Oxovanadium Complexes with Bidentate N, O Ligands: Synthesis, Characterization, DNA Binding, Nuclease Activity and Antimicrobial Studies

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Abstract

Novel oxovanadium(IV) complexes (1-4) with 2-methyl-3-(pyridine-2-ylmethyleneamino)quinazolin-4(*3H*)-one (L^1) or3-(2-hydroxy-3-methoxybenzylideneamino)-2-methylquinolin-4(*3H*)-one (L^2) were synthesized and characterized by elemental analysis, IR, ^{1}H-NMR, electronic spectra, molar conductance and thermal studies. Based on the above spectral studies, the complexes have the general formula $[VO(L^1)_2]$ (1), $[VO(L^1)phen]$ (2), $[VO(L^2)_2]$ (3) and $[VO(L^2)phen]$ (4). The synthesized compounds were tested for antimicrobial activity by disc diffusion method. The results indicate the enhanced activity of metal complexes over their parent ligands. The DNA binding and nuclease activity of the synthesized compounds were also studied. The investigation of the interaction of the complexes with calf thymus DNA has been performed with absorption spectroscopy which showed that the complexes are avid binders of calf thymus DNA. Also, the interaction of the oxovanadium(IV) complexes with plasmid DNA (pUC 19) was studied using agarose gel electrophoresis. The results revealed that these complexes could act as effective DNA cleaving agents resulting in the nicked form of DNA (P^{UC} 19) under physiological conditions. The gel was run both in the absence and in presence of the oxidizing agent.

Keywords: 4(*3H*)quinazolinone; oxovanadium complexes; antimicrobial activity; DNA interaction.

1. Introduction

Vanadium is actually known as a trace element, essential for higher organisms although deficiency symptoms in humans have not yet been clearly identified [1]. The coordination chemistry of vanadium is of great current interest because of the discovery of its presence in abiotic as well as biotic systems [2, 3]. Vanadium(V) complexes are known as potential inhibitors of various enzymes. Recent advances in catalytic and medicinal properties of vanadium complexes have stimulated their design and synthesis. Another important impetus to the coordination chemistry of vanadium in the context of medical application has arisen from the ability of vanadium complexes to promote the insulin mimetic activity in pathophysiological state of diabetes mellitus in humans [4-7]. This biological and catalytic relevance of vanadium has promoted the synthesis of model vanadium compounds containing O, N donor ligands.

Schiff bases and their metal complexes have a variety of applications in biological, clinical and analytical fields [8]. Recently, there has been a considerable interest in the chemistry of 4(*3H*)-quinazolinones because of their potential pharmacological applications [9, 10]. Quinazolinones are excellent reservoir of bioactive substances. The stability of the quinazoline nucleus has inspired medicinal chemist to introduce many bioactive moieties to this nucleus to synthesize new potential medicinal agents [11]. The interaction of Schiff base-transition metal complexes with nucleic acids is a major area of research due to the utility of these complexes in the design and development of synthetic restriction enzymes, chemotherapeutic agents, foot printing agents, spectroscopic probes, site specific cleavers and molecular photo switches. Especially, a number of metal complexes of a variety of ligands were studied in view of their affinity towards DNA and specificity for the DNA base sequence recognition [12-15]. Some kind of metal complexes interacted with DNA could induce the breakage of DNA strands by appropriate methods. In the case of cancer genes, after DNA strands are cleaved, the DNA double strands break. The replication ability of cancer gene is destroyed [16].

Literature search reveals that no research has centered on vanadium chemistry with 4-(*3H*)-quinazolinones ring systems. Therefore, considering the broad spectrum of potentially useful biological implications and the catalytic activity of the metal complexes, the work done in this area is being described in this paper, which deals with the oxovanadium(IV) complexes with two Schiff bases, viz., 2-methyl-3-(pyridine-2-ylmethyleneamine)quinazolin-4(*3H*)-one and 3-(4-hydroxy-3-methoxybenzylideneamino)-2-methylquinazolin-4(*3H*)-one with 1,10-phenanthroline.

2. Methods

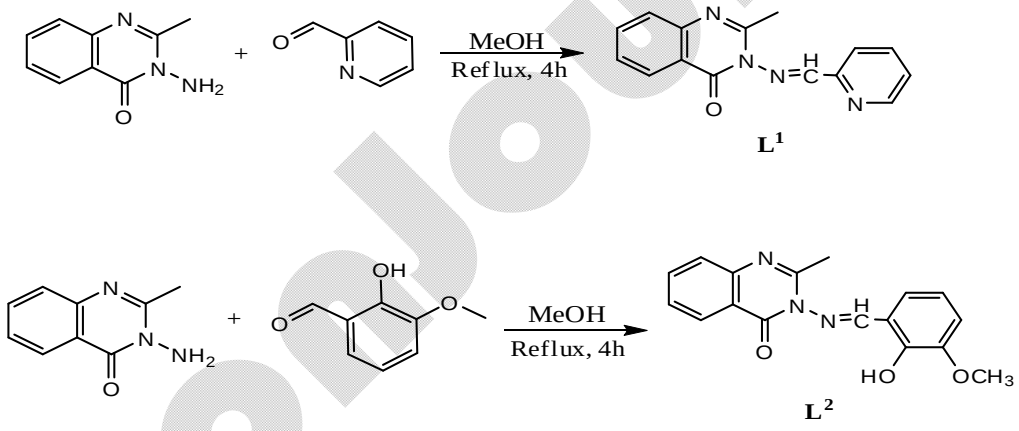
2.1. Chemistry

Solvents and reagents were obtained from commercial sources and used without further purification unless otherwise stated. 3-amino-2-methyl-4(*3H*)quinazolinone and calf thymus DNA were purchased from Sigma-Aldrich Chemicals, Germany. Plasmid pUC19 (0.25 µg/µL in 10 mM Tris and 1 mM EDTA, pH 8.0) was purchased from Roche Diagnostics, Germany. Melting points of the compounds were determined on an ELICO-3210 apparatus and are uncorrected. IR spectra of the compounds were recorded on a Shimadzu FT-IR-8300 instrument by Nujol mull method in the range of 4000-200 cm^{-1}. Elemental analyses (CHNO) were determined using a Perkin-Elmer 240 elemental analyzer. ^{1}H-NMR spectra of ligands and their VO(IV) complexes were recorded on Bruker DRX-300 spectrometer in DMSO-d_6 solution with TMS as the internal standard. Molar Conductivity Bridge with a cell having cell constant 1.Thermogravimetric analyses data were recorded on a Shimadzu TG-50 Thermobalance and were measured from room temperature to 600 ºC at a heating rate of 10 ºC/min.

2.2. Synthesis

2.2.1. Synthesis of Schiff base ligands (L^1 and L^2)

The ligands were synthesized by the condensation of 3-amino-2-methyl-4(*3H*)quinazolinone (1 mmol) with 2-pyridine carboxaldehyde (to obtain **L^1**) or 4-hydroxy-3-methoxybenzaldehyde (to obtain **L^2**) in 1:1 ratio. The reaction mixture was refluxed for 4 h with continuous stirring. The colorless product formed was then isolated by filtration, washed and recrystallized from methanol. The scheme of synthesis of two ligands (**L^1** and **L^2**) is presented in scheme 1.



Scheme 1. Synthetic route of ligands.

2.2.2. Synthesis of oxovanadium complexes

The complexes were prepared by mixing the aqueous solution of $VOSO_4.3H_2O$ (1 mmol) and the appropriate quinazolin-4(*3H*)-one (**L^1** or **L^2**) (2 mmol) in 1:2 ratio and with 1,10-phenanthroline in 1:1:1 ratio. The mixture was refluxed for about 5 h. the crystalline complexes were separated and collected by filtration, washed with hot methanol and air dried.

3. Biology

3.1. Antimicrobial activity

The *in vitro* antimicrobial screening effects of the synthesized compounds were tested against five bacterial strains namely *Bacillus subtilis, E.coli ,Staphylococcus aureus, Ralstonia solanacearum* and *Xanthomonas vesicatoria* and four fungal strains namely *Aspergillus niger, Aspergillus flavus, Fusarium oxysporum* and *Alternaria solani* by disc diffusion method using nutrient agar medium for antibacterial activity and potato dextrose agar medium for antifungal activity [17, 18].

The bacteria and fungi were subcultured in the agar and potato dextrose agar medium and were incubated for 24 h for bacteria and 48 h for fungi at 37 ºC. Standard antibacterial drug (gentamycine) and antifungal drug (fluconazole) were used for comparison. The discs having a diameter of 4 mm were soaked in the test solutions and were placed on an appropriate medium previously seeded with organisms in Petri plates and stored in an incubator at the above mentioned period of time. The inhibition zone around each disc was measured and the results have been recorded in the form of inhibition zones (in percentage).In order to clarify any effect of DMF on the biological screening, separate studies were carried out with solutions alone of DMF and they showed no activity against any microbial strains. The stock solution (1 mg/ml^{-1}) of the test compounds was prepared in DMF. Each test was performed in triplicate in individual experiments and the average is reported.

3.2. DNA binding and cleavage experiments

The DNA binding and cleavage experiments were performed at room temperature. A solution of CT-DNA in buffer solution gave a ratio of UV absorbance at 260 and 280 nm of 1.9:1, indicating that the DNA was sufficiently free from proteins [19]. The absorption titration of the VO(IV) complexes (**1-4**) in buffer (50 mM NaCl, 5 mM Tris-HCl, pH 7.0) was performed by using a fixed complex concentration (5 µM) to which increments of CT-DNA stock solution was added (5-25 µM). Complex-DNA solutions were allowed to incubate for 5 min before the absorption spectra were recorded. The data were then fitted into following equation to obtain the intrinsic binding constant, K_b [20].

$$[DNA]/(\varepsilon_a-\varepsilon_f) = [DNA]/(\varepsilon_b-\varepsilon_f)+ 1/ K_b (\varepsilon_a-\varepsilon_f)$$

Where ε_a, ε_f,ε_b are the apparent, free and bound metal complex extinction coefficients. A plot of [DNA]/ (ε_b-ε_f) versus [DNA] gave a slope of 1/ (ε_b-ε_f) and a '*y*' intercept equal to 1/K_b(ε_b-ε_f), where K_b is the ratio of the slope to the *y* intercept.

3.3. Viscosity measurements

The viscosity experiments were conducted using an Ubblodhe viscometer, immersed in a thermostatic water-bath maintained at 28 ºC. Each complex was introduced into the DNA solution (0.5 m mol L^{-1}) by a micro syringe. Flow time was measured with a digital stopwatch, and each sample was measured three times and average flow time was calculated. Data are presented as $(\eta/\eta_o)^{1/3}$ versus the binding ratio [21], where η is the viscosity of DNA in the presence of the complex and η_o is the viscosity of DNA solution alone.

3.4. Nuclease activity

The gel electrophoresis were performed by using pUC19 DNA supercoiled plasmid DNA. DMF solutions of the complexes were placed in clean Eppendorf tubes and 1 µg of pUC19 DNA was added. The contents were incubated for 1.5 h at 37 ºC and loaded on 1 % agarose gel after mixing 5 µl of loading buffer (25% bromophenol blue + 0.25 % xylene cyanol + 30 % glycerol (3 µl) + sterilized distilled water). Electrophoresis was performed at a constant voltage (70 V) until the bromophenol blue reached to three-fourth of the gel. The gel was then stained for 10 min by immersing it in ethidium bromide solution. It was then de-stained by keeping in sterile distilled water for 10 min. Plasmid bands were visualized by viewing the gel under UV light and photographed. The efficiency of the DNA cleavage was measured by determining the ability of the complex to form open circular (OC) or nicked circular (NC) DNA from its super coiled (SC) form. The reactions were carried out in oxidation conditions (H_2O_2).

4. Results and Discussion

All the complexes are stable at room temperature, non-hygroscopic, insoluble in water but slightly soluble in methanol and ethanol and soluble in DMF and DMSO. The analyses of the proposed $[VO(\mathbf{L^1})_2]H_2O$, $[VO(\mathbf{L^1})(phen)]H_2O$, $[VO(\mathbf{L^2})_2]H_2O$, $[VO(\mathbf{L^2})(phen)]H_2O$ are consistent with stoichiometry 1:1 and 1:1:1 (Figure 1) and are summarized in Table 1. The molar conductance values at the 10^{-3} concentration are too low to account for any dissociation of the complex in DMF. Hence, the synthesized VO(IV) complexes may be regarded as non-electrolytes.

4.1. IR spectra

The Schiff bases show prominent peaks at *ca* 1685 and 1691 cm^{-1} corresponding to ν(C=O) and another band at 1597 and 1605 cm^{-1} corresponding to ν(C=N). In addition, a broad weak band with fine structure in the region 2862-2871 cm^{-1} was observed and that can be attributed to ν(OH) as shown in Table 2.

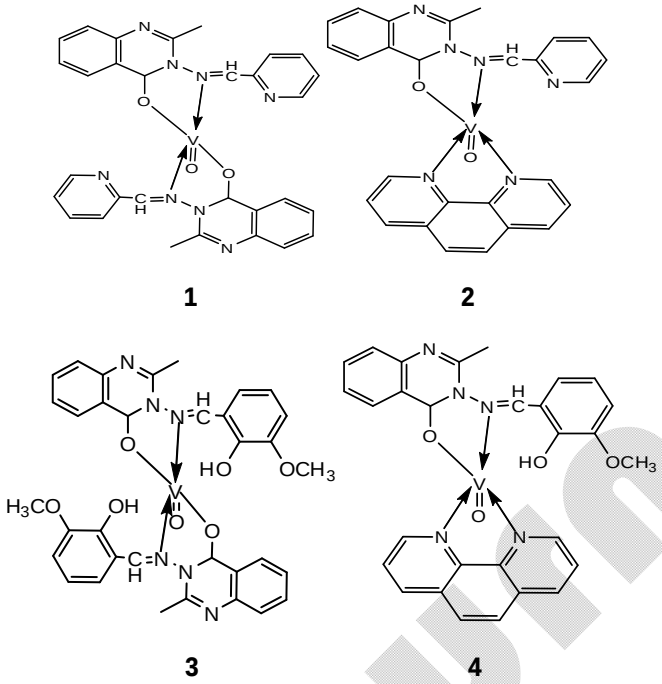


Figure 1. Proposed structures of the complexes.

Table 1. Analytical data and some physical properties of the Schiff bases and their complexes.

Compound	Mol. Formula	Yield %	ω_i(calc.)/% ω_i(found)/%				Molar conductivity Scm^2mol^{-1}
			C	H	O	N	
L^1	$C_{15}H_{12}N_4O$	84	68.17 (68.26)	4.75 (4.80)	6.05 (6.15)	21.20 (21.31)	-
L^2	$C_{17}H_{15}N_3O_3$	79	66.01 (66.22)	4.89 (4.93)	15.52 (15.64)	13.38 (13.49)	-
1	$C_{32}H_{24}N_8O_3V$	73	61.24 (61.51)	5.14 (5.20)	7.65(7.73)	17.85 (17.98)	4.13
2	$C_{28}H_{20}N_6O_2V$	82	63.76 (63.89)	4.59 (4.67)	6.07 (6.11)	15.93 (16.01)	8.20
3	$C_{36}H_{30}N_6O_7V$	72	60.25 (60.39)	5.34 (5.38)	15.61 (15.70)	11.71 (11.86)	5.31
4	$C_{30}H_{23}N_5O_4V$	80	62.94 (62.06)	4.75 (4.81)	11.18 (11.26)	12.23 (12.36)	8.63

In the spectra of all the complexes, the ν(C=O) band intensity decreases significantly as compared to the free ligands and new bands appear at 1658, 1665, 1669 and 1672 cm^{-1}. Two sharp peaks observed at 1582 and 971 cm^{-1} are due to ν(C=N) and ν(V=O), respectively. The observed down field shifts going from the free Schiff base ligands to the complexes, suggest coordination of the carbonyl group to the metal and also involvement of the azomethine nitrogen. The new less intense bands in the regions of 415 and 510 cm^{-1} are due to ν(V→N) and ν(V→O), which indicates the coordination of the ligand to the metal ion. All these complexes exhibit a broad band in the region 3325-3348 cm^{-1} indicating the presence of lattice or coordinated water molecule [22].

Table 2. Important infrared frequencies (cm^{-1}) of Schiff base ligands and theirVO(IV) complexes.

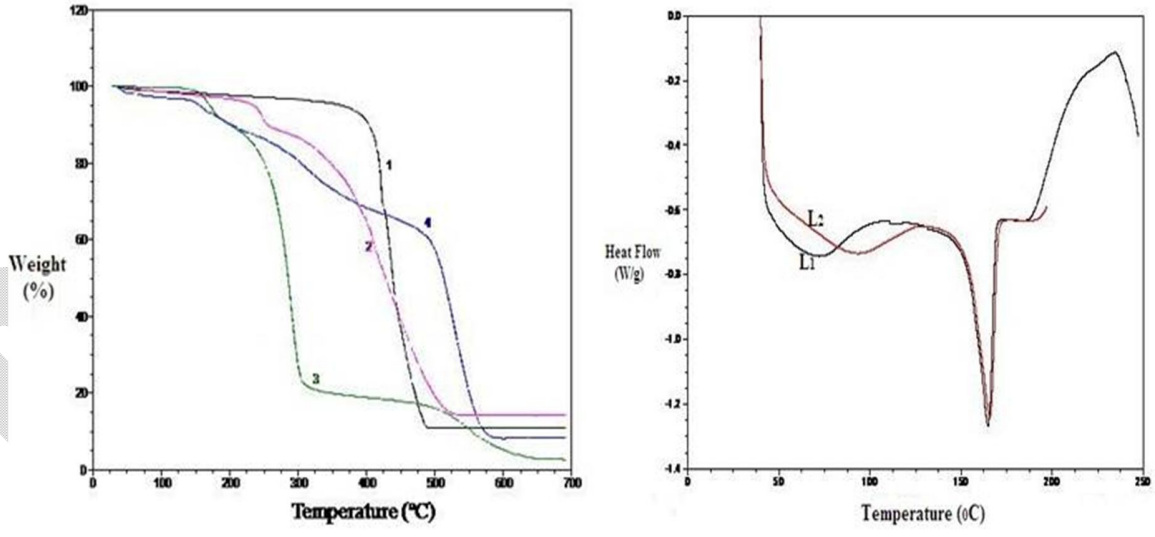
Compound	ν(C=N)	ν(C=O)	ν(M-N)	ν(M-O)
L^1	1597	1685	-	-
L^2	1605	1691	-	-
1	1582	1658	415	507
2	1584	1665	422	510
3	1579	1669	419	503
4	1577	1672	426	506

4.2. ^{1}H-NMR

The ^{1}H-NMR spectra of Schiff bases exhibit signals at 8.96, 2.74, and 7.42-8.36 ppm due to HC=N, methyl protons and aromatic protons, respectively. The signal at 10.6 ppm observed in **L^2**was assigned for OH proton. The signal due to azomethine proton of Schiff base (*ca* 8.96 ppm) shows a down field shift *ca* 9.17 ppm [23] in the spectra of complexes indicates the coordination of the azomethine group to the metal ion. This down field shift is due to deshielding of =CH proton. This was further supported by IR inferences. The resonance due to phenolic –OH *ca* 10.6 ppm in **L^2**was remained unchanged in the complexes, suggesting that the non-involvement of phenolic oxygen in the coordination. The signal due to a multipletca 7.42-8.36 ppm due to aromatic protons and signal around 2.74 ppm due to methyl protons are unaffected in the case of VO(IV) complexes.

4.3. Thermal studies

The thermal behavior of the free ligands and the four VO(IV) complexes was investigated by means of TG and DTA measurements under N_2 atmosphere up to 700 ºC (Figure 2). Thermograms of the complexes indicate that their decomposition occurs in two steps. The degradation starts at 110 ºC with a loss of trapped water molecule *ca.* 3. 02 % (in complex **1**), 4.9 % (in complex **2**), 2.6 % (in complex**3**) and 3.2 % (in complex**4**), while an exothermic weight loss of *ca.* 44.3 % (in complex **1**), 71.9 % (in complex **2**), 44.5 % (in complex **3**) and 54.8 %(in complex **4**) associated with removal of ligand moiety occurs in the temperature range of160-500 ºC. Further decomposition occurs in the temperature range of 520-680 ºC corresponds to the final residue estimated as free vanadium oxide with weight loss of 11.2 % (in complex **1**), 18.2 % (in complex **2**), 9.7 % (in complex**3**) and 12.0 % (in complex**4**). The experimental results were in good agreement with the theoretical calculations.



(a) (b)

Figure 2. a) Thermogravimetric (TGA) curve of VO(IV) complexes and (b) DTA curve of ligands.

4.4. Biological results

4.4.1. Antimicrobial activity

The *in vitro* antimicrobial screening results are given in Table 3. On the basis of observed zones of inhibition, it was found that the VO(IV) complexes are more active than their respective Schiff bases. It was found that all the complexes exhibit good antibacterial activity against *S.aureus*. Complexes **2** and **4** show potent antibacterial activity against all the microorganisms and rest of the complexes show moderate activity when compared to the standard. The antifungal activity of all the four complexes was moderate and that of ligands were weak. It was observed that complex **4** was particularly active against all the tested fungi. The higher activity of the metal complexes may be owing to the effect of metal ions on chelation. The polarity of the metal ion will be reduced to a greater extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. Thus, it enhances the penetration of the complexes into lipid membranes and blocking of the metal binding sites in the enzymes of microorganisms.

Table 3. *In vitro* antimicrobial activity of the Schiff base ligands and their metal complexes.

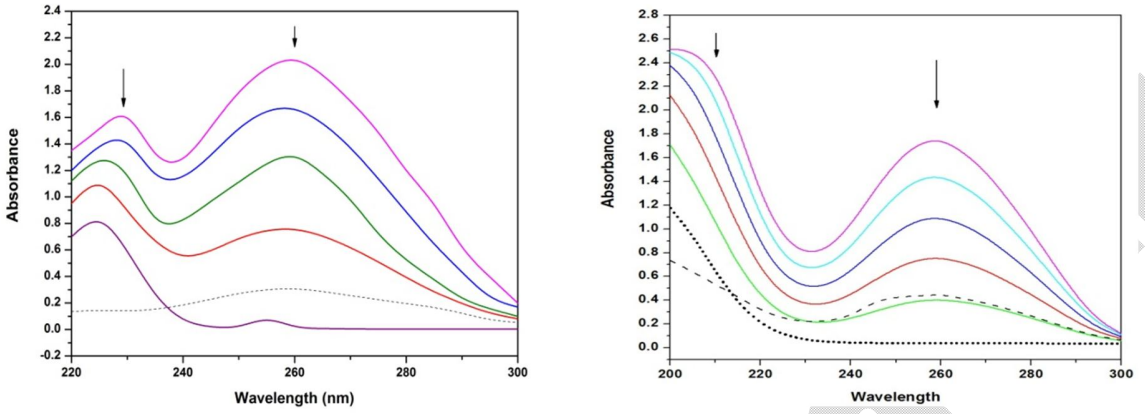
Compound	Conc.(μg mL^{-1})	Antibacterial activity (Zone of inhibition in %)*				Antifungal activity (Zone of inhibition in %)*			
		B. subtilis	*E.coli*	*S. aureus*	*X.vesicatoria*	*A.niger*	*A.flavus*	*F.oxysporum*	*A. solani*
L^1	100	45	48	44	49	35	43	47	49
L^2	100	43	49	47	54	39	41	51	54
1	100	63	65	73	62	46	54	61	63
2	100	77	76	81	79	61	67	70	69
3	100	67	72	77	73	47	53	65	68
4	100	82	87	85	84	75	77	81	73
standard[a]	100	100	100	100	100	-	-	-	-
standard[b]	100	-	-	-	-	100	100	100	100

[a]gentamycine, [b]fluconazole, *average of three replicates

4.4.2. DNA binding properties

The binding ability of the complexes **1** and **3** with DNA is characterized by measuring the effects on the UV spectra of calf thymus DNA (CT-DNA). With increase in the concentration of CT-DNA, the absorption bands of the complexes are affected, resulting in hyperchromism or hypochromism with a hypsochromic shift. In general, hypsochromism and red-shift are associated with the intercalative binding of the complex to the double helix, due to strong intercalation between the complex and the base pairs of DNA. The extent of hypochromism is commonly consistent with the strength of the intercalative interaction [24, 25]. The absorption spectra of both complexes **1** and **3** at different concentrations of CT-DNA are given in Figure 3. In the UV region, both complexes exhibit two absorption bands. Complex **1**shows absorption bands at 205 and 259 nm and complex **3** exhibit at 229 and 259 nm. This hypochromism can be attributed to the π-π* transition. With increase in the concentration of CT-DNA, complexes **1** and **3** show hypochromism and a 4 and 9 nm red-shift at 205 and 229 nm and hypochromism and a 1 and 2 nm red-shift at 259 and 258 nm, respectively. This result suggests that the interaction between the complexes (**1** and **3**) and CT-DNA is by intercalative mode. The

results indicated that the binding strength of the complex **3** is stronger than that of **1**. This may be due to the presence of 1,10-phenanthroline ligand in **3**which may provide an aromatic moiety extending from the metal centre through which overlapping would occur with the base pairs of the DNA by intercalation.



(a) (b)

Figure 3. Absorption spectra of (a) complex **1** and (b) complex **3**, in Tris-HCl buffer upon addition of DNA = 0.5 µM, 0-100 µl. Arrow shows the absorbance change upon increasing the concentration of DNA.

4.4.3. Viscosity measurements

The nature of binding of the complexes **1** and **3** to the CT-DNA was further investigated by viscometric studies. Viscosity measurements were carried out using an Ubbelodhe viscometer at room temperature. Flow time was measured by hand with digital stopwatch, each sample was measured three times and the average flow time was calculated. A significant increase in the viscosity of DNA on addition of complex results due to the intercalation which leads to the separation among the DNA bases to the increase in the effective size in DNA which could be the reason for the increase in the viscosity [26]. Plot of $(\eta/\eta_o)^{1/3}$ versus [complex]/[DNA] gives a measure of the viscosity changes (Fig. 4). A gradual increase in the relative viscosity was observed on addition of the complexes **1** and **3** to DNA solution suggesting mainly intercalation mode of binding nature of the complexes. The viscosity of both the ligands was least as we can see in the Figure 4.

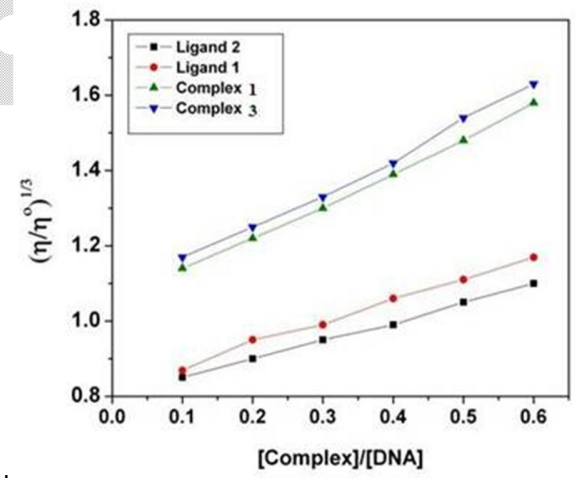


Figure 4. Effect of increasing amounts of VO(IV) complexes and ligands on the relative viscosities of CT-DNA at room temperature.

4.4.4. Nuclease activity

Gel electrophoresis experiments using pUC19 plasmid DNA were performed with free ligands and their complexes in the presence and absence of H_2O_2 as an oxidant. When super coiled DNA is conducted by electrophoresis, faster migration will be observed for DNA of closed circular confirmation (Form I). If one strand is cleaved, the supercoiled DNA will relax to produce a slower moving nicked circular form (Form II). If both strands are cleaved, a linear confirmation (Form III) that migrates between Form I and Form II will be generated [27]. This method using supercoiled pUC19 DNA in the presence of the ligands (L^1 and L^2) and their metal complexes (**2** and **4**) was carried out in a medium of 50 µM Tris-HCl/NaCl Buffer (pH 7.2). Both complexes showed remarkable cleavage in the presence of H_2O_2 (30 µM) as an oxidizing agent.

Figure 5 shows the gel electrophoretic results of the interaction of compounds with plasmid pUC19 DNA. From the figure 5 it is observed that, DNA cleavage by ligands (lane 3 and lane 5) does not occur, indicating the importance of the metal for nuclease activity. Both the complexes **2** and **4** can cleave DNA in the presence and absence of H_2O_2 (lane 6-10).The cleavage is more efficient in the presence of H_2O_2 which may be due to the reaction of hydroxyl radical with DNA [28]. Even in the absence of oxidant, the complexes exhibit significant DNA nuclease activity (lane 7 and lane 9). This may be due to hydrolytic cleavage of the DNA catalyzed by oxovanadium complexes.

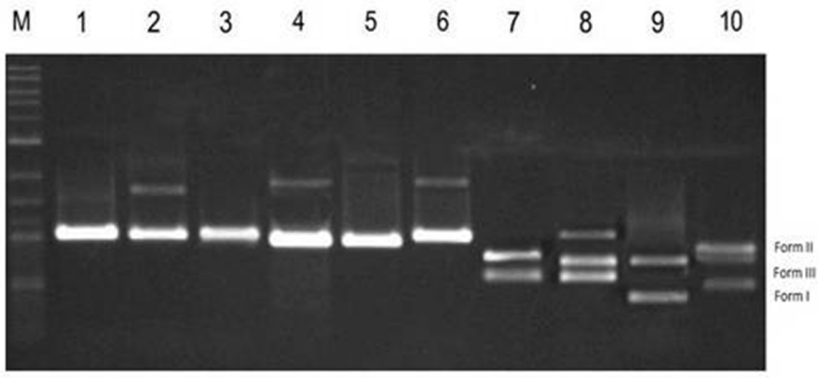


Figure 5. Cleavage of supercoiled pUC19 DNA (0.5 µg) by the ligands and complexes **2** and **4** in a buffer containing 50 mMTris-HCl at 37 ^{0}C (30 min): lane M: marker; lane 1: DNA control; lane 2: DNA + H_2O_2; lane 3: L^1 (10^{-3} M) + DNA; lane 4: L^1 + DNA + H_2O_2; lane 5: L^2 + DNA; lane 6: L^2 + DNA + H_2O_2; lane 7: complex**2** (10^{-3} M) + DNA; lane 8: complex**2** + DNA+ H_2O_2; lane 9: complex **4** + DNA; lane 10: complex **4** + DNA + H_2O_2.

5. Conclusion

In summary, we have synthesized and characterized two novel Schiff bases (**L^1 and L^2**) and their oxovanadium(IV) complexes (**1-4**). The DNA-binding propensity of complexes **1** and **3**were examined by absorption spectroscopy and viscosity measurements. Experimental results indicate that both the complexes can bind to CT-DNA in intercalative mode, and complex **3**have stronger binding affinity than **1**. Both the complexes can promote cleavage of plasmid DNA in the presence and absence of oxidizing agent (H_2O_2) and thus, they may be regarded as hydrolytic cleaving agents. The antimicrobial activity results revealed that oxovanadium complexes, especially complex **4**, was potently active than the ligands.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

HDR and KSP developed the project and were involved in the preparation of manuscript; LSK assisted with the spectral analysis; SCS carried out antimicrobial and anthelminthic experiments; MP carried out the DNA interaction studies.

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