Reactions of Oxidobis(quinolin-8-olato)vanadium(IV) with Hydroxamate Ligands: A Route Providing Mixed Ligand and Quinolin-8-olato-Free Vanadium(IV) Complexes[#]

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Complexes of composition $[VO(Q)_{2-n}(HL^{1,2})_n]$ (I–IV) (where Q: C₉H₆NO⁻ (quinolin-8-olato ion); HL¹: $[C_6H_4-(OH)CONHO]^-$ (salicyloylhydroxamate ion); HL²: $[C_6H_3(OH)(5-CI)CONHO]^-$ (5-chlorosalicyloylhydroxamate); n = 1 and 2) have been synthesized by the reactions of $[VO(Q)_2]$ with predetermined molar ratios of potassium salicyloylhydroxamate and 5-chlorosalicyloylhydroxamate in THF + MeOH solvent medium. The characterization of complexes has been accomplished by elemental analyses, molar conductivity, molecular weight determinations, magnetic measurements, electrochemical and IR, electronic, mass, and ESR spectral studies. The spectroscopic studies suggested bidentate nature of hydroxamate ligands involving O,O coordination. A square-pyramidal environment around vanadium has been proposed. The antimicrobial activity of newly synthesized complexes, ligands and precursor $[VO(Q)_2]$ have been assayed against some pathogenic bacteria *E. coli, S. aureus, S. typhi, S. paratyphi, S. epidermidis*, and *K. pneumoniae* and fungi *A. niger, B. fulva*, and *M. circinelloides* by MIC method. The complexes exhibited improved antimicrobial activity over the free ligands. Cytotoxicity of complexes was studied on mammalian transformed cell line Hep2C, a derivative of human cervix carcinoma HeLa cells by MTT assay.

Owing to the potential applications of vanadium complexes in diversified fields, vanadium presents a wealthy and fascinating chemistry with versatile coordination geometry. The pharmacological activities of vanadium complexes viz. insulinomimetic,¹ cholesterol biosynthesis,² effective inhibitors of human sperm mobility,³ anticancer,^{4,5} antimicrobial,⁶ and antiamoebic agents7 have further stimulated interest in vanadium chemistry. Of biologically important ligands, hydroxamic acids as organic bioligands have attracted much attention over the years,⁸ wherein the hydroxamic moiety (-NHOH) is a constituent of antibiotics, antifungal agents, food additives, drugs, tumor inhibitors, and growth factors.⁹⁻¹¹ The reactivity of hydroxamic acids toward nucleic acids and sulfhydryl groups of proteins has been established to be the reason for their inhibitory effect on various enzymes. The naturally occurring hydroxamic acids (siderophores) and some synthetic derivatives also display diverse ligating behavior toward metals.^{12–16}

The bulk of the literature on the synthesis of vanadium complexes has shown that VOSO₄•5H₂O, NH₄VO₃, [VCl₂-(acac)₂], and [VO(acac)₂]^{17–20} have been utilized as the starting materials. Literature also contains voluminous reports on the rich chemical and physiological profile of 8-hydroxyquinoline and its derivatives whereby the heterocycle itself and its complexes exhibit antiseptic, disinfectant, pesticidal, and antimicrobial activities.^{21–30} In view of these observations, in the present work, the potential of [VO(Q)₂] (Figure 1) as precursor toward its reactions with hydroxamate ligands viz. salicyloyl-hydroxamate and 5-chlorosalicyloylhydroxamate (Figure 2) which exhibit a broad spectrum of biological activities^{31–34}







Figure 2. Structures of salicyloylhydroxamate ion (HL¹) and 5-chlorosalicyloylhydroxamate ion (HL²).

has been undertaken. In order to obtain a wider insight into the antimicrobial activities of newly synthesized complexes we have screened these against pathogenic bacteria and fungi. The cytotoxicity of complexes has also been studied.

Experimental

Material and Methods. Reagent-grade solvents were dried and distilled prior to use. All other chemicals were reagent grade. $[VO(Q)_2]$ as yellow-brown solid was prepared from $[VO(acac)_2]$ by a reported method³⁵ under nitrogen atmosphere and its formation and purity was checked by C, H, and V microanalysis and IR spectral data. The potassium salicyloylhydroxamate and 5-chlorosalicyloylhydroxamate were synthesized by a reported method.³⁶ The vanadium content in complexes was determined as V2O5. The carbon, hydrogen, and nitrogen analysis were obtained on an Eager 300 NCH System Elemental Analyzer. The molar conductances $(10^{-3} M)$ solutions in methanol) were obtained on an Elico Conductivity Bridge Type CM-82T at 25 ± 0.1 °C. The room-temperature magnetic susceptibilities were measured by Gouy's method using Hg[Co(NCS)₄] as calibrant. IR spectra of complexes were recorded as KBr pellets on a Nicolet-5700 FTIR spectrophotometer. The pellets were prepared in a drv box to avoid the action of moisture. Electronic spectra of complexes were recorded on a Varian Cary-100 Bio UV-Vis spectrophotometer using methanol as solvent. X-band ESR spectra were recorded on a Varian E-112 ESR spectrometer with X-band microwave frequency (9.5 GHz) with sensitivity of $5 \times 10^{10} \Delta H$ spins using powdered sample. The DART-MS of compounds were recorded on a JEOL-AccuTOF JMS-T100LC mass spectrometer having a DART (direct analysis in real time) source. The samples were subjected as such in front of DART source. Dry helium was used with 4 LPM flow rate for ionization. The electrochemical studies were carried out at CH instrument electrochemical analyzer. All voltammetry experiments were performed in a single compartmental cell of volume 10-15 mL containing a three-electrode system comprising of a Pt-disk working electrode, Pt-wire as auxiliary electrode, and an Ag/ AgCl electrode as reference electrode. The supporting electrolyte was 0.4 M KNO₃ in Milli-Q water. The redox behavior was studied in methanol-H₂O (5:95) electrolyte system by means of cyclic voltammeter.

Synthesis. Preparation of $[VO(Q)_{2-n}(HL^{1,2})_n]$ (n = 1 and2): To a solution of $[VO(Q)_2]$ (1 g, 2.81 mmol) in THF (20 mL) were added equi- and bimolar amounts of potassium salicyloylhydroxamate/potassium 5-chlorosalicyloylhydroxamate (0.54 g, 2.81 mmol/1.08 g, 5.63 mmol)/(0.63 g, 2.81 mmol/1.26 g, 5.60 mmol) in methanol (20 mL), in separate experiments. The reaction mixture was stirred for 2 h and was then refluxed for 12-16 h during which the formation of a yellow solid anticipated as KC₉H₆ON was observed. It was filtered and the filtrate was distilled off to remove excess solvent. The concentrate was then dried under vacuum by treating it with petroleum ether whereupon black, green, and light brown colored complexes were obtained. These were recrystallized from dichloromethane giving yields [VO(Q)- (HL^{1})]/[VO(HL^{1})₂] (0.92 g, 82%/0.99 g, 80%); [VO(Q)- $(HL^{2})]/[VO(HL^{2})_{2}]$ (Yield: 0.91 g, 88.9%/1 g, 95.6%).

Antimicrobial Activity Test. The hydroxamate ligands, vanadium precursor $[VO(Q)_2]$, and synthesized complexes were tested in vitro for their antibacterial activity against different bacteria Gram +ve *Staphylococcus aureus* and *Staphylococcus epidermidis* and Gram –ve *Escherichia coli*, *Salmonella typhi*, *Salmonella paratyphi*, and *Klebsiella pneumoniae* and were also screened for antifungal activity against *Aspergillus niger*, *B. fulva*, and *M. circinelloides* to obtain MIC's at different concentrations in DMSO (1 mg mL⁻¹) employing the standard

method as recommended by National Committee for Clinical Laboratory Standard (NCCLS). MIC is the lowest concentration of the antimicrobial agents that prevents the development of visible growth after overnight incubation. All the samples were tested in triplicate. The results were compared with standard antibacterial and antifungal drugs viz. tetracycline hydrochloride and fluconazole (treated control), untreated control containing both broth and fungi and the control containing only broth (blank).

MIC Determination by Twofold Serial Dilution. The MIC assay³⁷ was performed in a 96-well micro-titer plate. For MIC assay of each test drug, a stock solution of 1 mg mL^{-1} of each drug was prepared in DMSO and a row of twelve wells was used out of which the last two wells were taken as untreated control (no drug added). Each of the ten wells received 100 µL of the Muller-Hinton broth, except the first well that received 200 μ L of broth containing 500 μ g mL⁻¹ concentration of the test drug. From the first well (containing test drug), 100 µL broth was withdrawn with a sterile tip, and same was added to the $100 \,\mu\text{L}$ of the broth in the second well; contents were mixed four times. Then 100 µL was withdrawn from the 2nd well and was added to the third well. This way a range of twofold serial dilutions were prepared (500-0.98 $\mu g m L^{-1}$) by performing twofold serial dilution. The broth in each of the wells was inoculated with $2\mu L$ of the bacterial culture (K. pneumoniae, S. epidermidis, S. aureus, E. coli, S. typhi, and S. paratyphi) and 5µL of the fungal culture (A. niger, B. fulva, and M. circinelloides) the contents were mixed by ten clockwise and ten anticlockwise rotations on a flat surface. The plate was incubated at 35 and 30 °C for bacteria and fungi respectively thereafter. The observations for growth of bacteria were recorded after 24 h and five days for bacteria and fungi respectively.

Cell Culture. Human Cervix carcinoma (HeLa) cells were trypsinized from a confluent monolayer culture obtained in a 25 cm^2 canted neck flask. The confluent monolayer of the cells was washed twice with phosphate-buffer saline (PBS), pH 7.2 followed by with exposure to Trypsin-EDTA (100 mg % EDTA and 125 mg % Trypsin 1:250; Sigma Chemical Co. St. Louis, USA) disaggregating solution for two minutes. The disaggregating solution treated flask was incubated at 37 °C for three minutes. The disaggregated cells were resuspended in appropriate volume of Dulbecco's modified Eagle's medium (DMEM) supplemented with fetal calf serum (FCS) (10%, v/v) and adjusted to a cell density of 4×10^3 cells/mL.

In Vitro Cytotoxicity Assay. The uniform volume of Hep2C cell suspension (200 μ L/well) was poured in the selected wells of a 96-wells tissue culture plate. The columns were marked, in wells under each of the columns the filter sterilized drug compound prepared in DMSO (0.1 M stock) was dispensed to achieve final concentration of 2, 4, 8, 20, and 28 mM. The cells treated with drug compound were incubated in a CO₂ incubator with 95% humidity at 37 °C for 16–18 h. Each of the drug compound concentrations were tested in quadruplicate and mean values were calculated after MTT assay (using 5 mg mL⁻¹ in PBS, 0.1 M pH 7.2 of MTT (1-(4,5-dimethyl-thiazol-2-yl)-3,5-diphenylformazan) compound. The appropriate controls with no drug compound but containing appropriate



(n = 1 and 2)

Scheme 1. Synthesis of oxidovanadium(IV) complexes.

amount of DMSO (used to prepare stock of drug compounds) were also incubated to see if DMSO alone has any effect on the viability of the proliferating cells cultured in vitro.

Results and Discussion

Complexes of composition $[VO(Q)_{2-n}(HL^{1,2})_n]$ have been synthesized in quantitative yields by the reaction of $[VO(Q)_2]$ with equi- and bimolar amounts of potassium salicyloylhydroxamate (KHL¹) and potassium 5-chlorosalicyloylhydroxamate (KHL²) in THF + methanol solvent medium according to Scheme 1.

The complexes are black, green, and light-brown in color and are soluble in common organic solvents such as methanol, chloroform, dichloromethane, and acetonitrile. The molar conductance values of complexes $(10^{-3} \text{ M solutions})$ (Table 1) in methanol in 2.62 to $4.21 \text{ S cm}^2 \text{ mol}^{-1}$ range suggested their nonelectrolytic nature. The cryoscopic molecular weight determinations of the complexes in water indicated these to exist as monomers. The room-temperature magnetic moment values of the complexes are in a $1.72-1.76 \mu_B$ range which suggest their paramagnetic nature and +4 oxidation state for vanadium.

IR Spectra. The formation of complexes has been inferred from a comparison of their IR spectra with those of the free ligands (KHL¹ and KHL²) and precursor $[VO(Q)_2]$ scanned in the $4000-250 \,\mathrm{cm}^{-1}$ region. The absorption band due to phenolic $\nu(OH)$ mode of KHL¹ and KHL² ligands occurring at ca. 3110 cm^{-1} has been observed to appear at ca. 3180 cm^{-1} in complexes indicative of weakening of intramolecular hydrogen bonding of phenolic group hydrogen to the carbonyl oxygen of the ligands upon complexation.³⁸ The retention of ν (OH) mode upon complexation is thus suggestive of nonparticipation of hydroxy group in bonding. The absorption bands occurring at 1606 and 1603 cm⁻¹ in KHL¹ and KHL² respectively attributed to ν (C=O) mode shifted to lower wavenumbers and appeared at ca. 1576 and ca. 1580 cm^{-1} in respective oxido-vanadium(IV) complexes. The absorption band due to ν (C–N) mode occurring at 1392 and 1378 cm⁻¹ in free ligands has been found to shift toward higher region at 1465 and 1467-1444 cm⁻¹ in complexes derived from KHL¹ and KHL² respectively. The bands at 3233 and 3212 cm⁻¹ assigned to ν (N–H) mode in KHL¹ and KHL² respectively did not undergo any change and appeared at 3225, 3210, 3212, and 3220 cm⁻¹ in respective (I), (II), (III), and (IV) complexes, suggesting thereby that -NH group is retained and coordination through nitrogen atom is excluded. The sharp band occurring at 999 and 997 cm^{-1} in KHL¹ and KHL² respectively ascribed to ν (N–O) mode has been observed to move toward higher wave numbers and appeared at ca. 1030 and ca. 1045, 1007 cm⁻¹ in respective complexes. A

shift in ν (C=O) mode to lower wavenumbers and ν (N–O) mode to higher wave numbers are suggestive of bonding of both the potassium salicyloylhydroxamate and potassium 5-chlorosalicyloylhydroxamate ions via oxygen atoms of carbonyl and hydroxyamine group. The absorption bands appeared in 985– 937 cm⁻¹ range in complexes under study have been assigned to ν (V=O) mode. The bands appearing at 369–350 cm⁻¹ in [VO(Q)(HL^{1,2})] have been assigned to ν (V–N) mode.²¹ The absorption bands occurring in the 502–460 cm⁻¹ region have been assigned to ν (V–O) mode in complexes.^{39,40}

Electronic Spectra. The electronic spectra of VO²⁺-oxine complexes are known to display strong intraligand transitions and ligand to metal charge-transfer transitions which obscure the typical d-d transitions of the oxocation. The electronic absorption spectra of KHL¹ and KHL² showed sharp bands at $\lambda = 226$ ($\varepsilon = 2890$), 257 (2410), 221 (2990), and 256 nm (2820) respectively attributed to intraligand $\pi \rightarrow \pi^*$ transitions. The precursor $[VO(Q)_2]$ in its spectrum is known to exhibit a low intensity band at $\lambda = 630 \text{ nm}$ ($\varepsilon = 390$) with a shoulder at $\lambda = 700 \,\mathrm{nm}$ ($\varepsilon = 30$). The black and deep green solutions of complexes of composition $[VO(Q)(HL^1)]$ and $[VO(HL^1)_2]$ displayed four bands at $\lambda = 277$ ($\varepsilon = 4080$), 309 (1170), 573 (128), 758 nm (40) and at $\lambda = 269$ ($\varepsilon = 3930$), 309 (1170), 584 (137), 830 nm (80) respectively. Likewise, the electronic spectra of black and dark brown solutions of $[VO(Q)(HL^2)]$ and $[VO(HL^2)_2]$ exhibited four bands at $\lambda =$ 275 ($\varepsilon = 3980$), 310 (880), 588 (146), 827 nm (20) and at $\lambda = 268$ ($\varepsilon = 4040$), 309 (2140), 583 (1310), 831 nm (60) respectively, implying thereby that these two series of complexes have shown similar features. The intense high energy bands appearing in the 268-277 nm range and at 309 and 310 nm may be assigned to intraligand and vanadium $(d\pi) \rightarrow \text{oxinate} (\pi^*)/\text{hydroxamate}$ ligand (π^*) transitions respectively. The less intense absorption bands that appeared in 588–573 nm range may be ascribed to ${}^{2}E_{g} \leftarrow {}^{2}T_{2g}$ transition characteristic of oxido-vanadium(IV) coordinating to good π donating ligands. The bands observed in the 831–758 nm range may be assigned to be originating from a lone pair of p orbitals on the hydroxamate/8-hydroxyquinolinate ligand (π) into an empty d orbital of vanadium to induce strong charge transfer to the metal center in complexes with high valence metal ions.

ESR Spectra. The room-temperature X-band ESR spectra of $[VO(Q)_{2-n}(HL^{1,2})_n]$ (I–IV) (Figure 3 and Table 2) displayed well-resolved typical eight lines due to the interaction of an unpaired electron of vanadium(IV) center with its own nucleus, I = 7/2 consistent with a single paramagnetic species of vanadium(IV). The *g* average values determined from the spectra

Comulex		Mn	Vield	EI	emental and	alysis % Fo	ound (Calcd	(⊿ in MeOH	Magnetic	Mol. wt.
(Molecular formula)	Color	/°C	1%	٨	С	Н	CI	Z	$/S \text{ cm}^2 \text{ mol}^{-1}$	moment $/\mu_{ m B}$	Found (Calcd)
[VO(C ₉ H ₆ ON)(C ₆ H ₄ (OH)(CO)NHO)] (I)	Black	116	89	14.41	52.04	3.40		7.66	4.21	1.74	375
$(VC_{16}H_{12}O_5N_2)$				(14.04)	(52.89)	(3.30)		(7.71)			(363)
$[VO(C_6H_4(OH)(CO)NHO)_2]$ (II)	Green	102	95	13.52	45.90	3.36		7.80	4.10	1.72	390
$(VC_{14}H_{12}O_7N_2)$				(13.71)	(45.28)	(3.23)		(7.54)			(371)
[VO(C ₉ H ₆ ON)(C ₆ H ₄ (OH)(CI)(CO)NHO)] (III)	Black	128	82	12.50	49.00	2.84	8.80	7.30	3.83	1.75	408
(VC ₁₆ H ₁₁ O ₅ N ₂ CI)				(12.83)	(48.30)	(2.76)	(8.93)	(7.04)			(397.5)
$[VO(C_6H_4(OH)(CI)(CO)NHO)_2]$ (IV)	Light brown	98	80	11.75	38.40	2.35	16.43	6.51	2.62	1.76	463
$(VC_{14}H_{10}O_7N_2Cl_2)$				(11.59)	(38.18)	(2.27)	(16.13)	(6.36)			(440)

Table 1. Analytical Data of Oxido-Vanadium(IV) Complexes



Figure 3. ESR spectra of $[VO(Q)(HL^2)]$ (III).

 Table 2. ESR
 Spectral
 Data
 of
 Oxido–Vanadium(IV)

 Complexes

Complex	$g_{ }$	g_\perp	$A_{ }/ imes 10^{-4} { m cm}^{-1}$	$\overset{A_{\perp}}{/\times 10^{-4}} \mathrm{cm}^{-1}$
$[VO(Q)(HL^1)]$ (I)	1.924	1.952	136	56
$[VO(HL^1)_2]$ (II)	1.941	1.975	139	59
$[VO(Q)(HL^2)]$ (III)	1.934	1.981	137	63
$[VO(HL^2)_2]$ (IV)	1.952	1.992	142	64

are ca. 1.98 similar to the spin only values (free electron value of 2.00) suggesting little spin–orbit coupling.

Mass Spectra. The ESI mass spectra of $[VO(Q)(HL^1)]$ (I), $[VO(HL^1)_2]$ (II), and $[VO(Q)(HL^2)]$ (III) did not show any molecular ion peaks but structurally important fragment ions clearly supported their formation. Complexes $[VO(Q)(HL^1)]$ (I) and $[VO(Q)(HL^2)]$ (III) displayed the most intense peak at m/e 146 corresponding to $[C_9H_7NO + H]^+$. The mass spectra of $[VO(Q)(HL^1)]$ (I) showed fragment ions at m/e 279 (66.66), 269 (86.11), 204 (80.55), 130 (88.88), and 77 (60.22) corresponding to $[C_6H_4(OH)C(O)NHOV(O)ONHC(O) + H]^+$, $[C_9H_6NOVO_2NHC(O) + H]^+,$ $[C_6H_5CONHOV(O) + H]^+,$ $[C_9H_7N + H]^+$, and $[C_6H_6 - H]$ respectively. The complex $[VO(HL^{1})_{2}]$ (II) exhibited base peak at m/e 152 corresponding to fragment ion $[C_6H_4(OH)C(O)NHO]^+$ and at m/e 279 (16.8), 138 (36.4), and 77 (30.8) corresponding to $[C_6H_4(OH)C(O)-$ NHOV(O)ONHC(O) + H]⁺, $[C_6H_4(OH)C(O)NH_2 + H]^+$, and $[C_6H_6 - H]$ respectively. Complex $[VO(Q)(HL^2)]$ (III) showed fragment ions at m/e 235 (50) and 204 (66.60) corresponding to $[C_6H_4(OH)C(O)NHOVO_2]^+$ and $[C_6H_5CONHOV(O) + H]^+$ respectively.

Electrochemical Studies. Electrochemical studies provide useful information not only on the thermodynamics of redox processes but also on the kinetics of heterogeneous electron-transfer reactions and coupled chemical reactions. The voltammetric data of complexes is taken as criterion of their stability as numerous reports describe the redox electrochemistry of coordination compounds.^{41,42} Owing to the variable oxidation states exhibited by vanadium, the reduction/oxidation is known to occur between different oxidation states of vanadium without any role played by the ligands.⁴³

In order to probe the electrochemical properties of newly synthesized oxido-vanadium(IV) complexes $[VO(Q)_{2-n}]$

 $(HL^{1,2})_n$] (I–IV) (Figure 4 and Table 3), the cyclic voltammetric measurements in MeOH/H₂O (5:95) at 300 mV s⁻¹ have been performed. The initial scan in the anodic direction in -1.75 to +1.75 V range displayed two cathodic and two anodic peaks corresponding to the formation of the VO³⁺/VO²⁺ and VO²⁺/V³⁺ redox couples demonstrating that these are associated with the monoelectronic transformation. The separations between cathodic and anodic peaks are indicative of quasireversible behavior of redox couples.⁴⁴ As the reduction of vanadyl complexes to the trivalent state involves loss of the



Figure 4. CV of precursor $[VO(Q)_2]$ and $[VO(Q)_{2-n}-(HL^{1,2})_n]$ (I–IV).

 Table 3. Cyclic Voltammetric Data of Oxido–Vanadium(IV) Complexes

vanadyl oxygen, such reductions are usually irreversible and no reversible reductions have been found for the vanadyl complexes under study.⁴⁵ The negative potentials of V^V/V^{IV} couple in the complexes are indicative of their stability suggesting thereby that oxido-vanadium(IV) complexes can be oxidised at distinctly lower potential. It is pertinent to mention here that the increased stability of complexes shifts the electrochemical reduction of the central ion toward a more negative potential. A blank CV run with the ligands in the potential range -1.75 to +1.75 gave one peak at +1.0 due to oxidation of the ligands. The nonoccurrence of this peak in complexes suggested that the redox processes are metal centered only.

The electrode process can therefore be represented as:

$$\mathbf{V}^{\mathrm{IV}} \rightleftharpoons \mathbf{V}^{\mathrm{V}} + \mathbf{e}^{-1} \tag{1}$$

$$\mathbf{V}^{\mathrm{IV}} + \mathbf{e}^{-1} \rightleftharpoons \mathbf{V}^{\mathrm{III}} \tag{2}$$

On the basis of IR, electronic, ESR and mass spectral data, a square pyramidal geometry for the complexes has tentatively been proposed (Figures 5–8).

Antimicrobial Activity. Antibacterial Activity: Literature contains reports that metal salts do not exhibit antimicrobial activity but complexation with metal leads to significant activity.^{46–49} In the present work, the hydroxamate ligands, vanadium precursor $[VO(Q)_2]$, and newly synthesized complexes were tested in vitro for their antibacterial activity (Table 4 and Figure 9) against Gram +ve bacteria *Staphylococcus aureus* and *Staphylococcus epidermidis* and Gram

Complex	Redox couple	$E_{\rm pc}/{ m V}$	$E_{\rm pa}/{ m V}$	$\Delta E/\mathrm{mV}$	$I_{\rm pc}/{\rm mA}$	I _{pa} /mA	$I_{\rm pa}/I_{\rm pc}$
[VO(Q) ₂]	VO ³⁺ /VO ²⁺	-0.441	-0.554	-113	-4.558	2.999	-0.688
	VO^{2+}/V^{3+}	0.276	0.139	-137	-1.506	6.625	-4.399
$[VO(Q)(HL^1)]$ (I)	VO^{3+}/VO^{2+}	-0.384	-0.674	-290	-3.566	4.424	-1.24
	VO^{2+}/V^{3+}	0.272	0.015	-257	1.584	6.496	4.10
$[VO(HL^1)_2]$ (II)	VO^{3+}/VO^{2+}	-0.376	-0.663	-286	-1.595	4.336	-2.71
	VO^{2+}/V^{3+}	0.226	-0.044	-269	-1.464	8.105	-5.536
$[VO(Q)(HL^2)]$ (III)	VO^{3+}/VO^{2+}	-0.380	-0.687	-306	-2.223	4.522	-2.034
	VO^{2+}/V^{3+}	0.234	-0.064	-298	-1.867	8.605	-4.608
$[VO(HL^2)_2]$ (IV)	VO^{3+}/VO^{2+}	-0.372	-0.644	-272	-1.251	4.397	-3.514
	VO^{2+}/V^{3+}	0.189	-0.032	-221	-5.598	8.512	-1.520



Figure 5. Proposed structure of $[VO(Q)(HL^1)]$ (I).



Figure 6. Proposed structure of $[VO(HL^1)_2]$ (II).



Figure 7. Proposed structure of $[VO(Q)(HL^2)]$ (III).



Figure 8. Proposed structure of $[VO(HL^2)_2]$ (IV).

Table 4. Antibacterial Activity of Ligands and Oxido–Vanadium(IV) Complexes by MIC Method Concentration (µg mL⁻¹)

Compound	E. coli	S. aureus	S. epidermidis	S. typhi	S. paratyphi	K. pneumoniae
$(C_6H_4(OH)C(O)NHOK)$ (KHL ¹)	125	125	62.5	62.5	62.5	62.5
$(C_6H_3(OH)(Cl)C(O)NHOK)$ (KHL ²)	62.5	62.5	62.5	125	125	62.5
$[VO(Q)_2]$	31.25	62.5	31.25	31.25	31.25	62.5
$[VO(Q)(HL^1)]$ (I)	31.25	62.5	31.25	62.5	62.5	62.5
$[VO(HL^1)_2]$ (II)	62.5	1.96	62.5	3.92	62.5	62.5
$[VO(Q)(HL^2)]$ (III)	62.5	62.5	62.5	3.92	62.5	3.92
$[VO(HL^2)_2]$ (IV)	62.5	62.5	62.5	7.84	62.5	62.5
Tetracycline hydrochloride	15.63	15.63	7.84	15.63	15.63	15.63



Figure 9. In vitro antibacterial spectrum of oxidobis(quinolin-8-olato)vanadium(IV) complexes.

–ve bacteria *Escherichia coli*, *Salmonella typhi*, *Salmonella paratyphi*, and *Klebsiella pneumoniae* employing MIC method recommended by National Committee for Clinical Laboratory Standard (NCCLS). The results were compared with treated control, commercial antibiotic tetracycline hydrochloride which inhibited bacteria under study in a 7.84–15.63 μ g mL⁻¹ range. The precursor [VO(Q)₂] was found to inhibit the growth of microorganisms in a concentration range of 31.25–62.5 μ g mL⁻¹ while free hydroxamate ligands inhibited in 62.5–125 μ g mL⁻¹ range.

 $[VO(Q)(HL^1)]$ (I) and $[VO(HL^1)_2]$ (II) exhibited significantly enhanced antibacterial activity at MIC in 1.96-62.5 $\mu g m L^{-1}$ range. Strikingly, [VO(HL¹)₂] (II) showed more pronounced activity than standard drug compound toward S. aureus and S. typhi at MIC value 1.96 and $3.92 \,\mu g \,m L^{-1}$ respectively. An explanation for the observed trend of significantly enhanced antibacterial activity of complex (II) may be ascribed to the penetration of complex (II) into the lipid membrane of S. aureus and thereby blocking of the metal binding sites in the enzymes of S. aureus more as compared to S. typhi. Complex [VO(Q)(HL²)] (III) displayed promising activity against S. typhi and K. pneumoniae at MIC 3.92 $\mu g m L^{-1}$ while [VO(HL²)₂] (IV) is more effective against S. typhi than standard drug compound at MIC 7.84 μ g mL⁻¹. All other bacteria were inhibited at MIC $62.5 \,\mu g \,m L^{-1}$ by III and IV.

Table 5. Antifungal Activity of Ligands and Oxido–Vanadium(IV) Complexes by MIC Method Concentration $(\mu g m L^{-1})$

Compound	A. niger	B. fulva	M. circinelloides
(C ₆ H ₄ (OH)C(O)NHOK)	125	125	125
(KHL ¹)			
(C ₆ H ₃ (OH)(Cl)C(O)NHOK)	125	125	125
(KHL ²)			
$[VO(Q)_2]$	125	125	125
$[VO(Q)(HL^1)]$ (I)	62.5	62.5	62.5
$[VO(HL^1)_2]$ (II)	62.5	31.25	62.5
$[VO(Q)(HL^2)]$ (III)	31.25	3.91	62.5
$[VO(HL^2)_2]$ (IV)	62.5	31.25	62.5
Fluconazole	3.91	3.91	3.91



Figure 10. In vitro antifungal spectrum of oxidobis(quinolin-8-olato)vanadium(IV) complexes.

An explanation for the observed promising antibacterial activity of complexes can be attributed to the biological significance associated with vanadium, hydroxamate and oxinate ligands and efficient diffusion of the metal complexes into bacterial cell.^{50,51}

Antifungal Activity: The potassium salicyloylhydroxamate and potassium 5-chlorosalicyloylhydroxamate ligands, $[VO(Q)_2]$ and $[VO(Q)_{2-n}(HL^{1,2})_n]$ (I–IV) were screened in vitro for their antifungal activity on selected fungi *A. niger*, *B. fulva*, and *M. circinelloides* using MIC method (Table 5 and Figure 10). The results were compared with standard antifungal

Test compound	Cell vi	iability (%) a	t the selecte	d test comp	ound concent	ration
Test compound	Control	2 mM	4 mM	8 mM	20 mM	28 mM
$VOSO_4 \cdot 5H_2O$	100	40	35	35	30	30
$(C_6H_4(OH)C(O)NHOK)$ (KHL ¹)	100	44.8	44.8	42.1	38.9	39.3
(C ₆ H ₃ (OH)(Cl)C(O)NHOK) (KHL ²)	100	30.8	28.6	25.8	24.9	21.1
$[VO(C_9H_6ON)_2]$	100	39.7	37.8	35.7	34.5	31.8
$[VO(Q)_2]$						
$[VO(Q)(HL^1)]$ (I)	100	55.9	55.0	51.2	50.2	42.7
$[VO(HL^1)_2]$ (II)	100	83.5	83.0	83.0	48.8	45.5
$[VO(Q)(HL^2)]$ (III)	100	38.1	28.8	27.0	21.1	19.3
$[VO(HL^2)_2]$ (IV)	100	33.0	31.9	25.1	24.7	24.7

Table 6. Cytotoxic Assay of Hydroxamate Ligands and Oxido-Vanadium(IV) Complexes against Hep2C Cell Line

drug fluconazole (treated control) which inhibits the fungi under study at $3.91 \,\mu g \,m L^{-1}$. A perusal of data has shown that both the hydroxamate ligands and precursor [VO(Q)₂] inhibited the fungal growth at $125 \,\mu g \,m L^{-1}$. Complex of composition $[VO(O)(HL^{1})]$ (I) inhibited all fungi at MIC 62.5 µg mL⁻¹ while, [VO(HL¹)₂] (II) exhibited enhanced activity against B. fulva at concentration $31.25 \,\mu g \,m L^{-1}$ and at $62.5 \,\mu g \,m L^{-1}$ for A. niger and M. circinelloides. The most pronounced activity toward *B. fulva* has been shown by $[VO(Q)(HL^2)]$ (III) at MIC $3.91 \,\mu g \,m L^{-1}$ comparable to that of standard antifungal drug fluconazole while A. niger and M. circinelloides inhibited at MIC value 31.25 and $62.50 \,\mu g \,m L^{-1}$ respectively. Although the exact molecular basis for the exceptional antifungal activity exhibited by complex (III) only for B. fulva cannot be given vet the intense cell permeable nature of the complex coupled with intrinsic metal chelating activity may be attributed to this observation. Complex $[VO(HL^2)_2]$ (IV) inhibited B. fulva at MIC value $31.25 \,\mu\text{g}\,\text{mL}^{-1}$ while *A. niger* and *M. circinelloides* were inhibited at MIC $62.5 \,\mu g \,m L^{-1}$. The observed effectiveness probably reflects the specificity of interaction of complexes with fungi and easier permeability toward the microbe cells.

In Vitro Cytotoxicity Assay. Cytotoxic assays of VOSO₄, potassium salicyloylhydroxamate, potassium 5-chlorosalicyloylhydroxamate ligands, $[VO(Q)_2]$, and newly synthesized complexes $[VO(Q)_{2-n}(HL^{1,2})_n]$ (I–IV) were performed at several concentrations by means of colorimetric microculture MTT assay (Table 6). Of four tested complexes, $[VO(Q)(HL^1)]$ (I) and $[VO(HL^1)_2]$ (II) exhibit appreciable viability of 55.9 and 83.5% respectively at 2 mM concentration. It has been observed that with increase in concentration of test complexes, cytotoxicity gets significantly enhanced. The cytotoxic study has shown that complexes derived from salicyloylhydroxamate are less toxic.

Conclusion

The use of $[VO(Q)_2]$ has led to new insight into the replacement of the 8-hydroxyquinolinate ion with a more polar hydroxamate group suggesting thereby that $[VO(Q)_2]$ can act as precursor to design new complexes with unique properties. A square-pyramidal geometry around vanadium involving (O,O coordination) of hydroxamate ligand and ON coordination of 8-hydroxyquinolinate ion has been inferred from various spectroscopic studies. An assay of antimicrobial activities of oxido-vanadium(IV) complexes showed promising antimicrobial activity against all the tested bacteria and fungi. Strikingly,

of tested drug compounds, $[VO(HL^1)_2]$ (II) has shown most encouraging results exhibiting promising antibacterial activity toward *S. aureus* and *S. typhi* even higher than standard drug compound. Complex $[VO(Q)(HL^2)]$ (III) has been found to exhibit most pronounced antifungal activity toward *B. fulva*. The cytotoxic study revealed I and II complexes to be more viable.

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