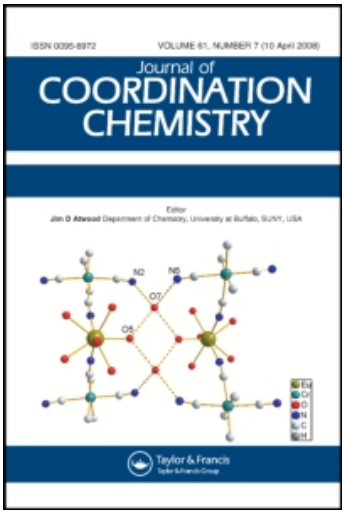

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Synthesis, characterization, and insulin-enhancing studies of unsymmetrical tetradentate Schiff-base complexes of oxovanadium(IV)

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A series of unsymmetrical tetradentate Schiff bases were synthesized by interaction of 2-hydroxy-1-naphthaldehyde, phenylenediamine and salicylaldehyde, or substituted salicylaldehyde in an ethanolic medium. The oxovanadium(IV) complexes and the ligands were synthesized and characterized by elemental analyses, ^{1}H NMR, infrared, electron paramagnetic resonance, electronic spectra, cyclic voltammetry, and room temperature magnetic susceptibility measurements. The elemental analyses for both the ligands and the metal complexes confirmed purity of the compounds as formulated. Electron paramagnetic resonance spectra of the complexes were measured as powder and in toluene/dichloromethane (9 : 1, v/v) solution at room and liquid N_2 temperatures. The g values, $g_o = 1.971$, $g_\perp = 1.978$, and $g = 1.950$, are the same for all the complexes examined. The vanadium nuclear hyperfine splitting, $A_o = 101$–99, $A_\perp = 65$–64, $A_\parallel = 179$–177, vary slightly with substituents on the salicylaldehyde. Infrared spectra reveal strong V=O stretching bands in the range 970–988 cm^{-1}, typical of monomeric five-coordinate complexes. The room temperature magnetic moments of 1.6–1.8 BM for the complexes confirmed that the complexes are V(IV) complexes, with d^1 configuration. Only one quasi-reversible wave is observed for each compound and they all showed redox couples with peak-to-peak separation values (ΔE_p) ranging from 78 to 83 mV, indicating a single step one electron transfer process. Insulin-mimetic tests on C2C12 muscle cells using Biovision glucose assay showed that all the complexes significantly stimulated cell glucose utilization with negligible cytotoxicity at 0.05 μg μL^{-1}.

Keywords: Oxovanadium(IV); Unsymmetrical Schiff base; Insulin-mimetic

1. Introduction

There has been a resurgence of interest in the synthesis and characterization of unsymmetrical Schiff bases and their use as ligands in metal complexation [1–3], partly due to the belief that systematic investigation of these complexes might shed light on the

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nature of complexes of biological relevance [4]. Unsymmetrical tetradentate Schiff-base complexes are required to model the irregular binding of peptides because trace metals have been found to occur in metalloenzymes bound to a macrocycle such as heme ring, or to donor atoms of peptide chains usually in a distorted environment [5]. Unsymmetrical ligands clearly offer advantages over their symmetrical counterparts in the elucidation of the composition and geometry of metal ion binding sites in metalloproteins and in duplication of enzymatic efficiency and selectivity of natural system with synthetic materials. A large percentage of enzymes have a metal at the active site. These metalloenzymes facilitate a variety of reactions, including redox reaction (carried out by oxidases and oxygenases), acid-catalyzed hydrolysis (hydrolases), and rearrangement of carbon–carbon bonds (synthases and isomerases) [6, 7]. Schiff-base complexes of transition metal ions display biological activities such as treatment of cancer, fungicides, viracides, bactericides, and function as catalysts in industrial processes [8–12].

Vanadium is a trace but essential element with well-defined biological activity and has acquired special status among the bio-metals [13]. Its presence in higher animals and some organisms has been well established [14, 15]. The coordination chemistry of vanadium also acquired renewed interest since the discovery of vanadium in organisms such as certain ascidians and amanita mushrooms and as a constituent of the cofactors in vanadate-dependent haloperoxidases and vanadium nitrogenase [15, 16]. Some Schiff-base complexes of oxovanadium(IV) display insulin-enhancing properties when administered as therapeutic agents [17–19]. In particular, Schiff-base complexes of oxovanadium(IV) with the N_2O_2 chromophore have been shown to normalize blood glucose level with high efficiency, even at low concentration, with low toxicity [20]. To date, only [VO(sal_2-en)] [21] and [VO(sal_2-1,3-pn)] [22], among complexes of the type reported in this work, have been reported. A detailed literature review on the insulin-mimetic properties of unsymmetrical Schiff-base metal complexes reveals dearth of information [1–4, 7, 12]. In view of the increasing importance of unsymmetrical Schiff-base complexes of oxovanadium(IV), we describe the synthesis, characterization, and insulin-enhancing studies of four new unsymmetrical tetradentate Schiff-base complexes of oxovanadium(IV), *N*-(naphthalidene)-*N*′-(5-chlorosalicylidene)orthophenylenediiminatooxovanadium(IV) [VOL^1], *N*-(naphthalidene)-*N*′-(5-nitrosalicylidene) orthophenylenediiminatooxovanadium(IV) [VOL^2], *N*-(naphthalidene)-*N*′-(3-ethoxylsalicylidene)orthophenylenediiminatooxovanadium(IV) [VOL^3], and *N*-(naphthalidene) *N*′-(salicylidene)orthophenylenediiminatooxovanadium(IV) [VOL^4]. The corresponding Schiff bases are labelled $H_2L^1 - H_2L^4$.

2. Experimental

2.1. *Materials*

All reagents and solvents were of analar/spectroscopic grades and used without purification. Ethanol, methanol, triethylamine, 5-chlorosalicylaldehyde, 5-nitrosalicylaldehyde, 3-ethoxysalicylaldehyde, 2-hydroxy-1-naphthaldehyde, salicylaldehyde, and vanadyl sulfate were purchased from Aldrich-Sigma.

2.2. *Preparation of the Schiff bases*

Solutions of 2-hydroxy-1-naphthaldehyde (3.444 g, 20 mmol), 1,2-phenylenediamine (2.163 g, 20 mmol), and salicylaldehyde or substituted salicylaldehyde (20 mmol) in absolute ethanol (75 mL) were prepared and chilled in a refrigerator at 4°C for 15 min. To a stirred solution of the cold 2-hydroxy-1-naphthaldehyde, a cold solution of 1,2-phenylenediamine was added dropwise followed by addition of cold solution of salicylaldehyde (or substituted salicylaldehyde) over a period of 2 min. The mixture was stirred at room temperature for 4 days, after which the mixture was warmed to, and kept at, 70°C for 20 min with stirring to dissolve any unreacted reactants. The product was filtered hot and washed twice with ice-cold ethanol. The orange product obtained was purified by digesting in hot ethanol, filtered hot, and dried in a desiccator over silica gel.

2.3. *Preparation of the oxovanadium(IV) complexes*

The following general procedure was used in the synthesis of all oxovanadium(IV) complexes [23]. To a mixture of triethylamine (1.214 g, 12 mmol) and the corresponding unsymmetrical Schiff bases (6 mmol), dissolved in methanol, a solution of oxovanadium(IV) sulfate (0.078 g, 6 mmol) dissolved in 300 mL hot methanol was added with stirring. An instant color change to green was observed in each case. The mixture was stirred for 3 h at 50°C and then concentrated to about half its volume using a rotary evaporator. The product was filtered and washed twice with cold absolute ethanol and allowed to dry in a desiccator over silica gel.

2.4. *Physical measurements*

Carbon, hydrogen, and nitrogen analyses were done on a Perkin-Elmer automated model 2400 Series II CHNS/O analyzer. The ^{1}H NMR spectra of the ligands, in $CDCl_3$ with tetramethylsilane (TMS) as an internal standard, were obtained using a Bruker Avance III 400 MHz spectrophotometer. All chemical shifts are given in ppm *versus* TMS. Infrared spectra were recorded on a Bruker ATR-FT-IR tensor 27 spectrophotometer directly on small samples of the compounds from 200 to 4000 cm^{-1}. Electronic absorption spectra were recorded in the 200–1100 nm range using a Cary Model 50 spectrophotometer. Melting points were determined on Barnstead/electrothermal digital melting point apparatus and are uncorrected. Magnetic susceptibility measurements were taken on powdered samples using a Sherwood Scientific magnetic susceptibility balance. $Hg[Co(NCS)_4]$ was used as the calibrant and corrections for diamagnetism were calculated from Pascal's constants. EPR spectra were measured using a Bruker EMX Micro Premium X spectrometer at X-band (9.4 GHz) on the powder and on the fluid and frozen solutions in toluene/dichloromethane (90/10 v/v). Cyclic voltammetry was run on CHI 832 electrochemical detector. Glassy carbon electrode, platinum wire, and Ag/Ag^+ were used as working, supporting and reference electrodes, respectively. Sample solutions were 10^{-3} M of each complex in spectroscopic grade DMSO containing 0.1 M tetrabutylammonium perchlorate as the supporting electrolyte. Each solution was degassed with ultra pure N_2 for 5 min before each measurement was made.

2.5. In vitro *studies*

2.5.1. Cell culture. C2C12 mouse skeletal muscle cells were obtained from the American Type Culture Collection (ATCC number CRL-1772). Cells were cultured in Dulbecco modified Eagle's medium (DMEM) with 4 mM L-glutamine adjusted to contain 1.5 g L^{-1} sodium bicarbonate, 4.5 g L^{-1} glucose (Lonza, USA), and 10% fetal bovine serum (Highveld Biological, South Africa) in a humidified atmosphere of 5% CO_2 and 95% air at 37°C. C2C12 cells were sub-cultured in log phase to 70% confluence and seeded at a density of 5000 cells per well into 96-well culture plates. To limit batch-to-batch variation, cell subcultures were limited to 10 passages. After 3 days culture myotube formation was induced by replacing the fetal bovine serum (FBS) in the medium with 10% horse serum (Highveld Biological, South Africa). All experiments were done in 5 days when more than 75% of the cells were differentiated morphologically.

2.5.2. Viable cell counts. The cells were suspended in a trypan blue (0.1% w/w) phosphate buffered saline solution and the ratio of stained to nonstained cells was determined after 5 min of incubation time. Viable cell counts were performed using a hemocytometer.

2.5.3. Glucose uptake determination. Three hours prior to glucose uptake, cells were incubated in glucose and serum-free media. On the fifth day, the medium was removed and replaced with 50 μL modified DMEM without phenol red, supplemented with 8 mM glucose and 0.1% BSA (Sigma, USA) containing either the oxovanadium(IV) complexes at concentration of 0.05 μg μL^{-1} or the positive controls, insulin, or metformin, at 1 μM were added to the 96-well plate. The plate was then incubated for 2 h at 37°C and 5% CO_2. After incubation, 4 μL media was removed from each well and transferred to a new 96-well plate to which 196 μL deionized water was added in each well. A total of 50 μL of this diluted medium was transferred to a new 96-well plate and 50 μL of the prepared glucose assay reagent (Biovision Inc., USA) was added per well and incubated for 30 min at 37°C. Absorbance was taken at 570 nm on a 96-well plate reader (Bio-Tek model ELx800, USA). The glucose concentration per well was calculated from a standard curve. Glucose utilization was determined by subtracting the glucose concentration left in the medium of the relevant wells following incubation to media not exposed to cells during incubation. All assays were performed in triplicate.

2.5.4. Cytotoxicity assay. 3-(4,5-Dimethylthiazo)-2-yl)-2,5-diphenyltetrazolium bromide, MTT, was dissolved in phosphate-buffered saline without phenol red at a concentration of 2 mg mL^{-1}. For the experiment, the DMEM in the 96-well plate was refreshed with 200 μL of fresh media followed by addition of 50 μL of MTT solution to each well. The plate was wrapped in aluminium foil to protect from light and the plate was incubated at 37°C for 4 h, after which the media with MTT was removed and replaced with 200 μL DMSO and 25 μL Sorensen's glycine buffer. Absorbance was read immediately at 570 nm in a plate reader.

2.5.5. Statistical analysis. Statistical analysis of data was performed by means of the student's *t*-test. Value is presented as means ± SD.

3. Results and discussion

A series of new structurally novel unsymmetrical Schiff-base ligands were prepared through condensation in a 1 : 1 : 1 molar ratio of 2-hydroxy-1-naphthaldehyde, *o*-phenylenediamine, and substituted salicylaldehyde or salicylaldehyde, and were stirred at room temperature for 4 days. All ligands formed were orange-yellow and melted at 134–195°C. They were also obtained in high yield and in high purity as shown in table 1. The procedure for the preparation of the unsymmetrical Schiff bases was developed in our laboratory as shown in figure 1. The following factors were found to affect the course of the synthesis: (i) the sequence of addition of the reagents, (ii) temperature of the solution of the starting reagents (solution chilled to about 4°C), (iii) the nature of the diamine (aliphatic or aromatic) used, and (iv) the reaction time. A change of amine from *o*-phenylenediamine to 1,3-diaminopropane led to the formation of symmetrical Schiff base, *N*,*N*′-*bis*(2-hydroxy-1-naphthalidene)-1,3-diaminopropane, following the same procedure. We believe that resonance stabilization energy arising from extended conjugation reinforces the formation of the *o*-phen-bridged Schiff base as against the symmetrical Schiff base formed with the aliphatic diamine. A change in reaction time from 4 days to just 3 h or the use of warm solutions of the starting reagents produced mixed products. From the above observations, it seems that the condensation reactions occurred stepwise.

The metal complexes were prepared by heating a mixture of each ligand and oxovanadium(IV) sulfate in 1 : 1 metal to ligand ratio, buffered with triethylamine at 50°C. All the oxovanadium(IV) complexes formed were green with melting points greater than 250°C as shown in table 1.

3.1. *^{1}H NMR spectra*

The ^{1}H NMR spectra of the free ligands were recorded in $CDCl_3$. The chemical shifts, expressed in ppm, are given in "Supplementary material". Signals for the methine proton of the azomethine were observed between 8.2 and 9.0 ppm. Peaks in the region 6.8–8.1 ppm are assigned to aromatic protons. The O–H protons were observed between 12.0 and 15.5 ppm, shifted downfield due to intramolecular hydrogen bonding [24]. The signal due to the methyl protons on the ethoxy substituent H_2L^3 appeared as a triplet at 1.5 ppm, while the signal at 4.1 ppm is assigned to the CH_2 proton. The chemical shifts obtained were similar to those of Schiff bases reported [12, 24, 25].

3.2. *Infrared spectra*

Important infrared spectral bands of the ligands and complexes are presented in table 2. The infrared spectra of both the ligands and complexes have no bands between 3100 and 4000 cm^{-1}, indicating the absence of uncondensed N–H and uncoordinated OH. Due to the unsymmetrical nature of ligands and the complexes, two bands were

Table 1. Physical properties, analytical data, and magnetic moments of the compounds.

Compound	Empirical formula	Formula mass	% Yield	Color	M.P. (°C)	Microanalysis (Anal. Calcd) %C	%H	%N	Magnetic moment (BM)
H_2L^1	$C_{24}H_{17}N_2O_2Cl$	400.86	91.5	Orange-yellow	194–195	70.20 (71.83)	4.27 (4.73)	6.98 (6.60)	–
VOL^1	$C_{24}H_{15}N_2O_3ClV$	465.79	81.6	Green	>250	62.22 (61.83)	3.15 (3.24)	6.11 (6.01)	1.73
H_2L^2	$C_{24}H_{17}N_3O_4$	411.41	86.2	Orange-yellow	134–135	69.69 (70.07)	4.23 (4.16)	10.12 (10.21)	–
VOL^2	$C_{24}H_{15}N_3O_5V$	476.34	75.4	Green	>250	60.72 (60.52)	3.38 (3.17)	8.67 (8.82)	1.73
H_2L^3	$C_{26}H_{22}N_2O_3$	410.47	71.9	Orange-yellow	135–136	76.34 (76.08)	5.51 (5.40)	6.79 (6.82)	–
VOL^3	$C_{26}H_{20}N_2O_4V$	475.40	79.2	Green	>250	65.69 (65.69)	5.51 (5.40)	5.91 (5.89)	1.60
H_2L^4	$C_{24}H_{18}N_2O_2$	366.41	78.4	Orange-yellow	187–188	78.41 (78.67)	4.81 (4.95)	7.62 (7.65)	–
VOL^4	$C_{24}H_{16}N_2O_3V$	431.34	92.8	Green	>250	66.29 (66.83)	3.86 (3.74)	6.38 (6.49)	1.81

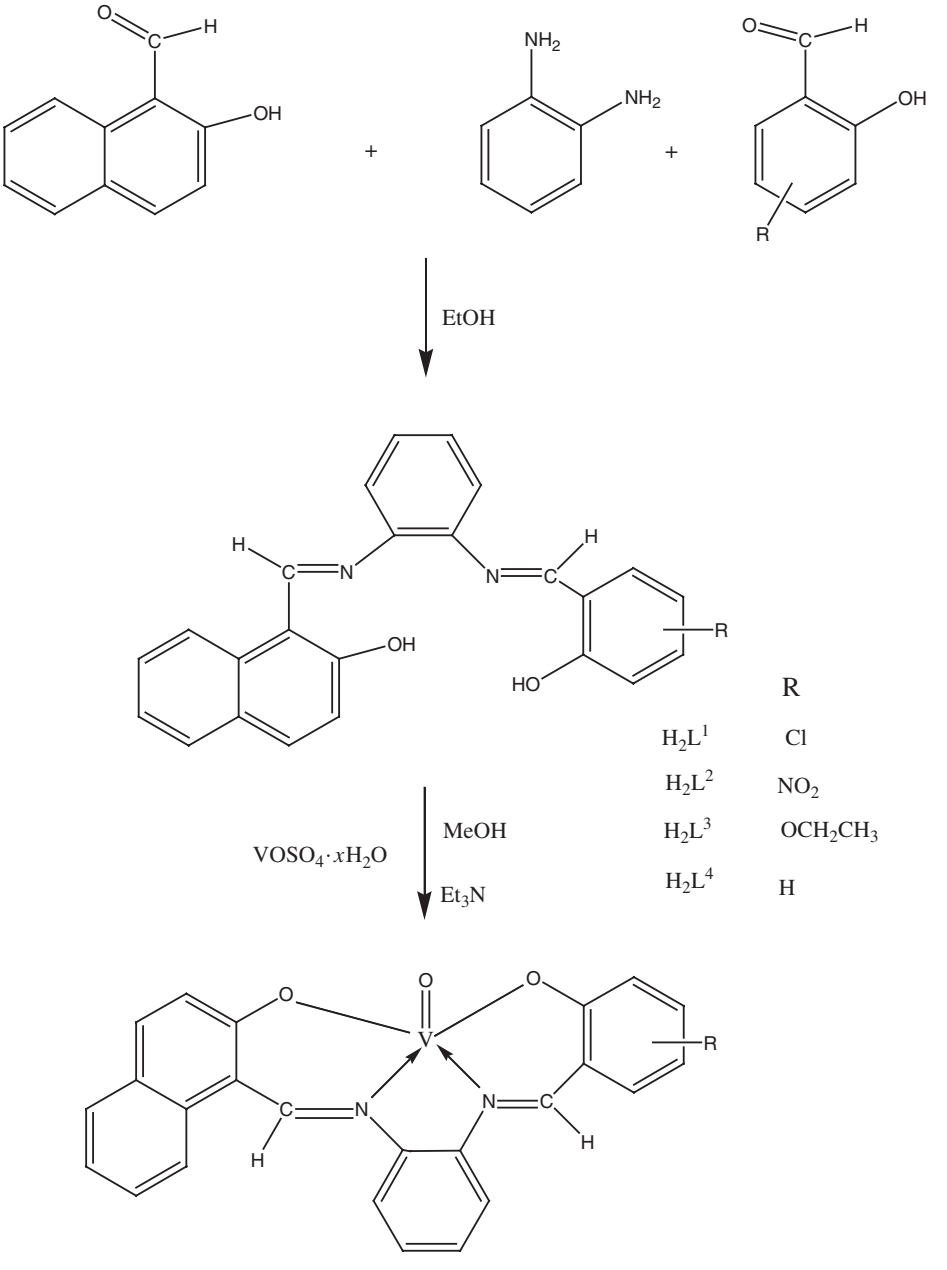

Figure 1. Preparation of the Schiff bases and complexes.

observed for ν(C=N), ν(C–O), ν(V–N), and ν(V–O) taking their origin from the different aldehydes. The ligands exhibited the characteristic C=N stretching frequency at 1610–1621 and 1567–1583 cm^{-1}, shifted to lower frequencies at 1604–1607 and 1573–1576 cm^{-1}, respectively, upon complexation indicating involvement of nitrogen of the azomethine in coordination [8]. Provisionally, we assign the lower frequency

Table 2. Selected infrared spectral bands of the compounds.

Compound	ν(C=N)	ν(C–O)	ν(V=O)	ν(V–N)	ν(V–O)
VOL[1]	1607 vs, 1574 s	1363 s, 1312 s	979 vs	573 m, 537 s	485 s, 456 m
H_2L^1	1611 vs, 1583 s	1313 s, 1276 s	–	–	–
VOL[2]	1607 vs, 1576 s	1362 s, 1315 s	982 vs	581 m, 541 s	483 m, 457 m
H_2L^2	1621 vs, 1567 s	1333 s, 1289 m	–	–	–
VOL[3]	1604 vs, 1573 s	1361 s, 1324 m	988 vs	572 m, 540 s	498 m, 455 m
H_2L^3	1610 vs, 1569 s	1316 s, 1283 s	–	–	–
VOL[4]	1607 vs, 1574 s	1366 s, 1321 m	970 vs	558 m, 536 s	488 s, 458 m
H_2L^4	1615 vs, 1568 s	1313 s, 1286 s	–	–	–

vs = very strong; s = strong; m = medium.

bands to $\nu C{=}N_{naph}$ and the higher frequency bands to $\nu C{=}N_{sal}$ of the unsymmetrical Schiff bases. The corresponding phenolic C–O stretching frequency occurs at 1313–1333 and 1276–1289 cm^{-1} for the ligands and at 1361–1366 and 1312–1324 cm^{-1} for the complexes. The shift in C–O stretching frequency confirms the participation of the phenolic O in C–O–M bond formation [26]. A significant change observed in infrared spectra of the complexes is the appearance of strong νV=O, which is absent in the ligands. The frequency spread due to νV=O observed for a large number of oxovanadium(IV) complexes was put at $985 \pm 50\ cm^{-1}$ [27]. All the complexes exhibited a strong V=O stretching band at 970–988 cm^{-1}, confirming the complexes are monomeric [28]. Bands observed in the complexes in the regions 558–581 and 536–541 cm^{-1} were assigned to ν(V–N), while 483–498 and 455–498 cm^{-1} are attributed to ν(V–O) [8].

3.3. *EPR spectra*

EPR spectroscopy has proven to be a powerful technique revealing three important types of information about: (i) the environment of the oxovanadium(IV), (ii) the nature of the ligand types, and (iii) the distortion of the complexes and degree of association within the system [29]. The unpaired electron responsible for EPR spectrum is confined largely to the oxovanadium(IV) center. This unpaired electron interacts with the nucleus spin of ^{51}V ($I = 7/2$), resulting in $(2I + 1)$ or eight lines separated by coupling constant with different intensities.

The EPR spectra of the compounds under consideration were obtained as solids and in toluene-dichloromethane (9 : 1, v/v) solutions at room temperature and at 120 K. The Hamiltonian parameters obtained for the oxovanadium(IV) complexes are presented in figure 2 and Supplementary material. The EPR spectrum of each oxovanadium(IV) complex consists of eight-line signals arising from the interaction of a single unpaired electron ($S = ½$) with the quenched orbital angular momentum of vanadium nucleus of spin $I = 7/2$. The g values, $g_o = 1.971$, $g_\perp = 1.978$, and $g_\parallel = 1.950$, are essentially the same for all the complexes examined. The vanadium nuclear hyperfine splitting, $A_o = 101$–99, $A_\perp = 65$–64, and $A_\parallel = 179$–177, vary slightly with substituents on the salicylaldehyde. The EPR data for these complexes are in agreement with previously published data for similar complexes with tetradentate bis(Schiff base) ligands [30]. The A_o values are satisfactorily matched with $A_{av} = 99.7$–100.7 in all cases, indicating that

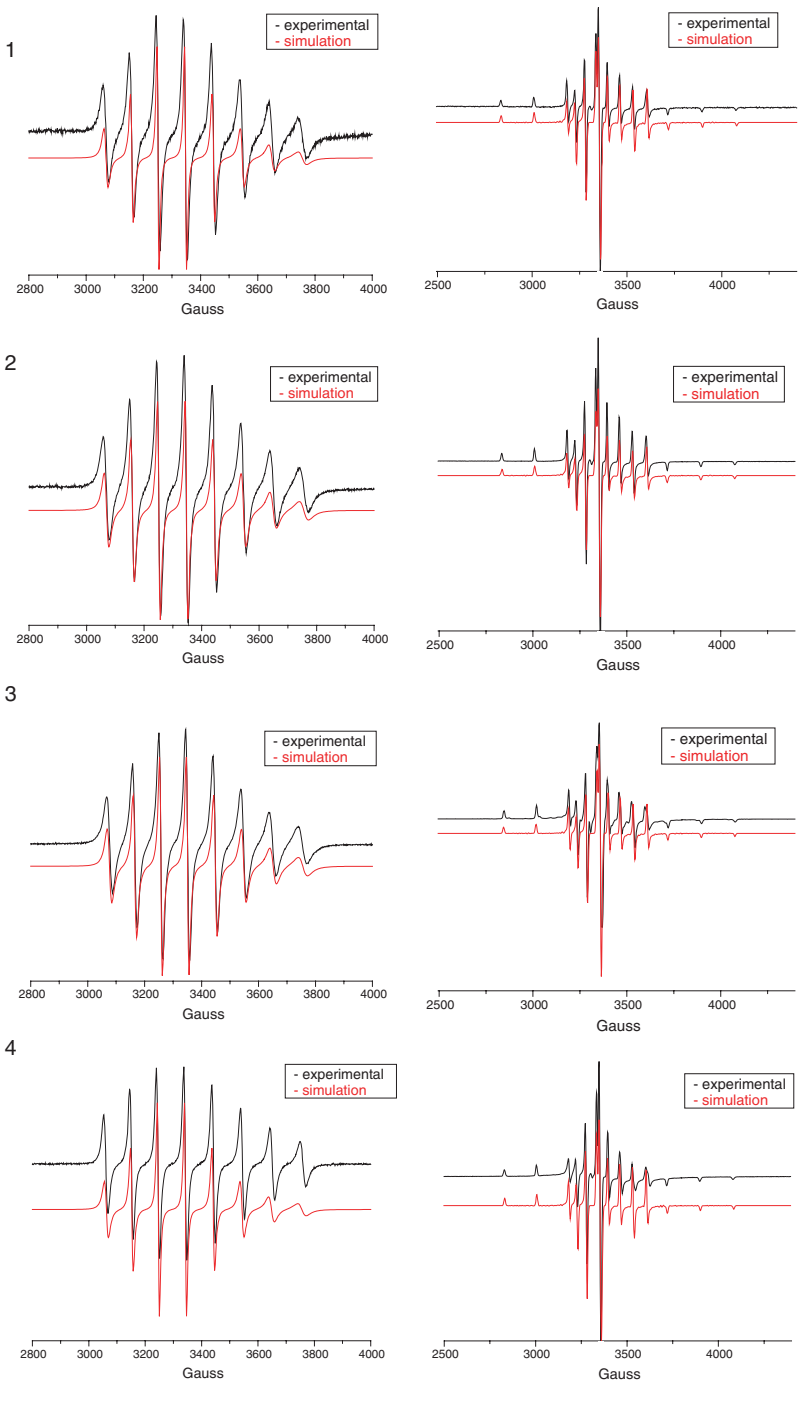

Figure 2. EPR spectra of **1** [VOL^1], **2** [VOL^2], **3** [VOL^3], and **4** [VOL^4] at 290 K (left) and 120 K (right) in toluene/CH_2Cl_2.

Table 3. Electronic spectral data of the oxovanadium(IV) complexes.

Complex	Solvent	λ_{max}, cm^{-1} (ε, $M^{-1}cm^{-1}$)			
		Band II	Band III	C.T.	Ligand
VOL^1	Chloroform	16,393 (99)	23,641 (2390)	27,701 (1380)*	31,447 (18,200) 40,323 (53,800)
	DMSO	16,129 (10)	23,753 (2180)		31,746 (1690)* 38,610 (1550)
VOL^2	Chloroform		21,598* (2375)	28,329 (860)	31,746 (890) 36,364 (1220)* 40,323 (1990)
	DMSO	15,748 (9)*		27,473 (4400)	31,746 (3640) 36,101 (4140)
VOL^3	Chloroform	16,207 (9)	23,256 (2090)	28,736 (2250)	41,322 (5000)
	DMSO	15,898 (9)	23,474 (2100)	29,155 (2230)	38,610 (3730)
VOL^4	Chloroform	16,234 (20)	24,096 (2270)	27,701 (1450)*	31,348 (1860) 40,323 (5000)
	DMSO	16,051 (10)	24,096 (2080)		31,153 (1700) 38,168 (3220)

*Shoulder.

the configuration of the complexes in solution at room temperature is the same as in frozen state at 120 K. The $g_{\parallel} < g_{\perp}$ and $A_{\parallel} > A_{\perp}$ relations are consistent with square pyramidal complexes with C_{4v} symmetry with the unpaired electron in the d_{xy} orbital [31].

Room temperature magnetic moments were observed in the range 1.6–1.8 BM for the complexes confirming that the complexes are monomeric with d^1 configuration (table 1).

3.4. *Electronic spectra*

Electronic absorption spectra are helpful in the evaluation of results furnished by other methods of structural investigation. Electronic spectra of the complexes were recorded in 10^{-3} and 10^{-5} molar DMSO and chloroform in the range 200–1100 nm, and their results are summarized in table 3 and Supplementary material. Interpretation of the electronic spectra of oxovanadium(IV) complexes is a subject of continuing investigation and discussion [27]. Ballhausen and Gray [32] provided a convenient energy level scheme for these complexes. In general, oxovanadium(IV) complexes display three low intensity bands in the 10,000–30,000 cm^{-1} range attributable to $b_2 \rightarrow e_{\pi}^*$ (band I), $b_2 \rightarrow b_1^*$ (band II), and $b_2 \rightarrow a_1^*$ (band III).

Three structural types can be identified from the solution spectra. VOL^1 and VOL^4 have similar spectra in both solvents. VOL^2 and VOL^3 are distinctively different in chloroform and DMSO. The electronic spectra in both solvents for 10^{-3} M solution displayed a broad d–d transition similar to the one observed by Kolawole *et al.* [33] for symmetric napthaldiimine complexes. The authors proposed the possibility of pseudo-aromatization of the rings around V when the azomethine nitrogens are bridged with aromatic groups. In such instances the d^1 electron of V could be delocalized into the ring system. We suspect a similar scenario in these complexes because the salicylidiimine group is also bridged with a phenyl ring in each case. Such conjugation would cause

Table 4. Cyclic voltammetric data for oxovanadium(IV) complexes.

Compound	Redox couple	E_{pc} (mV)	E_{pa} (mV)	i_c ($\times 10^6$)	i_a ($\times 10^6$)	i_a/i_c	$E_{1/2}$ (mV)	ΔE_p (mV)
VOL[1]	V^{IV}/V^V	613	530	6.91	5.31	0.77	571.5	83
VOL[3]	V^{IV}/V^V	552	469	7.87	6.26	0.80	510.5	83
VOL[4]	V^{IV}/V^V	568	490	6.91	5.45	0.79	529	78

mV = millivolt. E_{pc} = cathodic peak. E_{pa} = anodic peak. ΔE_p = potential for cathodic and anodic peak separation. $E_{1/2}$ = half-sum of the cathodic and anodic peaks.

ligand-based electronic transition to shift to red overlapping with the d–d transition resulting in complex spectra, like the one recorded here. At 10^{-3} and 10^{-5} M solutions only bands II and III could be observed in these complexes. The high extinction co-efficient observed for band III corroborates borrowing of intensities from the ligand (as a result of the hyperconjugation) by the vanadium. The d–d bands are assigned as: $b_2 \rightarrow b_1^*$: 16,207–16,393 ($CHCl_3$), 15,748–16,129 (DMSO), and $b_2 \rightarrow a_1^*$: 21,598–24,096 ($CHCl_3$), 23,474–24,096 (DMSO). The bands in the region 27,701–41,322 ($CHCl_3$) and 27,473–38,610 (DMSO) are assigned to either a metal-ligand charge transfer band or to an electronic transition within the ligand.

3.5. *Cyclic voltammetry*

Electrochemical properties of three complexes have been studied using cyclic voltammetry in order to monitor spectral and structural changes accompanying electron transfer. The cyclic voltammetric data for three of the complexes are presented in Supplementary material and table 4. At a scan rate of 100 mV s^{-1} on a 10^{-3} M solution of each complex in DMSO containing 0.1 M tetrabutylammonium perchlorate as supporting electrolyte, all complexes display one well-defined oxidation–reduction wave at positive potentials. The complexes showed redox couples with peak-to-peak separation values (ΔE_p) ranging from 78 to 83 mV, indicating a single-step one-electron transfer process [34]. None of the complexes showed completely reversible behavior, but pseudo-reversible behavior (as judged from the peak-current ratios) was observed ($i_a/i_c = 0.77$–0.80). The positive $E_{1/2}$ values of 510.5–571.5 mV may be assigned to metal-centered oxidation of V^{IV} to V^V in which there appears not to be any change in the structure when oxidized [35] and falls within the range reported in the literature for similar complexes [36]. The electrode process can therefore be represented as:

$$[VO^{IV}L]^0 \rightleftharpoons [VO^{V}L]^+.$$

3.6. *Glucose uptake in the presence of oxovanadium(IV) complexes*

A wide range of vanadium complexes are known to inhibit enzymes including phosphatises, ATPases, nucleases, kinases, and other enzymes [37]. Vanadate, a phosphate analog, has been shown to be an insulin mimetic for a number of *in vitro* insulin target responses [38]. Its ability to inhibit tyrosine phosphatases results in increased

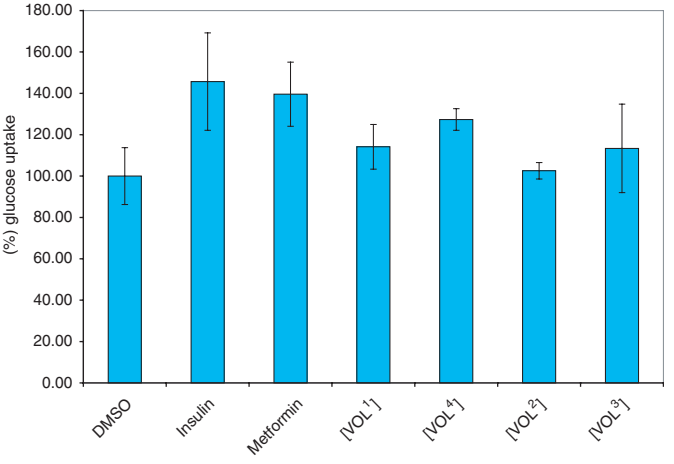

Figure 3. Glucose uptake in C2C12 cell line. The graph shows the uptake of glucose from the culture media containing 8 mM glucose by C2C12 cells over one 1 h. C2C12 cells were pre-exposed to the compounds, insulin and metformin, respectively, in glucose and serum-free media for 3 h before the glucose uptake experiments. Basal glucose uptake, i.e., solvent vehicle only (DMSO), is represented as 100% and the subsequent increase or decrease induced by the compounds is reflected as ±100%.

Table 5. Glucose uptake data.

Compound	% in glucose utilization	SD
DMSO	100	14
Insulin	146	24
Metformin	140	15
VOL^1	114	11
VOL^2	103	4
VOL^3	113	21
VOL^4	127	5

SD = standard deviation.

protein phosphorylation leading to insulin-like effects [39]. Glucose level is a key diagnostic parameter for many metabolic disorders. Biovision glucose assay kit provides direct measurement of glucose in various biological samples. The glucose enzyme mix specifically oxidizes glucose to generate a product, which reacts with a dye to generate color. The generated color is proportional to the glucose amount. The method is rapid, simple, sensitive, and suitable for high throughput.

The insulin-like capacity of vanadium compounds is usually related to their ability to lower the blood glucose level by activating the glucose transport into the cell of the peripheral tissues. In this study, we have investigated the *in vitro* glucose uptake by C2C12 muscle cells following exposure to four unsymmetrical tetradentate Schiff-base complexes of oxovanadium(IV). Insulin-mimetic test on C2C12 muscle cells shows that all the complexes significantly stimulated cell glucose utilization with negligible cytotoxicity at 0.05 $\mu g\,mL^{-1}$ (figure 3 and table 5), but not at the same level as insulin.

Substitution on the salicylaldehyde lowers the insulin enhancing activity as VOL^4 gave the largest effect. We also observe that the electronic state of the substituents has

some effects on the effectiveness. The order of activity, $VOL^4 > VOL^1 \sim VOL^3 > VOL^2$, corresponds to H, Cl (−I, +M), OEt (+I, +M), NO_2 (−I, −M) substituents on the salicylaldehyde ring. The lowest percentage glucose utilization for VOL^2 may therefore be attributed to the greater negative electron withdrawing effect (−I and −M) of the nitro group on the salicylaldehyde when compared to the other substituents.

4. Conclusion

A series of unsymmetrical tetradentate Schiff bases containing the N_2O_2 chromophore and their corresponding oxovanadium(IV) complexes have been synthesized and characterized. The room temperature magnetic moments of 1.6–1.8 BM for the complexes confirm monomeric V(IV) complexes with d^1 configuration. This is further corroborated by the solution ESR spectra of the complexes at room temperature, which have the characteristic eight-line pattern due to coupling of the unpaired electron with ^{51}V. The g values, $g_o = 1.971$, $g_\perp = 1.978$, and $g_\parallel = 1.950$, are the same for all the complexes examined. The vanadium nuclear hyperfine splitting, $A_o = 101$–99, $A_\perp = 65$–64, $A_\parallel = 179$–177, vary slightly with substituents on the salicylaldehyde. IR data suggest that the metal is bonded to the Schiff base through the phenolic oxygen and the imino nitrogen. From the position of $\nu V{=}O$, all the complexes are monomeric assuming a square pyramidal shape. Insulin-mimetic tests on C2C12 muscle cells showed that all the complexes significantly stimulated cell glucose utilization with negligible cytotoxicity at $0.05\,\mu g\,\mu L^{-1}$.

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