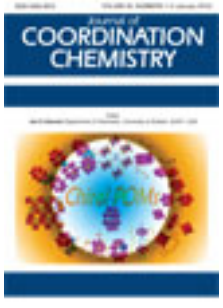

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Puja Kapoor [a], R.V. Singh [a] & Nighat Fahmi [a]
[a] Department of Chemistry, University of Rajasthan, Jaipur, Rajasthan, India
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Coordination chemistry of rare earth metal complexes with coumarin-based imines: ecofriendly synthesis, characterization, antimicrobial, DNA cleavage, pesticidal, and nematicidal activity evaluations

PUJA KAPOOR, R.V. SINGH and NIGHAT FAHMI*

Department of Chemistry, University of Rajasthan, Jaipur, Rajasthan, India

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Coordination and biocidal aspects of lanthanide(III) complexes with 3-formyl-4-chlorocoumarin hydrazinecarbothioamide (L^1H) and 3-formyl-4-chlorocoumarin hydrazinecarboxamide (L^2H) are described with the support of elemental analyses, molecular weight, melting point, and molar conductance determinations along with the IR, ^1H NMR, EPR, UV spectral, and X-ray powder diffraction studies. The spectral data suggest that the ligands are monobasic bidentate, coordinating through nitrogen and sulfur/oxygen. The isolated products are colored solids, soluble in DMSO, DMF, and methanol. All the complexes are monomeric, as indicated by their molecular weight determinations. Conductivity measurements in dry DMF show them to be non-electrolytes. The antimicrobial, DNA cleavage, pesticidal, and nematicidal activities were examined and are discussed.

Keywords: 3-Formyl-4-chlorocoumarin hydrazinecarbothioamide; 3-Formyl-4-chlorocoumarin hydrazinecarboxamide; DNA cleavage activity; Pesticidal and nematicidal activities

1. Introduction

The expanding development in the chemistry of coordination compounds is due to their applications in agriculture and medicine. Coordination complexes of sulfur and nitrogen donors have been widely investigated and show novel structural features [1], unusual spectral and catalytic properties [2], and relevance to biological systems [3]. Schiff base metal complexes act as active nematicidal agents [4]. Metal complexes of thiosemicarbazones and semicarbazones have aroused interest in industrial and biological applications [5]. Schiff bases derived from coumarin and its metal complexes exhibit antibacterial, antifungal, anticoagulation, and plant growth regulating activities [6–8]. 4-Hydroxycoumarin derivatives exhibit an inclination for complexation [9–14] with transition and rare earth complexes of hydroxycoumarin derivatives of increasing interest in bioinorganic and coordination chemistry. Applications of trivalent lanthanide complexes as contrast agents for Nuclear Magnetic Resonance (NMR) imaging [15],

*Corresponding author. Email: nighat.fahmi@gmail.com

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stains for fluorescence imaging [16], responsive luminescent systems [17], catalysts for specific cleavage of RNA hydrolysis [18], or as active agents in cancer radiotherapy [19] have prompted considerable interest in lanthanide coordination chemistry [20]. This study deals with synthesis, characterization, and biological evaluation of lanthanide(III) complexes of coumarin-based ligands. Emphasis has been put on biological evaluation of the complexes.

2. Experimental

All the chemicals were of reagent grade, and the solvents of analytical grade were distilled from appropriate drying agents immediately prior to use. 4-Hydroxycoumarin, samarium(III), and neodymium(III) salts were purchased from Alfa Aesar. Gadolinium(III) salt was procured from Sigma Aldrich. The metal chlorides used were in hydrated form. Molecular weight determinations were carried out by the Rast Camphor method [21]. Sulfur, chlorine, and nitrogen were estimated by Messenger's [22], Volhard's and Kjeldahl's [22] methods, respectively. Metal contents were estimated complexometrically with EDTA using Erichrome Black T as an indicator. Melting points were determined by using capillaries in an electrical melting point apparatus. The conductivity values measured on 10^{-3} mol dm^{-3} solution in DMF at room temperature on a Century Digital Conductivity Meter model CC601. Magnetic moment measurements were taken on a model 155 vibrating sample magnetometer. Infrared (IR) spectra of the ligands and their complexes were recorded on a Nicolet Magna FTIR-550 spectrophotometer as KBr pellets. ^{1}H NMR spectra were recorded on a JEOL-AL-300 FT NMR spectrometer in DMSO-d_6 using TMS as the internal standard. Electron Paramagnetic Resonance (EPR) spectra of the complexes were monitored on a Varian Make E Line Century X-band EPR spectrometer (Model E-112). The electronic spectra were recorded on a Varian–Cary/5E spectrophotometer.

2.1. *Preparation of the ligands*

2.1.1. Synthesis of 3-formyl-4-chlorocoumarin. 3-Formyl-4-chlorocoumarin is prepared as described [23]. Phosphorus oxychloride (10 mL) was added dropwise to a solution of DMF (20 mL) keeping the temperature below 50°C. Solution of 4-hydroxycoumarin (4.0 g) in DMF (10 mL) was then gradually added to the mixture with constant stirring and temperature was maintened below 5°C. The reaction mixture was then allowed to stand at room temperature for 2 h and heated on a steam bath for 1 h. After cooling, the reaction mixture was poured onto crushed ice and neutralized with sodium carbonate. A solid product was immediately formed and was crystallized from ethanol to give a yellow solid, m.p. 115°C, yield 3.2 g; 80%.

2.1.2. Microwave-assisted synthesis. The ligands were prepared by the condensation of 3-formyl-4-chlorocoumarin (2.68 g, 0.01 mol) with thiosemicarbazide (1.55 g, 0.01 mol) or semicarbazide hydrochloride (1.90 g, 0.01 mol) in the presence of sodium acetate. The reaction mixture was irradiated using 2–3 mL solvent. The reactions were

Table 1. Analytical data and physical properties of the ligands and their complexes.

Compound	Color	Melting point (°C)	Found (Calcd) (%) N	Cl	S	Ln	μ_{eff} (B.M)	Mol. Wt. Found (Calcd)
1	Yellow	127	14.72 (14.92)	12.04 (12.58)	11.12 (11.38)	–	–	278.15 (281.71)
2	White	133	15.56 (15.82)	13.02 (13.35)	–	–	–	261.32 (265.65)
3	Wine red	205(d)	11.96 (12.11)	10.04 (10.22)	9.08 (9.24)	13.15 (13.86)	3.62	1031.430 (1040.419)
4	Brown	218	11.95 (12.05)	10.16 (10.71)	9.85 (10.20)	14.03 (14.37)	1.63	1034.251 (1046.539)
5	Orange	225	11.45 (11.97)	9.85 (10.10)	9.11 (9.13)	14.33 (14.93)	7.98	1037.269 (1053.429)
6	Green	195(d)	12.54 (12.70)	10.63 (10.72)	–	14.02 (14.54)	3.51	976.655 (992.224)
7	Cream	228(d)	12.52 (12.75)	10.74 (10.76)	–	15.05 (15.21)	1.52	965.573 (998.344)
8	Light green	237	12.45 (12.54)	10.48 (10.58)	–	156.15 (15.64)	7.90	993.021 (1005.234)

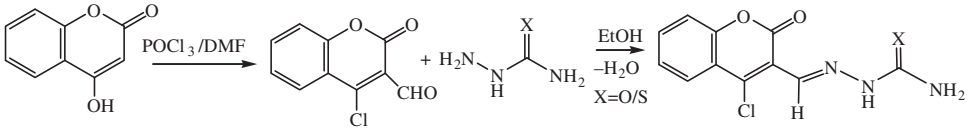

Figure 1. Synthetic scheme of the ligands.

completed in 5–6 min. After completion, the reaction was cooled to afford a solid product, which was recrystallized in alcohol, characterized and analyzed before use. Elemental analyses (N and S) were conducted using the methods mentioned above and their results were found to be in good agreement with the calculated values (table 1). The systematic scheme for preparation of the ligands is shown in figure 1.

2.1.3. Conventional thermal method. For comparison, the above ligands were also synthesized by thermal methods. In this method, instead of a few drops of ethanol, 100 mL of ethanol was used to dissolve the starting materials of the ligands and the contents were refluxed for 3.5–4 h. The residue formed was separated out, filtered off, washed with water, recrystallized from ethanol, and finally dried in vacuum over fused calcium chloride. A comparison between the thermal method and microwave method is given in table 2.

2.2. *Synthesis of the sodium salt of the ligands*

Sodium metal and the ligand were dissolved in minimum of methanol separately. Ultimately, these two solutions had been dissolved to prepare sodium salt of the ligand. In this process the sodium metal first reacts with methanol and forms sodium methoxide.

Table 2. Comparison between microwave and thermal method.

Sl. No.	Compound	Yield (%)		Solvent (mL)		Time	
		Thermal	Microwave	Thermal	Microwave	Thermal (h)	Microwave (min)
1	L^1H	85	92	100	3	3.5	5
2	L^2H	84	90	100	2	4	6
3	$Nd(L^1)_3 \cdot 3H_2O$	70	82	35	3	13	8
4	$Sm(L^1)_3 \cdot 3H_2O$	72	86	40	4	14	5
5	$Gd(L^1)_3 \cdot 3H_2O$	74	82	45	2	14.5	6
6	$Nd(L^2)_3 \cdot 3H_2O$	73	85	40	3.5	13.5	5
7	$Sm(L^2)_3 \cdot 3H_2O$	78	84	35	2.5	15	5.5
8	$Gd(L^2)_3 \cdot 3H_2O$	75	83	45	3	16	6

This sodium methoxide in the next step reacts with the ligand and replaces acidic proton from the enolic form of the ligand with the sodium metal and forms sodium salt of the particular ligand.

2.3. *Preparation of the metal complexes*

2.3.1. Microwave-assisted synthesis. In microwave-assisted synthesis, the complexes were prepared by irradiating the reaction mixture of $LnCl_3 \cdot 6H_2O$ (0.01 mol) and the respective sodium salt of the ligands (0.03 mol) in 1 : 3 molar ratios. The products were recovered and dissolved in a few mL of dry methanol, where the precipitate of sodium chloride formed during the course of the reaction was removed by filtration and the filtrate was concentrated under reduced pressure. The resulting compounds were washed and recrystallized with cyclohexane.

2.3.2. Thermal method. These complexes were also synthesized by thermal methods where instead of 5–8 min, reactions were completed in 13–16 h, and the yield of the products was also less than that obtained by the microwave-assisted synthesis. In this method, $LnCl_3 \cdot 6H_2O$ (0.01 mol) was added with sodium salt of the ligands (0.03 mol) in 1 : 3 molar ratios. The resulting mixture was heated under reflux for 13–16 h, the sodium chloride formed during the course of the reaction was removed by filtration and the solvent was removed under reduced pressure. The product was dried in *vacuum*. The complexes were washed with dry *n*-hexane and recrystallized with cyclohexane.

2.4. *Pharmacology*

2.4.1. Anti-microbial studies

2.4.1.1. *Antifungal activity*. The antifungal activities were evaluated against *Candida albicans*, *Aspergillus niger*, and *Fusarium oxysporum* using Czapek's agar medium [24] having the following composition: glucose 20 g, starch 20 g, agar–agar 20 g, and distilled

water 1000 mL. The requisite amount of the compounds was added to this medium after being dissolved in methanol so as to obtain three different concentrations (50, 100, and 200 ppm). The medium then was poured into Petri plates, and the spores of fungi were placed on the medium with the help of an inoculum needle. These Petri plates were wrapped in polyethene bags containing a few drops of alcohol and were placed in an incubator at $30 \pm 2^{\circ}$C. The controls were also run, and three replicates were used in each case. The antifungal activity of a common standard flucanazole was also recorded using the same procedure as above at the same concentrations and solvent. The linear growth of the fungus was recorded by measuring the diameter of the fungal colony after 96 h, and the percentage inhibition was calculated by the following equation:

$$\% \text{ Inhibition} = (C - T)100/C$$

where C and T are the diameters of the fungal colony in the control and the test plates, respectively.

2.4.1.2. *Antibacterial activity*. Antibacterial activity was tested against *E. coli*, *P. aeruginosa*, *B. subtilis*, and *S. aureus* using the paper disc plate method [24]. Each compound was dissolved in methanol and solutions of the concentrations (500 and 1000 ppm) were prepared separately. Paper discs of Whatman filter paper (No. 42) of uniform diameter (2 cm) were cut and sterilized in an autoclave. The paper discs soaked in the desired concentration of the complex solutions were placed aseptically in the Petri dishes containing nutrient agar media (agar 20 g + beef extract 3 g + peptone 5 g) seeded with *E. coli* and *P. aeruginosa* bacteria separately. The Petri dishes were incubated at 37°C and the inhibition zones were recorded after 24 h of incubation. The antibacterial activity of a common standard antibiotic Ciprofloxacin was also recorded using the same procedure as above at the same concentrations and solvent.

2.4.1.3. *Determination of minimum inhibitory concentration*. Minimum Inhibitory Concentration (MIC) is the lowest concentration of test agent that inhibited visible growth of bacteria after 18 h incubation at 37°C. The determination of the MIC involves a semi quantitative test procedure which gives an approximation to the least concentration of an antimicrobial needed to prevent microbial growth. The MIC was determined by liquid dilution method [25]. Stock solutions of Ln(III) complexes with 10–50 $\mu g\,mL^{-1}$ were prepared with aqueous methanol. Inoculum of the overnight culture was prepared. In a series of tubes, 1 mL each of metal complex solutions with different concentrations was taken and 0.4 mL of the inoculum was added to each tube. Further, 3.5 mL of the sterile water was added to each of the test tubes. These test tubes were incubated for 24 h and observed for the presence of turbidity. The absorbance of the suspension of the inoculum was observed with a spectrophotometer at 555 nm. The end result of the test was the minimum concentration of antimicrobial (test materials) which gave a clear solution, i.e., no visual growth [26, 27].

2.4.2. Pesticidal activity. Adult red flour beetles *Tribolium castaneum* were used for the experiment. Rearing of insects was maintained at the storage section of Division of Entomology, Agricultural Research Institute, Durgapura, Jaipur, in a laboratory for bioassay. All compounds were weighed and dissolved in methanol to prepare 500, 200, and 100 ppm solutions. One mL of each concentration of various compounds was

directly poured in each petri plate (90 mm) with the help of a micropipette. Petri plates with test solution were rotated vigorously to prepare uniform film and allowed to dry for 3–5 min.

A thin layer was prepared in the petri dishes (10 cm in diameter) using solution after drying at room temperature. Thirty insects were released in treated Petri dishes. These were kept in an incubator at a fixed temperature ($30 \pm 0.5^{\circ}$C). Along with a control, experiment was replicated thrice. The mortality of these beetles was observed after 96 h. A simple microscope was used to check natural movement of the pests. The mortality percentage was calculated by using Abbott's formula [28]:

$$P_t = \frac{P_o - P_c}{100 - P_c} \times 100$$

where P_t = corrected mortality (%), P_o = observed mortality (%), and P_c = controlled mortality (%).

2.4.3. Nematicidal activity. Nematicidal activity was carried out on *Meloidogyne incognita*. Nematode *M. incognita* is known to attack more than 3000 host plants [29]. The yields of okra, tomato, and brinjal typically suffer 90.9%, 46.2%, and 2.3% losses, respectively, due to *M. incognita*. *Meloidogyne incognita* produces galls on the roots of many host plants and is responsible for 44.87% yield loss in brinjal. The root-knot nematode produces galls on the roots of many vegetables, pulses, some fruit crops, tobacco, and ornamental crops and causes severe losses.

Clean *M. incognita* eggs were obtained by the reported method [30]. Egg masses were separated from heavily infected brinjal roots and washed under running water. After cutting the roots, 1% of sodium hypochlorite solution was added, shaken, and then sieved through 150 and 400 sieves. Then the eggs of nematode were counted. A total of 150 eggs of nematode *M. incognita* were used per replicate sample and each treatment was replicated three times. The experiment was conducted at $30 \pm 2^{\circ}$C. The eggs were treated with complexes dissolved in 25, 50, and 100 ppm for 24 h and observations in relation to hatching of eggs were noted. The nematicidal activity was calculated using the following formula:

$$N_A(\%) = \frac{H_T}{H_C} \times 100$$

where N_A is the nematicidal activity, H_C is the amount of hatching in the control and H_T is the amount of hatching in the test plate.

2.4.4. DNA cleavage analysis

2.4.4.1. *Preparation of culture media.* Nutrient broth used for the growth of the organism was prepared, then autoclaved for 15 min at 121°C and 15 lb pressure. The autoclaved media were inoculated with the seed culture and incubated at 37°C for 24 h on a shaker with 180 rpm.

2.4.4.2. *Isolation of DNA.* DNA was isolated using the following procedure [31]. Fresh bacterial culture (1.5 mL) was centrifuged to obtain the pellet. The pellet was

dissolved in 0.5 mL of lysis buffer (100 mmol L^{-1} tris pH 8.0, 50 mmol L^{-1} EDTA, 50 mmol L^{-1} lysozyme). 0.5 mL saturated phenol was added and incubated at 55°C for 10 min, then centrifuged at 10,000 rpm for 10 min. To the supernatant, equal volume of chloroform : isoamyl alcohol (24 : 1) and 1/20th volume of 3 mol L^{-1} sodium acetate (pH 4.8) was added and again centrifuged at 10,000 rpm for 10 min; 3 volumes of chilled absolute alcohol were added to the supernatant. The precipitated DNA was separated by centrifugation. The pellet was dried and dissolved in TAE buffer (100 mmol L^{-1} tris pH 8.0 adjusted with glacial acetic acid, 10 mmol L^{-1} EDTA) and stored in cold.

2.4.4.3. *Treatment of DNA with the samples.* The compounds (500 ppm) were added separately to the DNA sample and mixtures were incubated at 37°C for 2 h.

2.4.4.4. *Agarose gel electrophoresis.* Following the treatment of DNA samples, electrophoresis of the samples was done according to the following procedure [31]. 200 mg of agarose was weighed and dissolved in 25 mL of TAE buffer (4.84 g Tris base, pH 8.0, 0.5 mol L^{-1} EDTA/1 L) by boiling. When the gel attained ~55°C, it was poured into the gel cassette fitted with comb. The gel was solidified. The comb was removed carefully and the gel was placed in the electrophoresis chamber flooded with TAE buffer. 20 μL of DNA sample (mixed with bromophenol blue dye in 1 : 1 ratio) was loaded carefully into the wells, along with control and constant 50 V of electricity was passed for 30 min. The gel was removed, carefully stained with ethidium bromide solution (10 μg mL^{-1}) for 10–15 min, and the bands were observed under UV transilluminator (UVP, Germany).

3. Results and discussion

The reactions of hydrated lanthanide chlorides with monobasic bidentate ligands have been shown by the following general equation:

$$LnCl_3 \cdot xH_2O + 3LNa \xrightarrow{MeOH} [Ln(L)_3] \cdot nH_2O + 3NaCl + (x-3)H_2O$$

where LNa is the sodium salt of the ligand, Ln = Nd, Sm, and Gd, $n = 3$ and $x = 6$. The newly synthesized complexes have been obtained as colored solids with solubility in methanol, DMSO, and DMF. The molar conductance values of 10^{-3} mol L^{-1} solutions of metal complexes are 10–15 Ohm^{-1} cm^2 mol^{-1} in dry DMF, indicating their non-electrolytic behavior. The complexes are monomers as revealed by their molecular weight determinations.

3.1. *IR spectra*

Absorption frequencies of the ligands and their metal complexes show that both ligands are monobasic bidentate, coordinating through thiol sulfur/ketone oxygen, and azomethine nitrogen. IR spectra of the free ligands show a medium intensity band at 3150–3240 cm^{-1} due to νNH, which is absent in spectra of the complexes. The bands due to ν(C=O) and ν(C=S) in the spectra of the ligands are observed

at 1715 and 1080 cm^{-1}, respectively. Bands for ν(C–S) and ν(C−O) in spectra of the complexes support the involvement of sulfur and oxygen in coordination. For L^1H and L^2H, the most significant band at 1630–1635 cm^{-1} assigned to ν(C=N) shifts to lower frequency in the complexes, suggesting the coordination of azomethine nitrogen. There are no changes in the ν_{sym} (3340–3490) and ν_{asym} (3350–3500) of NH_2, indicating non-involvement of this group in chelation. The band at 1735 cm^{-1} due to (>C=O) remains unchanged in the complexes, indicating that the lactone oxygen is not involved in coordination. The complexes exhibit new bands at 510–550 and 360–420/580–620 cm^{-1} which may be attributed to the different vibrational modes of ν(M–N) and ν(M–S)/ν(M–O), respectively, and support the coordination of the azomethine nitrogen and bonding of the thiol sulfur/ketone oxygen.

3.2. *^{1}H NMR spectra*

The ^{1}H NMR spectra of both ligands were recorded in DMSO-d_6. Signal at δ 2.83–2.85 ppm is observed for $-NH_2$ and the signal of $-NH$ is observed at δ 9.90–9.62 ppm. The free ligands show multiplets at δ 6.45–8.20 ppm attributable to aromatic protons.

3.3. *Electronic spectra*

The electronic spectra of the ligands and their complexes were recorded in distilled DMSO. The spectra of L^1H and L^2H show a broad band at 365–375 nm which can be assigned to n–π^* transitions of the azomethine group which undergoes a blue shift in the complexes due to polarization within the >C=N chromophore caused by the metal–ligand interaction. Two bands at 275–285 nm and 295–310 nm due to π–π^* transitions in the ligands remain at the same positions in spectra of the metal complexes.

Absorptions of Nd(III) and Sm(III) are due to transitions from the ground levels, $^4I_{9/2}$, and $^6H_{5/2}$ to the excited "J" levels of 4f configuration. The nephelauxetic parameter (β) [32], bonding parameter ($b^{1/2}$) [33], Sinha's covalency parameter (δ) [34], and angular covalency (η) for the Nd(III) and Sm(III) complexes are presented in table 3. Sinha's parameter (δ) suggests the degree of covalency and is obtained by the equation

$$\delta = (1 - \beta_{av})/\beta_{av} \cdot 100$$

Table 3. Electronic spectral data of lanthanide complexes.

Complex	Assignment		V_{max} of Ln^{+3} ion (cm^{-1})	V_{max} of complexes (cm^{-1})	β	$1-\beta$	$b^{1/2}$	δ	η
$[Sm(L^1)_3]\cdot 3H_2O$	$^6H_{5/2}$	$-^4I_{13/2}$	21,455	21,272	0.9915	0.0085	0.0652	0.8572	0.0926
		$-^4F_{9/2}$	25,830	25,650	0.9930	0.0070	0.0592	0.7049	0.0840
		$-^4I_{9/2}$	26,525	26,320	0.9923	0.0077	0.0620	0.7760	0.0881
		$-^6P_{3/2}$	28,735	28,565	0.9941	0.0059	0.0543	0.5935	0.0770
$[Nd(L^2H)_3]\cdot 3H_2O$	$^4I_{9/2}$	$-^4G_{5/2}$, $^2G_{7/2}$	16,822	16,660	0.9904	0.0096	0.0693	0.9693	0.0985
		$-^2G_{9/2}$	19,813	19,630	0.9908	0.0092	0.0678	0.9285	0.0964
		$-^4G_{11/2}$	21,915	21,740	0.9920	0.0080	0.0632	0.8064	0.0897

where β_{av} is the average value of the ratio of $\nu_{complex}/\nu_{metal\ ion}$. The magnitude of the bonding parameters ($b^{1/2}$) suggests the degree of involvement of 4f orbitals in metal–ligand bonding and is related to nephelauxetic ratio (β) by the equation

$$b^{1/2} = [(1 - \beta_{av})/2]^{1/2}$$

$$\text{Angular covalency } (\eta) = (1 - \beta_{av})^{1/2}/\beta_{av}^{1/2}$$

According to Karraker [35], the shape and intensity of these transitions indicate the geometry of the complex. In the present complexes, nephelauxetic ratio (β) being less than one and positive values of $b^{1/2}$ and δ indicate slight covalent bonding between metal and ligand. On comparison of the spectra with that of the known compounds [36, 37], it is concluded that the coordination number of the present complexes is six.

3.4. *Magnetic studies*

Magnetic moment data are summarized in table 1. The paramagnetic behavior of the lanthanide complexes is consistent with the presence of unpaired electrons. The magnetic susceptibility measurements of the complexes show little deviation from the Van Vleck values, indicating nonparticipation of the 4f electrons in bonding. However, in the case of Sm(III) complexes, the agreement between calculated and experimental values is not good [36].

3.5. *EPR spectra*

EPR spectra of some of the synthesized compounds were recorded (both at RT and LNT) and similar g values of 1.98 have been observed, nearly equal to the free electron value ($g = 2.00277$). Similar line widths at both temperatures indicate spin–lattice and spin–spin relaxation processes contribute equally to line width.

3.6. *X-ray powder diffraction spectra*

Lattice dynamics of the finely powdered product, $[Nd(L^2)_3 \cdot 3(H_2O)]$, have been deduced on the basis of X-ray powder diffraction studies. The observed interplanar spacing values ('d' in Å) have been measured from the diffractogram of the compound and the Miller indices h, k, and l have been assigned to each d value and 2θ angles are reported. The results show that the compound belongs to "Monoclinic" crystal system having unit cell parameters $a = 11.260$, $b = 12.458$, $c = 14.890$, maximum deviation of $2\theta = 0.046$ and $\alpha = 90$, $\beta = 103.58$, $\gamma = 90$ at the wavelength = 1.54178 (table 4 and figure 2).

3.7. *Mass spectra*

The mass spectrum of $Sm(L^1)_3 \cdot 3H_2O$ (**2**) was studied as a representative case. Peaks of appreciable intensity were observed at m/z values 1046.2581, 992.2909, and 261.3233 (figure S1, Supplementary material). The molecular ion peak for $Sm(L^1)_3 \cdot 3H_2O$ at m/z 1046.2581 is in good agreement with its molecular weight, which suggests the monomeric

Table 4. X-ray powder diffraction data of $Nd(L^2)_3 \cdot 3H_2O$.

h	*k*	*l*	2θ (Exp.)	2θ (Calcd)	2θ (Diff.)	*d* (Exp.)	*d* (Calcd)	Intensity (Exp.)
2	0	3	27.324	27.363	−0.039	3.26383	3.25926	51.46
0	1	5	31.752	31.734	0.018	2.81807	2.81963	206.92
0	6	2	45.436	45.443	−0.007	1.99612	1.99581	171.08
3	4	5	53.606	53.595	0.011	1.70958	1.70991	16.34
3	1	7	56.540	56.560	−0.020	1.62762	1.62709	72.95
3	8	1	66.120	66.150	−0.030	1.41314	1.41258	45.82

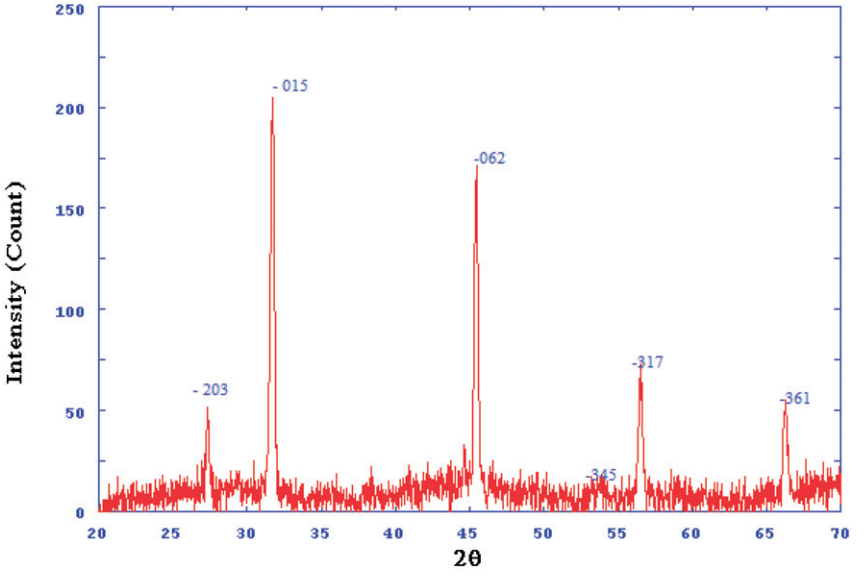

Figure 2. X-ray powder diffraction of $Nd(L^2)_3 \cdot 3H_2O$.

nature of the complex and confirms the proposed formula. The peak at *m*/*z* 992.2909 indicates that the molecular ion gives a fragment ion $[Sm(L^1)_3]$ by losing three water molecules. This fragment ion undergoes demetallation to form $[L + H]^+$, resulting in a peak at *m*/*z* 261.3233. The mass spectra of all the complexes are summarized in table 5.

4. Pharmacology

4.1. *Antimicrobial assay*

The ligands and their metal complexes were evaluated for antimicrobial activity against four bacteria, *E. coli*, *P. aeruginosa*, *B. subtilis*, and *S. aureus*, and three fungi, *C. albicans*, *A. niger*, and *F. oxysporum*. The results, summarized in tables 6 and 7, are compared with those of the standard drug ciprofloxacin for bacteria and flucanazole for fungi. The results show that the complexes are more active than their parent ligands against the same microorganisms and as the concentration increases the activity

Table 5. Mass spectra of the complexes.

Name of the compound	Molecular ion peak $[ML_3 \cdot 3H_2O]^+$		$ML_3 \cdot 3H_2O–3H_2O \rightarrow [ML_3]^+$	
	Observed	Calculated	Observed	Calculated
$Nd(L^1)_3 \cdot 3H_2O$	1040.1514	1040.4186	986.1896	986.3742
$Sm(L^1)_3 \cdot 3H_2O$	1046.2581	1046.5386	992.2909	992.4942
$Gd(L^1)_3 \cdot 3H_2O$	1053.3458	1053.4286	999.1592	999.3842
$Nd(L^2)_3 \cdot 3H_2O$	992.1225	992.2236	938.0598	938.1792
$Sm(L^2)_3 \cdot 3H_2O$	998.2958	998.3436	944.1916	944.2992
$Gd(L^2)_3 \cdot 3H_2O$	1005.0456	1005.2336	951.0398	951.1892

Table 6. Antifungal screening data for the ligands and their corresponding metal complexes.

	(Antifungal activity) Percentage inhibition after 96 h (conc. in ppm)					
	F. oxysporum		*C. albicans*		*A. niger*	
Compound	100	200	100	200	100	200
1	39 ± 0.4	43 ± 0.7	42 ± 0.3	47 ± 0.5	45 ± 0.3	48 ± 0.5
2	32 ± 1.1	40 ± 1.1	34 ± 0.4	44 ± 0.3	38 ± 0.3	42 ± 0.2
3	79 ± 1.4	83 ± 0.2	78 ± 0.7	80 ± 0.6	72 ± 1.4	75 ± 0.4
4	71 ± 0.4	77 ± 1.1	61 ± 0.6	67 ± 0.6	50 ± 0.4	57 ± 1.1
5	84 ± 0.6	89 ± 0.5	69 ± 0.7	77 ± 0.5	63 ± 1.4	68 ± 0.2
6	50 ± 0.2	65 ± 0.2	59 ± 0.7	65 ± 0.2	55 ± 0.6	61 ± 0.5
7	53 ± 0.6	63 ± 0.2	60 ± 0.4	78 ± 0.4	50 ± 0.7	64 ± 0.3
8	69 ± 0.2	73 ± 0.5	60 ± 0.2	65 ± 0.2	54 ± 0.4	59 ± 1.4
Flucanazole	100 ± 1.1	100 ± 0.8	100 ± 0.5	100 ± 1.1	100 ± 0.9	100 ± 0.6

also increases. Chelation theory [38] accounts for the increased activity of the metal complexes. The enhanced activity could also be due to inherent properties of the metal ion in precipitating or denaturing proteins. Since enzymes are proteins, it would be expected that the heavy metal would inactivate these catalysts [39]. The mode of action of antimicrobials may involve various targets in microorganisms, e.g., interference with cell wall synthesis, damage to the cytoplasmic membrane, causing an alteration of the cell permeability or a disorganization of the lipoproteins leading to cell death. Antimicrobials can bind to ribosome and may interfere with peptide chain formation in microorganisms or with the transcription mechanisms. At lower concentration, inhibition is less severe because the activities of the organisms will be slowed down, while at higher concentration, more enzymes will become inhibited leading to a quicker death of the organisms [40]. Revankar *et al*. [41] and Alghool [42] have reported similar results. Revankar *et al*. synthesized Co(II), Ni(II), and Cu(II) complexes of Schiff base derived from the condensation of 2,6-diaminopyridine and 3-acetylcoumarin and screened them for their antimicrobial activity, and observed that all the complexes were more active against bacteria and fungi. Alghool screened Cu(II), Co(II), Ni(II), Cd(II), and Zn(II) complexes of 6-(2-phenyldiazenyl)-7-hydroxy-4-methyl coumarin (PAHC) for antibacterial activity and found that all the complexes show better activity than the ligand but less activity than the standard drug.

Table 7. Antibacterial screening data for the ligands and their corresponding metal complexes.

	(Antibacterial activity) Diameter (mm) of inhibition zone after 24 h (conc. in ppm)							
	E. coli		*P. aeruginosa*		*B. subtilis*		*S. aureus*	
Compound	500	1000	500	1000	500	1000	500	1000
1	8.4 ± 0.03	9.6 ± 0.06	7.1 ± 0.03	9.0 ± 0.02	6.4 ± 0.04	7.6 ± 0.06	10.0 ± 0.11	10.3 ± 0.04
2	7.2 ± 0.14	8.3 ± 0.06	5.6 ± 0.03	8.1 ± 0.05	5.4 ± 0.03	7.1 ± 0.04	8.2 ± 0.10	8.8 ± 0.04
3	13.8 ± 0.10	16.0 ± 0.03	13.4 ± 0.03	16.2 ± 0.01	13.1 ± 0.03	15.8 ± 0.04	15.0 ± 0.03	19.1 ± 0.03
4	11.5 ± 0.10	14.3 ± 0.02	10.8 ± 0.03	12.7 ± 0.03	9.5 ± 0.03	13.2 ± 0.04	13.9 ± 0.09	17.0 ± 0.04
5	13.5 ± 0.08	14.9 ± 0.18	12.2 ± 0.14	15.4 ± 0.01	12.9 ± 0.03	14.5 ± 0.05	14.4 ± 0.10	18.0 ± 0.02
6	10.7 ± 0.3	13.9 ± 0.14	11.4 ± 0.03	14.5 ± 0.03	9.6 ± 0.03	13.0 ± 0.03	12.6 ± 0.09	15.6 ± 0.05
7	11.0 ± 0.1	13.1 ± 0.11	12.0 ± 0.03	13.2 ± 0.04	8.9 ± 0.03	11.6 ± 0.03	10.7 ± 0.02	13.9 ± 0.08
8	11.1 ± 0.04	12.8 ± 0.02	10.5 ± 0.09	14.2 ± 0.03	10.5 ± 0.02	12.9 ± 0.04	10.9 ± 0.03	14.1 ± 0.10
Ciprofloxacin	17.6 ± 0.06	19.2 ± 0.05	18.5 ± 0.10	20.0 ± 0.04	17.9 ± 0.05	21.1 ± 0.03	18.6 ± 0.03	22.0 ± 0.11

Table 8. MIC ($\mu g\,mL^{-1}$) of the ligands and their corresponding complexes.

Compound	*E. coli*	*P.aeruginosa*	*B.subtilis*	*S.aureus*	*C.albicans*	*F. oxysporum*	*A. niger*
1	20	25	35	10	10	10	10
2	25	30	40	25	20	25	25
3	15	20	25	10	10	10	10
4	10	15	20	10	10	10	10
5	15	15	25	15	10	25	10
6	20	25	30	15	15	10	25
7	15	20	25	15	10	10	10
8	20	20	30	20	15	10	10

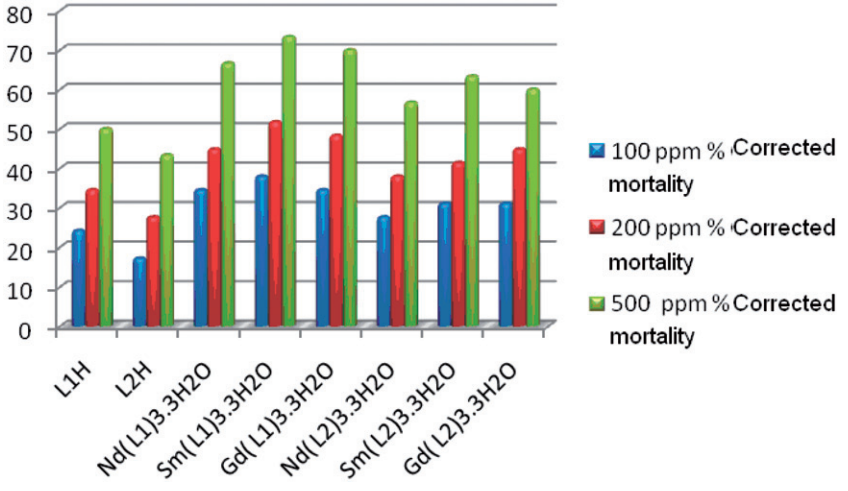

Figure 3. Pesticidal activity (% corrected mortality) for the ligands and their corresponding metal complexes.

4.2. *Minimum inhibitory concentration*

MIC values calculated for the ligands and their lanthanide complexes are shown in table 8. The ligands and their metal complexes were most active in inhibiting growth of the tested organisms between 10–40 MIC ($\mu g\,mL^{-1}$) against selected bacteria and fungi. The MIC values for Ciprofloxacin against *E. coli* and *S. aureus* are 0.009 and 0.016, respectively, and for Flucanazole against *C. Albicans*, the value is 0.079 [43].

4.3. *Pesticidal and nematicidal activity*

The results of pesticidal activity reveal that the ligands and their metal complexes showed good pesticidal activity (figure 3). All the compounds are fairly active in killing *T. castaneum*. An appreciable increase in the pesticidal activities in the case of metal complexes compared to the uncomplexed ligands was observed. Sm(III) chelates were most effective as evidenced from the comparative study of their percentage mortality data with other metal chelates. Dawara and Singh [44] have also screened Bi(III) and

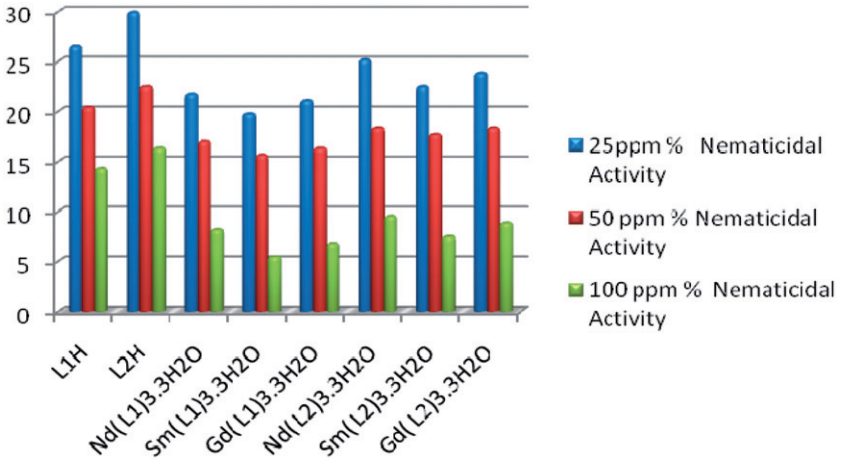

Figure 4. Nematicidal activity for the ligands and their corresponding metal complexes.

Sb(III) complexes of 3-acetylcoumarin-based ligands for their antimicrobial as well as pesticidal activity and observed that all complexes are active in inhibiting the growth of microorganisms and pests.

The objective of nematode control is to improve growth and yield of plants, which can be achieved through reduction of the nematode population in soil or in plants, or through reduction of their damage. From the results of nematicidal activity, maximum hatching was recorded in the control but in the eggs treated with the ligands as well as metal complexes, low hatching was recorded. The results in figure 4 reveal that the activity increases on complexation and chelation, i.e., the newly synthesized complexes have been found to be more active in lowering the hatching of eggs than the parent ligand itself.

4.4. *DNA cleavage activity*

DNA cleavage studies of both the synthesized ligands and corresponding lanthanide metal complexes have been carried out against *E. coli* ATCC 35218 by agarose gel electrophoresis. Gel electrophoresis works on the migration of DNA under the influence of electric potential. Gel electrophoresis in control experiments clearly reveal that the untreated DNA does not show any cleavage (figure S2 (Lane C), Supplementary material), whereas all the metal complexes along with the ligand exhibit cleavage activity on DNA. The difference in migration was observed in the Lanes L, N1, N2, N3 of ligand and lanthanide complexes, respectively, compared to the control DNA of *E. coli* (Lane C). N2 clearly showed the absence of the marker band, thus providing proof for their DNA cleaving ability, whereas N1 and N3 displayed partial cleavage. L is the least active among the four. The results indicate the important role of coumarin-based ligand coordinated with metal ions in isolated DNA cleavage. Kostova and Stefanova [45] have synthesized Sm(III) and Gd(III) metal complexes of acenocumarol and screened them for their cytotoxic study. The Sm(III) and Gd(III) complexes exhibit good activity against melanoma B16 and fibrosarcoma L929 and are stronger inhibitors of tumor cell proliferation than the ligand. In this work, the comparative data of DNA cleavage of lanthanide complexes showed that Sm(III)

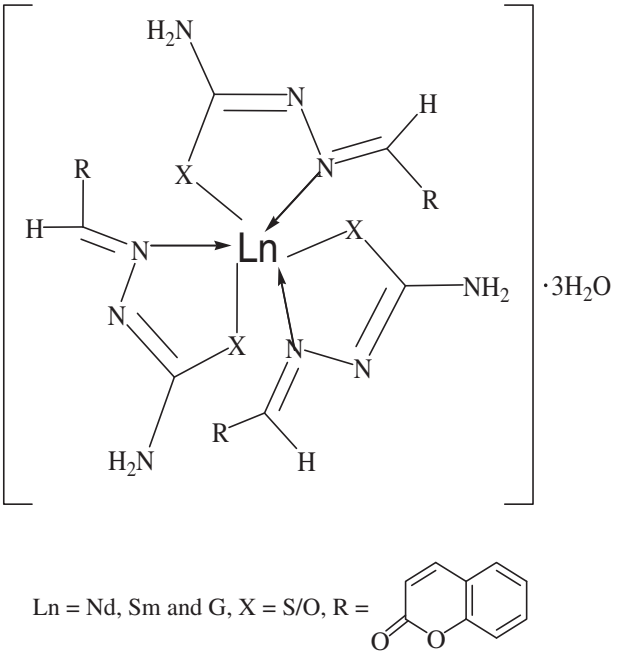

Figure 5. Proposed structure of the synthesized metal complexes.

complex with thiosemicarbazone has the highest activity towards cleavage of DNA and all the metal complexes exhibited better results than free ligand 3-formyl-4-chlorocoumarin thiosemicarbazone.

5. Conclusion

Microwave irradiation is an efficient and environmentally-benign method to accomplish various syntheses in higher yields in shorter reaction periods. From analytical data and spectral studies, the ligands coordinate in a monobasic bidentate manner and the complexes possess octahedral geometry. The analytical and spectral studies suggest the structure shown in figure 5. The ligand and metal complexes were active for pesticidal, nematicidal, antibacterial, and antifungal activities. The compounds are more active than the ligands but less active than Flucanazole and Ciprofloxacin as standard drugs. The nematicidal activity shows that the metal complexes are more active in lowering the hatching of eggs than the parent ligand. The DNA cleavage studies revealed that the metal complexes showed good efficiency towards DNA cleavage.

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