Synthesis, structure and reactivity of *azosalophen* complexes of vanadium(IV): studies on cytotoxic properties†

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The free ligands, 2-{(*o*-hydroxyaryl)azo}-1-*N*-salicylidene phenylamine, H_2L [where H_2L = $RC_6H_4N{=}NC_6H_4N{=}CH\text{-}C_6H_4OHR$ = *p*-H for H_2L^1, *p*-Me for H_2L^2 and *p*-Cl for H_2L^3], were prepared by the condensation of salicylaldehyde with 2-{(*o*-hydroxy aryl)azo} aniline. Reaction of H_2L with $VOSO_4$ afforded the oxovanadium complex, (L)V(O)(H_2O). The (L^1)V(O)(H_2O) complex displays two reversible responses at 0.7 V and –0.65 V *vs.* SCE in cyclic voltammetry. Catalytic activity of (L^1)V(O)(H_2O) toward H_2O_2 induced oxidation of organic thioethers to sulfoxide and sulfones have been examined. The cytotoxicity of (L^1)V(O)(H_2O) has also been examined on human lung cancer cells.

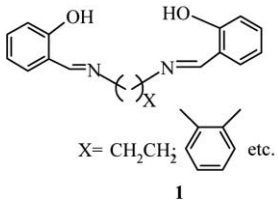
Introduction

Synthesis of target molecules *via* metal assisted routes is an important area of current chemical research.[1] Interest on the chemistry of transition metal complexes incorporating *salen* type of ligands, **1**, has accelerated during the last few years due to the discovery of their catalytic properties and towards alkene epoxidation in particular.[2,3]

Studies on tuning the catalytic activity of such complexes by modifying the ligand backbone have been ongoing. As a consequence developments in the area of asymmetric epoxidation of unfunctionalised alkenes are remarkable.[3]

Recently we have reported the synthesis and properties of Ni(II) complexes incorporating new *azosalophen* ligands, **2**.[4] The Ni(II)–*azosalophen* complexes could be prepared by O-insertion into the Ni–C(aryl) bond with peroxo reagents.[4] The *azosalophen* ligands are new *salen* like ligands in terms of coordination mode (O, N, N, O) and charge (*i.e.* –2 after dissociation of two phenolic protons).

Isolation or syntheses of free authentic *azosalophen* ligands, H_2L, were necessary to explore the possibility of preparing new complexes with the potential to exhibit new and interesting properties. Therefore we intended to isolate the free H_2L ligands and the chemistry of corresponding vanadium complexes. Several V(IV) and V(V) complexes were used as catalysts for oxygenation



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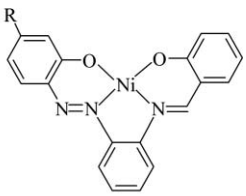

2

of organic substrates.[5] The oxovanadium complexes of tetradentate (O, N, N, O) *salen* ligands bearing electron withdrawing substituents catalyse the epoxidation of cyclohexene.[6] Thus it was expected that the *azosalophen* complexes of $[VO]^{2+}$ may be an interesting alternative catalyst for oxygenation reactions since coordinated diazo (-N=N-) nitrogen possesses considerable π acid character.[4,7]

Further, the oxidative properties of *salen* complexes have attracted biological chemists to search for a novel class of agents that are capable of triggering apoptosis in human cells.[8c] As a result, we were encouraged to examine the cytotoxity for the resultant vanadium complexes of *azosalophen* ligands. It is important to mention that several studies have been reported where the Fe(II) and Fe(III), Mn(III) and Ni(II) *salen* complexes have been examined towards *in vitro* DNA damage and human cell cytotoxicity.[8]

Herein we report the synthesis and characterization of *azosalophen* complexes of V(IV). The electrochemical properties, catalytic properties toward oxidation of thioethers and cytotoxicity on human lung cancer cells have been described.

Results and discussion

Preparation of authentic H_2L ligand and new V(IV)-azosalophen complexes

The 2-{(2-hydroxyaryl)azo}aniline, **3**, were prepared following Ni(II) assisted route as described earlier (Scheme 1).[4] The condensation of **3a**, **3b** and **3c** with salicylaldehyde in refluxing diethyl ether afforded H_2L^1(**4a**), H_2L^2(**4b**) and H_2L^3 (**4c**) respectively in

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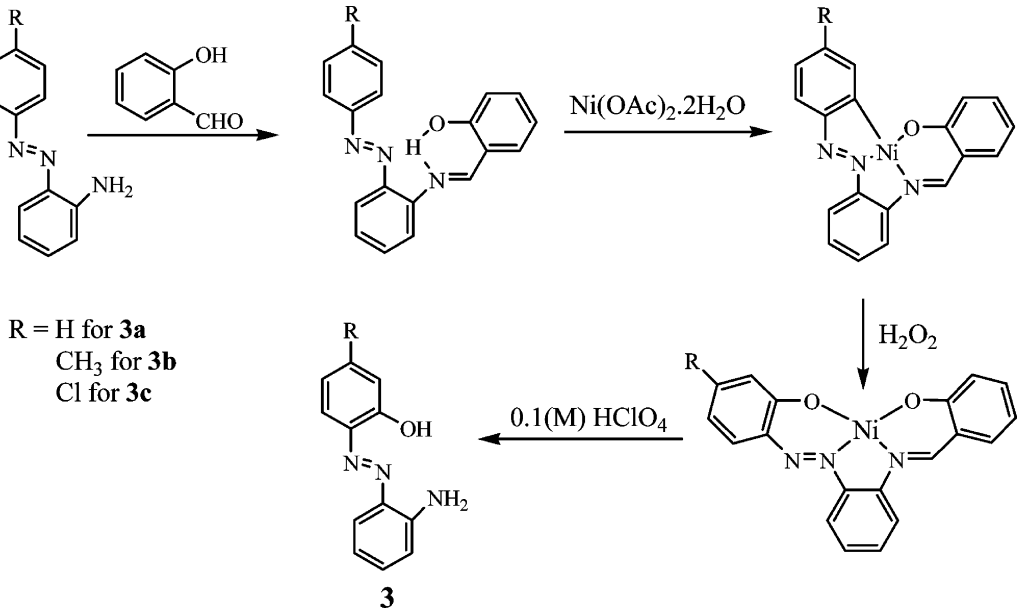
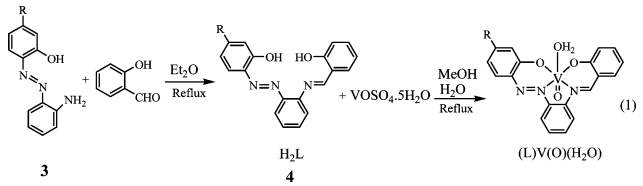
† Electronic supplementary information (ESI) available: Fig. S1–S5, UV/Vis, ^{1}H NMR of H_2L ligands, Fig. S6–S7, UV/Vis of (L)V(O)(H_2O) complexes, Fig. S8–S13 all the IR spectra of H_2L ligands and (L)V(O)(H_2O) complexes and Fig. S14 mass spectrum for complex (L^1)V(O)(H_2O). CCDC reference number 671690 for **6**. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/b903352a

‡ Collaborator for experiments related to cytotoxicity.



Scheme 1

good yield (eqn (1)). The H_2L ligands displayed characteristic UV/Vis spectra.



(1)

A representative UV/Vis spectrum of H_2L^1 (**4a**) has been given in Fig. 1. The other two spectra are given in Fig. S1 and Fig. S2 (see ESI†) for H_2L^2 (**4b**) and H_2L^3 (**4c**) respectively and the data are collected in the Experimental section.

^{1}H NMR spectra of the H_2L ligands exhibited two singlets at $\delta = 12.88$ and $\delta = 12.77$ ppm, respectively which are characteristic of two phenolic protons. Azomethine (-N=CH-) proton resonance appeared near δ 8.62. Other aromatic proton signals are consistent with the structure. The spectra are given in Fig. S3–S5† and the data are collected in the Experimental section.

Reaction of H_2L with $VOSO_4{\cdot}5H_2O$ in refluxing aqueous methanol afforded the complex of composition $(L)V(O)(H_2O)$, (eqn (1)). The $(L)V(O)(H_2O)$ complexes displayed characteristic UV/Vis spectra in dichloromethane solution where five absorption bands appeared within the range of 494–244 nm. A representative UV/Vis spectrum of $(L^1)V(O)(H_2O)$ has been shown in Fig. 1. The other two spectra are given in Fig. S6 and

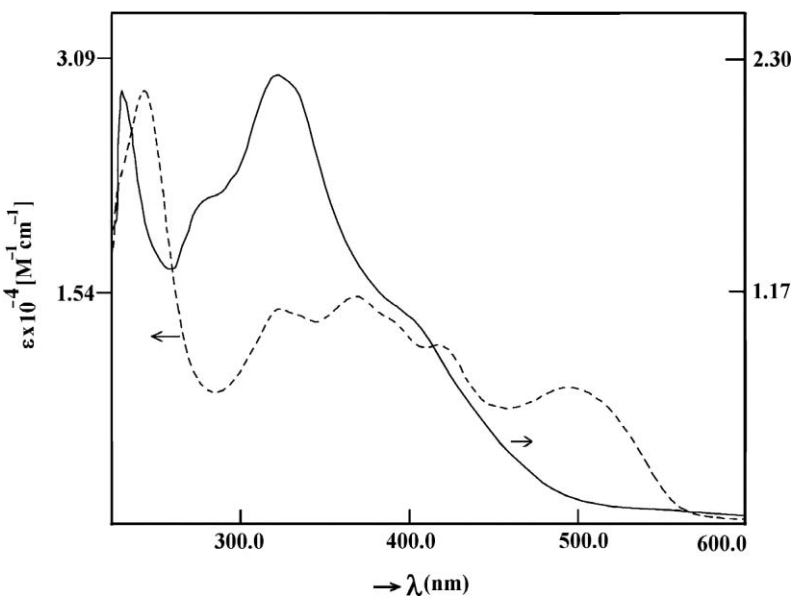


Fig. 1 UV/Vis spectra of H_2L^1 (- - -) and $(L^1)V(O)(H_2O)$ (—). The arrows indicate scales of the corresponding spectra.

Fig. S7† for $(L^2)V(O)(H_2O)$ and $(L^3)V(O)(H_2O)$ respectively and data are collected in the Experimental section.

IR spectra of H_2L ligands exhibited $\nu_{O\text{-}H}$, $\nu_{C=N}$ and $\nu_{N=N}$ near 3375, 1612 and 1473 cm^{-1} respectively. Whereas in the case of $(L)V(O)(H_2O)$ complexes, $\nu_{O\text{-}H}$ is absent indicating the binding of phenoxide oxygen. The $\nu_{C=N}$ and $\nu_{N=N}$ of $(L)V(O)(H_2O)$ shifted to lower energy (1601 and 1384 cm^{-1} respectively) than the corresponding ligands signifying the coordination of azo (-N=N-) and imine (-CH=N-) nitrogens.[4,7] The IR spectra of ligands and complexes are given in Fig. S8–S13.† The absorption near 984 cm^{-1} for all the complexes is characteristic of ν_{VO} consistent with a vanadyl, $[VO]^{2+}$, centre.[9]

The magnetic susceptibility measurements indicated that the moment ($\mu \approx 1.68$ BM) is consistent with one unpaired electron and the EPR spectrum of $(L^1)V(O)(H_2O)$ exhibited eight lines (Fig. 2) for $I = 7/2$ confirming the tetrapositive oxidation state of vanadium having d^1 configuration.

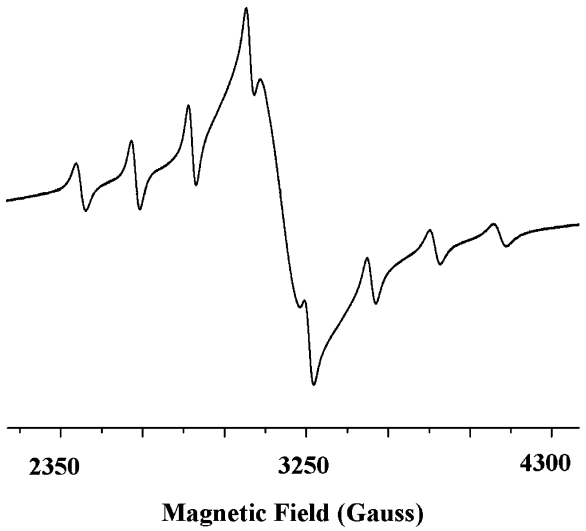


Fig. 2 EPR spectrum of $(L^1)V(O)(H_2O)$ in dichloromethane at 298 K.

ESI-MS spectrum of $(L^1)V(O)(H_2O)$ was recorded as representative for characterization. The molecular ion peak $[M + H]^+$ (without water molecule) appeared at m/z 383.2282.[10]

X-Ray structure of $(L^1)V(O)(H_2O)$

The X-ray structure of $(L^1)V(O)(H_2O)$, has been determined. Fig. 3 shows the perspective view of the molecule in which the octahedral vanadium centre is equatorially coordinated by tetradentate (O, N, N, O) dianionic $(L^1)^{2-}$ ligand. The oxo (O) and aqua (H_2O) ligands bind axially completing the hexacoordination about V(IV). Selected bond distances and angles are collected in Table 1.

V=O (1.573(8) Å) bond length is typical,[9] whereas the V–O (H_2O) distance is longer compared to other aqua complexes of VO^{2+} where H_2O is *trans* to oxo ligand.[11] The coordinated $(L^1)^{2-}$ ligand backbone is planar (mean deviation 0.086 Å). The vanadium centre is displaced toward the oxo ligand by 0.313 Å from the least square plane of (O, N, N, O) the coordination sphere of $(L^1)^{2-}$. Two molecules of $(L^1)V(O)(H_2O)$ exhibit dimeric association in the crystal lattice through hydrogen bond interactions of coordinated H_2O as shown in Fig. 4.

Electrochemistry of $(L^1)V(O)(H_2O)$

The redox property of $(L^1)V(O)(H_2O)$ complex has been studied by cyclic voltammetry. The cyclic voltammogram (Fig. 5) displays two reversible responses at 0.7 V and –0.65 V *vs* SCE in dichloromethane–acetonitrile mixed solvent. The oxidative response at 0.7 V was assignable to the $[V^{V}O(L)]^+/[V^{IV}O(L)]$ redox process[12] while the reductive response may be assigned to either $[V^{IV}O(salen)]/[V^{III}O(salen)]^-$ redox couple or for the ligand centered redox process. Though the reductive process, $[V^{IV}O(salen)]/[V^{III}O(salen)]^-$, has never been reported for similar $(VO)^{2+}$ (*salen*) complexes, but these types of redox couples were reported in some other kind of vanadium complexes.[11] However, the possibility of the involvement of a delocalized metal–ligand mixed redox orbital could not be ruled out unequivocally.

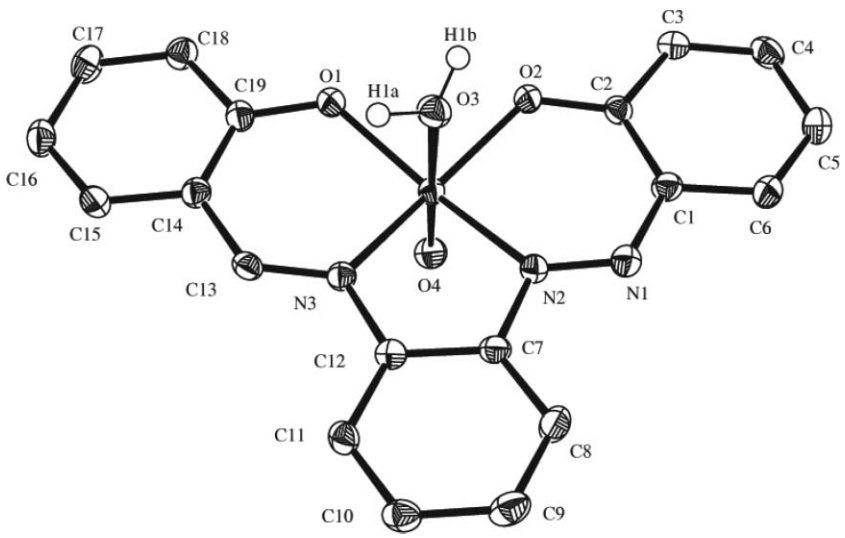


Fig. 3 Perspective view of molecular structure of $(L^1)V(O)(H_2O)$ with atom numbering scheme. The hydrogen atoms except on O3 have been omitted for clarity.

Table 1 Selected bond distances (Å) and angles (°) for compound $(L^1)V(O)(H_2O)$

Bond distances			
V–O1	1.9451(17)	N2–C7	1.433(3)
V–O2	1.9499(15)	N1–N2	1.271(3)
V–O3	2.3536(19)	N3–C13	1.296(3)
V–O4	1.6112(16)	C1–C2	1.439(3)
V–N2	2.069(2)	C2–C3	1.414(3)
V–N3	2.0673(18)	C4–C5	1.406(3)
O1–C19	1.320(3)	C1–C6	1.411(3)
C2–O2	1.308(3)	C8–C9	1.376(3)
O3–H1B	0.78(4)	C3–C4	1.364(3)
O3–H1A	0.86(4)	C5–C6	1.366(3)
N1–C1	1.389(3)	C7–C8	1.389(3)
Bond angles			
O1–V–O2	93.07(6)	N1–C1–C6	113.8(2)
O1–V–O3	81.01(6)	N1–C1–C2	126.7(2)
O1–V–O4	104.26(8)	C1–C2–C3	116.9(2)
O1–V–N2	156.81(7)	O2–C2–C3	118.7(2)
O1–V–N3	90.56(7)	O2–C2–C1	124.49(19)
O2–V–O3	81.17(6)	C2–C3–C4	121.7(2)
O2–V–O4	103.93(7)	C3–C4–C5	121.5(2)
O2–V–N2	87.75(7)	O2–V–N3	155.95(7)
O3–V–O4	172.28(8)	O3–V–N2	76.21(7)
N2–C7–C8	123.3(2)	O3–V–N3	75.93(7)
N2–C7–C12	115.7(2)	O4–V–N2	98.00(8)
O4–V–N3	98.17(7)	N2–V–N3	79.81(8)
V–O1–C19	127.64(14)	V–O2–C2	127.16(14)
V–O3–H1A	114(2)	N1–N2–C7	115.68(19)
V–O3–H1B	112(3)	N2–N1–C1	119.97(19)
H1A–O3–H1B	116(4)		

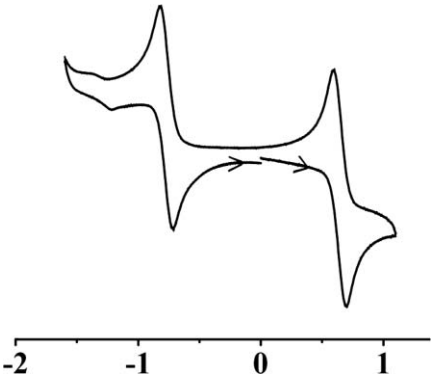
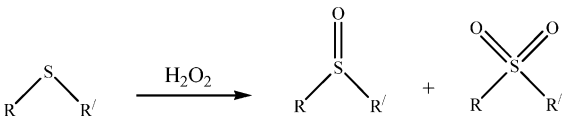


Fig. 5 Cyclic voltammogram of $(L^1)V(O)(H_2O)$ in CH_2Cl_2–CH_3CN and TEAP (0.1 M).

Meticulous experiments of spectroelectrochemistry may provide clues to rationalise the reductive couple.[13] To gain further insight, single point DFT calculations were performed using the crystallographic coordinates of $(L^1)V(O)(H_2O)$. Two frontier orbitals HOMO and LUMO, are shown in Fig. 6(a) and (b). From these orbital diagrams it appears that the HOMO is metal centered while the LUMO has considerable ligand character. Thus, from the DFT results, the oxidative and reductive couples may be inferred to be a metal centred oxidation (V^{IV}/V^{V}) and ligand reduction respectively.

Catalytic oxygenation of thioethers

Selective catalytic oxygenation of organic thioethers to sulfoxides and sulfones (eqn (2)) is an efficient area in catalysis.[14] Use of H_2O_2 as an oxidant has been extensively studied for this purpose.[15]



(2)

Oxidation of several organic sulfides with H_2O_2 in the presence of $(L^1)V(O)(H_2O)$ as catalyst was examined (Table 2). In most of the cases, the conversion was calculated on the basis of isolated yields where both the products (sulfoxide and sulfones) were obtained. The oxidation of 1-benzylthio-2-(arylazo) benzene, **2** of Table 2, afforded the sulfinyl derivative specifically. It was believed

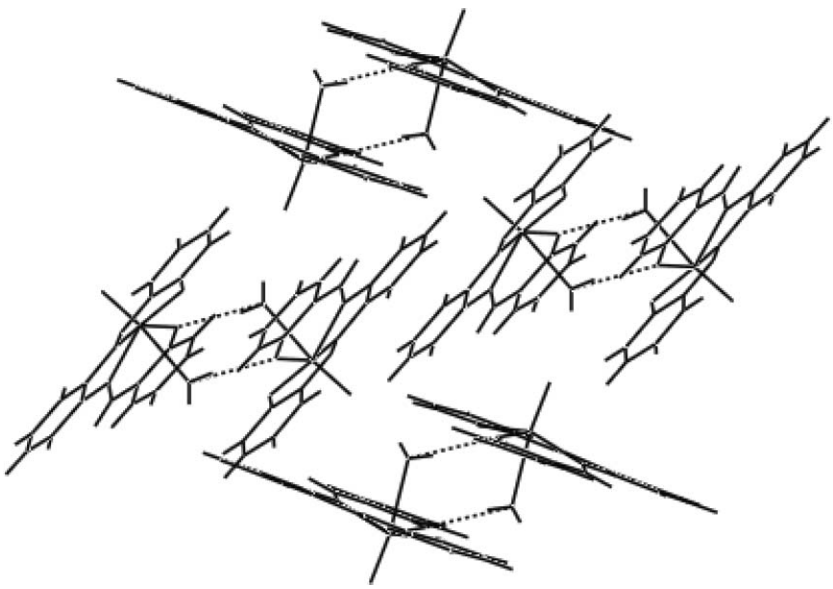
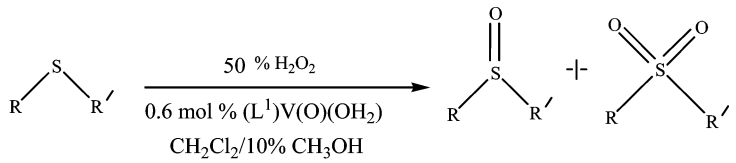


Fig. 4 H-bonded dimer of $(L^1)V(O)(H_2O)$. The dotted line represents a H-bond.

Table 2 Oxidation of sulfides catalyzed by $(L^1)V(O)(H_2O)$



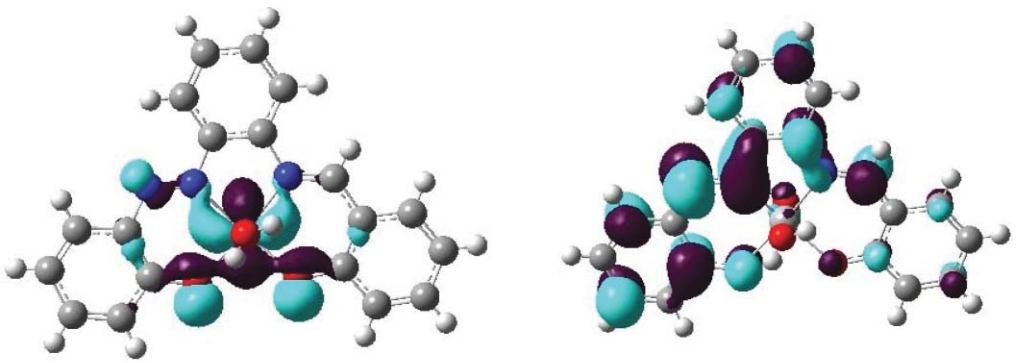
1: R = Ph; $R^{/}$ = CH_2-Ph **2:** R = 2-(PhN=N)-C_6H_4-; $R^{/}$ = CH_2-Ph

3: R = Ph; $R^{/}$ = CH_3 **4:** R = Ph; $R^{/}$ = -CH_2-CH=CH_2

5: R = *o*-$NH_2C_6H_4$-; $R^{/}$ = -CH_2-Ph

Entry	Sulfide	H_2O_2 (equiv)	Time[a] (h)	Yield (%)[b] Sulfoxide	Sulfone
(i)	**1**	5	0.5	35	45
(ii)	**2**	5	2	60	—
(iii)	**3**	5	2.5	40	48
(iv)	**4**	5	3	37	40
(v)	**5**	5	3	30	35

[a] Time required for optimal conversion. [b] Isolated yields.



6a **6b**

Fig. 6 (a) HOMO of $(L^1)V(O)(H_2O)$ and (b) LUMO of $(L^1)V(O)(H_2O)$.

that the catalytic oxidation of thioethers went on according to the proposed mechanism using analogous catalysts (Scheme 2).[16]

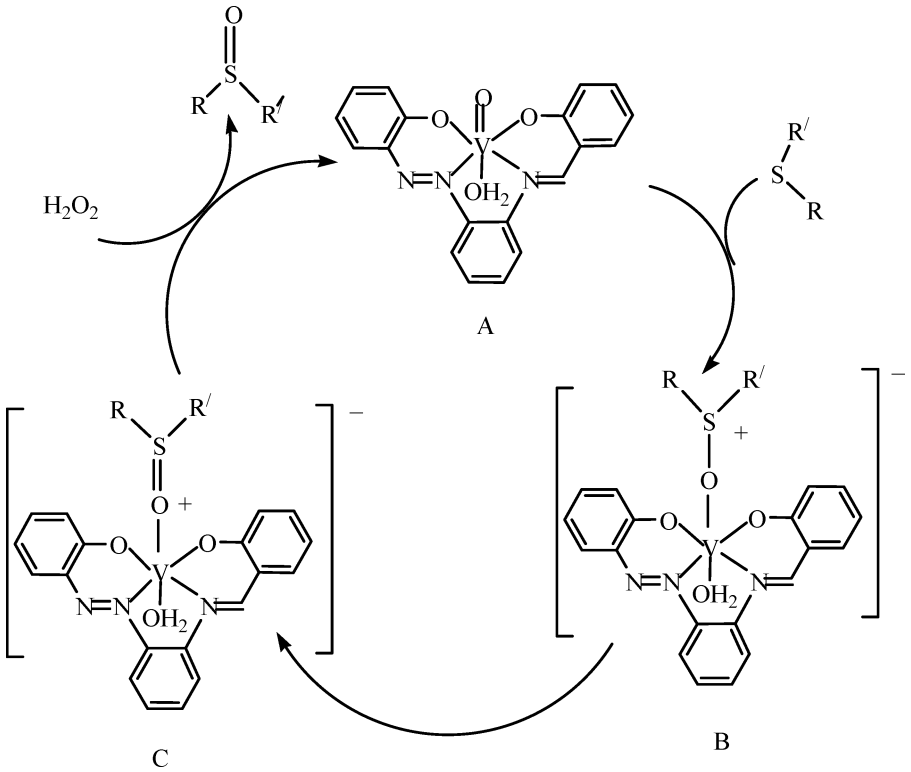
The reaction conditions and the results are collected in Table 2. The products were isolated after separation and characterized by monitoring the appearance and nature of $\nu_{S=O}$ (for sulfoxides, $\nu_{S=O}$ ~ 1040–1060 cm^{-1}; for sulfones, $\nu_{S=O}$ ~1310–1350 and 1120–1160 cm^{-1}) in their IR spectra.

Cytotoxicity and death pattern of human cancer cell

The ability of the $(L^1)V(O)(H_2O)$ complex to activate hydrogen peroxide toward the catalytic oxygenation of thioethers has been described (*vide supra*). In biology, the production of reactive oxygen species (ROS) *i.e.* incremental in the oxidative stress in the cell was identified as one of the putative mechanisms of tumoricidal action of vanadium compounds.[17] ROS is the various reduced products of O_2 (uptaken during respiration) within cells which include peroxides and superoxides. This ROS *i.e.* peroxide and superoxide, accumulation within the cell appears to be a common event that occurs during cell death pathways.[18] Therefore we were encouraged to examine the effect of agents, which are able to activate peroxide or superoxide, which here is $(L^1)V(O)(H_2O)$ on human cancer cells.

The effect of $(L^1)V(O)(H_2O)$ in the human lung cancer cell line A549 was assessed. These cells were exposed to $(L^1)V(O)(H_2O)$, within the dose range 5 to 25 μM. The Tryptan blue assay, measured after 12 and 24 h, exhibited a time dependent reduction in cell viability. The cell line was susceptible to the antiproliferative effect after 24 h with the IC_{50} value of 15 μM (Fig. 7) though no significant cell death was observed after 12 h.

Flow cytometric cell cycle analysis was carried out to determine the DNA breakdown within the cell. The cell cycle phase distribution exhibited that the content of hypoploid DNA was increased in A549 lung cancer cells upon treatment with $(L^1)V(O)(H_2O)$ as given in Fig. 8. This indicated the DNA damage within the cell upon treatment with $(L^1)V(O)(H_2O)$ which is one of the features of apoptosis.[19] Further, the cells were co-stained with PI in addition to Annexin V to distinguish between apopotic and necrotic cell death. The results of flow cytometric analysis are shown in Fig. 9.



Scheme 2

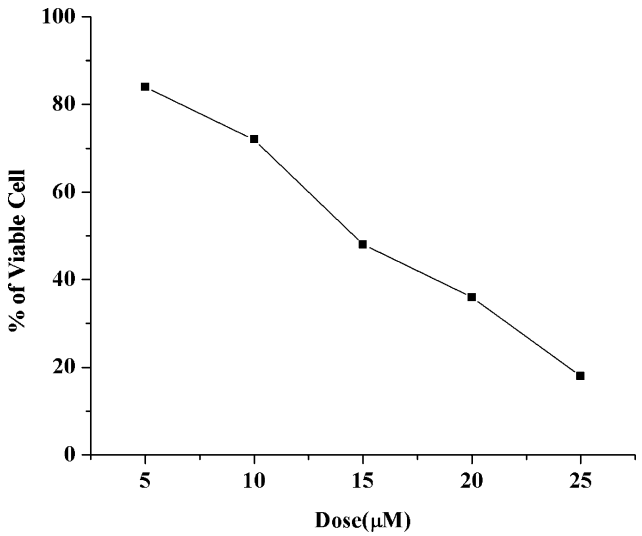


Fig. 7 Effect of $(L^1)V(O)(H_2O)$ on survivability of A549 cells. Plot of dose *vs.* viable cells to determine the IC_{50}.

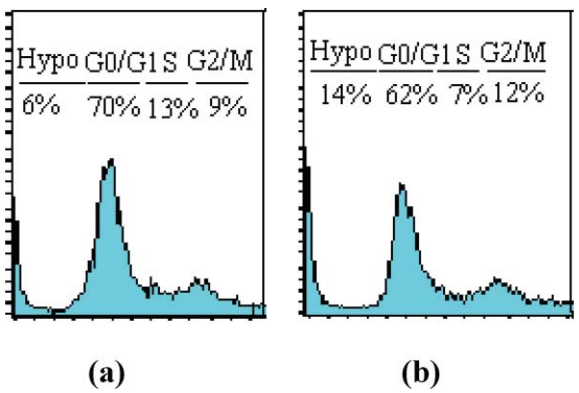


Fig. 8 Flow cytometric cell cycle analysis (a) before treatment and (b) after treatment with $(L^1)V(O)(H_2O)$.

The bottom-right quadrant of Fig. 9 represents the apoptotic cells. The results show that the death pattern is apoptotic since the population is considerable in the bottom right quadrant after treatment (Fig. 9b) while the untreated cells did not undergo apoptosis considerably (Fig. 9a).

After observing the apoptotic cell death pattern and antiproliferative ability of $(L^1)V(O)(H_2O)$ we intended to examine the oxidative stress within the cell before and after treatment. Intracellular ROS accumulation was assessed by staining the cells with ROS sensitive fluorescent dye DCFDA (dichlorofluorescein diacetate). Fig. 10(a) and (b) shows the image of the cells through a confocal microscope before and after treatment with $(L^1)V(O)(H_2O)$ respectively.

Enhanced fluorescent intensity of DCFDA in the $(L^1)V(O)(H_2O)$ treated cells (Fig. 10(b))[20] indicated the increase in ROS level within the treated cell after 12 h signifying the fact that the enhanced accumulation of ROS may be necessary for apoptotic death of A549 cells. Treatment of the A549 cell with $(L^1)V(O)(H_2O)$ in the presence of NAC (a common antioxidant)[19e] reduced the ROS level, as expected, but surprisingly the extent of cell death did not alter significantly. Therefore we inferred that $(L^1)V(O)(H_2O)$ created such an environment within the cell that the death program went on either at a very low concentration of ROS or in a ROS independent pathway.

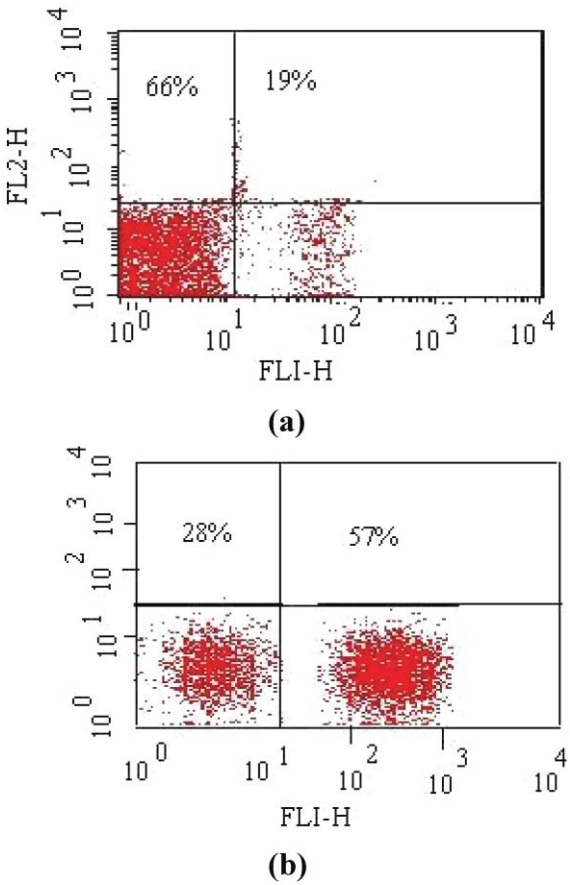


Fig. 9 Flow cytometric measurements on A549 human lung cancer cell using Annexin V/PI (a) Represents the plot of untreated cells; (b) Represents the plot for the cells treated with $(L^1)V(O)(H_2O)$.

To gain further insight into the mechanism of $(L^1)V(O)(H_2O)$ induced apoptosis of A549 cells, the expressions of related proteins were examined. It is well recognized that the expressions of pro-apoptotic proteins play a crucial role in programmed cell death.[21] Herein, the effects on the expressions of *Bad* and *Caspase-3* (pro-apoptotic proteins), upon treatment with $(L^1)V(O)(H_2O)$ have been examined. Western blot analysis showed that the *Bad* and *Caspase-3* protein expressions were upregulated considerably in $(L^1)V(O)(H_2O)$ treated cells. The expression of *Bad* as per western blot analysis is shown in Fig. 11(a).

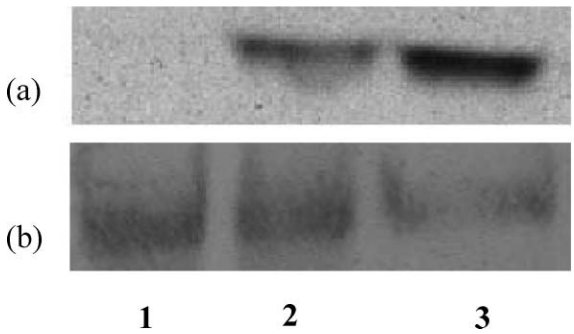


Fig. 11 Western blot of (a) *Bad* and (b) *procaspase-3* expressions.

Lane 1 represents untreated cells, lane 2 for the cells treated after 12 h and lane 3 is for the treated cells after 24 h. The increase in intensity and size of the band from lane 1 to lane 3 is consistent with its gradual upregulation of *Bad* after treatment. On the other hand, *procaspase-3* protein expression from lane 1 to lane 3 decreased as shown in Fig. 11(b) but remains unchanged at 12 h after treatment indicating the upregulation of *Caspase-3* after 12 h.

From the results, reported in this paper, we can summarize that the apoptotic death of A549 cancer cells may occur either at a very low ROS level or due to activation of pro-apoptotic proteins upon treatment with $(L^1)V(O)(H_2O)$. A concerted mechanism, *i.e.* activation of ROS at a low concentration *vis-à-vis* the upregulation of proapoptotic proteins could not be ruled out. A detailed study will allow a more definite conclusion to be reached.

Conclusions

Azosalophen oxovanadium complex, $(L)V(O)(H_2O)$, could be prepared after the successful synthesis of free *azosalophen* ligand, H_2L-which is the latest *salen* analogue. The scope of studies on the catalytic oxygenations of organic substrates using $(L^1)V(O)(H_2O)$ complexes as catalyst have emanated from the preliminary studies on oxidations of organic thioethers to sulfoxides and sulfones. Studies on the cytotoxicity of $(L^1)V(O)(H_2O)$ toward human

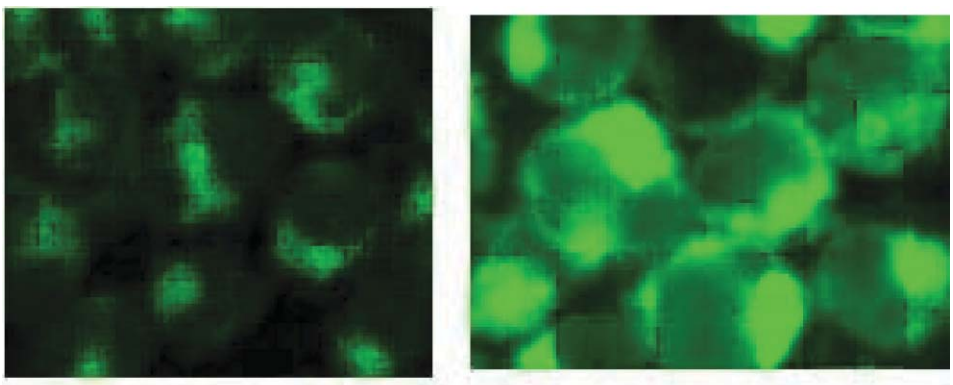


Fig. 10 Images of (a) untreated cells and (b) $(L^1)V(O)(H_2O)$ treated cells through a confocal microscope.

lung cancer cells (A549) showed that the pattern of cell death is apoptotic. Enhancement of ROS accumulation and upregulation of *Bad* and *Caspase-3* pro apoptotic proteins, within the cell, upon treatment with $(L^1)V(O)(H_2O)$ were important observations to search for a plausible mechanism of cell death pathway.

Experimental

General details

The solvents used in the reactions were of reagent grade obtained from E. Merck, Kolkata, India and purified and dried by reported procedures.[22] Vanadyl sulfate was purchased from E. Merck, Kolkata, India. DMEM, FBS, penicillin, streptomycin, 3-(4,5-dimethylthiazol-2,5-yl)-2,5-diphenylterazolium bromide were purchased from Himedia. PI and annexin were purchased from Pharmingen. A549 was purchased from NCCS, Pune. DCFDA was procured from Sigma. The FACS caliber instrument is of Becton Dickinson, USA. CO_2 incubator is Hillforce, China made. Fluorescence (FL2-A) detector is of Becton Dickinson. The Gel kit for western blot was procured from Genei, Bangalore (India). 2-(Arylazo)aniline and 2-(2-aminophenylazo)phenol, **3** were prepared according to the procedure described earlier.[4,23] Electronic spectra were recorded on a Shimadzu UV-2401 PC spectrophotometer. IR spectra were taken on a Perkin-Elmer L120–00A FT IR spectrometer (4000–225 cm^{-1}) on KBr pellets. C, H, N analyses were performed on a Perkin-Elmer 2400 C,H,N,S/O series II system, NMR spectra were drawn on a Bruker Avance RPX 500 MHz spectrometer in $CDCl_3$ using TMS as the internal standard. X-band EPR spectra was recorded on a Varian E-109C spectrometer (magnetic field up to ~6000 G) using quartz sample tubes of 3 mm diameter. DPPH was used to calibrate the spectra. Electrochemical measurements were made under a dinitrogen atmosphere using a PAR model VERSASTAT-II potentiostat. A glassy carbon working electrode, platinum wire auxiliary electrode and standard saturated calomel electrode (SCE) were used in a three-electrode configuration. 0.1 M Tetraethyl perchlorate was used as the supporting electrolyte. All electrochemical data were collected at 298 K and are uncorrected for junction potentials. ESI mass spectra were recorded on a micro mass Q-TOF mass spectrometer (serial no YA 263).

Synthesis of 2-{(2-hydroxyaryl)azo}aniline, 3. Three compounds, 2-{(2-hydroxyphenyl)azo}aniline, **3a**, 2-{(2-hydroxy-4-methyl phenyl)azo}aniline, **3b** and 2-{(2-hydroxy-4-chloro phenyl)azo}aniline, **3c** were prepared by a similar general procedure as described earlier and the characterization data for **3a** have been reported earlier.[4] The characterization data for **3b** and **3c** are furnished below.

2-{(2-hydroxy-4-methyl phenyl)azo}aniline, 3b. Yield: 32.17 mg (55%). IR (KBr, cm^{-1}): $\nu(NH_2)$; 3406 ν(OH); 3287 ν(N=N); 1456. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) = 2.42 (s, 3H); 6.10 (s, NH_2); 6.73 (d, 1H); 6.82 (t, 3H); 7.16–7.20 (m, 1H); 7.65–7.62 (m, 2H); 12.89 (s, OH). UV-Visible spectrum (dichloromethane) [λ_{max}, nm (ε, M^{-1} cm^{-1})]: 450 (10236); 323 (12150); 230 (10392). Elemental analysis: found C 68.70, H 6.20, N 18.48; $C_{13}H_{14}N_3O$ (mol wt 227) requires C 68.72, H 6.16, N 18.50%.

2-{(2-hydroxy-4-chloro phenyl)azo}aniline, 3c. Yield: 30.23 mg (50%). IR (KBr, cm^{-1}): $\nu(NH_2)$; 3446 ν(OH); 3300 ν(N=N); 1460. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) = 6.19 (s, NH_2,); 6.75 (d, 1H); 6.83 (t, 1H); 7.04–6.99 (m, 2H); 7.20 (t, 1H); 7.29 (t, 1H); 7.67 (d, 1H); 7.76 (d, 1H). UV-Visible spectrum (dichloromethane) [λ_{max}, nm (ε, M^{-1} cm^{-1})]: 455 (115892); 323 (17981); 231 (15196). Elemental analysis: found C 58.20, H 4.02, N 16.99; $C_{12}H_{10}N_3OCl$ (mol wt 247.5) requires C 58.18, H 4.04, N 16.96%.

Synthesis of H_2L

Three ligands, H_2L^1, H_2L^2 and H_2L^3 were prepared by a similar general procedure. The detailed procedure for the preparation of H_2L^1 is given below.

H_2L^1

2-{(*o*-Hydroxy aryl)azo} aniline, **3a**, (100 mg, 0.46 mmol) was dissolved in diethyl ether (40 mL), and to it salicylaldehyde (63 mg, 0.50 mmol) was added. The mixture was then heated to reflux for 8 h and then solvent was evaporated *in vacuo* for 2 h to obtain the solid product eliminating the excess of salicylaldehyde. It was further kept in a vacuum desiccator for 24 h before use and characterization. Yield: 141.38 mg (95%). IR (KBr, cm^{-1}): ν(C=N) 1612; ν(N=N) 1473; ν(OH) 3375. ^{1}H NMR (500 MHz, $CDCl_3$,): δ (ppm) = 6.95 (t, 1H); 7.06 (t, 2H); 7.11 (d, 1H); 7.25–7.26 (m, 1H); 7.34 (t, 1H); 7.40 (t, 3H); 7.94 (t, 2H); 8.62 (s, 1H); 12.88 (s, OH); 12.77 (s, OH). UV-Visible spectrum (dichloromethane) [λ_{max}, nm (ε, M^{-1} cm^{-1})]: 323 (23458); 231 (22760). Elemental analysis: found C 71.95, H 4.75, N 13.20; $C_{19}H_{15}N_3O_2$ (mol wt 317) requires C 71.92, H 4.73, 13.24%.

H_2L^2

Yield: 138.52 mg (95%). IR (KBr, cm^{-1}): ν(C=N) 1614; ν(N=N) 1474; ν(OH) 3386. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) = 2.37 (s, 3H); 6.82 (d, 2H), 6.95 (t, 1H); 7.11 (d, 1H); 7.24 (s,1H); 7.37–7.43 (m, 3H); 7.51 (t, 1H); 7.81 (d, 1H); 7.91 (d, 1H); 8.66 (s, 1H); 12.79 (s, OH); 12.99 (s, OH). UV-Visible spectrum (dichloromethane) [λ_{max}, nm (ε, M^{-1} cm^{-1})]: 334 (17191); 232 (14894). Elemental analysis: found C 72.47, H 4.15, N 12.70; $C_{20}H_{17}N_3O_2$ (mol wt 331) requires C 72.50, H 4.13, N, 12.68%.

H_2L^3

Yield: 134.91 mg (95%).IR (KBr, cm^{-1}): ν(C=N) 1617; ν(N=N) 1473; ν(OH) 3375. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.96 (t, 1H); 7.06 (t, 2H); 7.11 (d, 1H); 7.25–7.26 (m, 1H); 7.34 (t, 1H); 7.40 (t, 3H); 7.94 (t, 2H); 8.67 (s, 1H); 12.77 (s, OH); 12.88 (s, OH). UV-Visible spectrum (dichloromethane) [λ_{max}, nm (ε, M^{-1} cm^{-1})]: 330 (22000); 232 (18750). Elemental analysis: found C 64.89, H 4.00, N 11.92; $C_{19}H_{14}N_3O_2Cl$ (mol wt 351.5) requires C 64.86, H 3.98, N, 11.94%.

Synthesis of $(L)V(O)(H_2O)$

Three complexes, $(L^1)V(O)(H_2O)$, $(L^2)V(O)(H_2O)$ and $(L^3)V(O)(H_2O)$ were prepared by a similar general procedure. The detailed procedure for the preparation of $(L^1)V(O)(H_2O)$ is given below.

$(L^1)V(O)(H_2O)$

$H_2OL^1{}_{sal}$ (125 mg, 0.39 mmol) was dissolved in methanol (40 mL), and to it an aqueous solution of $VOSO_4 \cdot 5H_2O$ (100 mg, 0.39 mmol) was added. The mixture was then heated to reflux for 1 h to obtain a dark precipitate of $(L^1)V(O)(H_2O)$. The solid product was then filtered and washed with water and a little methanol. The volume of the filtrate was reduced to ~20 mL and kept in a beaker covered with a watch glass. After 12 h a second crop of product was collected by filtration. All the products were combined and recrystallized from dichloromethane–petroleum ether to obtain pure $(L^1)V(O)(H_2O)$. Yield: 126.27 mg (80%). IR (KBr, cm^{-1}): ν(C=N) 1601; ν(N=N) 1384; ν(V=O) 984. UV-Visible spectrum (dichloromethane) [λ_{max}, nm (ε, M^{-1} cm^{-1})]: 494 (12900); 418 (17120); 369 (21867); 324 (20720); 244 (42090). Magnetic moment (μ, BM): 1.68. Elemental analysis: found C 57.00, H 3.79, N, 10.45; $C_{19}H_{15}N_3VO_4$ (mol wt 400.28) requires C 56.96, H 3.74, N 10.49%. MS (EI) m/z, (%): 383.2282 $[M - H_2O + H]^+$.

$(L^2)V(O)(H_2O)$

Yield: 117.33 mg (75%). IR (KBr, cm^{-1}): ν(C=N) 1606; ν(N=N) 1360; ν(V=O) 979. UV-Visible spectrum (dichloromethane) [λ_{max}, nm (ε, M^{-1} cm^{-1})]: 495 (11250); 420 (14500); 387 (18500); 326 (14400); 245 (31000). Magnetic moment (μ, BM): 1.70. Elemental analysis: found C 57.89, H 4.13, N 10.16; $C_{20}H_{17}N_3VO_4$ (mol wt 414.28) requires C 57.93, H 4.10, N 10.13%.

$(L^3)V(O)(H_2O)$

Yield: 123.69 mg (80%). IR (KBr, cm^{-1}): ν(C=N) 1610; ν(N=N) 13865; ν(V=O) 982. UV-Visible spectrum (dichloromethane) [λ_{max}, nm (ε, M^{-1} cm^{-1})]: 490 (14750); 420 (16700); 370 (21100); 324 (17000); 244 (36250). Magnetic moment (μ, BM): 1.69. Elemental analysis: found C 52.46, H 3.19, N 9.70; $C_{20}H_{17}N_3VO_4$ (mol wt 434.78) requires C 52.44, H 3.22, N 9.66%.

DFT calculations

Using the X-ray coordinates of the $(L^1)V(O)(H_2O)$ complex, ground state electronic structure calculations have been carried out using DFT[24] methods with the Gaussian 03 program.[25] Becke's hybrid function[26] with the Lee–Yang–Parr (LYP) correlation function[27] was used throughout the study. We employed a triple-ζ quality all-electron basis set for vanadium (TZP),[28] a double-ζ all-electron basis set[29] for C, N, and O, and a 6-31G basis set for H. LANL2DZ valence and effective core potential functions were used. All energy calculations were performed using the self-consistent field "tight" option of the Gaussian 03 program to ensure sufficiently well converged values for the state energies.

Catalytic oxygenation of thioethers

All the catalytic reactions were performed by a similar general procedure. The detailed procedure for the conversion of PhSMe to PhSOMe and $PhSO_2Me$ is given below.

To a solution of catalyst, $(L^1)V(O)(H_2O)$ (6.8 mg, 0.0175 mmol) in methanol–dichloromethane (10:90) mixed solvent, PhSMe (340 mg, 2.8 mmol) and 1 mL of 50% H_2O_2 were added at 0 °C. The reaction mixture was stirred for 2.5 h within the temperature range 0–4 °C. The solution was then dried in vacuum. The products were separated and purified by preparative TLC using Benzene–acetonitrile (95:5) mixed solvent. The solid PhSOMe and $PhSO_2Me$ were isolated as the third and second fraction respectively. The isolated yields were 40% and 48% respectively. The products were characterized by IR spectroscopy. For sulfoxides, $\nu_{S=O}$ appeared at 1052 cm^{-1} for sulfones, $\nu_{S=O}$ appeared at 1313 and 1135 cm^{-1} ranges. The results of the conversions of other thioethers to sulfoxides and sulfones are given in Table 2.

Cell culture

The A549 human lungs cancer cell lines were cultured in DMEM supplemented with 10% fetal bovine serum along with 100 U/mL penicillin and 100 μg/mL streptomycin. All cell types were cultured at 37 °C in a humidified incubator containing 5% CO_2. Cells in the log phase growth were harvested by trypsinization for use in viability assays.

Cell viability assay

Cell viability was assessed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenylterazolium bromide which is commonly known as MTT assay[30] in three replicates. Twenty-four hours later, cells were treated with $(L^1)V(O)(H_2O)$ in the concentration range 5 μM to 25 μM in 10% FBS–supplemented DMEM. The medium was removed and MTT dye was solubilized in 200 μL/well DMSO. Absorbance was determined at 570 nm. Concentrations of compounds that inhibited viability by 50% (IC_{50}) were determined from the plot of viable cell *vs.* dose.

Cell cycle analysis and studies on cell death pattern by flow cytometry

For the determination of cell-cycle phase distribution of nuclear DNA, A549 (1X 10^6 cells in each case) as harvested from untreated or treated sets were fixed with 3% *p*-formaldehyde for 30 min, trypsinised, and nuclear DNA was labeled with propidium iodide (PI, 125 μg/mL) after RNase treatment using the Cycle TEST PLUS DNA reagent kit. Cell-cycle phase distribution of nuclear DNA was determined on FACS Calibur using CellQuest software, fluorescence (FL2-A) detector equipped with 488 nm Argon laser light source and 623 nm band pass filter (liner scale). A total of 10,000 events were acquired for analysis. Treated and untreated cells (1×10^6 in each case) were harvested, in a double labeling system. PI and Annexin V fluos were added directly to the culture medium. The mixture was then incubated for 30 min at 37 °C. Excess PI and Annexin V Fluos were then washed off; cells were fixed and then analyzed on FACS Calibur (equipped with 488 nm Argon laser light source 515 nm band pass filter, FL1-H, and 623 nm band pass filter, FL2-H) after fixing the cells using CellQuest software. Electronic compensation of the instrument was performed to exclude overlapping of the emission spectra. A total of 10,000 events were acquired, the cells were properly gated and a dual parameter dot plot of FL1-H (*x*-axis; Fluos-fluorescence) *versus* FL2-H (*y*-axis; PI-fluorescence) was shown in logarithmic fluorescence intensity.[31]

Table 3 Crystal data for complex $(L^1)V(O)(H_2O)$

Formula	$C_{19}H_{15}N_3VO_4$
M	400.28
Crystal system	Monoclinic
Space group	$P21/c$
a/(Å)	12.149(2)
b/(Å)	11.6449(14)
c/(Å)	12.0062(14)
α/°	90
β/°	102.807(2)
γ/°	90
λ (Å)	0.71073
Unit cell vol./Å^3	1656.3(4)
Z	4
T/K	150
ρ (Mg/m^{-3})	1.605
μ/mm^{-1}	0.632
$R1$ [$I>2\sigma(I)$]	0.0415
wR_2 [all data]	0.1000
R (int)	0.082
Uniq. Data	4269
Uniq Data with [$I>2\sigma(I)$]	2840
GoF	1.01

Measurement of reactive oxygen species production with DCFH-DA

To measure the production of reactive oxygen species, cells (5×10^5 cells/mL of culture medium) were incubated for 1 h at 37 °C in the dark with 10 mM/L of DCFH-DA molecular probes (Sigma). The 2,7-Dichlorofluorescein (DCF) fluorescence resulting from the oxidation of DCFH-DA was measured in cells on a fluorescence microscope.

Western blotting

For western blot analysis of *Bad*[32] and *Caspase-3*,[33] cell lysate was loaded into a 10% SDS-polycrylamide gel.[34] After electrophoresis the gel was transferred to a nitrocellulose membrane and blocked with nonfat milk and was monitored by measuring the chemiluminescence at different intervals (12 h and 24 h).

Crystallography

Crystals of $(L^1)V(O)(H_2O)$ were grown by slow diffusion of dichloromethane–petroleum ether at 298 K respectively. Data were collected on a Bruker SMART CCD diffractometer using a Mo-Kα monochromator ($\lambda = 0.71043$). Structure solutions were performed using the SHELXL 97 PC version program.[35] The refinement was performed using the full matrix least squares method; with the non-hydrogen atoms modeled using anisotropic displacement parameters. Hydrogen atoms were included at calculated positions. The data collection parameters and relevant crystal data are collected in Table 3.

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