

Coordination Compounds of Hydroxamatooxovanadium(IV) Complexes with Nitrogenous Bases and Their Antimicrobial Activities

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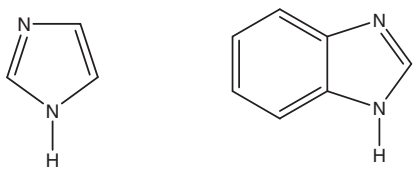
The coordination compounds of composition $[VO(HL^{1,2})_2B]$ (I–IV) (where B = imidazole ($C_3H_4N_2$) and benzimidazole ($C_7H_6N_2$); HL^1: $C_6H_5OCH_2C(O)NHO^-$ (phenoxyacetohydroxamate), HL^2: $C_6H_5CH{=}CHC(O)NHO^-$ (cinnamohydroxamate) have been synthesized from the reactions of $[VO(HL^{1,2})_2]$ with equimolar amounts of imidazole and benzimidazole in ethanol. The compounds have been characterized by elemental analyses, molar conductivity, magnetic measurements, IR, UV–vis, ESR, and FAB mass spectral studies. The spectral studies and molecular modeling dynamics suggest a distorted octahedral geometry around vanadium in coordination compounds. The antibacterial and antifungal activities of the newly synthesized coordination compounds, parent complexes, and respective potassium hydroxamate ligands have been screened in vitro against three bacterial strains viz. *E. coli*, *S. aureus*, and *B. subtilis* and two fungal strains viz. *C. albicans* and *A. niger*. The MIC values of newly synthesized coordination compounds have shown enhanced activity over parent complexes and respective potassium hydroxamate ligands. The cytotoxicity of the coordination compounds was studied on mammalian transformed cell line Hep2C, a derivative of human cervix carcinoma HeLa cells by MTT assay.

There has been enormous research interest in the coordination chemistry of oxovanadium(IV) complexes owing to their pronounced catalytic,[1–3] industrial and immense pharmacological activities.[4–10] Compared to voluminous documentation on oxovanadium(IV) complexes derived from a variety of ligands,[11–13] only a few scattered reports describe hydroxamatooxovanadium(IV) and hydroxamatooxovanadium(V) complexes.[14–17] The synthetic significance of hydroxamate derivatives essentially stems from the broad spectrum of biological activities associated with hydroxamic acids, an important family of organic bioligands and strong chelators.[18–20] The chemistry of vanadium–hydroxamate interactions has gained special interest owing to their use as bioinorganic model compounds to study their enzymatic interactions, growth inhibiting and insulin-mimetic properties.[21–27] As part of our research interest on the synthesis of biologically important hydroxamatovanadium(IV) complexes,[28–30] we have synthesized new oxovanadium(IV) complexes incorporating phenoxyacetohydroxamate and cinnamohydroxamate ligands. With an aim to have further insight into the coordination chemistry of these hydroxamatooxovanadium(IV) complexes, the present work describes their reactions with nitrogenous bases viz. imidazole and benzimidazole as secondary ligands (Figure 1). It deserves mention here that a particular interest in coordination chemistry of these nitrogenous bases lies in multitudinal and significant applications of their complexes as fungitoxic, antibacterial, and insecticidal, as stabilizers for polymers, corrosion inhibitors, hypnotics, and analgesic as well as their remarkable key role in various enzymatic catalysis and bioenergetics.[31–33] It has also been established that imidazole is biologically most important since imidazole nitrogens of



Imidazole Benzimidazole

Figure 1. Structures of imidazole ($C_3H_4N_2$) and benzimidazole ($C_7H_6N_2$).

histidyl residues coordinate to metal ions in many metalloproteins.[34] The existence of a benzimidazole group in B_{12} has also been long known.[35] In this context, anticipating that the isolated compounds may exhibit improved biological properties, the antimicrobial activities of coordination compounds against three bacterial strains viz. *E. coli*, *S. aureus*, and *B. subtilis* and two fungal strains viz. *C. albicans* and *A. niger* have been assayed and the results are described. The in vitro cytotoxicity assay of the newly synthesized compounds has been performed on mammalian transformed cell line Hep2C.

Experimental

Material and Methods. Reagent-grade solvents were dried and distilled prior to use. All other chemicals were reagent grade. The potassium phenoxyacetohydroxamate and cinnamohydroxamate were synthesized by an earlier reported method.[36] Complexes of composition $[VO(HL^{1,2})_2]$ were synthesized from the reaction of $[VO(acac)_2]$ with potassium phenoxyacetohydroxamate and potassium cinnamohydroxamate in 1:2 metal:ligand ratio in THF + methanol.[30] The

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vanadium content in complexes was determined as V_2O_5.[37] 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 at 25 ± 0.1 °C on an Elico Conductivity Bridge Type CM-82T. The room-temperature magnetic susceptibilities were measured by Guoy's method using $Hg[Co(NCS)_4]$ as calibrant.[38] IR spectra of compounds were recorded as KBr pellets on a Nicolet-5700 FTIR spectrophotometer. The pellets were prepared in a dry 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 5×10^{10} ΔH spins using powdered samples. 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 the DART source. Dry helium was used at a 4 LPM flow rate for ionization. The molecular model calculations using HyperChem 7.5 (student version) have been performed to visualize the probable geometry acquired by the complexes by applying MM^+ force field with Polak–Ribiere algorithm and RMS gradient 0.01 kcal mol^{-1}. Molecular dynamic simulation was done up to 727 °C (relaxation time, ps). All bacteria used were clinical isolates and were taken from the Post-Graduate Institute of Medical Education and Research, Chandigarh, India.

Synthesis. Preparation of $[VO(HL^{1,2})_2B]$ (B = Imidazole and Benzimidazole): To an ethanolic solution of $[VO(HL^1)_2]$/$[VO(HL^2)_2]$ (0.44 g, 1.10 mmol)/(0.47 g, 1.20 mmol), equimolar amounts of imidazole (0.075 g, 1.10 mmol)/(0.082 g, 1.20 mmol) and benzimidazole (0.13 g, 1.10 mmol)/(0.14 g, 1.20 mmol) in ethanol (25 mL) were added in separate experiments. The reaction mixture was initially stirred for 4–5 h and was then refluxed for 1–2 h whereupon a change in color of the reaction mixture was observed. The solvent was removed by distillation and the concentrate was dried under vacuum by repeatedly treating with petroleum ether. The dark green colored solids obtained were recrystallized from dichloromethane.

Anal. Calcd for $VC_{19}H_{20}O_7N_4$: C, 48.81; H, 4.28; N, 11.99; V, 10.92%. Found: C, 48.76; H, 4.15; N, 11.73; V, 10.23%. Λ_m ($PhNO_2$): 4.02 S cm^2 mol^{-1}; μ_{eff} (293 K): 1.73 μ_B (yield: 0.39 g, 76%).

Anal. Calcd for $VC_{23}H_{22}O_7N_4$: C, 53.38; H, 4.25; N, 10.83; V, 9.86%. Found: C, 53.09; H, 4.07; N, 10.59; V, 9.85%. Λ_m ($PhNO_2$): 4.11 S cm^2 mol^{-1}; μ_{eff} (293 K): 1.70 μ_B (yield: 0.48 g, 85%).

Anal. Calcd for $VC_{21}H_{20}O_5N_4$: C, 54.89; H, 4.36; N, 12.20; V, 11.11%. Found: C, 54.67; H, 4.17; N, 12.04; V, 10.96%. Λ_m ($PhNO_2$): 4.21 S cm^2 mol^{-1}; μ_{eff} (293 K): 1.72 μ_B (yield: 0.46 g, 83%).

Anal. Calcd for $VC_{25}H_{22}O_5N_4$: C, 58.92; H, 4.32; N, 10.99; V, 10.02%. Found: C, 58.51; H, 4.21; N, 10.61; V, 10.00%. Λ_m ($PhNO_2$): 4.16 S cm^2 mol^{-1}; μ_{eff} (293 K): 1.70 μ_B (yield: 0.56 g, 91%).

Antimicrobial Activity Test. Antibacterial Activity Test: The parent complexes $[VO(HL^{1,2})_2]$, potassium phenoxyacetohydroxamate (KHL^1), potassium cinnamohydroxamate (KHL^2), and coordination compounds derived from imidazole and benzimidazole were screened in vitro for their antibacterial activity on selected bacteria *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis* using minimum inhibitory concentration (MIC). All the samples were tested in triplicate.

MIC Determination by Twofold Serial Dilution: The MIC assay[39] was performed in a 96-well microtiter 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 control (no drug added). Each of the ten wells received 100 µL of Muller-Hinton broth, except the first well that received 200 µL of broth containing 500 µg mL^{-1} concentration of the test drug in DMSO. From the first well (containing test drug), 100 µL broth was withdrawn with a sterile tip, and the same was added to the 100 µL of 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 µg mL^{-1}). The broth in each of the wells was inoculated with 2 µL of the bacterial culture and the contents were mixed by ten clockwise and ten anticlockwise rotations on a flat surface. The plate was incubated at 35 °C thereafter. The observations for growth of bacteria were recorded after 24 h.

Antifungal Activity Test: The ligands; HL^1 and HL^2, oxovanadium(IV)hydroxamate complexes $[VO(HL^{1,2})_2]$ and their coordination compounds with imidazole and benzimidazole were screened for their antifungal activity against *Candida albicans* and *Aspergillus niger* by using twofold serial dilution similar to antibacterial assay using Sabouraud's dextrose broth[40] following the incubation at 37 ± 1 °C for a period of 36 h for *C. albicans* and at 25 ± 1 °C for a period of 7 days for *A. niger*. The "MIC" level was assessed visually thereafter. Standard antibacterial (Tetracyclin, Chloramphenicol) and antifungal drug (Cycloheximide, Fluconazole) was used for comparison under similar conditions.

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 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 was completely removed by decantation and the enzyme solution treated flask was incubated at 37 °C for three minutes. The disaggregated cells were resuspended in an 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: A uniform volume of Hep2C cell suspension (200 µL/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 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 CO_2 incubator with 95% humidity at 37 °C for 16–18 h. Each of the drug compound concentrations were tested in quadruplicate and

$$[VO(HL^{1,2})_2] + C_3H_4N_2 \xrightarrow[\text{Reflux}]{\text{EtOH}} [VO(HL^{1,2})_2(C_3H_4N_2)]$$

$$[VO(HL^{1,2})_2] + C_7H_6N_2 \xrightarrow[\text{Reflux}]{\text{EtOH}} [VO(HL^{1,2})_2(C_7H_6N_2)]$$

Scheme 1. Preparation of coordination compounds of hydroxamatooxovanadium(IV) complexes.

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-dimethylthiazol-2-yl)-3,5-diphenylformazan) compound). Appropriate controls with no drug compound but containing an appropriate amount of DMSO (used to prepare stock solutions) were also incubated to see if DMSO alone has any effect on the viability of the proliferating cells cultured in vitro.

Results and Discussion

The interaction of $[VO(HL^{1,2})_2]$ with equimolar amounts of imidazole and benzimidazole in ethanol solvent medium led to the formation of coordination compounds $[VO(HL^{1,2})_2B]$ (B = imidazole and benzimidazole) in quantitative yields according to Scheme 1.

The compounds are dark green fine powders and are soluble in common organic solvents such as methanol, chloroform, dichloromethane, and acetonitrile. The molar conductance values of the compounds (10^{-3} M solutions) in methanol of 4.02 to 4.21 S cm^2 mole^{-1} suggested their nonelectrolytic nature. The room-temperature magnetic moment values of the complexes of 1.70–1.73 μ_B suggested their paramagnetic nature. The lack of single crystals has prevented structure determinations of newly synthesized complexes. Nonetheless, chemical analysis by IR, UV, ESR, and mass spectra demonstrate the coordination of nitrogenous bases in the isolated compounds.

IR Spectra. A comparison of IR spectra of coordination compounds recorded from 4000 to 200 cm^{-1} with those of free ligands i.e., imidazole and benzimidazole, parent complexes and IR spectral information available on metal derivatives of these ligands in the literature has provided useful information on their formation. The diagnostic bands of imidazole and benzimidazole of interest are due to ν(N–H), ν(C=C), ν(C=N), and ν(C–H) modes. In imidazole, the band due to ν(N–H) mode is reported to occur at 3206 cm^{-1}. The coordination with a metal is known to result in a decrease of the ν(N–H) mode despite the nonparticipation of the NH group in bonding with metal. Nevertheless, depending upon the linkage of the unsaturated nitrogen of the imidazole ring to metal, one might expect some type of electronic interaction through the imidazole ring system that may in some way affect the strength of the N–H bond. In imidazole, the absorption bands are known to appear at 1828, 1675, 1582, 1548, 1499, 1488, and 1451 cm^{-1} due to ν(C=C) and ν(C=N) and N–H in-plane deformation modes.[31] The IR spectra of benzimidazole, is reported to exhibit a band at 3117 cm^{-1} assigned to ν(N–H) mode and three weak bands near 1620, 1600, and 1500 cm^{-1} attributed to ν(C=C) and ν(C=N) modes.

The IR spectra of the coordination compounds of phenoxyacetohydroxamatooxovanadium(IV), $[VO(HL^1)_2]$ with imidazole and benzimidazole displayed bands at 3137 and 3112 cm^{-1} ascribed to ν(N–H) mode. The coordination compounds of cinnamohydroxamatooxovanadium(IV), $[VO(HL^2)_2]$ with imidazole and benzimidazole exhibited bands at 3155 and 3115 cm^{-1} respectively due to ν(N–H) mode. These observations are suggestive of small shift in ν(N–H) mode to lower wavenumber in compounds with imidazole while the coordination compounds with benzimidazole exhibited a significant lowering in ν(N–H) mode relative to those occurring at 3297–3192 cm^{-1} in parent hydroxamate complexes. The absorption bands due to ν(C=C) and ν(C=N) modes appeared at 1665–1705 cm^{-1} in coordination compounds. A shift in ν(C=C) and ν(C=N) modes to higher wavenumber is suggestive of coordination through unsaturated nitrogen to vanadium.[31] The coordination through nitrogen has further been ascertained by the observance of bands ≈320 cm^{-1} in far-IR spectra of coordination compounds attributed to ν(V←N) mode in agreement with reports on bis(8-quinolinato)oxovanadium(IV) adducts with pyridines and substituted pyridines.[41] A perusal of IR spectra showed a significant decrease in ν(V=O) mode from ≈973 cm^{-1} in parent complexes to 964–922 cm^{-1} in coordination compounds. It is pertinent to mention here that oxovanadium(IV) complexes are known to exhibit a sharp band at 985 ± 50 cm^{-1} due to ν(V=O) mode[42] and the coordination of a ligand at the position trans to V=O bond may bring about a drop of ≈50 cm^{-1} in ν(V=O) stretching frequency of the parent complexes. The observed extent of lowering in ν(V=O) mode in coordination compounds derived from both the bases is indicative of an octahedral environment around vanadium.

Electronic Spectra. The electronic spectra of oxovanadium(IV) complexes has been a subject of continuing investigation and discussion.[42] The energy level scheme for these complexes has critically been described by Ballhausen and Gray.[43] The oxovanadium(IV) complexes, in general, are reported to display three prominent bands in 800–625 ($b_2 \rightarrow e_\pi^*$), 690–520 ($b_2 \rightarrow b_1^*$), and 470–330 nm ($b_2 \rightarrow a_1^*$) regions (ε 10–100, 5–50, and 5–100 M^{-1} cm^{-1}) respectively attributed to $d \rightarrow d^*$ transitions. However, the electronic spectra of complexes $[VO(HL^{1,2})_2]$ have been observed to exhibit two bands in 800–700 and 560–400 nm regions ascribed to LMCT transition from lone-pair of p orbital of acetylacetonato or the hydroxamato oxygen atoms to vanadium and d–d transition respectively.[44,45] The absorption spectra of the coordination compounds of $[VO(HL^{1,2})_2]$ with imidazole and benzimidazole depicted a red shift of the low energy band appeared at 755 and 760 nm in respective parent complexes. The occurrence of absorption bands at 810 and 812 nm in $[VO(HL^1)_2(imid)]$ (imid: $C_3H_4N_2$ (imidazole)) and $[VO(HL^1)_2(benzimid)]$ (benzimid: $C_7H_6N_2$ (benzimidazole)) respectively and bands at 815 and 818 nm in $[VO(HL^2)_2(imid)]$

Table 1. Mass Spectral Data

Compound $[VO(HL^2)_2(imid)]$	m/z (%)	Compound $[VO(HL^2)_2(benzimid)]$	m/z (%)
$[VO(HL^2)_2(imid)]^+$	459.36 (1.51)	$[VO(HL^2)_2(benzimid) + C_6H_6 + 2H]^+$	589.31 (3.03)
$[VO(HL^2)_2 - 2H]^+$	389.30 (1.51)	$[V(HL^2)_2 - C_6H_6]^+$	297.16 (1.510)
$[VO(HL^2)(imid) - 2H]^+$	295.18 (3.03)	$[V(HL^2)_2 - C_6H_6 - H]^+$	296.16 (13.63)
$[VO(HL^2) + NHO + H]^+$	261.14 (9.84)	$[V(HL^2)_2 - C_6H_6 - 2H]^+$	295.16 (71.21)
$[VO(HL^2) + NH + H]^+$	245.14 (24.24)	$[V(HL^2)(benzimid) - C_6H_6 - 3H]^+$	266.14 (7.5)
$[V(HL^2) + 3H]^+$	216.13 (100)	$[VO(HL^2) + H_2O]^+$	247.16 (1.51)
$[V(imid)_2 - 4H]^+$	183.13 (7.5)	$[HL^2 + NHO - 4H]^+$	189.12 (3.5)
$[VO(HL^2) - CONHO - 3H]^+$	167.13 (18.18)	$[benzimid + 2H]^+$	120.09 (7.5)
$[(HL^2) - NH]^+$	148.10 (25.75)	$[benzimid + H]^+$	119.08 (100)
$[VO(imid) + 2H]^+$	137.10 (23.48)		
$[(imid) + NHO]^+$	99.11 (6.81)		
$[(imid) + NH]^+$	83.09 (12.12)		
$[(imid) + H]^+/[VO + 2H]^+$	69.07 (3.03)		

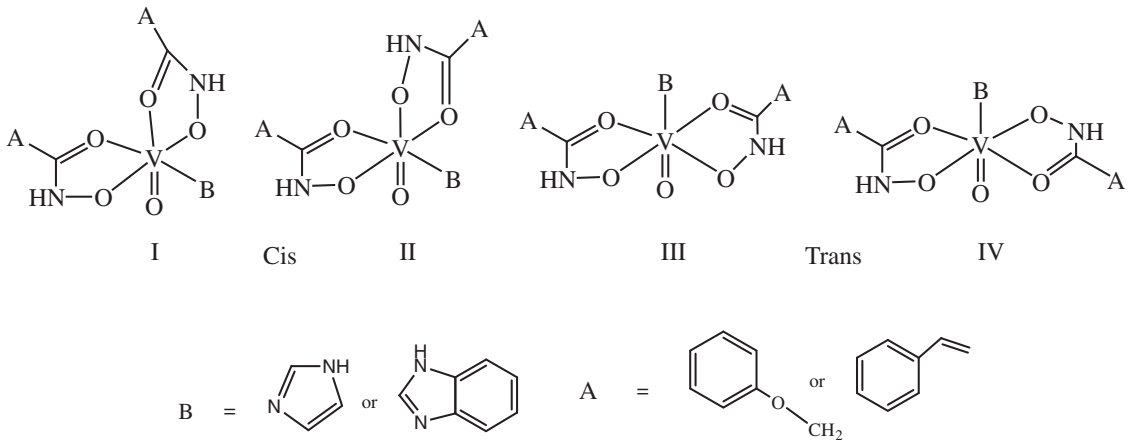

Figure 2. Perspective structures for coordination compounds of hydroxamatooxovanadium(IV) complexes with nitrogenous bases.

and $[VO(HL^1)_2(benzimid)]$ are suggestive of a change from five-coordinate square-pyramidal geometry in parent complexes to six-coordinate octahedral geometry upon coordination.[46]

ESR Spectra. The room-temperature X-band ESR spectra of hydroxamatooxovanadium(IV) compounds displayed eight well resolved 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_{av} values determined from the spectra are $\approx$1.98 similar to the spin only value (free electron value of 2.00) suggesting little spin orbit coupling.

Mass Spectra. The DART-MS spectral data of coordination compounds, $[VO(HL^2)_2B]$ is given in Table 1. The mass spectra of $[VO(HL^2)_2(imid)]$ displayed molecular ion peak at m/z 459.36 thereby confirming its formation. The most intense peak in $[VO(HL^2)_2(imid)]$ appeared at m/z 216.13 corresponded to $[V(HL^2) + 3H]^+$. The other important fragment ions appeared at m/z 389.30, 295.18, 137.10, 99.11, 83.09, and 69.07 corresponded to $[VO(HL^2)_2 - 2H]^+$, $[VO(HL^2)(imid) - 2H]^+$, $[VO(imid) + 2H]^+$, $[imid + NHO]^+$, $[imid + NH]^+$, and $[imid + H]^+/[VO + 2H]^+$ respectively. The mass spectra of $[VO(HL^2)_2(benzimid)]$ did not display any molecular ion peak but the appearance of the most intense peak at 119.08 corresponding to $[benzimid + H]^+$ further substantiated its formation. The other important fragment ions appeared at m/z 589.31, 297.16, 266.14, and 120.09 corresponded to $[VO(HL^2)_2(benzimid) + C_6H_6 + 2H]^+$, $[V(HL^2)_2 - C_6H_6]^+$, $[V(HL^2)(benzimid) - C_6H_6 - 3H]^+$, and $[benzimid + 2H]^+$ respectively.

Molecular Modeling. The molecular mechanical adjustments for energy optimization from strained structures to the likely geometry of complexes were attempted. The molecular mechanics were repeated five to six times to ensure that the structure with minimized energy has been attained. The structure with minimized energy is assumed to be closer to the stable geometry in consonance with physicochemical and spectral data. For each molecule, theoretically four structures (Figure 2), two cis (I, II) and two trans (III, IV) are possible. From molecular modeling calculations, the strain energy (kcal mol^{-1}) obtained for different structures is given in Table 2. The magnitude of strain energy showed that for all the synthesized coordination compounds trans structure having lowest strain energy seemed to be the most probable (Figures 3–6).

Based upon IR, electronic, ESR, and mass spectral data combined with molecular modeling calculations, a hexacoordinate environment around vanadium has been proposed.

Table 2. Strain Energy (kcal mol^{-1}) from Molecular Modeling Calculations

Compound	Structure			
	I	II	III	IV
$[VO(C_6H_5OCH_2C(O)NHO)_2(C_3H_4N_2)]$	34.528	36.288	27.651	37.276
$[VO(C_6H_5OCH_2C(O)NHO)_2(C_7H_6N_2)]$	30.847	40.944	27.639	34.357
$[VO(C_6H_5CH{=}CHC(O)NHO)_2(C_3H_4N_2)]$	45.851	45.018	44.119	46.787
$[VO(C_6H_5CH{=}CHC(O)NHO)_2(C_7H_6N_2)]$	47.740	52.139	46.614	51.911

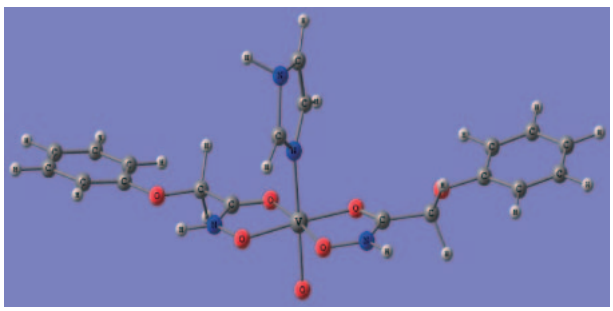

Figure 3. Proposed structure of $[VO(C_6H_5OCH_2C(O)NHO)_2(C_3H_4N_2)]$.

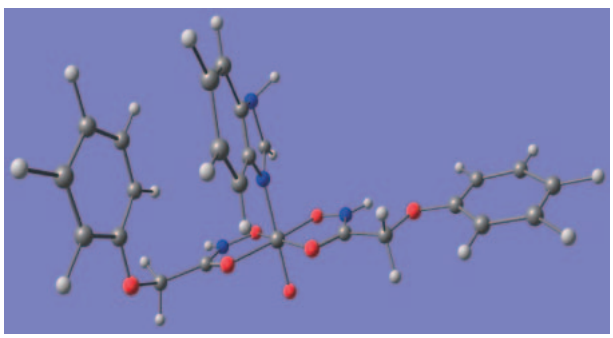

Figure 4. Proposed structure of $[VO(C_6H_5OCH_2C(O)NHO)_2(C_7H_6N_2)]$.

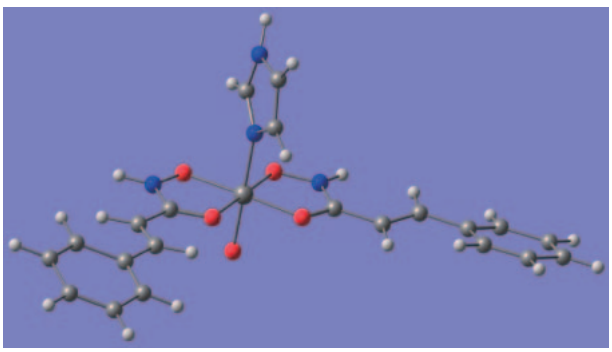

Figure 5. Proposed structure of $[VO(C_6H_5CH{=}CHC(O)NHO)_2(C_3H_4N_2)]$.

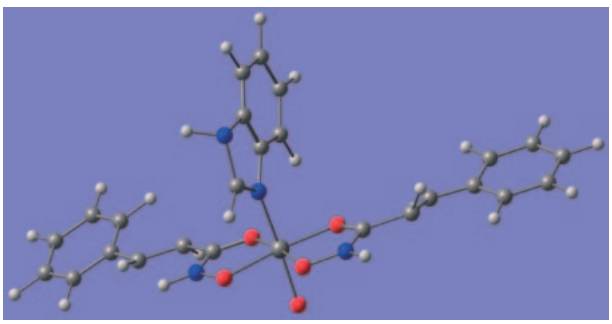

Figure 6. Proposed structure of $[VO(C_6H_5CH{=}CHC(O)NHO)_2(C_7H_6N_2)]$.

Antimicrobial Activity. Literature contains reports that metal salts do not exhibit antimicrobial activity but complexation leads to show significant activity.[47,48] The antimicrobial activity (antibacterial and antifungal activity) of HL^1 and HL^2, nitrogenous bases imidazole and benzimidazole, parent complexes $[VO(HL^{1,2})_2]$ and their coordination compounds with nitrogenous bases were compared. The results (Table 3 and Figure 7) show that the complex $[VO(HL^1)_2(imid)]$ exhibits MIC values at 7.81–31.2 µg mL^{-1} while $[VO(HL^1)_2(benzimid)]$ has MIC value at 15.6 µg mL^{-1} for *C. albicans* and 31.2 µg mL^{-1} for other microorganisms. The coordination compound, $[VO(HL^2)_2(imid)]$ is most effective against *C. albicans* at an MIC value 3.90 µg mL^{-1}. It also inhibits other microorganisms at 15.6–7.81 µg mL^{-1} which is lower than other compounds. The $[VO(HL^2)_2(benzimid)]$ shows lowest value of MIC against *S. aureus* at 7.81 µg mL^{-1} and *A. niger* at 15.6 µg mL^{-1}. The parent complexes inhibited the growth of microorganisms at 62.5–15.6 µg mL^{-1}. The free imidazole and benzimidazole did not show any activity against *E. coli* at the concentrations studied (500–0.98 µg mL^{-1}) but inhibit the growth of *S. aureus* and *S. subtilis* and fungi; *C. albicans* and *A. niger* in the range of 62.5–250 µg mL^{-1}. The variation in the effectiveness of the complexes against different organisms may be attributed to either the difference in permeability of the cells of the microbes or on difference in ribosomes of the microorganisms.[49,50] The increase in activities of complexes may be explained on the basis of chelation theory; chelation reduced the polarity of the metal atom mainly because of partial sharing of its positive charge with the donor groups and possible electron delocalization within the whole chelate ring. Also, chelation increased the lipophilic nature of the central atom which subsequently favored its permeation through the lipid layer of the cell membrane.[51,52] The mechanism of action is generally considered to be the disturbance of the cytoplasmic memberane, disrupting the proton motive force (PMF), electron flow, active transport, and coagulation of cell contents.[53,54] The ligands and their vanadium(IV) coordination compounds have shown different antimicrobial activity toward tested microorganisms. The coordination compounds have exhibited appreciable activity relative to MIC of standard drugs selected for antibacterial activity (Tetracycline, Chloramphenicol,) and antifungal activity (Cycloheximide, Fluconazole) (MIC $<$3.90 µg mL^{-1}).

Table 3. Antibacterial and Antifungal Activity Data ($\mu g\,mL^{-1}$)

Compound	Bacteria			Fungi	
	E. coli	*S. aureus*	*B. subtilis*	*C. albicans*	*A. niger*
$C_6H_5OCH_2C(O)NHOK$ (HL^1)	125	250	125	125	125
$C_6H_5CH{=}CHC(O)NHOK$ (HL^2)	125	250	62.5	125	62.5
Imidazole[a)]	—	125	62.5	125	125
Benzimidazole[b)]	—	250	125	250	125
$[VO(HL^1)_2]$	31.2	62.5	31.2	31.2	31.2
$[VO(HL^1)_2$(imid)]	15.6	31.2	7.81	31.2	31.2
$[VO(HL^1)_2$(benzimid)]	31.2	31.2	31.2	15.6	31.2
$[VO(HL^2)_2]$	31.2	62.5	15.6	15.6	31.2
$[VO(HL^2)_2$(imid)]	15.6	7.81	15.6	3.90	15.6
$[VO(HL^2)_2$(benzimid)]	31.2	7.81	31.2	31.2	15.6

a) Imidazole: $C_3H_4N_2$. b) Benzimidazole: $C_7H_6N_2$.

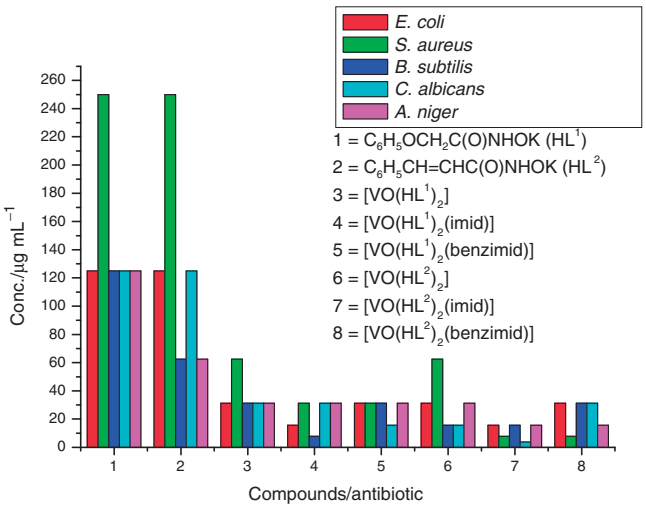

Figure 7. In vitro antimicrobial spectrum of coordination compounds.

Table 4. Cytotoxic Assay of $VOSO_4$, Bases, and Hydroxamatooxovanadium(IV) Compounds against Hep2C Cell Line

Test compound	Cell viability (%) at the selected test compound concentration					
	Control	2 mM	4 mM	8 mM	20 mM	28 mM
$VOSO_4$	100	40	35	35	30	30
Imidazole	100	100	70	5	5	5
Benzimidazole	100	70	50	35	32	30
$[VO(HL^1)_2$(imid)]	100	70	45	20	18	15
$[VO(HL^1)_2$(benzimid)]	100	42	36	35	33	30
$[VO(HL^2)_2$(imid)]	100	68	40	20	15	15
$[VO(HL^2)_2$(benzimid)]	100	40	35	35	32	30

In Vitro Cytotoxicity Assay. Cytotoxic assays of $VOSO_4$, imidazole, benzimidazole, $[VO(HL^1)_2$(imid)], $[VO(HL^1)_2$(benzimid)], $[VO(HL^2)_2$(imid)], and $[VO(HL^2)_2$(benzimid)] were performed at several concentrations by means of colorimetric microculture MTT assay (Table 4). The $VOSO_4$, imidazole, and benzimidazole exhibit cell viability of 40, 100, and 70% respectively at concentration 2 mM. Of four tested coordination compounds, $[VO(HL^1)_2$(imid)] and $[VO(HL^2)_2$(imid)] exhibit appreciable viability of 70 and 68% respectively at 2 mM concentration, compared to 42 and 40% cell viability shown by analogous compounds derived from benzimidazole. With increase in concentration of test compounds cytotoxicity gets significantly enhanced. The cytotoxic study shows that at low-concentration coordination compounds derived from imidazole are less toxic.

Conclusion

Coordination compounds of hydroxamatooxovanadium(IV) complexes with imidazole and benzimidazole have been prepared and characterized by various physicochemical and spectral techniques. A distorted octahedral geometry around vanadium in coordination compounds has been suggested. An assay of antimicrobial activities of newly synthesized compounds has shown substantial increase in antimicrobial activity compared to parent complexes.

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