



Original article

Synthesis, characterization, DNA cleavage and *in vitro* antimicrobial studies of La(III), Th(IV) and VO(IV) complexes with Schiff bases of coumarin derivativesAjaykumar Kulkarni^a, Sangamesh A. Patil^{a,*}, Prema S. Badami^b^a P.G. Department of Chemistry, Karnatak University, Dharwad-580 003, Karnataka, India^b Department of Chemistry, Shri Sharanabasaveswar College of Science, Gulbarga-585102, Karnataka, India

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ABSTRACT

A series of La(III), Th(IV) and VO(IV) complexes have been synthesized with Schiff bases derived from 8-formyl-7-hydroxy-4-methylcoumarin and *o*-phenylenediamine/ethylenediamine. The structure of the complexes has been proposed in the light of analytical, spectral (IR, UV–vis, ESR and FAB-mass), Magnetic and thermal studies. The complexes are soluble in DMF and DMSO. The measured molar conductance values indicate that, the complexes are non-electrolytes in nature. The redox behavior of the complexes was investigated by electrochemical method using cyclic voltammetry. The Schiff bases and their complexes have been screened for their antibacterial (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella typhi*) and antifungal activities (*Aspergillus niger*, *Aspergillus flavus* and *cladosporium*) by Minimum Inhibitory Concentration method. The DNA cleavage activity of La(III) and VO(IV) metal complexes is studied by agarose gel electrophoresis method.

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1. Introduction

Coumarin (1,2-benzopyrone) is structurally the least complex member of a large class of compounds known as benzopyrones [1]. The biological activities of coumarin derivatives are multiple and include antithrombotic [2], antimicrobial [3], antiallergic [4], anti-inflammatory [5], antitumor [6] and anticoagulants [7]. Recently, coumarin derivatives have been evaluated in the treatment of human immunodeficiency virus, due to their ability to inhibit human immunodeficiency virus integrase [8,9]. The coumarin nucleus is also present in the antibiotics such as novobiocin, clorobiocin and coumermycin A₁. These antibiotics are potent catalytic inhibitors of DNA gyrase. In addition to that, these antibiotics have been shown to be active against Gram-positive bacteria, especially against methicillin-resistant *Staphylococcus aureus* [3]. Further it is evident from the literature [10–13] that, the transition and rare earth complexes of hydroxycoumarin derivatives are also subjects of increasing interest in bioinorganic and coordination chemistry [14,15]. Lanthanides(III) show antitumor activity [16]. Some interesting lanthanide complexes of coumarin derivatives like bis(4-hydroxy-3-coumarinyl)-acetic acid [17], *N,N'*-bis(8-aceto-7-

hydroxy-4-methylcoumarin)-ethylenediamine [18,19] and coumarin-3-carboxylic acid [20] have been reported. Recently some transition metal complexes with coumarin Schiff bases have been reported from our laboratory [13,21].

Thus, the aim of present work is to synthesize and characterize La(III), Th(IV) and VO(IV) metal complexes with newly synthesized Schiff bases derived from 8-formyl-7-hydroxy-4-methylcoumarin and *o*-phenylenediamine/ethylenediamine. The electron transfer mechanism of the Schiff bases and their complexes is investigated by the aid of cyclic voltammetry. The Schiff bases and their metal complexes are screened for their *in vitro* antimicrobial activity. DNA cleavage property of the complexes is also studied.

2. Chemistry

2.1. Methods

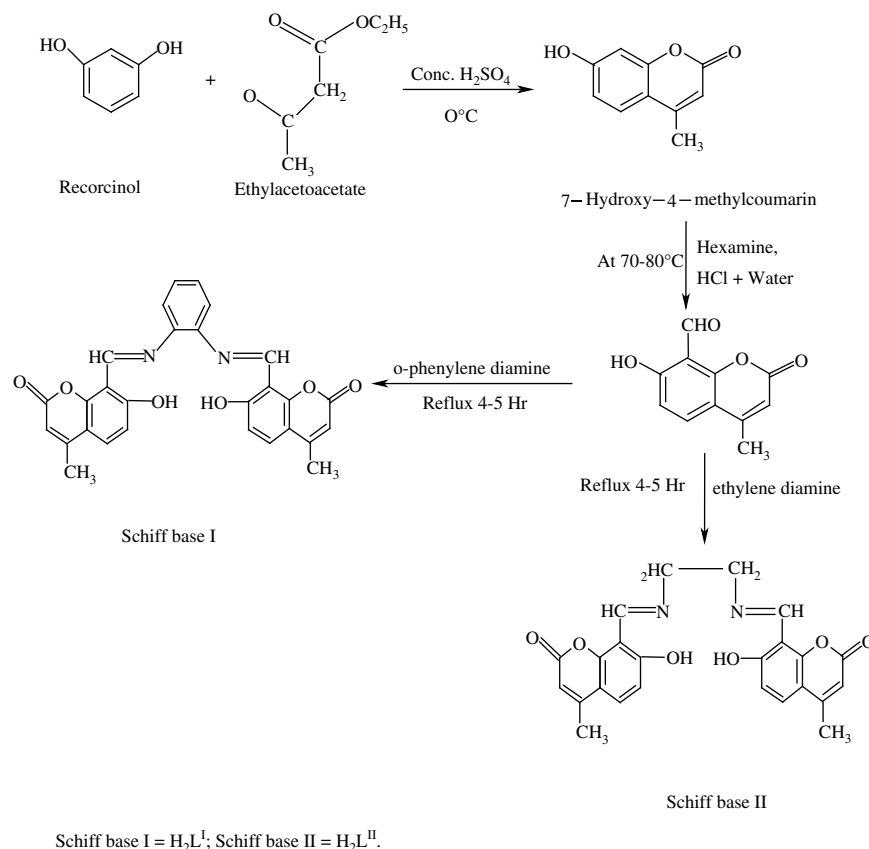
All the chemicals used were of reagent grade. 7-Hydroxy-4-methylcoumarin was synthesized according to the literature procedure [22].

2.1.1. Synthesis of 8-formyl-7-hydroxy-4-methylcoumarin

8-Formyl-7-hydroxy-4-methylcoumarin is prepared by the known method [18] with slight modification. 7-Hydroxy-4-methylcoumarin (5.28 g, 0.03 mol) and hexamine (9.8 g, 0.07 mol) in

* Corresponding author. Fax: +91 0836 2771275.

E-mail address: patil1956@rediffmail.com (S.A. Patil).



Scheme 1. Synthesis of Schiff bases H_2L^I and H_2L^{II} .

glacial acetic acid (50 mL) were heated for 6–7 h, then, 20% HCl (75 mL) was added and further heated for 45 min, then cooled to 20–25 °C and extracted with ether. Ether is evaporated and the remaining solution is poured into ice with constant stirring, pale yellow colored solid of 8-formyl-7-hydroxy-4-methylcoumarin formed was filtered and was recrystallized from hot ethanol.

2.1.2. Synthesis of Schiff bases [H_2L^I – H_2L^{II}]

The Schiff bases have been synthesized by refluxing the reaction mixture of hot ethanol solution (30 mL) of *o*-phenylenediamine/ethylenediamine (1.08/0.6 g, 0.01 mol) and hot ethanol solution (30 mL) of 8-formyl-7-hydroxy-4-methylcoumarin (4.08 g, 0.02 mol) for 4–5 h with addition of a 3–4 drops of concentrated hydrochloric acid. The precipitate formed during refluxion was filtered, washed with cold EtOH and recrystallized from hot EtOH (yield: 72.5%).

2.1.3. Synthesis of La(III), Th(IV) and VO(IV) complexes [1–6]

The La(III)/Th(IV) nitrate and vanadyl sulphate (4.3/5.7/1.81 g, 0.01 mol) in super-dry alcohol (10 mL) were treated with Schiff

bases (4.8/4.3 g, 0.01 mol) in super-dry alcohol (40 mL). The reaction mixture was refluxed for 4–5 h. After cooling, the pH of the reaction mixture was adjusted to *ca.* 7 by adding dilute ammonia with constant stirring. The precipitate of the complex formed during the addition of dilute ammonia was filtered, washed thoroughly with super-dry alcohol and ether and finally dried over fused $CaCl_2$ in vacuum (yield: 61–65%).

3. Pharmacology

3.1. DNA cleavage experiment

3.1.1. Preparation of culture media

DNA cleavage experiments were done according to the literature [23]. Nutrient broth [peptone, 10; Yeast extract, 5; NaCl, 10; in (g/l)] was used for culturing of *Escherichia coli* and potato dextrose broth [potato, 250; dextrose, 20; in (g/l)] was used for the culture of *Aspergillus niger*. The 50 mL media was prepared, autoclaved for 15 min at 121 °C under 15 lb pressure. The autoclaved media were

Table 1

Elemental analyses of Schiff bases and their La(III), Th(IV) and VO(IV) complexes along with molar conductance and magnetic moment data.

Comp. No.	Empirical formula	M%		C%		H%		N%		Molar conductance $\text{Ohm}^{-1} \text{cm}^{-2} \text{mole}^{-1}$	μ_{eff} (BM)
		Obsd	Calcd	Obsd.	Calcd	Obsd.	Calcd	Obsd.	Calcd		
H_2L^I	$C_{28}H_{20}N_2O_6$	–	–	69.994	70.0	4.161	4.166	5.841	5.83	–	–
H_2L^{II}	$C_{24}H_{20}N_2O_6$	–	–	66.742	66.66	4.634	4.629	6.479	6.481	–	–
1	$[La(H_2L^I)(NO_3)] \cdot H_2O$	19.821	19.827	48.271	48.275	2.877	2.873	6.035	6.034	21.3	–
2	$[La(H_2L^{II})(NO_3)] \cdot H_2O$	21.289	21.296	44.440	44.444	3.081	3.086	6.483	6.481	22.6	–
3	$[Th(H_2L^I)(NO_3)_2] \cdot 3H_2O$	26.120	26.126	37.829	37.837	2.709	2.702	6.305	6.306	19.8	–
4	$[Th(H_2L^{II})(NO_3)_2] \cdot 3H_2O$	27.625	27.619	34.291	34.285	2.851	2.857	6.664	6.666	23.5	–
5	$[VO(H_2L^I)]$	12.299	12.293	61.659	61.651	3.306	3.302	5.139	5.137	22.9	1.73
6	$[VO(H_2L^{II})]$	13.488	13.481	57.941	57.947	3.626	3.621	5.639	5.633	24.1	1.72

Table 2The important infrared frequencies (in cm^{-1}) of Schiff bases and their metal complexes.

Compound	Lattice water ν (OH)	Lactonyl ν (C=O)	ν (C=N)	H-bonded –OH Stretching	Phenolic ν (C–O)	ν (M–N)	ν (M–O)
H ₂ L ^I	–	1735	1613	3062	1306	–	–
H ₂ L ^{II}	–	1720	1632	3051	1305	–	–
1	3447	1736	1602	–	1397	516	378
2	3430	1724	1622	–	1371	510	375
3	3402	1733	1604	–	1390	524	410
4	3442	1723	1624	–	1389	513	405
5	3438	1736	1603	–	1395	486	395
6	3439	1724	1626	–	1392	480	398

inoculated with the seed culture. *E. coli* is incubated for 24 h and *A. niger* for 48 h at 37 °C.

3.1.2. Isolation of DNA

The fresh bacterial culture (1.5 mL) is centrifuged to obtain the pellet which is then dissolved in 0.5 mL of lysis buffer (100 mM tris pH 8.0, 50 mM EDTA, 10% SDS). To this 0.5 mL of saturated phenol was added and incubated at 55 °C for 10 min. Then centrifuge at 10,000 rpm for 10 min and to the supernatant, equal volume of chloroform:isoamyl alcohol (24:1) and 1/20th volume of 3 M sodium acetate (pH 4.8) was added. Then, centrifuge at 10,000 rpm for 10 min and to the supernatant, 3 volumes of chilled absolute alcohol was added. The precipitated DNA was separated by centrifugation and the pellet was dried and dissolved in TAE buffer (10 mM tris pH 8.0, 1 mM EDTA) and stored in cold condition.

3.1.3. Agarose gel electrophoresis

Cleavage products were analyzed by agarose gel electrophoresis method [23]. Test samples (1 mg/mL) were prepared in DMF. The samples (25 μg) were added to the isolated DNA of *E. coli* and *A. niger*. The samples were incubated for 2 h at 37 °C and then 20 μL of DNA sample (mixed with bromophenol blue dye at 1:1 ratio) was loaded carefully into the electrophoresis chamber wells along with standard DNA marker containing TAE buffer (4.84 g Tris base, pH 8.0, 0.5 M EDTA/1 L) and finally loaded on agarose gel and passed the constant 50 V of electricity for around 30 min. Removed the gel and stained with 10.0 $\mu\text{g/mL}$ ethidium bromide for 10–15 min and the bands observed under Vilberlourmate Gel documentation system and photographed to determine the extent of DNA cleavage. Then the, results are compared with standard DNA marker.

3.2. In vitro antibacterial and antifungal assays

The biological activities of synthesized Schiff bases and their La(III), Th(IV) and VO(IV) complexes have been studied for their antibacterial and antifungal activities by agar and potato dextrose agar diffusion method respectively in DMF solvent against *E. coli*, *S. aureus*, *Pseudomonas aeruginosa* and *Salmonella typhi* bacterial and *A. niger*, *Aspergillus flavus* and *cladosporium* fungi species [24]. The stock solution (1 mg mL^{-1}) of the test chemical was prepared by dissolving 10 mg of the test compound in 10 mL of dimethyl formamide (DMF) solvent. The stock solution was suitably diluted with sterilized distilled water to get dilution of 100, 50 and 25 $\mu\text{g mL}^{-1}$. Control for each dilution was prepared by diluting

10 mL of solvent instead of stock solution with sterilized distilled water.

The bacteria were subcultured in agar medium. The petridishes were incubated for 24 h at 37 °C. Standard antibacterial drug, *Gentamycin* was also screened under similar conditions for comparison. The fungi were subcultured in potato dextrose agar medium. Standard antifungal drug, *Fluconazole* was used for comparison. The petridishes were incubated for 48 h at 37 °C. Sterile disks were used in the agar media. Activity was determined by measuring the diameter of the zone showing complete inhibition. Compounds showing promising antibacterial/antifungal activity were selected for minimum inhibitory concentration (MIC) studies [24].

4. Results and discussion

The synthesis of Schiff bases H₂L^I and H₂L^{II} is schematically presented in Scheme 1. All the La(III), Th(IV) and VO(IV) complexes are colored, stable and non-hygroscopic in nature. The complexes are insoluble in common organic solvents but soluble in DMF and DMSO. The elemental analyses show that, the La(III), Th(IV) and VO(IV) complexes have 1:1 stoichiometry. The molar conductance values are too low to account for any dissociation of the complexes in DMF, indicating the non-electrolytic nature of the complexes (Table 1).

4.1. Infrared spectra

The prominent infrared spectral data of Schiff bases and their La(III), Th(IV) and VO(IV) complexes are presented in Table 2.

The IR spectra of the Schiff bases exhibit characteristic high intensity band at 1613–1632 cm^{-1} which is attributed to the ν (C=N) vibration. Another strong band at 1735–1720 cm^{-1} is assigned to ν (C=O), lactonyl carbon of the coumarin moiety. In addition to this, the broad band around 3062 cm^{-1} is assigned to phenolic H-bonded –OH stretching; a high intensity band in the 1306–1305 cm^{-1} region with an additional band around 1500 cm^{-1} is assigned to the phenolic ν (C–O) vibrations and a medium intensity band at 1585 cm^{-1} is regarded as an aromatic C=C stretching vibrations.

In comparison with the spectra of the Schiff bases, all the complexes exhibit the band of ν (C=N) in the region 1602–1626 cm^{-1} ; showing the shift of band to lower wave numbers [25] indicating that, the azomethine nitrogen is coordinated to the

Table 3The ¹H NMR and ¹³C NMR data of Schiff bases.

Schiff base	¹ H NMR (CDCl ₃) (ppm)	¹³ C NMR (CDCl ₃) (ppm)
H ₂ L ^I	14.7 (s, 2H, OH), 9.42 (s, 2H, HC=N), 6.1–7.6 (m, 10H, Ar-H), 2.4 (s, 6H, Ar-CH ₃)	111.3, 118.6, 123.6, 125.5, 126.7, 131.5, 133.6, 138.2, 148.6, 152.2, 158.6 (aromatic carbon), 144.3 (C=N), 172.8 (C=O), 38.4 (CH ₃)
H ₂ L ^{II}	14.3 (s, 2H, OH), 9.1 (s, 2H, HC=N), 6.1–7.7 (m, 6H, Ar-H), 2.5 (s, 6H, Ar-CH ₃), 2.1 (t, 4H, CH ₂ –CH ₂)	113.1, 123.6, 125.5, 126.7, 131.5, 133.6, 138.2, 148.6 (aromatic carbon), 142.3 (C=N), 165.6 (C=O), 36.8 (CH ₃), 21.4 (CH ₂)

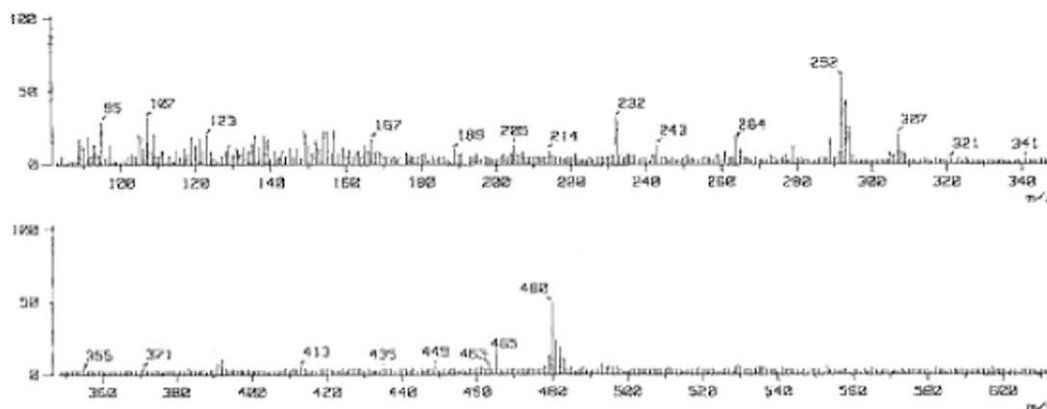


Fig. 1. FAB-mass spectrum of H_2L^I .

metal ion. The high intensity band due to phenolic C–O appeared at $1306\text{--}1305\text{ cm}^{-1}$ in the Schiff bases appeared as a medium to high intensity band in the $1371\text{--}1397\text{ cm}^{-1}$ region in the complexes. These observations support the formation of M–O bonds via deprotonation.

The broad band of medium intensity occurring in the $3447\text{--}3402\text{ cm}^{-1}$ region is due to the symmetric and antisymmetric O–H stretching vibrations of the lattice water [26]. The new bands in the region of $410\text{--}375$ and $524\text{--}480\text{ cm}^{-1}$ in all the complexes are assigned to stretching frequencies of (M–O) and (M–N) bonds respectively.

The La(III) and Th(IV) nitrate complexes (**1–4**) contain bands characteristic of both coordinated and ionic nitrates. The bands around 1481 cm^{-1} and around 1330 cm^{-1} are due to the $\nu(N=O)(\nu_1)$ and $\nu_{\text{asym}}(NO_2)(\nu_5)$ respectively, of the coordinated nitrate. The $\nu_{\text{sym}}(NO_2)$ vibration (ν_2) at 1020 cm^{-1} is characteristic of bi-dentate chelating nitrate. The separation ($\Delta\nu$) of the nitrate stretching fundamentals ($\nu_1\text{--}\nu_5$) has been used as a criterion to distinguish between mono- and bi-dentate chelating nitrates [27], increasing as the coordination changes from mono- to bi-dentate and bridging. The magnitude of this separation ($\Delta\nu=151$) is inductive of bi-dentate coordination of the nitrate ion.

In the VO(IV) complexes (**5–6**), a characteristic medium to high intensity band in the region $944\text{--}936\text{ cm}^{-1}$ is attributed to the $\nu(V=O)$ vibrations [28].

Thus, the IR spectral data provide strong evidence for the complexation of the tetradentate Schiff bases with ONNO sequence.

4.2. 1H NMR spectra

The Schiff bases have been characterized by 1H , ^{13}C NMR spectra and also by 2D $^1H\text{--}^1H$ HOMOCOSY to ensure ligand purity *in solution* and elucidate the differently positioned proton and carbon. The 1H NMR and ^{13}C NMR spectral data are produced in Table 3.

In 1H NMR spectrum of the Schiff base H_2L^I , signals at 14.7 and 2.4 ppm are ascribed to phenolic –OH and Aromatic- CH_3 respectively. A characteristic singlet proton signal at 9.42 ppm is assigned to $-CH=N$. In addition to this the multiplet signals around 6.1–7.6 ppm are due to aromatic protons. In case of Schiff base H_2L^{II} , the singlet at 14.3, 9.1 and 2.5 ppm are attributed to phenolic –OH, $-CH=N$ and Aromatic- CH_3 respectively. In addition to this the multiplet signals around 6.1–7.7 ppm and triplet around 2.1 ppm is due to aromatic and $CH_2\text{--}CH_2$ protons respectively. In both the Schiff bases, phenolic –OH proton signals disappeared after the addition of D_2O [29].

In ^{13}C NMR spectra of the Schiff bases, the signals appeared in the region 111.3–158.6 ppm are assigned to aromatic carbons. The signals at 172.8–165.6, 144.3–142.3 and 38.4–36.8 ppm are due to $C=O$, $C=N$ and CH_3 respectively. In case of Schiff base (II) an additional signal at 21.4 ppm is ascribed to $CH_2\text{--}CH_2$.

The two dimensional $^1H\text{--}^1H$ homocosity NMR spectra of Schiff bases clearly includes all the protons of the aromatic region observed as a cross peaks in the region 6.1–7.7 ppm. The peaks due to $CH=N$, CH_3 , and $CH_2\text{--}CH_2$ are well resolved from the spectrum.

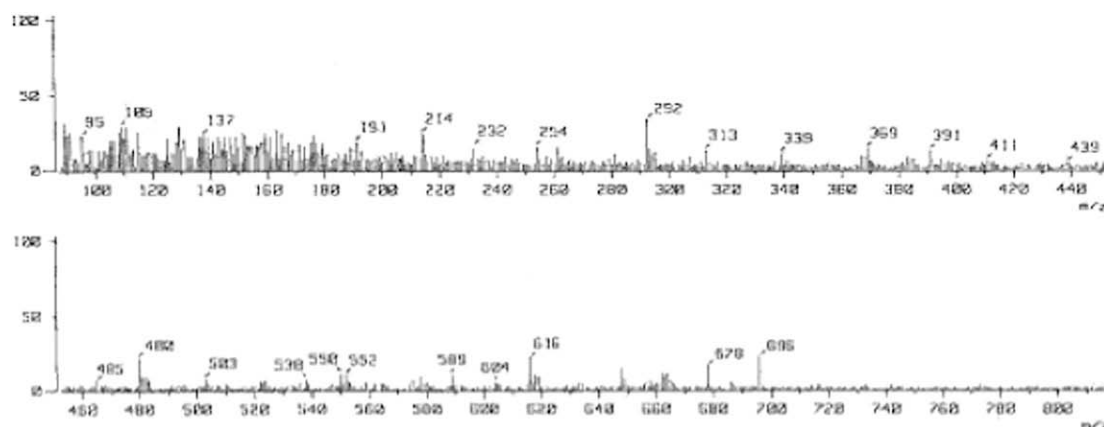


Fig. 2. FAB-mass spectrum of La(III) (**1**) complex.

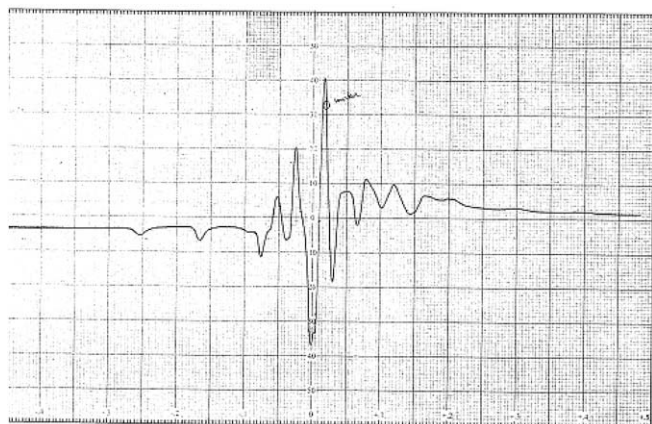


Fig. 3. ESR spectrum of VO(IV) (5) complex.

In ^1H NMR spectra of the La(III) and Th(IV) complexes, the signal due to azomethine proton of Schiff bases (9.42–9.1 ppm) appears in the region 10.2–9.8 ppm indicating the coordination of the azomethine nitrogen to the metal ion. This downfield shift is due to deshielding of the $\text{N}=\text{CH}$ proton. Also the slight downfield shift is observed for aromatic and aliphatic protons. The resonance due to phenolic $-\text{OH}$ around 14.7–14.3 ppm in the Schiff bases disappears in the spectrum of complexes; this confirms that, the hydroxyl group reacted with metal ion via deprotonation. All these observations supported the IR inferences.

4.3. FAB-mass spectral studies of Schiff bases and its complexes

The FAB-mass spectrum of $\text{H}_2\text{L}^{\text{I}}$ has been depicted in Fig. 1. The spectrum showed a molecular ion peak at m/z 480 which is equivalent to its molecular weight. In addition, the fragment peaks at m/z 465 and 292 are due to the cleavage of CH_3 and $\text{C}_{10}\text{H}_8\text{O}_3$ respectively. In case of $\text{H}_2\text{L}^{\text{II}}$ the molecular ion peak at m/z 432 is ascribed to $\text{C}_{24}\text{H}_{20}\text{N}_2\text{O}_6$. The peaks at 417 and 241 are assigned to the cleavage of CH_3 and $\text{C}_{10}\text{H}_8\text{O}_3$ respectively.

The FAB-mass spectra of La(III), Th(IV) and VO(IV) complexes with Schiff base $\text{H}_2\text{L}^{\text{I}}$ have also been studied. In the spectrum of

La(III) (1) complex (Fig. 2) the molecular ion peak M^+ is observed at m/z 696 which is equivalent to its molecular weight. This molecular ion undergoes fragmentation with the loss of one water molecule, gave a species $[\text{La}(\text{H}_2\text{L}^{\text{I}})(\text{NO}_3)]^+$ at m/z 678. Further, the fragment ion by the loss of one nitrate molecule gave a fragment ion at m/z 616. In case of the Th(IV) (3) complex, the spectrum shows a molecular ion peak M^+ at m/z 888 which is equivalent to its molecular weight $[\text{Th}(\text{H}_2\text{L}^{\text{I}})(\text{NO}_3)_2] \cdot 3\text{H}_2\text{O}$. This species gave a fragment ion at m/z 834, by the loss of three water molecules. This fragment ion lost the two nitrate molecules and gave a fragment ion $[\text{Th}(\text{H}_2\text{L}^{\text{I}})]^+$ at m/z 710. Finally, these complexes undergo demetallation to form the species $[\text{L}^{\text{I}} + 2\text{H}]^+$ gave a fragment ion at m/z 480. All these fragmentation patterns are well observed in the FAB-mass spectra.

In the spectrum of VO(IV) (5) complex (Fig. S1) the molecular ion peak M^+ , which is equivalent to its molecular weight is observed at m/z 545. This molecular ion undergoes demetallation to give fragment ion peak at m/z 480 which corresponds to the $[\text{L}^{\text{I}} + 2\text{H}]^+$ species.

4.4. Electronic spectra and magnetic studies

The electronic absorption spectra of the complexes were recorded for freshly prepared solution in DMF at room temperature.

Electronic absorption spectra of oxovanadium complexes show two d–d bands around ca. 11,710 and 18,100 cm^{-1} corresponding to $^2\text{B}_2 \rightarrow ^2\text{E}$ and $^2\text{B}_2 \rightarrow ^2\text{B}_1$ transitions, respectively, which is in consistent with that of square pyramidal geometry [30,31]. The magnetic moment values (Table 1) are found to be 1.72–1.73 slightly less than the spin only value.

The La(III) and Th(IV) complexes exhibited a broad band around 31000 cm^{-1} which was assigned to $\text{L} \rightarrow \text{M}$ charge transfer transition. All the La(III) and Th(IV) complexes are found to be diamagnetic in nature.

4.5. ESR spectroscopy

The ESR spectrum of VO(IV) (5) complex has been studied under liquid nitrogen temperature using TCNE as a g marker. The spectrum (Fig. 3) is a typical eight-line pattern, which shows that

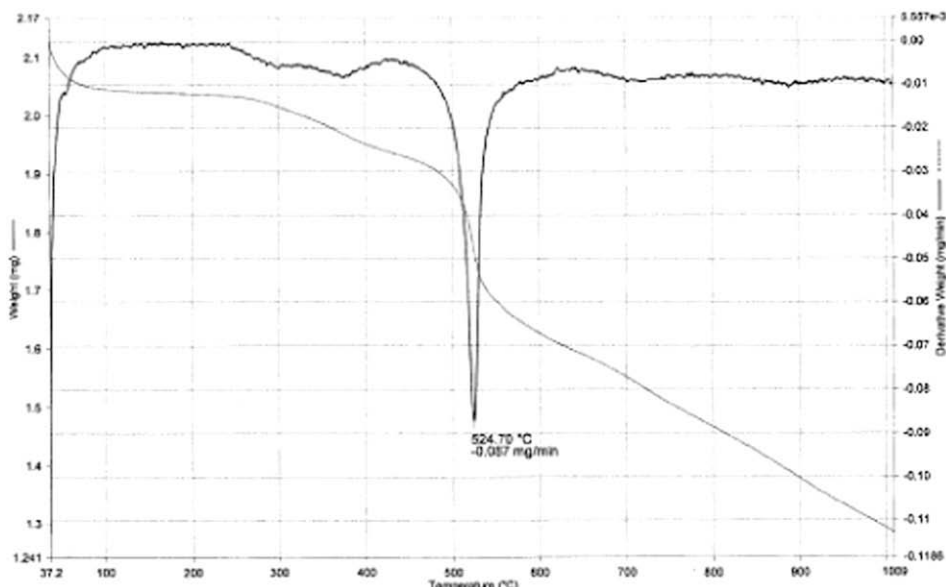


Fig. 4. Thermogram of La(III) (1) complex.

Table 4
Thermogravimetric data of La(III) (**1**), Th(IV) (**3**) and VO(IV) (**5**) complexes.

Empirical formula	Metal oxide%		Decomposition temperature °C	% Weight loss		Inference
	Obsd.	Calcd.		Obsd.	Calcd.	
[La(H ₂ L ¹)(NO ₃)]·H ₂ O	22.122	22.126	50–60	2.572	2.586	Loss of lattice water
			355–375	8.901	8.908	Loss of coordinated nitrate
			505–565	68.672	68.678	Decomposition of organic part
			45–95	6.075	6.081	loss of lattice water
[Th(H ₂ L ¹)(NO ₃) ₂]·3H ₂ O	27.923	27.928	310–340	13.966	13.964	Loss of coordinated nitrate
			505–585	53.821	53.829	Decomposition of organic part
			300–345	2.931	2.935	Loss of oxygen atom
			460–535	87.701	87.706	Decomposition of organic part
[VO(H ₂ L ¹)]	12.290	12.293				

a single vanadium is present in the molecule, i.e., it is a monomer. The g_{\parallel} and g_{\perp} values have been found to be 2.0093 and 2.0294 respectively. The g_{av} was calculated to be 2.0227. The parameter G , is found to be 0.3163. The A_{\parallel} and A_{\perp} values have been found to be $144.79 \times 10^{-4} \text{ cm}^{-1}$ and $46.1035 \times 10^{-4} \text{ cm}^{-1}$ respectively. The A_{av} was calculated to be $78.99 \times 10^{-4} \text{ cm}^{-1}$. The observed order of the parameters ($A_{\parallel} > A_{\perp}$ and $g_{\perp} > g_{\parallel}$) indicates that the complexes are of square-pyramidal geometry, characteristic of the oxovanadium m(IV) complexes [32,33].

4.6. Thermal studies

The thermal behavior studies of all the complexes are almost same. Hence, the representative La(III) (**1**), Th(IV) (**3**) and VO(IV) (**5**) complexes have been discussed. The representative thermogram of La(III) (**1**) is depicted in Fig. 4.

The La(III) (**1**) and Th(IV) (**3**) complexes decompose gradually with the formation of respective metal oxide above 600 °C. In the thermograms of the La(III) (**1**) complex and Th(IV) (**3**) complexes, the lattice water molecules decomposed between 50–60 and 45–95 °C respectively; coordinated nitrate decomposes in the region 355–375 and 310–340 °C respectively. The La(III) (**1**) complex decomposed significantly from 505 to 565 °C centered at 524 °C and Th(IV) (**3**) complex from 505 to 585 °C centered at 540 °C due to the loss of organic part.

The thermal decomposition of VO(IV) (**5**) complex took place from 300–345 °C corresponding to the decomposition of oxygen atom. The organic part decomposed from 460 to 535 °C in two steps centered at 466 and 510 °C corresponding to the loss of aminophenol and formyl coumarin moieties respectively. Finally the metal oxide formed above 720 °C. The nature of the proposed

chemical change with the temperature range and the percentage of metal oxide obtained are given in Table 4.

4.7. Electrochemistry

Electrochemical behaviour of the VO(IV) (**5–6**) complexes was studied on a CHI110A-Electrochemical analyzer in dimethyl formamide (DMF) containing 0.05 M *n*-Bu₄NClO₄ as the supporting electrolyte. The nature of cyclic voltammogram is similar for both the complexes. Hence the representative VO(IV) (**5**) is discussed here. A cyclic voltammogram of VO(IV) (**5**) (Fig. 5) displays one reduction peak at $E_{pc} = 0.6553 \text{ V}$ with a corresponding oxidation peak at $E_{pa} = 0.7926 \text{ V}$. The peak separation (ΔE_p) is 0.1373 V at 100 mV/s. The most significant feature of the VO(IV) complex is the one electron transfer redox process corresponding to the VO(IV)/VO(III) couple. The analyses of cyclic voltametric responses with the varying scan rates from 100 to 400 mV/s give the evidence for quasi-reversible nature (Fig. S2). [Cyclic voltammetric figures of VO(IV) (**6**) is presented in Supplementary information; Figs S3 and S4].

4.8. Pharmacological results

4.8.1. In vitro antibacterial and antifungal activities

The antibacterial and antifungal studies suggested that, both the Schiff bases were found to be biologically active and some of their metal complexes showed significantly enhanced antibacterial and antifungal activities. It is, however, known [34,35] that, chelating tends to make the Schiff bases act as more powerful and potent bacteriostatic agents, thus inhibiting the growth of bacteria and fungi more than the parent Schiff bases.

In case of antibacterial studies it was observed that, both the Schiff bases were found to be potentially active against *E. coli* and *Sa. typhi*. La(III), Th(IV) and VO(IV) metal complexes shown much enhanced activity against *E. coli*, *P. aeruginosa* and *Sa. typhi* and some of these complexes shown almost similar activity as that of standard drug (Table 5, Fig. 6). Most interesting finding is both the Schiff bases and their complexes shown higher activity compared to standard drug against *S. aureus* however the activity is less.

Both the Schiff bases and their La(III), Th(IV) and VO(IV) complexes are found to possess high antifungal activity than the antibacterial activity. Antifungal activity of synthesized Schiff bases and some of their complexes is almost nearer to the standard drug *Flucanazole* (Table 5, Fig. 7).

The minimum inhibitory concentration (MIC) of some selected compounds, which showed significant activity against selected bacterial and fungi species, was also determined (Table 6). The MIC of these compounds varies from 10–100 µg/mL. The results indicated that these compounds were the most active in inhibiting the growth of the tested organisms at a 10 µg/mL concentration.

The ligands generally showed moderate antibacterial activity against one or two species. However, they showed good antifungal

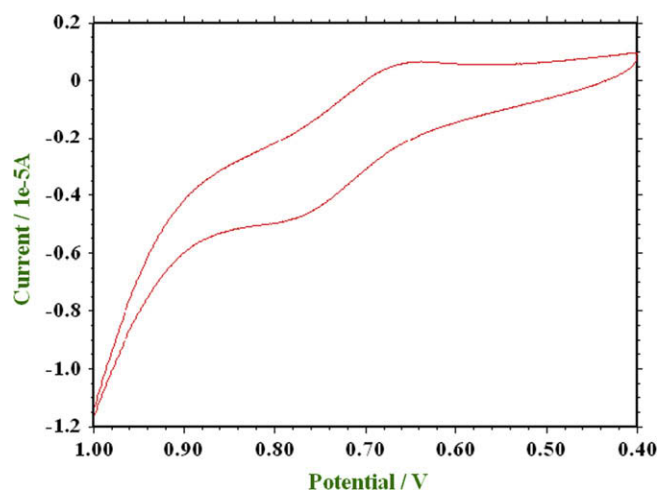


Fig. 5. Cyclic voltammogram of VO(IV) (**5**) complex at scan rate 100 mV/s.

Table 5
Antimicrobial results of Schiff bases and their metal complexes.

Compound	Conc. ($\mu\text{g mL}^{-1}$)	% Inhibition against bacteria				% Inhibition against fungi		
		<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>Salmonella typhi</i>	<i>Aspergillus flavus</i>	<i>Cladosporium</i>	<i>Aspergillus niger</i>
$\text{H}_2\text{L}^{\text{I}}$	25	6.97	17.14	2.63	26.19	84.31	53.84	47.61
	50	23.25	23.52	44.73	45.23	92.15	76.92	76.19
	100	95.34	38.2	50.00	59.52	96.07	87.17	88.09
$\text{H}_2\text{L}^{\text{II}}$	25	8.26	16.35	6.35	28.96	82.64	52.86	54.36
	50	25.36	25.36	41.98	44.96	88.96	71.56	75.93
	100	92.55	44.26	52.63	61.59	94.56	82.94	83.67
1	25	27.9	5.88	55.26	42.85	86.27	58.97	78.57
	50	67.44	10.29	65.76	69.04	90.19	64.01	83.33
	100	81.39	16.17	94.73	80.71	98.03	74.35	92.85
2	25	29.63	9.36	53.67	44.59	83.69	56.95	79.38
	50	65.32	25.63	71.48	67.83	91.59	66.79	81.37
	100	84.23	29.35	91.35	80.49	94.86	78.19	94.59
3	25	44.66	16.17	46.39	54.76	72.53	56.41	42.85
	50	70.69	22.05	66.05	64.28	78.43	74.35	69.04
	100	78.34	25.00	78.06	76.19	84.31	79.48	71.42
4	25	43.59	14.59	40.59	44.89	68.96	55.76	44.85
	50	71.29	21.03	65.98	61.55	72.36	68.96	68.97
	100	79.29	22.36	74.89	76.36	78.99	80.97	74.55
5	25	37.20	23.52	18.42	61.9	39.34	53.84	80.95
	50	48.83	27.94	71.05	69.04	50.81	79.48	88.09
	100	76.74	35.29	73.68	71.42	54.09	97.43	95.23
6	25	41.26	25.96	26.43	55.96	44.59	55.86	82.67
	50	55.63	35.89	67.89	68.35	55.87	82.57	86.96
	100	81.32	44.56	72.99	74.59	61.59	96.38	94.67
Gentamycin	25	86.04	5.88	81.57	61.9	–	–	–
	50	88.37	7.35	86.84	71.4	–	–	–
	100	88.37	14.7	89.47	80.95	–	–	–
Flucanazole	25	–	–	–	–	84.31	92.3	88.09
	50	–	–	–	–	94.1	94.87	92.85
	100	–	–	–	–	96.08	97.43	97.61
Control	100	100	100	100	100	100	100	100

activity against most of the species. It was evident from the data that this activity significantly increased on coordination. This enhancement in the activity of both the Schiff bases may be rationalized on the basis that their structures mainly possess C=N bond. It has been suggested that the ligands with nitrogen and oxygen donor systems inhibit enzyme activity, since the enzymes which require these groups for their activity appear to be especially more susceptible to deactivation by metal ions on coordination. Moreover, coordination reduces the polarity [36,37] of the metal ion mainly because of the partial sharing of its positive charge with the donor groups [38–40] within the chelate ring system formed during coordination. This process, in turn, increases the lipophilic nature of the central metal atom, which favors its permeation more efficiently through the lipid layer of the micro-organism [41–44] thus destroying them more aggressively.

4.8.2. Electrophoretic analysis

The La(III) (**1**) and VO(IV) (**5**) complexes were studied for their DNA cleavage activity by agarose gel electrophoresis method and presented in Fig. 8.

The gel after electrophoresis clearly revealed that, both the complexes have acted on DNA as there was molecular weight difference between the control and the treated DNA samples. The difference was observed in the bands of complexes (Lane 1–4) compared to the control DNA of *E. coli* and *A. niger*. This shows that the control DNA alone does not show any apparent cleavage where as complexes shown. However, the nature of reactive intermediates involved in the DNA cleavage by the complexes has not been clear. The results indicated the important role of metal in these isolated DNA cleavage reactions. As the compound was observed to cleave

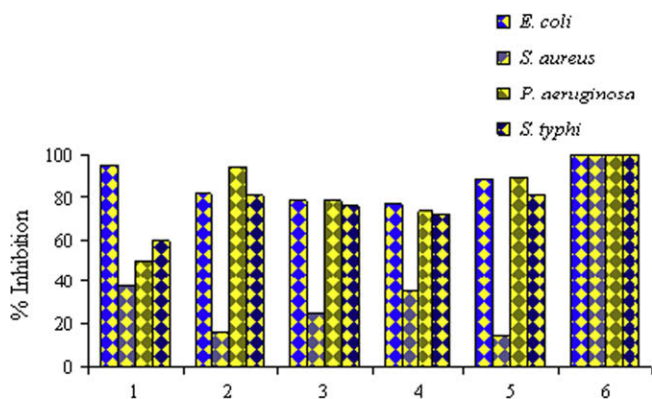


Fig. 6. Antibacterial results of $\text{H}_2\text{L}^{\text{I}}$ and its complexes.

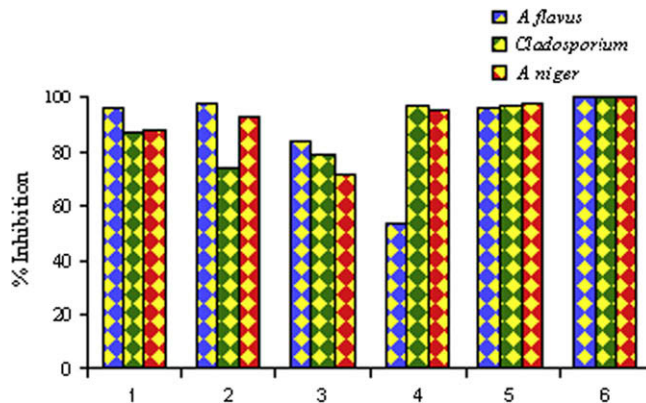


Fig. 7. Antifungal results of $\text{H}_2\text{L}^{\text{I}}$ and its complexes.

Table 6

Minimum inhibitory concentration ($\mu\text{g/mL}$) of selected compounds against selected bacteria.

Compound	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>Sa. typhi</i>	<i>A. Flavus</i>	<i>Cladosporium</i>	<i>A. Niger</i>
$\text{H}_2\text{L}^{\text{I}}$	10	22	10	10	10	10
$\text{H}_2\text{L}^{\text{II}}$	10	24	10	10	10	10
1	10	15	15	10	20	10
2	10	20	10	15	20	15
3	30	20	50	30	50	20
4	50	40	40	30	40	15
5	10	20	20	15	15	10
6	15	30	10	10	25	10

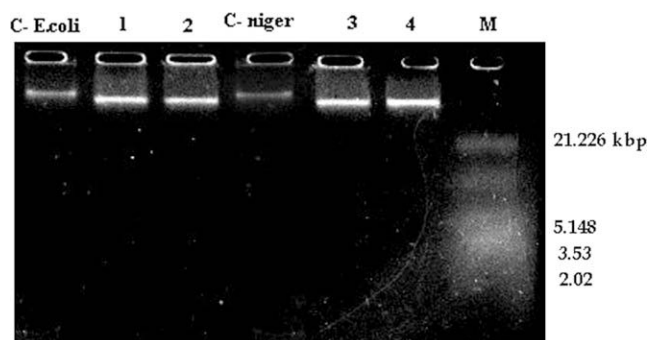


Fig. 8. DNA cleavage activity of La(III) (**1**) and VO(IV) (**5**) complexes.

the DNA, it can be concluded that the compound inhibits the growth of the pathogenic organism by cleaving the genome.

5. Conclusions

The newly synthesized Schiff bases act as tetradentate ligands. The metal ion is coordinated through the azomethine nitrogen and phenolic oxygen atoms via deprotonation. The bonding of ligand to metal ion is confirmed by the analytical, spectral, magnetic and thermal studies.

The electrochemical properties of the metal complexes, investigated in DMF shown the most significant feature of the VO(IV) complex is the VO(IV)/VO(III) couple.

Antibacterial study reveals that, Schiff bases and some metal complexes were found to be highly active against *E. coli*, *P. aeruginosa* and *Sa. typhi*. In case of antifungal studies, both Schiff bases and some of their complexes are found to be highly active. The DNA cleavage studies revealed that, the La(III) (**1**) and VO(IV) (**5**) complexes shown non-specific cleavage of DNA.

All these observations put together lead us to propose the following structure (Figs. 9–11).

6. Experimental

Carbon, hydrogen and nitrogen were estimated by using C, H, N analyzer. The IR spectra of the Schiff bases and their La(III), Th(IV) and VO(IV) complexes were recorded on a HITACHI-270 IR spectrophotometer in the $4000\text{--}250\text{ cm}^{-1}$ region in KBr disc. The electronic spectra of the complexes were recorded in DMF and DMSO on a VARIAN CARY 50-BIO UV-spectrophotometer in the

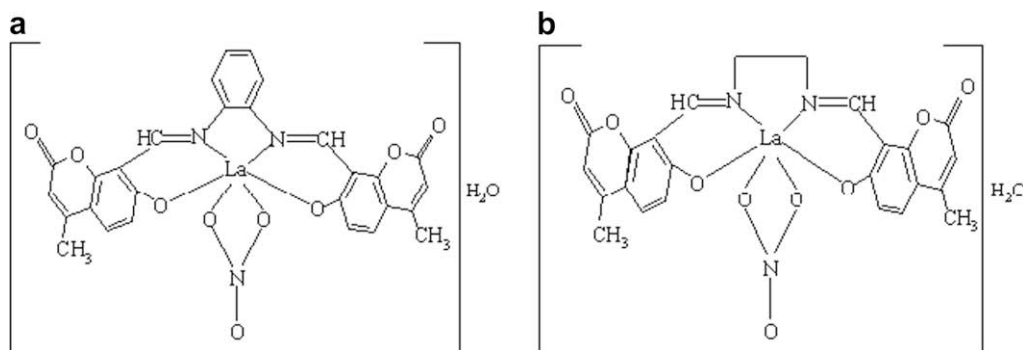


Fig. 9. Proposed structure of La(III) (**1,2**) complexes.

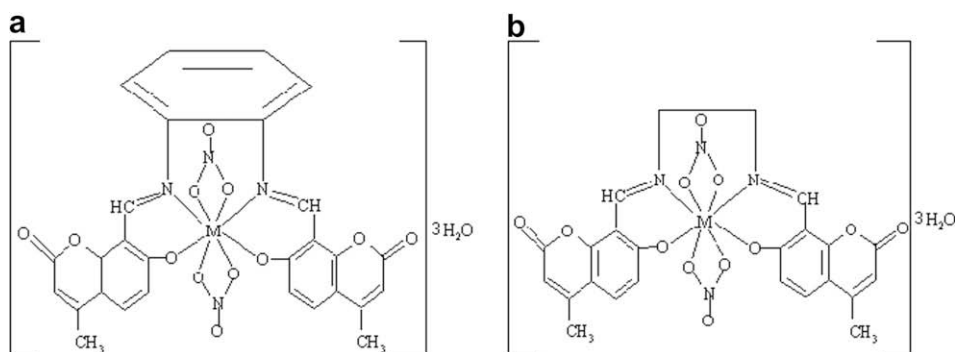


Fig. 10. Proposed structure of Th(IV) (**3,4**) complexes.

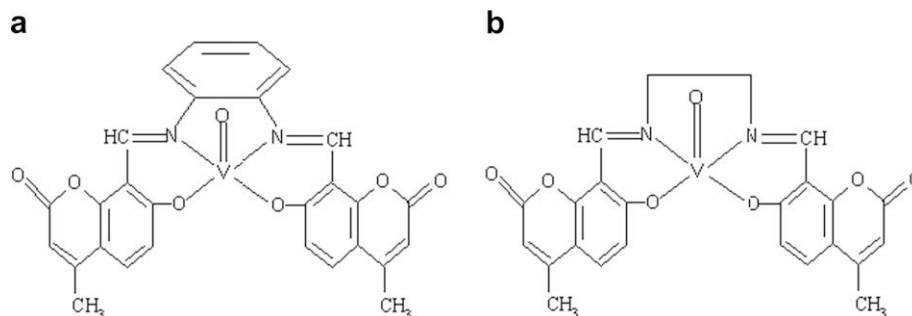


Fig. 11. Proposed structure of VO(IV) (5,6) complexes.

region of 200–1100 nm. The ^1H NMR, ^{13}C NMR and two dimensional homocopy spectra of the Schiff bases were recorded in CDCl_3 and ^1H NMR spectra of La(III), Th(IV) complexes were recorded in $\text{DMSO}-d_6$ on a BRUKER 300 MHz spectrometer at room temperature using TMS as an internal reference. The ESR spectrum was recorded on Varian-E-4X-band EPR spectrometer and the field set is 3200 G at modulation frequency of 100 kHz under liquid nitrogen temperature using TCNE as g marker. FAB-mass spectra were recorded on a JEOL SX 102/DA-6000 mass spectrometer/data system using argon/xenon (6 kV, 10 A) as the FAB gas. The accelerating voltage was 10 kV and the spectra were recorded at room temperature and m -nitrobenzyl alcohol was used as the matrix. The mass spectrometer was operated in the positive ion mode. The electrochemistry of VO(IV) complexes was studied on CHI1110A-electrochemical (HCH Instruments) analyzer (Made in U.S.A). Thermogravimetric analyses 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. Molar conductivity measurements were recorded on ELICO-CM-82T Conductivity Bridge with a cell having cell constant 0.51 and magnetic moment was carried out by using Faraday balance.

Appendix. Supporting information

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejmech.2008.12.012

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