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Synthesis, characterization, fluorescent and antimicrobial properties of new Lanthanide(III) complexes derived from coumarin Schiff base

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ABSTRACT

A series of La(III), Pr(III), Nd(III), Sm(III), Eu(III), Gd(III), Tb(III), Dy(III) and Yb(III) complexes have been synthesized with the Schiff base (PHC) derived from the condensation of 8-formyl-7-hydroxy-4-methyl-coumarin with 3-amino pyridine. The prepared Schiff base and their lanthanide complexes have been characterized by elemental analysis, molar conductance, magnetic susceptibilities electronic, IR, ^{1}H-NMR, ESR, FAB-mass and thermal studies. The complexes have stoichiometry of the type $[Ln(PHC)_2(NO_3)(H_2O)_2].2H_2O$, where the ligand PHC coordinates to the lanthanide ion through azomethine nitrogen and phenolic oxygen of coumarin moiety via deprotonation .The Fluorescence properties of the synthesized compounds have also been studied. The compounds have been screened in vitro for antibacterial and antifungal activities. Active compounds have been assayed with MIC values. The antitubercular activities for the compounds have been carried out against, $H_{37}Rv$ Strain by middle Brook Method. The brine shrimp bioassay was also carried out to study the in vitro cytotoxicity properties for the ligand and their corresponding complexes.

Keywords: Coumarin, Lanthanide, fluorescent probe, biological studies, cytotoxicity.

INTRODUCTION

Coumarin derivatives are known for their physiological, photodynamic, anti- coagulant, bacteriostatic and antitumor activity [1]. A large number of structurally novel coumarin derivatives have ultimately been reported to show substantial cytotoxic and anti-HIV activity *in*

vitro and *in vivo* systems [2]. Recently, coumarin derivatives have been evaluated in the treatment of human immunodeficiency virus, due to their ability to inhibit human immunodeficiency virus integrase [3,4]. Several authors have reported the use of 7-hydroxycoumarin as the treatment of human carcinomas [5] and the inhibition of growth of cell lines of various types of cancer [6–8]. The coumarin derivatives play the vital role in designing of new cytotoxic agents [9, 10].

Metal complexes are being used as diagnostic agents and have opened relatively new area of medicinal research and have flourished rapidly from last 4-5 decades. The optical and fluorescent properties of coumarins have attracted wide range of researchers [11,12]. Coumarins have proven their applications in optical brighteners, laser dyes, sensitizers in phototherapies [13].

I.P.Kostova et.al [14] have prepared the Lanthanide(III) complexes with 4-methyl-7-hydroxy coumarin. Cerium complexes have been analyzed and Characterized by elemental analysis, conductometry, IR, NMR, DTA and TGA studies.

T.R. Goudar et.al [15] have reported the synthesis and physico chemical properties of some Lanthanide(III) complexes with 2-(3-comaryl) imidazole pyridine. Biological activity of ligands increased upon chelateion. Chelateion with Lanthanides have increased the optical properties of coumarins [16].

I. Manlolov Few lanthanide complexes of coumarins with carboxylic acid group have shown antiproliferative activities [17]. Neodymium(III) Complexes of 4-Hydroxycoumarins have exhibited anticoagulant activity [18].

Going through the literature survey and considering the vital role of the coumarin derivatives in biological applications and diagnostic parameters with fluorescent activities, the present work was undertaken to prepare lanthanide(III) complexes with a coumarin Schiff base (PHC), further to know the coordination behavior and the varying biological and fluorescent properties of the synthesized complexes.

EXPERIMENTAL SECTION

Materials:
All the chemicals were of A.R. grade and were used without further purification. Hydrated lanthanide (III) nitrates were prepared by dissolving lanthanide(III) oxides in excess of aqueous nitric acid.

Measurements:
Elemental Analyses (C, H and N) were performed on a Perkin- Elmer 2400 CHN elemental Analyzer Model 1106. The IR spectra of the ligands and their Lanthanide(III) complexes were recorded on a HITACHI-270 IR spectrophotometer in the 4000-400 cm^{-1} region in KBr disks. Molar conductivity measurements were recorded on an ELICO-CM-82 T conductivity bridge with a cell having cell constant 0.51. The electronic spectra of the complexes were recorded in DMF on a VARIAN CARY 50-BIO UV-spectrophotometer in the region of 200-1100 nm. The proton ^{1} HMR spectra of ligands were recorded in $CDCl_3$ on BRUKER 300 MHz spectrometer at

room temperature using TMS as an internal reference. FAB mass spectra were recorded on a JEOL SX 102/DA-6000 mass spectrometer/data system using Argon/Xenon (6 KV, 10Am) as the FAB gas. The accelerating voltage was 10 KV and the spectra were recorded at room temperature m-Nitrobenzyl alcohol was used as the matrix. The mass spectrometer was operated in the +ve ion mode. Thermogravimetric analysis data were measured from room temperature to 1000°C at a heating rate of 10°C/min. The data were obtained by using a PERKIN-ELMER DIAMOND TG/DTA instrument. Fluorescence spectra were measured with F-2000 Hitachi fluorescence spectrophotometer.

Synthesis of ligand and complexes

***Synthesis of ligand* (PHC)**

The one of the compound viz., 8-formyl-7-hydroxy-4-methyl-coumarin required for the synthesis of PHC was prepared by the reported procedure [19]. Equimolar ratios of 8-formyl-7-hydroxy-4-methyl-coumarin (0.01mol) and 3-amino-pyridine(0.01 mol) were refluxed for a period of 2 h in ethanol, orange colored Schiff base obtained was filtered, washed with excess of ethanol, dried and recrystallised from dioxane.

Yield -72%; Colour: Light orange; m.p. 172-174^{0}C.

Synthesis of Lanthanide(III) complexes

All the lanthanide (III) complexes were synthesized by the following procedure, Lanthanide (III) nitrates (0.001mol) were dissolved in dry alcohol. The ligand (0.001mol) in the same solvent (10mL) was added to the above solution with constant stirring and was further refluxed for 1h. The pH was then raised to 6.5 to 7.5 by adding sodium acetate (dissolved in minimum amount of alcohol) and the solution was further refluxed for 4-5h on a water bath. The solution was then concentrated to a small volume and the precipitate obtained was washed with absolute alcohol and dried under vacuum at room temperature.

RESULTS AND DISCUSSION

The synthesized complexes are stable at room temperature, insoluble in water, partially soluble in methanol and ethanol, completely soluble in DMF and DMSO. The elemental analyses, magnetic moments and molar conductance data given in (Table.1) are consistent with the general formula $[Ln(PHC)_2(NO_3)(H_2O)_2].2H_2O$. The ligand is a bidentate coordinating through azomethine nitrogen, phenolic oxygen of coumarin moiety via deprotonation. The molar conductance values in DMSO in 10^{-3}M fall in the range 22-30 Ω^{-1} indicating non-electrolytic nature of the complexes [20].

Magnetic studies:

All the complexes except La(III) are paramagnetic in nature. The magnetic moments of the complexes do not deviate much from Van Vleck [21] and Hund's values [22]. Non-deviation of magnetic moment values also indicates that there is no metal-metal interaction between the adjacent metal atoms either through bridging or super-exchange mechanism. However, Sm(III) and Eu(III) showed slight higher values which originated due to low ***J-J*** separation leading to thermal population of next higher energy ***J*** levels and susceptibility due to first order Zeeman effect [23]. The magnetic moment values agree with those reported for typical lanthanide

complexes and indicate the non involvement of 4f electrons in bonding due to their very effective shielding by the electrons in $5s^2\ 5p^6$ orbitals [24].

Table.1. Analytical, magnetic and conductance data of the PHC and its Lanthanide (III) complexes.

Comp. code	Complex	C	H	N	M	Magnetic	Molar conductance Ohm^{-1} cm2 mol^{-1}	Molar conductance Ohm^{-1} cm2 mol^{-1}
PHC	$C_{16}H_{12}O_3N_2$	68.31 (68.57)	4.03 (4.29)	9.84 (10.00)	-	-	-	-
L_1	$[La(PHC)_2(NO_3)(H_2O)_2].2H_2O$.	47.93 (48.19)	3.23 (3.51)	8.59 (8.78)	17.29 (17.43)	Dia	45.19	45.19
L_2	$[Pr\ (PHC)_2(NO_3)(H_2O)_2].2H_2O$.	47.78 (48.07)	3.21 (3.50)	8.54 (8.76)	17.48 (17.64)	3.84	41.98	41.98
L_3	$[Nd(PHC)_2(NO_3)(H_2O)_2].2H_2O$.	47.61 (47.83)	3.19 (3.49)	8.49 (8.72)	17.86 (18.05)	3.63	47.56	47.56
L_4	$[Sm(PHC)_2(NO_3)(H_2O)_2].2H_2O$.	47.34 (47.50)	3.17 (3.46)	8.41 (8.66)	18.37 (18.60)	1.87	47.81	47.81
L_5	$[Eu(PHC)_2(NO_3)(H_2O)_2].2H_2O$.	47.14 (47.41)	3.15 (3.46)	8.38 (8.64)	18.49 (18.76)	3.79	48.53	48.53
L_6	$[Gd(PHC)_2(NO_3)(H_2O)_2].2H_2O$.	46.88 (47.10)	3.11 (3.43)	8.34 (8.59)	19.07 (19.29)	7.92	47.39	47.39
L_7	$[Tb(PHC)_2(NO_3)(H_2O)_2].2H_2O$.	46.73 (47.00)	3.09 (3.43)	8.31 (8.57)	19.22 (19.45)	9.93	49.36	49.36
L_8	$[Dy(PHC)_2(NO_3)(H_2O)_2].2H_2O$.	46.55 (46.80)	3.08 (3.41)	8.27 (8.53)	19.64 (19.81)	10.89	49.72	49.72
L_9	$[Yb(PHC)_2(NO_3)(H_2O)_2].2H_2O$.	46.04 (46.21)	3.04 (3.37)	8.18 (8.42)	20.63 (20.82)	4.39	47.38	47.38

Electronic spectra:

The electronic spectral data of the two representative complexes are given in Table 2. The free ligand exhibits an intense bands at 290 and 330 nm which are assigned to the n-π* transitions. On comparison with the respective aqua ions, it has been found that in the spectra of the complexes, these bands have been shifted to lower energy levels. In the present complexes most of the absorption bands due to the f-f transitions of the lanthanide ions in the visible region are obscured. Only in the case of Nd(III) and DyIII) complexes, the f-f bands were observed and are given in Table 2.

The various spectral parameters, viz., Nephelauxetic ratio (β), bonding parameter ($b^{1/2}$), Sinha's covalency parameter (% δ) and angular covalency parameter (η) have been calculated [25-26]. The Sinha's parameter(δ) is taken as a measure of covalency and was given by $\boldsymbol{\% \delta = [(1-\beta_{av})/\beta_{av}]\times 100}$, where β_{av} is the average value of the ratio of the bonding parameter $b^{1/2}$, magnitude of which suggests the comparative involvement of the *4f* orbitals in metal-ligand bonding and is related to the Nephelauxetic ratio β and is given by the expression, $\boldsymbol{b^{1/2} = [(1-\beta_{av})/2]^{1/2}}$.

The β values obtained are less than one and positive values of $b^{1/2}$ and δ indicate weak covalent bonding between the metal and the ligand. The covalency decreases from Pr(III) to Sm(III) complexes which is due to lanthanide contraction [27]. According to Karraker, the shape of the hypersensitive transition reflects the environment of the metal ion. On comparison of the spectra

$\nu_{complex}$

ν_{aqua}

with that of known compounds, it is concluded that the present complexes have a coordination number eight [28].

Table. 2. Electronic spectra data (in cm^{-1}) and related bonding parameters of a few Lanthanide (III) complexes of PHC.

Complex	Assignments	λ_{max} of Ln^{+3} ion	λ_{max} of complex	β	Related parameters
$[Nd(PHC)_2(NO_3)(H_2O)_2].2H_2O$.	$^4I_{9/2} \rightarrow {}^4F_2$ $^4I_{9/2} \rightarrow {}^2H_{9/2}$ $^4I_{9/2} \rightarrow {}^4F_{7/2}$ $^4I_{9/2} \rightarrow {}^3P_{1/2}$ $^4I_{9/2} \rightarrow {}^4G_{11/2}$	11487 12369 13516 20188	11409 12296 13438 20096	0.9932 0.9941 0.9942 0.9954 β_{av}=0.9942	δ= 0.58338 $b^{1/2}$=0.05385 η= 0.076379
$[Dy(PHC)_2(NO_3)(H_2O)_2].2H_2O$.	$^6H_{15/2} \rightarrow {}^6F_{5/2}$ $^6H_{15/2} \rightarrow {}^4I_{15/2}$ $^6H_{15/2} \rightarrow {}^4G_{11/2}$	11968 22287 23509	11894 22154 23418	0.9938 0.9940 0.9961 β_{av}=0.9946	δ= 0.54293 $b^{1/2}$=0.05196 η= 0.073684

Infrared Spectra

For the ligand PHC a high intensity band observed *ca*. at 1618 cm^{-1} is assigned to $\nu_{(C=N)}$ vibration suggesting the formation of Schiff base. Broad weak band at 2854 cm^{-1} is assigned to H-bonded – OH in the Schiff base. The band at 1576 cm^{-1} is assigned to the combination of $\nu_{(C=C)}$ and $\nu_{(C=N)}$ of the pyridine ring. A high intensity band in the region 1305 cm^{-1} is assigned to phenolic $\nu_{(C-O)}$ vibration and 1734 cm^{-1} for lactone carbonyl.

The $\nu_{(C=N)}$ of PHC appeared at 1618 cm^{-1} has shifted to 1600-1607 cm^{-1} in the complexes. This lower shift supports the coordination of metal ion with azomethine nitrogen.

The disappearance of the band at 1305 cm^{-1} and appearance of new medium intensity bands in the region 1384 cm^{-1} for $\nu_{(C-O)}$ supports the coordination of phenolic oxygen to the metal ion via deprotonation. The unaltered position of $\nu_{(C=O)}$ at *ca.*1734 cm^{-1} confirms its non-involvement in the coordination. The complexes show six absorption bands near 1470, 1290, 1040, 815, 740 and 695 cm^{-1} which are assigned, respectively to ν_4, ν_1, ν_2, ν_6, ν_3 and ν_5, vibrations of the coordinated (C_{2v}) nitrate group. The magnitudes of ν_4- ν_1, and ν_3- ν_5 lie in the range 186-200 and 55-65 cm^{-1}, respectively indicating the coordination of nitrate groups in bidentate fashion [29].

A broad band at 3360-3400 cm^{-1} and a peak at *ca.*950 cm^{-1} in the complexes indicates the presence of coordinated water molecules [30]. Further the presence of coordinated and non-coordinated water molecules is confirmed by the TGA studies. The appearance of two strong bands at 530-510 cm^{-1} and at 470-440 cm^{-1} are assignable to ν(Ln-N) and ν(Ln-O) vibrations respectively [30]. The important infrared spectral data of the ligand PHC and its complexes are given in Table 3.

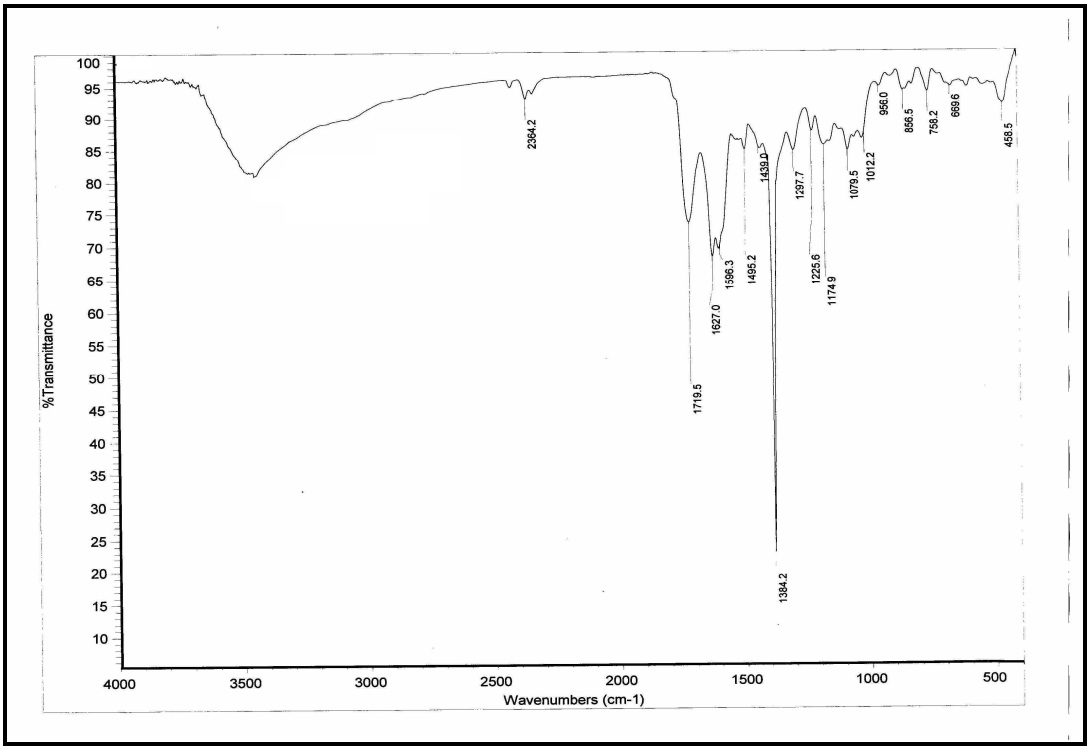


Fig. 2. IR Spectrum of PHC

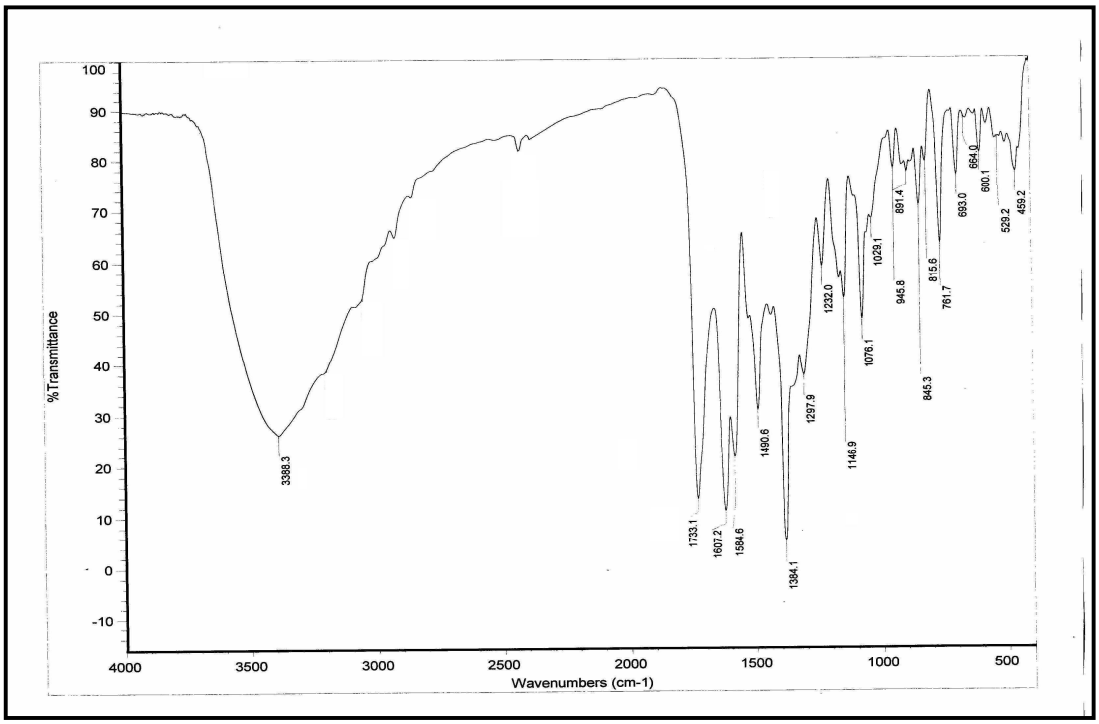


Fig. 3. IR Spectrum of $[Tb(PHC)_2(NO_3)(H_2O)_2].2H_2O$.

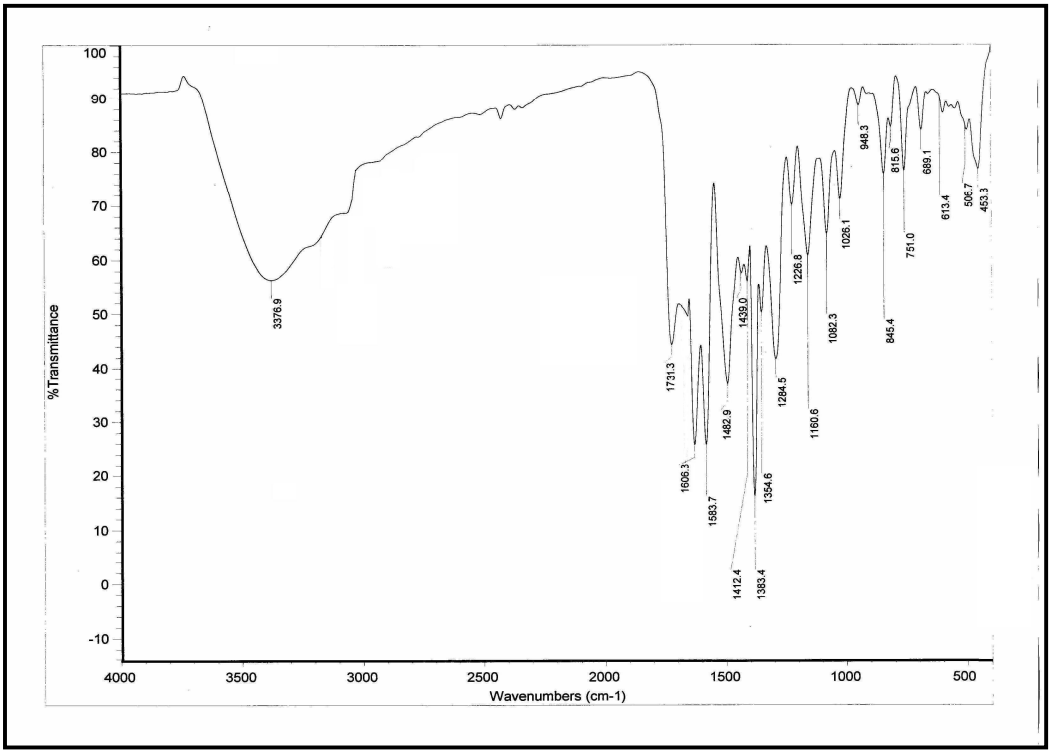


Fig. 3. IR Spectrum of $[Tb(PHC)_2(NO_3)(H_2O)_2].2H_2O$.

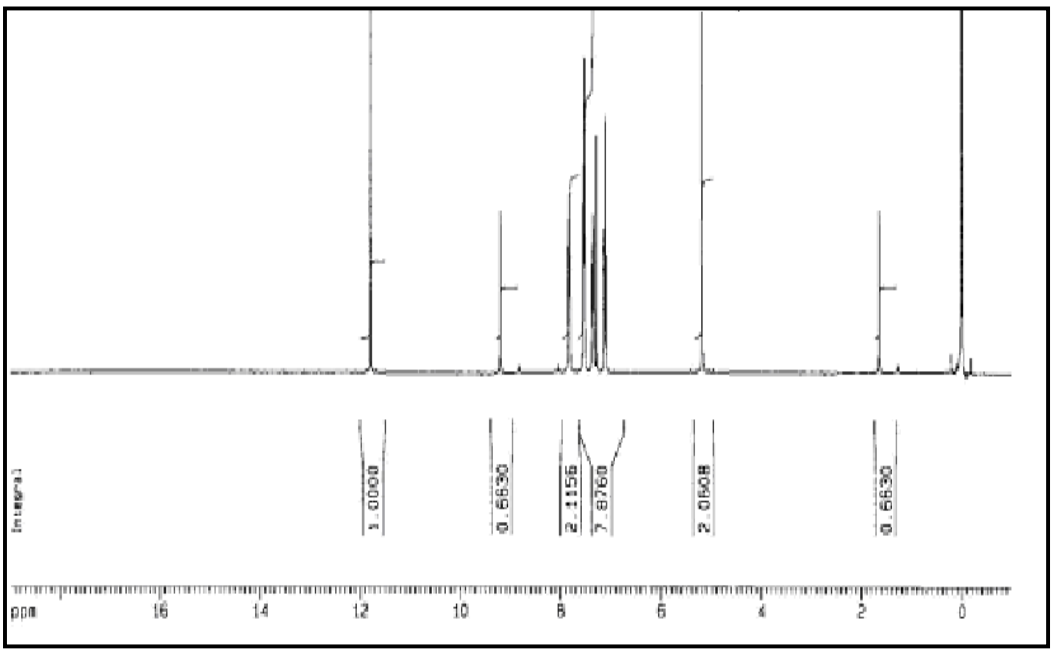


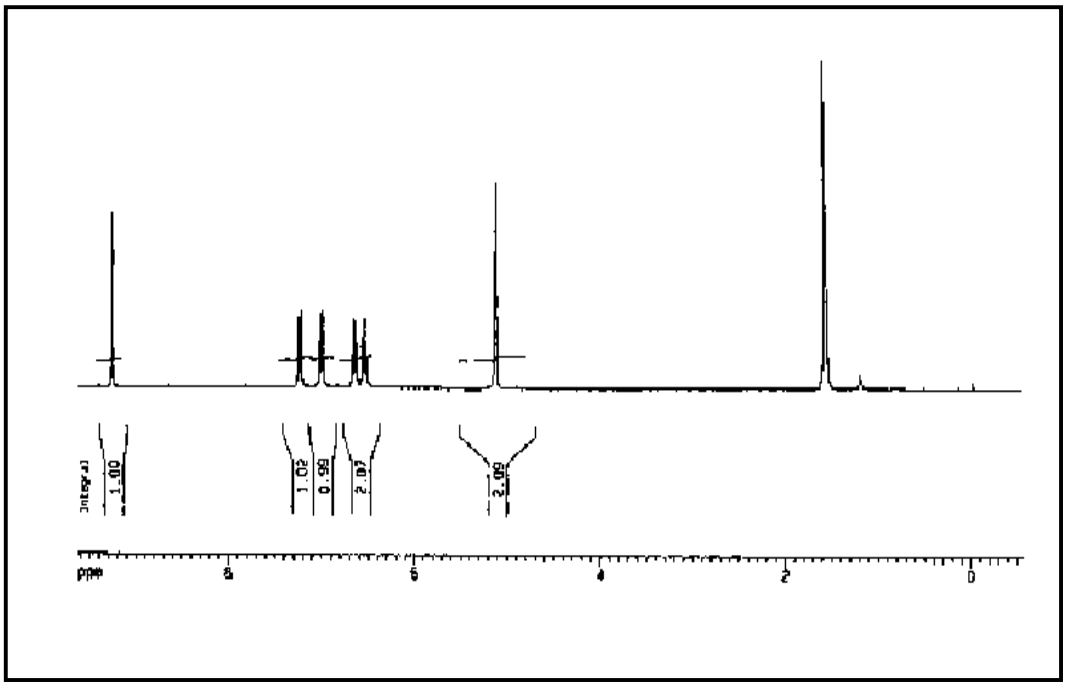
Fig. 4. ^{1}H NMR Spectrum of PHC.

Table. 3. Infrared spectral data (in cm^{-1}) of PHC and its Lanthanide(III) complexes.

Code.	Phenolic $\nu_{(OH)}$	$\nu_{(OH)}$ of water	$\nu_{(C=O)}$	$\nu_{(C=N)}$	ν_4	ν_1	ν_2	ν_6	ν_3	ν_5	$\nu_{(M-N)}$	$\nu_{(M-O)}$
PHC	3118b	-	1731s	1625s	-	-	-	-	-	-	-	-
L_1	-	3401b	1729s	-	1468s	1277m	1038w	816w	730w	697w	490m	428w
L_2	-	3394b	1730m	1608s	1470s	1280m	1041w	814w	731w	695m	478m	430w
L_3	-	3397b	1728s	1612s	1469s	1279m	1040w	815w	728w	694m	485m	427w
L_4	-	3392b	1730s	1610s	1470s	1281m	1041w	817w	729w	695m	490m	418w
L_5	-	3410b	1731s	1604s	1468s	1280m	1038w	813w	730w	693m	489m	426w
L_6	-	3398b	1730s	1600s	1470s	1279m	1040w	815w	731w	695m	490w	428w
L_7	-	3404b	1731m	1609s	1468s	1280m	1039w	813w	728w	695m	470m	430w
L_8	-	3396b	1727m	1611s	1471s	1282m	1040w	817w	727w	696w	476w	424w
L_9	-	3391b	1730s	1608s	1470s	1281m	1042w	815w	731w	694m	485m	429w

^{1}H NMR studies:

The ^{1}H NMR spectrum of the ligand PHC taken in $CDCl_3$ and its La(III) complex taken in DMSO-d_6 exhibits resonance at 9.3 ppm due to the azomethine proton. A singlet corresponding to one proton observed at 12.4 ppm is due to phenolic OH group. The observed increase in the chemical shift is due to deshielding of the hydrogen bonded proton. The sharp multiplet signals of the phenyl protons are found in the region 6.2-7.8 ppm. The methyl protons of the ligand are observed as a sharp peak at 2.3 ppm. The peak due to phenolic OH at 12.4 ppm is not observed in the complex. This confirms the involvement of phenolic oxygen in coordination with the metal via deprotonation. The downfield shift of the azomethine proton from 9.3 ppm in the ligand spectrum to 9.8 ppm in the complex indicates the participation of azomethine nitrogen in the coordination [31].



***FAB mass spectrum*:**

The FAB mass spectrum of one of the representative Tb(III) complex **(L7)** showed a molecular ion peak M^+ at m/z 853 which is equivalent to its molecular weight of the complex $[Tb(PHC)_2(NO_3)(H_2O)_2].2H_2O$. This molecular ion undergoes fragmentation with the loss of two lattice water molecules and one coordinated nitrate molecule gave a species *i.e.* $[Tb(PHC)_2(H_2O)_4]^+$ at m/z 756. Further, this fragment ion by the loss of two coordinated water molecules gave a fragment ion at m/z 720. Finally it undergoes demetallation to form the species $[2\ (PHC) + H]^+$ gave a fragment ion at m/z 560. The fragment patterns are well observed in the Figure. 6.

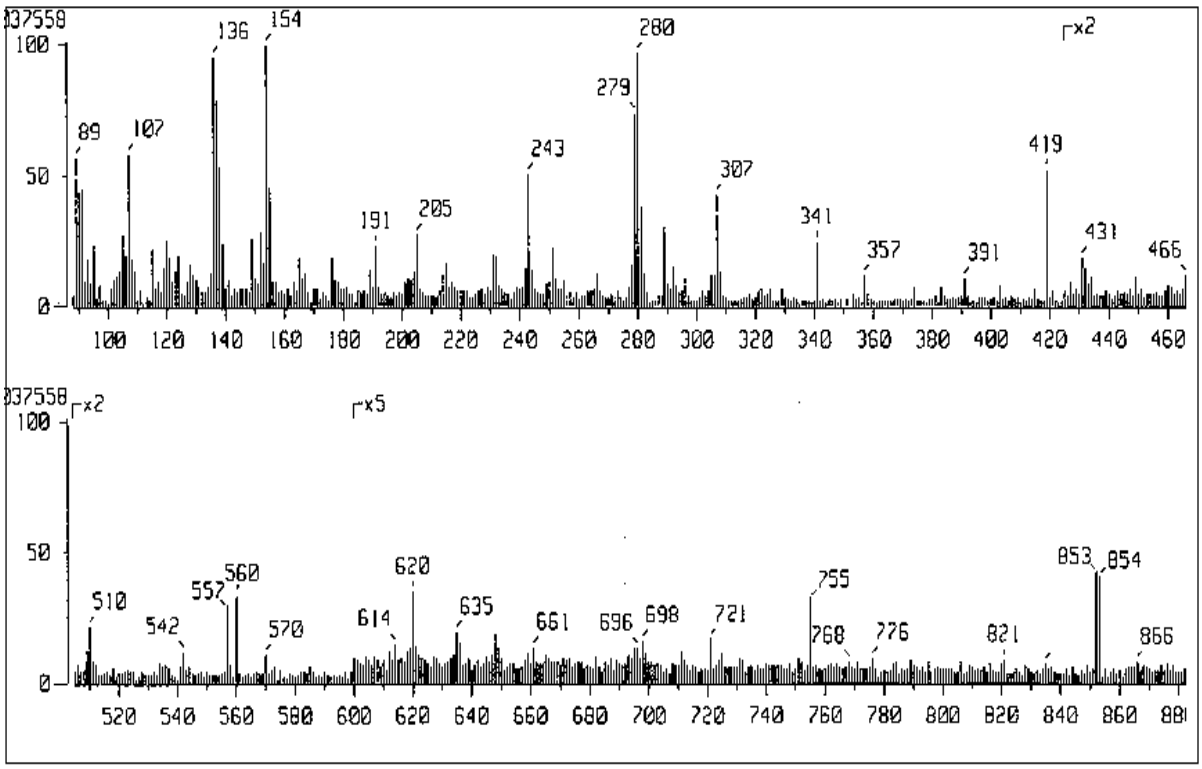


Figure. 6. FAB-mass Spectrum of one of the representative complex (L_7)

***3.5. EPR spectra*:**

Gd (III) has an $^8S_{7/2}$ single ion ground state and a spin value (*I*) of 3/2. The energy level of lowest excited state is very high with there being no contribution from orbital angular momentum and the anisotropic effect [32]. The EPR spectrum of the present Gd(III) complex exhibits a single broad band with (g) value of 2.01 (at RT) and 2.03 at (LNT), taking the (g) value of free-ion TCNE being 2.00277. It is observed that the fine structure, as a consequence of Gd^{+3} zero-field splitting is lacking, instead a broad band is obtained which indicates that the Gd^{+3} complex is located in a rather disordered environment. Thus, the spectrum was an average overall possible realization of the crystal field, which can be influenced by distribution of hydrogen bonds. The random H-bonds between water molecules and complex induce small distortions, which lead to line broadenings. This phenomenon called g-strain for the g-tensor distribution and D-strain for the zero-field splitting (ZFS) distribution, leads to broad asymmetric EPR line shapes [33]. Same band widths and almost similar g values at both the temperatures indicates line widths are

independent of temperature with equal contributions from spin-lattice relaxation processes and spin-spin relaxation processes [34]. The ESR spectrum of the Gd complex (**L_6**) at RT is represented in the Figure 7.

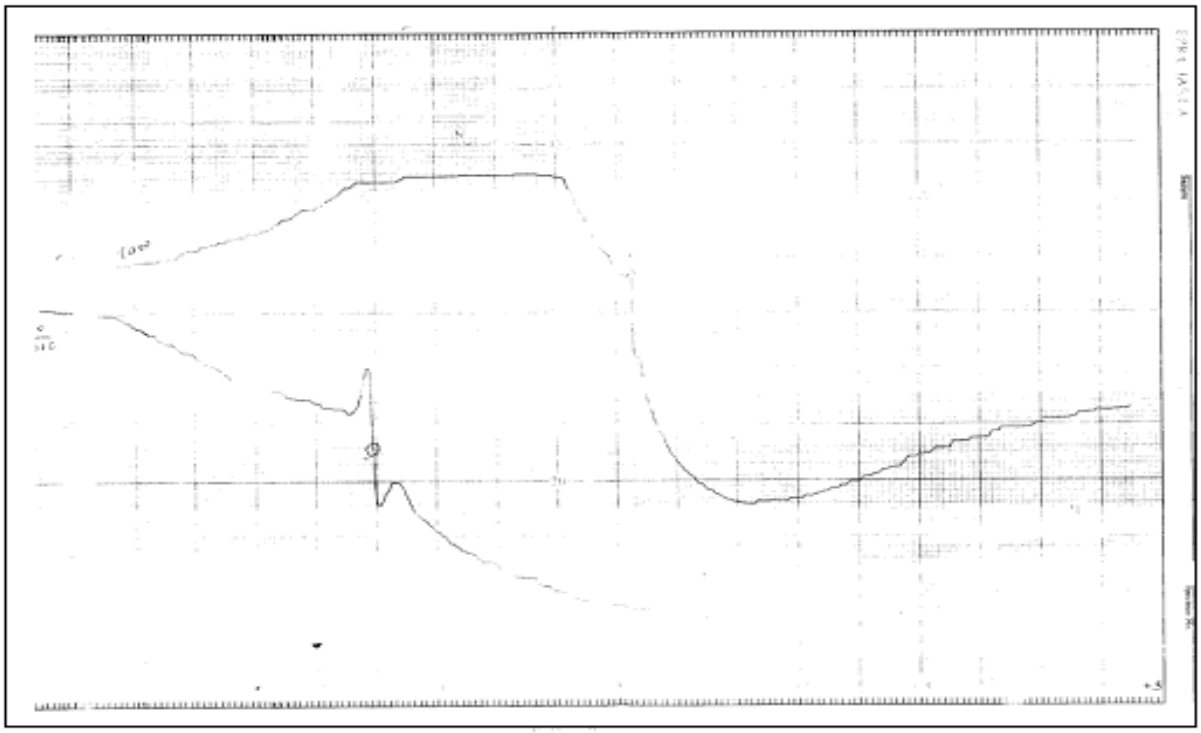


Figure 7. ESR Spectrum of one of the representative (L_6) complex.

Table 4. Thermal data of representative Lanthanide(III) complexes

Code	Complex	Temp (^oC)	% weight loss	Proposed chemical change	%Metal
L_5	$[Eu((PHC)_2(NO_3)(H_2O)_2].2H_2O$.	60-75 240-245 317-320 436-440	4.24 (4.45) 4.29 (4.45) 7.48 (7.65) 68.89 (69.14)	Two lattice water molecules Two coordinated water molecules One ionic nitrate molecule Two Ligand moieties.	18.57 (18.76)
L_9	$[Yb(PHC)_2(NO_3)(H_2O)_2].2H_2O$.	78-80 239-242 316-320 436-440	4.11 (4.33) 4.07 (4.33) 7.29 (7.46) 67.18 (67.39)	Two lattice water molecules Two coordinated water molecules One ionic nitrate molecule Two Ligand moieties	20.65 (20.82)

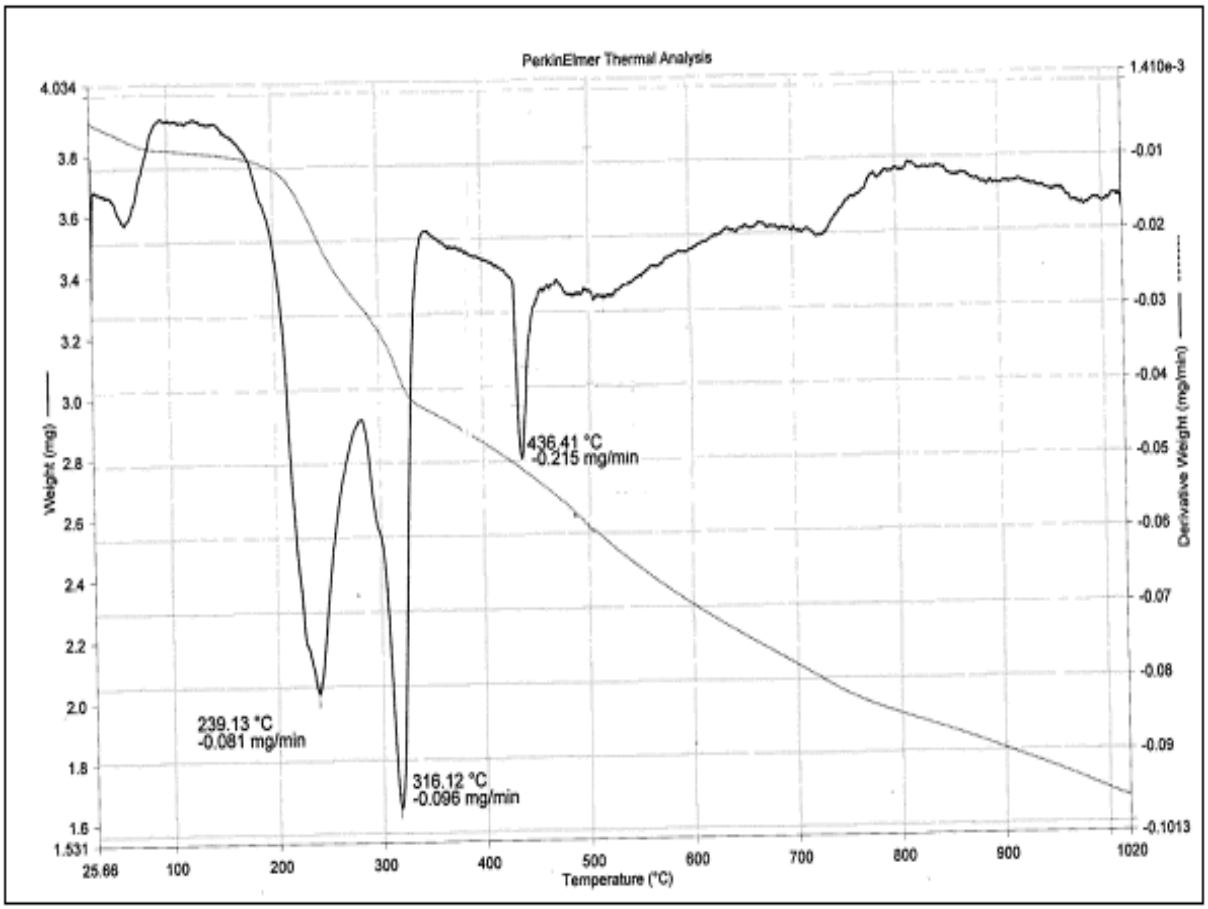


Figure. 8. Thermogram of one of the representative (L_9) complex.

***Thermal studies*:**

The thermal decomposition of the representative $[Yb(PHC)_2(NO_3)(H_2O)_2].2H_2O$. is studied as function of temperature. The decomposition of the complex proceeds with an endothermic peak in the temperature range 60-80 ^{0}C decomposing two lattice water molecules. In the temperature range 239-242 ^{0}C the decomposition of two coordinated water molecules is predicted. Further in the temperature range 316-320 ^{0} C the decomposition of one coordinated ionic nitrate molecule takes place. The temperature range of 436-440 ^{0} C decomposes the two ligand moieties. Finally the most stable metal oxide form is obtained. The percentage weight loss, nature of decomposed chemical change with the temperature range and percentage of metal oxide obtained are in good agreement with calculated values (table 4). Thermogram of one of the representative (**L_9**) complex. Presented in Figure 8.

Fluorescence Spectra

The emission spectra of the Schiff base (PHC) and its Ln(III) complexes are studied in different solvents like DMF, THF, Acetonitrile and DMSO at (EX-370 nm). Among the solvents, the better fluorescent properties of the ligand and the complexes are found in DMF. The most intense transitions are observed at 515 nm and 525 nm for L7 and **L_8** complexes respectively. Lanthanide (III) ions are weakly luminescent in aqueous solution as such their molar absorptivity

are low and the excited states are effectively quenched by solvent molecules with weak light emission. Coordinated aromatic ligands may greatly enhance the luminescence by absorbing energy and transferring it to the central ion, and by extruding water molecules from the Inner-coordination sphere of the Lanthanide (III) ion. In case of $\mathbf{L_7}$ and $\mathbf{L_8}$ chelates, the energy transfer from the excited single state of the ligand to its triplet state and further to the metal ion is effective, as shown by the fact that the strongest emission of these chelates are long-lived luminescence emanating from the metal ion [35]. With the chelates of the other lanthanide ions, the excited ligand may return to the ground state either with concomitant emission of a prompt ligand luminescence, or without light emission. $\mathbf{L_7}$ and $\mathbf{L_8}$ chelates also have the properties that make their usage as luminescent markers with the advantage of larger difference between the wavelength of excitation and emission. [36-37].

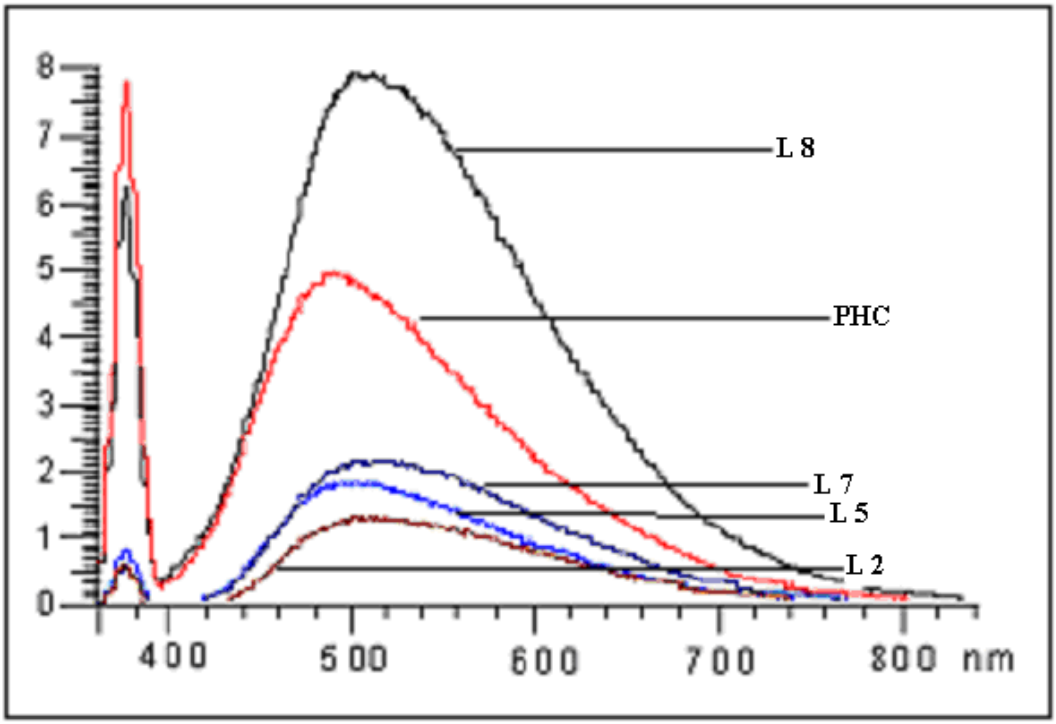


Figure. 9. Fluorescent spectra of the compounds

Biological Studies

Antibacterial and antifungal activities:

Antibacterial and antifungal activities of ligand and its complexes are tested against two bacteria such as *S.Aurease*, *Escherichia.Coli* and two fungi *A.Niger*, *C.Albicans*. Norfloxacin for bacteria and Grisiofulvin for fungi are used as standard drugs. The zone of inhibition in mm for the ligand and their Lanthanide(III) complexes are presented in Table 5. From the data it is clear that the metal chelates exhibit higher antimicrobial activity than that of the free ligand molecule. The compounds are found to be more susceptible towards the bacterial strains as compared to the fungal strains .The complexes $\mathbf{L_1}$, $\mathbf{L_4}$ and $\mathbf{L_8}$ are most active among the other complexes.

Table 5. Antibacterial and antifungal activity of representative Schiff base and its Lanthanide(III) complexes (zone of inhibition in mm)

Compuond Code	Antibacterial		Antifungal	
	S..Aurease	*E.coli*	*A.niger*	*C. Albicans*
PHC	17	16	14	14
L_1	20	18	16	16
L_2	17	14	15	14
L_3	18	17	15	15
L_4	21	19	17	17
L_5	18	17	15	14
L_6	17	17	15	14
L_7	18	16	15	15
L_8	19	18	15	16
L_9	17	16	15	14
Norfloxacin	24	24	--	--
Grisiofulvin	--	--	24	24
DMF	04	04	04	04

Less than 10mm----Inactive; Less than 10-15mm----Weakly active
Less than 15-20mm----Moderately active; More than 20mm----Highly active

Figure. 10. Antibacterial data of the Ligands and their Complexes on *E.Coli*.

The MICs of the active compounds are carried out as described by Clause [38] with minor modifications. Antifungal activities of the yeast are performed by following the guidelines in NCCLs document M27-A using the micro dilution broth method [39]. Solutions of the test compounds and reference drug are dissolved in DMF as a concentration of 12.5 µg ml^{-1}. The twofold dilution of the compounds and reference drug are prepared (12.5, 6.25, 3.12, 1.56) μg ml^{-1}. The broths are maintained at pH 7.2 with an innoclum of (1-2) X 10^3 cells ml^{-1} by the spectrophotometric method and an aliquot of 100 μl is added to each tube of the serial dilution. The chemical compounds-broth medium serial level dilutions inoculated with each bacterium are incubated on a rotary shaker at 37 ºC for 24 h at 150 rpm. The minimum inhibitory concentrations of the active compounds are recorded as the lowest concentrations of each chemical compounds in the tubes with no growth (i.e. no turbity) of inoculated bacteria and yeast.

The MICs values are shown in Table 6. Only the active compounds **PHC, L_1, L_4** and **L_8** are evaluated for their minimum inhibitory concentrations. Compound **L_4** is most active exhibiting a MIC value of 3.12 μg / mL active compounds in medium against the bacterial strains. The activities of other compounds are in the range of 6.25-12.5 µg / mL for the strains.

The activity of any compound is a complex combination of steric, electronic and pharmacokinetic factors. A possible explanation for the toxicity of the complexes is postulated in the light of chelation theory. It is suggested that the chelation considerably reduces the charge of the metal ion mainly because of partial sharing of its positive charge with the donor groups and possible π- electron delocalization over the whole chelate ring. This increases the lipophilic character of the metal chelate which favors its permeation through lipoid layers of cell membranes. Furthermore, the mode of action of the compounds may involve the formation of a hydrogen bond through the –N=C group of the chelate or the ligand with the active centers of the cell constituents resulting in interference with the normal cell process. The higher bacterio toxicity experienced by the compounds may be ascribed to the fact that the ligand and metal ions are more susceptible towards the bacterial cells than fungicidal cells. From the above studies, it is concluded that these compounds serve as good fungicides and better bactericides.

Table. 6. MICs* values of the some active compounds.

Compound Code	Antibacterial		Antifungal	
	S..Aurease	*E.coli*	*A.niger*	*C. Albicans*
PHC	6.25	12.5	12.5	12.5
L_1	6.25	6.25	12.5	12.5
L_4	3.12	3.12	6.25	6.25
L_8	6.25	12.5	12.5	12.5
Grisiofulvin	--	--	1.56	1.56
Norfloxacin	1.56	1.56	--	--

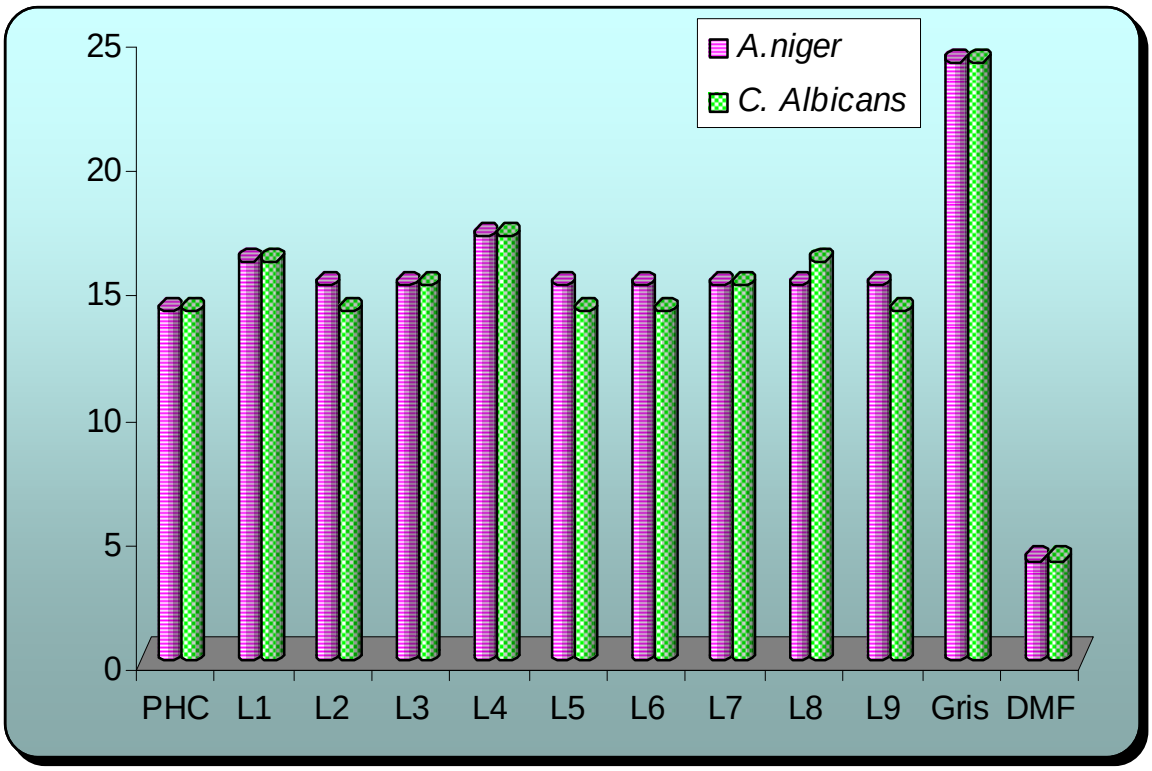


Figure. 11. Antifungal data of the Ligands and their Complexes on A.Niger.

Antitubercular assays

The ligand and its Ln(III) complexes are screened for antitubercular activity which is carried out by Middle brook 7H9 agar medium against $H_{37}Rv$ Strain[40,41]. Middle brook 7H9 agar medium containing different derivatives**,** standard drug as well as control. Only **L_4** has shown promising activity in Middle brook 7H9 agar medium against $H_{37}Rv$ Strain for this assay. All the other compounds are inactive for this assay (Table 7).

Table. 7. Antitubercular Activity*of the compounds. (zone of inhibition in mm)

Code	50 µg/ml	100 µg/ml	150 µg/ml
PHC	R	R	S
L_1	R	S	S
L_2	R	R	R
L_3	R	R	R
L_4	S	S	S
L_5	R	R	S
L_6	R	R	R
L_7	R	R	R
L_8	R	R	S
L_9	R	R	R
Standard	S	S	S

**Standard drug: Streptomycin; R-Resistance ; S-Sensitive.*

Cytotoxicity Bioassay (in vitro studies)

In the present study brine shrimp (*A.Salina. L)* eggs are hatched in a shallow rectangular plastic dish (22X32 cm), filled with artificial seawater, which is prepared [42] with commercial salt mixture and double distilled water. An unequal partition was made in the plastic dish with the help of a perforated device. 50mg of eggs are sprinkled approximately into the large compartment, which is darkened while the matter compartment was opened to ordinary light. After two days, nauplii are collected by a pipette from the lighter side. By dissolving 20mg of each compound in 2ml DMF the samples are prepared. From the stock solutions, 500,50,5µg/mL are transferred to vials (three for each dilution were used for each test sample and LD_{50} is the mean of the three values) one vial is used as a control with only 2mL DMF and another with the above concentrations of *Bleomycin* as a standard. The solvent was allowed to evaporate overnight. After two days, when shrimp larvae are ready, 1mL of seawater and 10 shrimps are added to each vial (25 shrimps/dilution) and the volume is adjusted with seawater to 5 ml per vial. After 24 h, the numbers of survivors are counted. Data is analyzed by Finney computer program to determine the LD_{50} values [43].

For the active compounds are screened for their cytotoxicity (brine shrimp bioassay) using protocol of Meyer *et al*. Only $\mathbf{L_4}$ exhibited potent cytotoxicity activity against *Artemia salina* while all the other compounds have exhibited higher values (Table 8).

Table. 8. Brine shrimp bioassay data of the some active compounds.

Compound Code	LD_{50} (M/ml)
PHC	5.432×10^{-3}
L_1	2.987×10^{-3}
L_4	6.422×10^{-4}
L_8	3.261×10^{-3}

CONCLUSION

The ligand (PHC) is bidentate molecule coordinating through azomethine nitrogen and phenolic oxygen of coumarin moiety via deprotonation . The bonding of ligand to metal ion is confirmed by the analytical, IR, ^{1}H-NMR, electronic, magnetic, FAB mass and thermal studies. The following structure shown in (Figure 1) may be proposed by the above physico-chemical and spectral observations.

In biological studies, the compounds $\mathbf{L_1}$, $\mathbf{L_4}$ and $\mathbf{L_8}$ are active but $\mathbf{L_4}$ has shown promising results. $\mathbf{L_4}$ is most active exhibiting a MIC value of 3.12 µg / mL active compounds in medium against the *S..Aurease* strain. $\mathbf{L_4}$ has exhibited promising result as antitubercular agent by Middle brook 7H9 agar medium against $H_{37}Rv$ Strain. Only $\mathbf{L_4}$ exhibited potent cytotoxic activity against *Artemia salina* with a LD_{50} value of 6.422×10^{-4} M/ml. The compounds $\mathbf{L_5}$ and $\mathbf{L_8}$ show better fluorescent properties with high stoke's shifts.

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