	9765	13301	16825	3.10
C^3	9970	14215	17295	2.98
C^4	9330	12798	17008	2.88
C^5	9525	13434	16601	3.01

FAB Mass Spectra

Fast atom bombardment (FAB) mass spectra and its fragmentation scheme of the C^1 complex given in supplementary material. This spectrum reveals that isotropic peak at m/z 789 (molecular ion peak) of complex (without water of crystallization) where as several peaks observed at 779.07, 743, 664, 579, 489, 449, 379, 328 and 283 m/z value. Thus the m/z of all the fragments of complex with the relative intensity confirms the stoichiometry of the complex.

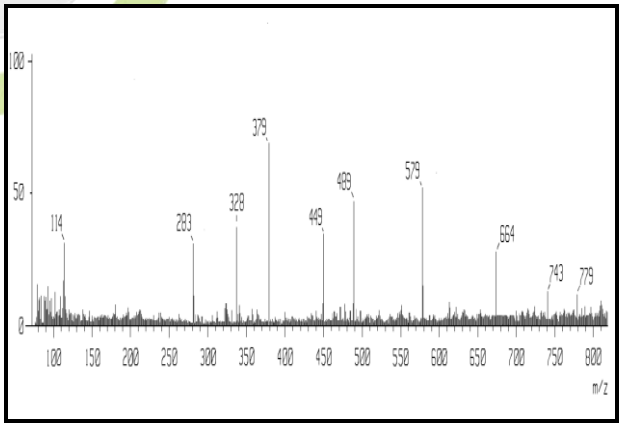


Figure 3: Fab mass spectra of complexes C^1

Antimicrobial Bioassay

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).

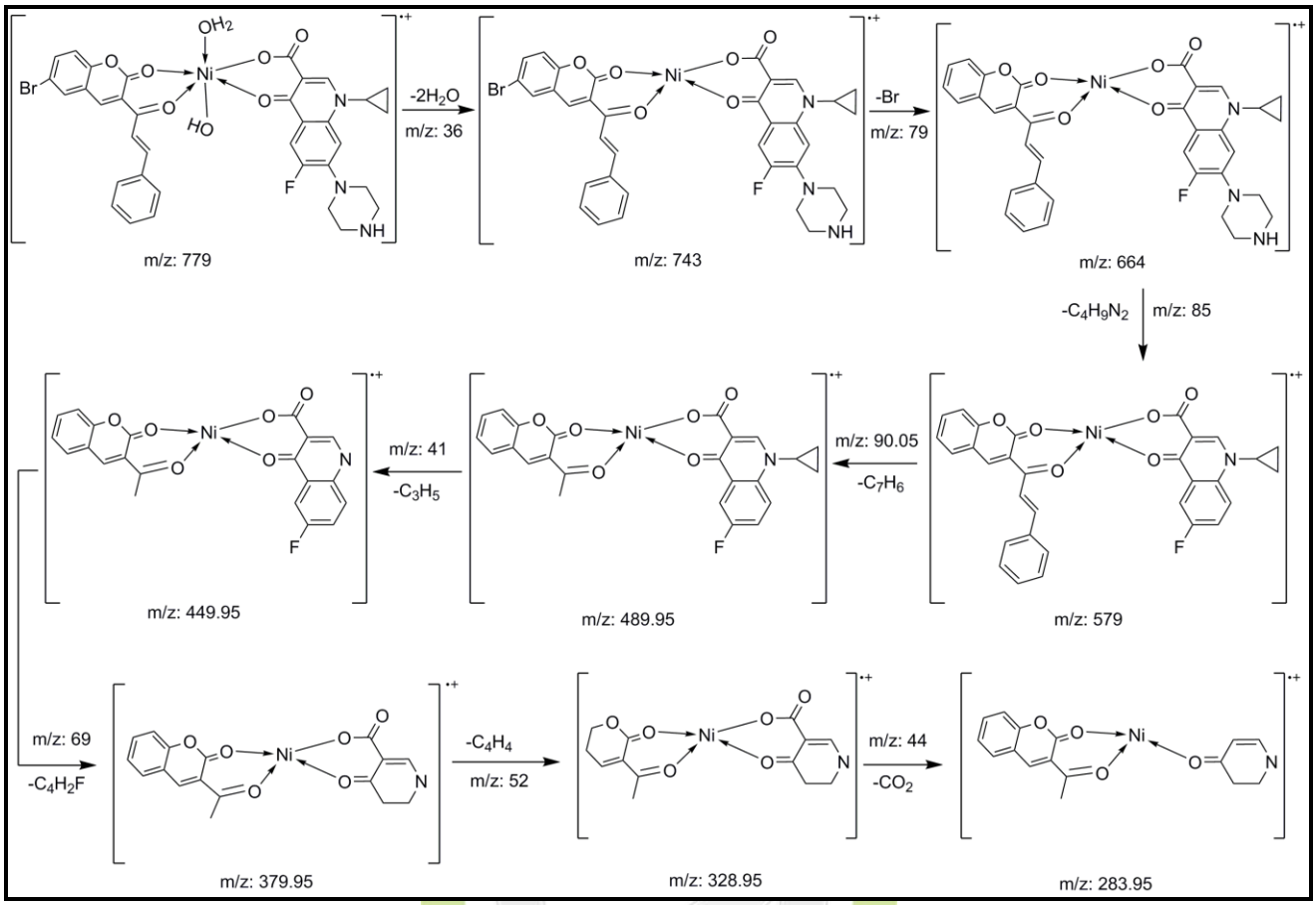


Figure 4: FAB mass fragmentation Scheme of complex C^1

Table 4: Antimicrobial results of compounds

Compounds	Minimal Inhibition Concentration [a] of microorganisms (µg/mL)					
	Bacteria				Fungi	
	S.P.	***B.S.***	***E.C.***	***P.A.***	***C. A.***	***A. N.***
L^1	50	100	50	100	50	100
L^2	50	100	25	100	25	50
L^3	100	50	25	50	50	100
L^4	50	50	100	50	100	50
L^5	100	50	100	100	50	25
C^1	50	50	25	50	25	50
C^2	12.5	12.5	12.5	25	25	25
C^3	3.125	3.125	6.25	6.25	6.25	3.25
C^4	6.25	25	12.5	25	12.5	25
C^5	12.5	6.25	25	25	12.5	12.5
Streptomycin	0.025	0.025	0.020	0.020	NT	NT
Flucanazole	NT	NT	NT	NT	0.05	0.05

[a] Average value of triplicate results
NT = Not Tested

The ligand and its metal complexes were screened for their antibacterial activities according to the respective literature protocol[25] and the results obtained are presented in Table 4. The results were compared with those of the standard drug. All the metal complexes were more potent bactericides than the ligand.

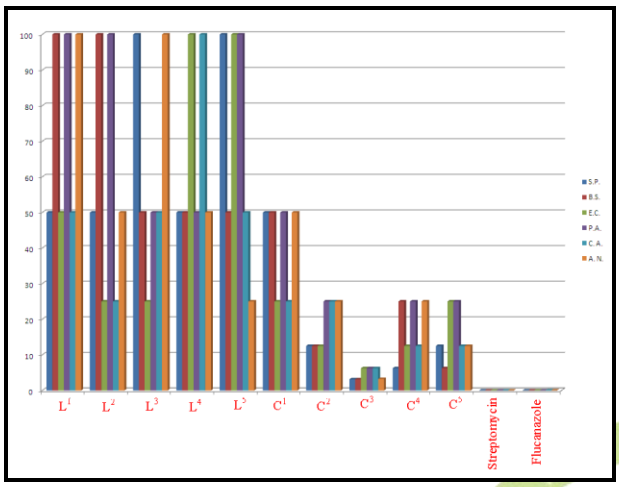


Figure 5: Statistical representation for biological activity of ligand and its complexes

Fluorescence Activity

The ligands and the Ni(II) complexes show no fluorescence at room temperature in solution. The vials contain a clear lyophilized powder of ligand and its complexes. Immediately prior to assaying, dissolve the contents of one vial with DMSO with a concentration of 1×10^{-5}M at room temperature and then add diluted Assay buffer. The ligands (A^n) and their complexes are stable for some time and increased background fluorescence is occurring shown in figs. 3 and 4, respectively.

All the ligands (L^n) exhibit fluorescent properties in the green region at room temperature, upon excitation at $\lambda_{max,\ ex}$ = 315 nm, having maximum emission peaks are at 380, 383, 386, 388 and 390nm, respectively. The four ligands are shown insignificant red shifts of the emission energies. Fluorescence emission intensities depend upon the introduction of different substitutions in the ligand molecules. Electro-donating groups and electro-withdrawing groups on the position of the coumarin ring can drastically alter the electron density and the electron conjugate system of the ligands A^n [26, 27]. The Fluorescence emission intensities of the their Ni(II) complexes are excited at the same wavelength, the spectra of the complexes $[Cu(A^n)(CF)OH(H_2O)]$ (where n = 1–5) have a dimly red shift as compared to their ligands as shown in Fig. 3, which probably was led by the charge transfer that was caused by the alternation of the structure of the ligand during the formulation of complexes. The maximum emission of complexes $[Cu(A^1)(CF)OH(H_2O)]$ is at 450 nm in the blue region. The shapes of the emission spectra of ligands and their complexes are similar, so the emission properties of complexes are believed to originated from $\pi^* \rightarrow \pi$ transitions in the ligands. The significant red shift of the fluorescence spectra of the complexes compared to ligands may be due to the chelating of the ligand to the Cu(II) ion, which enhances the ligands ability to accept electrons and decreases the electron transition energy.

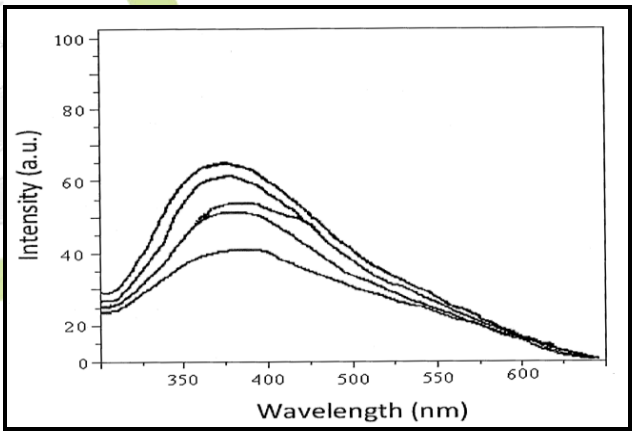


Figure 6: Fluorescence activity (A^1-A^5)

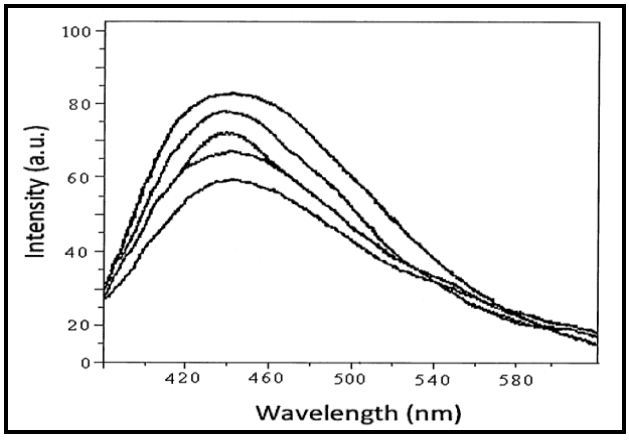


Figure 7: Fluorescence activity (C^1-C^5)

CONCLUSION

Here elucidate the synthesis of biological active coumarin derivatives (A^1-A^5) and their Ni(II) complexes (C^1-C^5). The structures of the ligands were investigated and confirmed by the elemental analysis, ^{1}H-NMR, ^{13}C-NMR and mass spectral studies while structure of metal complexes were investigated and confirmed by FT-IR and FAB-mass spectral studies. Octahedral geometry were allocate for Ni(II) complexes on the basis of electronic and magnetic moment. *In vitro* antimicrobial activity of all synthesized compounds show good results with an enhancement of activity on complexation with metal ions. This enhancement in the activity may be attributed to increased lipophilicity of the complexes. Comparative fluorescence studies of coumarin-based derivatives [A^n] (where n = 1-5) and their Ni(II) heterochelates in the solution state were carried out. The fluorescence spectra of the heterochelates showed a red shift, which may be due to the chelating of ligands to metal ion. This enhances the ligand ability to accept electrons and decreases the electron transition energy.

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