



Synthesis, Characterisation and Anti amoebic activity of 1E-1-(1H-benzimidazol-2-yl)-N-hydroxy ethanimine and 1E-1-(1H-benzimidazol-2-yl)-N-hydroxy-1-phenyl methanimine with Oxovanadium (IV), Mn(II), Fe(II).

Madhu Bala* and L.K Mishra^a

*Department of chemistry, NIT, Patna

^aDepartment of Chemistry, Science College Patna, Patna University, Patna

Abstract: The solid complexes of 1E-1-(1H-Benzimidazol-2-yl)-N-hydroxy ethanimine (LH) and 1E-1-(1H-Benzimidazol-2-yl)-N-hydroxy-1-phenyl methanimine (L'H) with VO(II), Mn(II) and Fe(II) have been prepared and characterised from the studies of magnetic susceptibility and spectral studies. The ligands coordinate as either neutral chelate or mono anionic bidentate complexing molecules forming complexes of compositions $[Mn(BH)_2Cl_2]$, $[Fe(BH)_3]SO_4 \cdot 2H_2O$, (BH = LH or L'H) $VO(BH)_2SO_4 \cdot H_2O$, (BH = LH or L'H) and $MB_2 \cdot nH_2O$ (M = VO^{II}, Mn^{II} or Fe^{II}, BH = L'H or LH and n = 1 or 2). The magnetic and spectral properties of complexes were consistent with octahedral structure. The anti-amoebic activity of LH, L'H and their bis chelates $MB_2 \cdot nH_2O$ (M = VO^{II}, Mn^{II} or Fe^{II}, BH = L'H or LH) were screened in vitro by micro dilution technique against *Entamoeba Histolytica*. The IC₅₀ value of VO (II) and Cu (II) complexes showed important Anti- amoebic properties.

Key Words: Synthesis, Characterisation, Anti-amoebic activity, Benzimidazolyl oxime derivatives, their metal complexes.

I. Introduction

The current interest in the development of biological evaluation of metal complexes can be attributed to the considerably enhanced physiological activity of metal complexes than those of pure drug¹⁻⁴. The increasing emergence of bacterial resistivity towards currently used antibiotics and various marketed medicines for treatment of various diseases and to counteract newly emerging unknown pathogens, is a matter of great concern, therefore the search for alternative drugs and antibacterial pharmacophore is the need of present research activity⁵. Wide range spectrum of physiological activity of various benzimidazole derivatives interested us to synthesise metal complexes of new derivative and to evaluate their microbial activity. The current biochemical investigation of benzimidazole derivatives have shown that they can act as bacteriostates⁵, bacteriocides⁶, fungicides⁷, anticarcinogens⁸, antiparasitic⁹, antihelmintic¹⁰, anti-inflammatory, antiamoebic¹¹, antiviral¹², anti- HIV¹³, and anti hypertensive¹⁴, anti ulcer¹⁵ substance. Hydroxy substituted benzimidazole derivatives show promising activity against parasites and intestinal amoebic as well as hepatitis-B infection^{14, 15}. In pursuance of our interest of structural and synthesis of various metal complexes with benzimidazole derivatives¹⁶⁻²⁰, we are now reports the preparations, characterisation and anti-amoebic activity of two oxime derivatives of benzimidazole with bivalent metal ions.

II. Methods and Material

The ligands 1E-1-(1H-benzimidazol-2-yl)-N-hydroxyethanimine (Hbhe) and 1E-1-(1H-benzimidazol-2-yl)-N-hydroxy-1-phenyl methanimine (Hbhpm) were prepared by condensing 2- Acetylbenzimidazole[1-(1H-benzimidazol-2-yl)ethanone] with hydroxylamine hydrochloride (NH₂OH HCl) in aqueous ethanol keeping basic medium by K₂CO₃ as well as by condensing 2-benzoylbenzimidazole[1-(1H-benzimidazol-2-yl)phenone] as above. 2- Acetylbenzimidazole and 2- benzoylbenzimidazole were prepared by reported procedure²¹⁻²². Both Hbhe and Hbhpm were recrystallized with minimum volume of ethanol. M.pt found Hbhe -243⁰c and that of Hbhpm - 195⁰c (uncorrected). Metal salts were obtained from BDH or E. Merck. solvents and organic chemicals used were obtained from E. Merck, (India) Nice or Sd fine chemicals. Metal and halogen or sulphate contents of complexes were estimated by standard methods²³. The molar conductance (qualitatively) of complexes were measured at room temperature by a digital conductivity meter as model 611E having cell constant 1.0 using 10⁻³ molar solutions of complexes in DMF. Magnetic susceptibility measurement were carried by Gouy's method at room temperature (304K) . IR spectra of compounds were recorded on SHIMADZU FTIR spectrophotometer at IIT Patna in range 4000 – 400 cm⁻¹ as KBr disc. The U-V spectra over the region 200 – 850 nm were recorded in ethanol of DMF at IIT Patna. The result of Mass and NMR spectra were obtained from CDRI Lucknow. The NMR Spectra were recorded on Bruker DRXFT-NMR spectrometer

(300MHz) in CDCl_3 or DMSO. Mass spectra were taken on JEOL – SX – 1021 (PA – 600 Et) spectrometer. Anti-amoebic activity was screened in vitro by micro dilution technique as reported in literature²⁴⁻²⁵.

Preparation of Complexes: $\text{Mn}(\text{HB})_2\text{Cl}_2$, $\text{VO}(\text{HB})_2\text{SO}_4\cdot\text{H}_2\text{O}$ and $[\text{Fe}(\text{HB})_3]\text{SO}_4\cdot 2\text{H}_2\text{O}$, (HB = Hbhe or Hbhpm). About 10 millimole of metal sulphate was dissolved in 30 – 40 ml aqueous ethanol ($\text{MnCl}_2\cdot 6\text{H}_2\text{O}$ in absolute ethanol) were added with constant stirring to 20 millimole of appropriate ligand (30 millimole for Fe^{II}) dissolved in 30 ml hot ethanol. The resulting solution was refluxed on a steam bath for half an hour when desired complex separated. Mn(II) separated on to a small volume and cooling at room temperature. The products were collected on a filter washed with cold ethanol. The purity of samples were checked by TLC.

COMPLEXES $[\text{ML}_2(\text{H}_2\text{O})_2]$, (M = Mn^{II} , VO^{IV} or Fe^{II} and HL = Hbhe or Hbhpm)

About 5 millimole of metal chloride or sulphate was dissolved in aqueous ethanol (25 ml) and added with stirring to hot ethanolic solution of appropriate ligand and refluxed on steam bath. The p^{H} of resulting solution was raised to 8 – 9 by adding aqueous sodium acetate when neutral bis chelate separated. The product was digested for 25 – 30 minutes by diluting with 40 – 50 ml water. The complexes collected on cooling washed with cold aqueous ethanol and dried over CaCl_2 and finally in an air oven at 50 – 60°C. The results of elemental analysis and physical data are recorded in table I.

Table I. Elemental analysis and Physical data of complexes

Compound	Colour	% analysis found (calc)				Magnetic moment μ_{eff} in BM	χ_{M} $\text{ohm}^{-1} \text{mol}^{-1} \text{cm}^2 30^\circ \text{C}$
		M	C	N	H		
Hbhe = LH	Cream	-	61.51(61.71)	23.73(24.01)	5.31(5.14)	-	-
Hbhpm = L H	Cream	-	70.93(71.18)	17.48(17.72)	4.91(4.64)	-	-
$[\text{VO}(\text{LH})_2(\text{H}_2\text{O})]\text{SO}_4$	Cream yellow	9.31(9.46)	40.71(40.83)	15.70(15.88)	4.01(3.78)	1.79	138
$[\text{VO}(\text{L H})_2(\text{H}_2\text{O})]\text{SO}_4$	Cream yellow	7.91(7.82)	51.31(51.45)	12.59(12.86)	3.91(3.67)	1.76	145
$[\text{Mn}(\text{LH})_2\text{Cl}_2]$	Light yellow	11.42(11.60)	45.41(45.56)	17.48(17.72)	3.92(3.79)	5.89	12
$[\text{Mn}(\text{L H})_2\text{Cl}_2]$	Light yellow	8.41(8.52)	56.21(56.18)	14.16(14.04)	3.88(3.68)	5.91	10
$[\text{Fe}(\text{LH})_3]\text{SO}_4\cdot 2\text{H}_2\text{O}$	Vermilion red	8.11(8.27)	47.71(47.86)	18.43(18.61)	4.12(3.98)	4.79	136
$[\text{Fe}(\text{L H})_3]\text{SO}_4\cdot 2\text{H}_2\text{O}$	Vermilion red	6.51(6.27)	60.41(60.74)	14.91(15.13)	4.12(3.96)	4.82	138
$[\text{FeL}_2(\text{H}_2\text{O})_2]$	Brick red	12.58(12.72)	49.18(49.09)	18.81(19.09)	4.67(4.53)	4.73	-
$[\text{FeL}_2(\text{H}_2\text{O})_2]$	Brick red	9.71(9.92)	59.77(59.57)	14.61(14.89)	4.31(4.25)	4.81	8
$[\text{MnL}_2(\text{H}_2\text{O})_2]$	Cream yellow	12.41(12.52)	49.46(49.20)	19.31(19.13)	4.61(4.55)	5.91	-
$[\text{MnL}_2(\text{H}_2\text{O})_2]$	Cream yellow	9.52(9.76)	59.51(59.68)	14.81(14.92)	4.41(4.26)	5.87	6
$[\text{VOL}_2(\text{H}_2\text{O})_2]$	Light brown	11.48(11.77)	49.62(49.88)	19.21(19.39)	4.15(3.81)	1.74	5
$[\text{VOL}_2(\text{H}_2\text{O})_2]$	Light brown	9.01(9.15)	59.76(60.32)	14.86(15.08)	4.21(3.95)	1.76	-

The ligands IE-1-(1H-Benzimidazol-2-yl)-N-hydroxy-ethanimine (Hbhe) and 1E-1-(1H-benzimidazol-2-yl)-N-hydroxy-1-phenyl methanimine (Hbhpm) coordinates in weakly acid medium as neutral bidentate ligand forming complexes of composition $[\text{Mn}(\text{HB})_2\text{Cl}_2]$, $[\text{VO}(\text{HB})_2\text{H}_2\text{O}]\text{SO}_4$ and $[\text{Fe}(\text{HB})_3]\text{SO}_4\cdot 2\text{H}_2\text{O}$ (HB = Hbhe or Hbhpm). In basic medium the ligands coordinates as monoanionic chelating molecule forming complexes of composition $[\text{VO B}_2 \text{H}_2\text{O}]$ and $[\text{M B}_2(\text{H}_2\text{O})_2]$, (M = Mn^{II} or Fe^{II} and HB = Hbhe or Hbhpm). These neutral complexes are stable to heat and atmospheric air below 100-110°C indicating water molecule to be bonded with metal atom. In case of $[\text{Fe}(\text{BH}_3)]\text{SO}_4\cdot 2\text{H}_2\text{O}$, the water is lost below 110°C suggesting that water is not coordinated to metal atom. The molar conductance value of complexes in DMF at room temperature ($30 \pm 0.5^\circ \text{C}$) indicated that $[\text{Mn}(\text{HB})_2\text{Cl}_2]$ and $\text{MB}_2\cdot n\text{H}_2\text{O}$ are non-ionic (Table-I). The conductance value of $[\text{VO}(\text{HB})_2\text{H}_2\text{O}]\text{SO}_4$ and $[\text{Fe}(\text{HB})_3]\text{SO}_4\cdot 2\text{H}_2\text{O}$ (HB = Hbhe or Hbhpm) occur in the range 136-145 $\text{ohm}^{-1} \text{mol}^{-1} \text{cm}^2$ supported ionic nature of sulphate for these complexes in DMF solution²⁷. All aqua complexes on heating in air oven, losses H_2O molecule between 120-200°C, supporting the presence of lattice or coordinated water molecule. The room temperature magnetic moment value of complexes (Table-I) occur in range of spin free octahedral structure of VO(IV), Mn(II), and Fe(II) complexes²⁸. The electronic absorption spectra of Mn(II) complexes did not show distinct electronic transition in DMF or ethanol probably due to negligible ϵ_{max} value of spin forbidden d-d transitions expected for Mn(II). The VO(IV) and Fe(II) complexes show strong absorption below 380 nm (High energy) due to charge transfer transition²⁸. The electronic absorption band at 490-510 nm observed for Fe(II) complexes is assigned to ${}^5\text{T}_{2\text{g}} \rightarrow {}^5\text{E}_{\text{g}}$ transition in octahedral field²⁹. Oxovanadium (IV)

display medium absorption band in DMF at 430-460 nm and 540-550 nm. The electronic band can be assigned to ${}^2B_{2g} \rightarrow {}^2B_{1g}$, ${}^2A_{1g}$ and ${}^2B_{2g} \rightarrow {}^2E_g$ in distorted octahedral field²⁹. The HNMR, ${}^{13}\text{C}$ NMR, Mass and IR spectra of ligands and some of the complexes were recorded to evaluate nature of bonding of Hbhe and Hbhpm with metal atom. The IR spectra of Hbhe shows $\nu(\text{OH})$, $\nu(\text{NH})$, $\nu(\text{CH})$ of phenyl and methyl group at 3350, 3190, 3060 and 2940, 2820 cm^{-1} . These vibrations are retained in complexes with neutral ligand in $[\text{Mn}(\text{Hbhe})_2\text{Cl}_2]$, $[\text{VO}(\text{Hbhe})_2\text{H}_2\text{O}] \text{SO}_4$ and $[\text{Fe}(\text{Hbhe})_3]\text{SO}_4 \cdot 2\text{H}_2\text{O}$. The (=N-OH) hydrogen is probably hydrogen bonded with imidazole ring (NH) which display broad band at 3550 cm^{-1} and 2940-2820 cm^{-1} . The strong band at 1652 cm^{-1} is attributed to imine part $\nu(\text{C}=\text{N})$ vibration. The ligand display a number of prominent IR bands located at 1595, 1535, 1492, 1452, 1380, 1132, 1023 and 931, 842, 747, 693 and 540 cm^{-1} . The IR band located at 1595 and 1492 cm^{-1} are assigned to ring $\nu(\text{C}=\text{N})$ and (NH) bending bands. The $\nu(\text{N}-\text{O})$ vibration of ligand can be assigned to strong IR band at 1132 cm^{-1} . This band shifted to 1310-1330 cm^{-1} in Bis-chelated neutral complexes $[\text{M}(\text{Hbhe})_2] \cdot n\text{H}_2\text{O}$ ($\text{M}=\text{VO}(\text{II})$, $\text{Mn}(\text{II})$ and $\text{Fe}(\text{II})$ $n=1$ or 2) suggesting bonding of $\text{C}=\text{N}-\text{OH}$ through nitrogen by deprotonation. The ring (C=N) stretch is shifted to lower frequency by 10-15 cm^{-1} in complexes. The imine (C=N) stretch located at 1652 cm^{-1} also shifted by 15-25 cm^{-1} complexes suggesting the bonding through hydroxyl imine (C=N) nitrogen.

The aqua or hydrated complexes show broad band between 3400-2950 cm^{-1} due to hydrogen bonded H_2O in complexes. The IR spectra of Hbhpm displays prominent IR bands at 3443, 3190, 3064, 2888 and 2840 cm^{-1} attributed to $\nu(\text{OH})$, $\nu(\text{NH})$, phenyl ring $\nu(\text{C}-\text{H})$ vibrations. The hydrogen bonded $\nu(\text{C}=\text{N})$ of N-hydroxy-1-phenyl methanimine was located at 1655 cm^{-1} as strong and sharp band and ring (C=N) stretch at 1595 cm^{-1} and these are shifted to lower frequency on complexation (Table-II). The IR bands located in free ligand at 1485 cm^{-1} is attributed to $\nu(\text{NH})$ of benzimidazole ring. The prominent and strong IR bands located at 1429 cm^{-1} , 1385 cm^{-1} , 1320, 1280, 1232, 1180, 1120, 1043, 935, 930, 752, 739, 693, 652, 470 and 430 cm^{-1} are observed due to various mode of IR vibration of phenyl and benzimidazole ring. The IR band at 1043 cm^{-1} is attributed to $\nu(\text{N}-\text{O})$ and it is shifted to higher wave number indicating deprotonation of (N-OH) and bonding through nitrogen atom in neutral bis chelates $[\text{M}(\text{bhpm})_2 \cdot n\text{H}_2\text{O}]$. The $\nu(\text{N}-\text{O})$ in complexes $[\text{Cu}(\text{HL})\text{X}_2]$, $[\text{Mn}(\text{HL})_2\text{Cl}_2]$ and complexes sulphates are not affected appreciably but $\nu(\text{C}=\text{N})$ is shifted to lower wave number due to coordination. The complexes sulphate display strong and broad band at 1105 ± 5 and 618 ± 3 cm^{-1} (ν_1 & ν_4) stretches of ionic sulphate group³⁰. The Vanadyl complex sulphates display $[\text{VO}(\text{LH})_2\text{H}_2\text{O}]\text{SO}_4$ ($\text{LH}=\text{Hbhe}$ or Hbhpm) display very strong IR bands at 1108 ± 8 cm^{-1} supporting ionic nature of sulphate group³¹. The new band in the spectra located between 932 and 890 cm^{-1} in $\text{VO}(\text{II})$ complexes is assigned to ($\text{V}=\text{O}$) stretch. The force constant of ($\text{V}=\text{O}$) group was calculated and given in (Table-II). The force constant value of complex sulphate was higher than those of neutral complex.

Complexes	$\nu(\text{VO})$	Force constant
$\text{VO}(\text{Hbhe})_2\text{SO}_4$	932	6.19×10^5 dyne
$\text{VO}(\text{Hbhpm})(\text{H}_2\text{O})$	925	6.10×10^5 dyne
$\text{VO}(\text{bhe})_2(\text{H}_2\text{O})$	896	5.72×10^5 dyne
$\text{VO}(\text{bhpm})_2(\text{H}_2\text{O})$	892	5.67×10^5 dyne

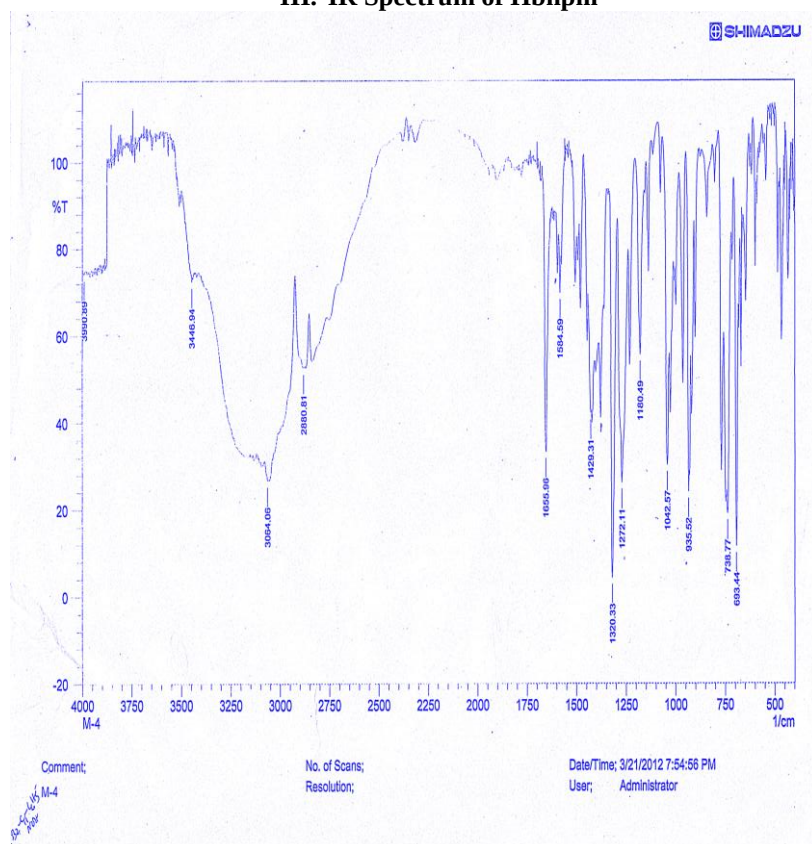
The aqua complexes display a broad band at 680 ± 5 cm^{-1} (rocking band of coordinated water molecules³⁰). The complexes $[\text{FeL}_3]\text{SO}_4 \cdot 2\text{H}_2\text{O}$ do not show rocking IR band (ρ_w) of coordinated water molecules. The elimination of water molecules below 60-70°C for $[\text{FeL}_3\text{SO}_4] \cdot 2\text{H}_2\text{O}$ supports the presence of lattice water.

Prominent diagnostic IR bands of ligands and some of their complexes in cm^{-1}

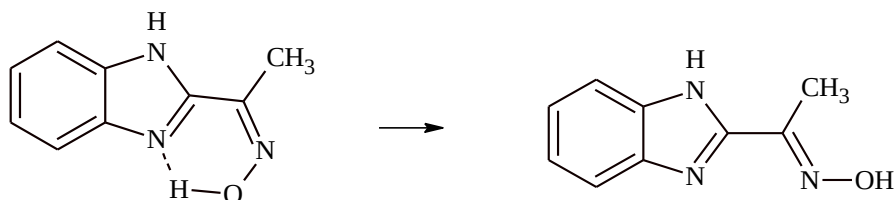
Compound	$\nu(\text{OH}), \nu(\text{NH}), \nu(\text{CH})$	$\nu(\text{C}=\text{N})$ imine	$\nu(\text{C}=\text{N})$ ring	$\delta(\text{NH})$	$\nu(\text{N}-\text{O})$	Anions vibrations
Hbhe=HL	3350, 3190, 3060, 2940	1665	1600	1492	1132	-
$\text{Mn}(\text{HL})_2\text{Cl}_2$	3340, 3185, 3040,	1652	1590	1485	1130	-
$\text{MnL}_2(\text{H}_2\text{O})_2$	3400-3100 br, 3045, 2920	1648	1585	1490	1305	-
$\text{VO}(\text{LH})_2\text{SO}_4 \cdot \text{H}_2\text{O}$	3350-3100 3060, 2880	1655	1590	1495	1140	$\nu(\text{SO}_4)$ 1105br 618
$[\text{VO}(\text{LH})_2(\text{H}_2\text{O})]$	3400-3100br, 3050, 2900	1648	1585	1480	1330	ν_4 2618
$\text{FeL}_2(\text{H}_2\text{O})$	3350-3100, 3060, 2890	1640	1580	1485	1340	-
$\text{Fe}(\text{LH})_3\text{SO}_4 \cdot 2\text{H}_2\text{O}$	3350-3120, 3044, 2890	1650	1595	1490	1145	1105, str 617
Hbhpm=HL	3443, 3190, 3064, 2888	1655	1595	1485	1043	--
$\text{Mn}(\text{HL})_2\text{Cl}_2$	3440, 3190, 3063	1643	1590	1480	1056	--
$\text{VO}(\text{HL})_2\text{SO}_4 \cdot \text{H}_2\text{O}$	3429, br 3180, 3065	1638	1585	1485	1053 br	1108, str 615
$\text{VO}(\text{LH})_2\text{H}_2\text{O}$	3400-3100, br 3040	1150	1590	1480	1350 br	
$\text{Fe}(\text{HL})_3\text{SO}_4 \cdot 2\text{H}_2\text{O}$	3420-3120, 3055, 2880	1645	1585	1485	1056	1110str 618

Fe(L ₂)(H ₂ O) ₂	3400-3100, 3050, 2900	1650	1590	1484	1340br	--
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III. IR Spectrum of Hbhpmm

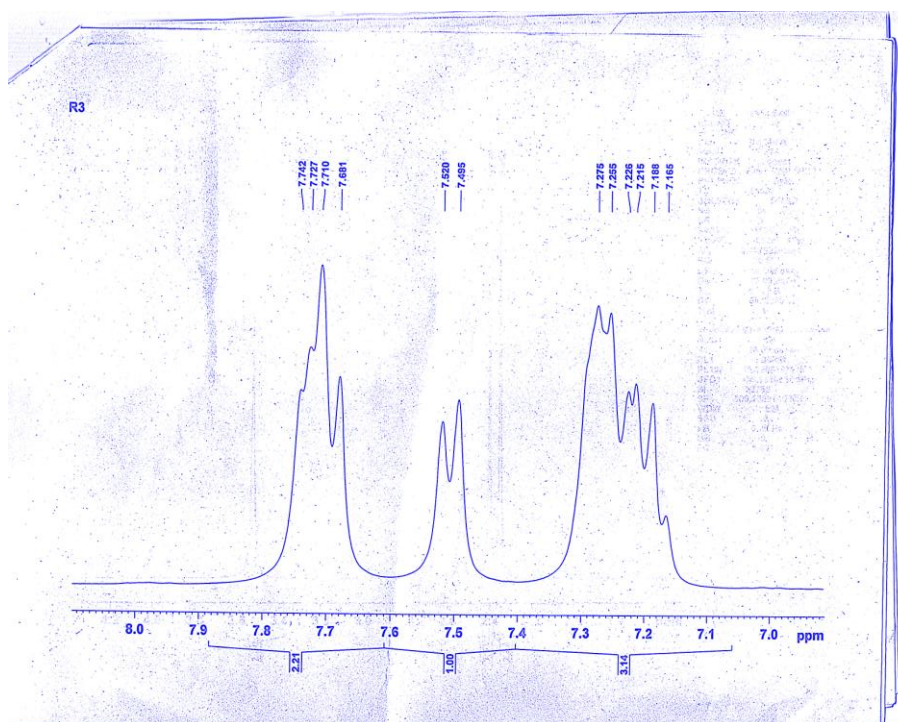
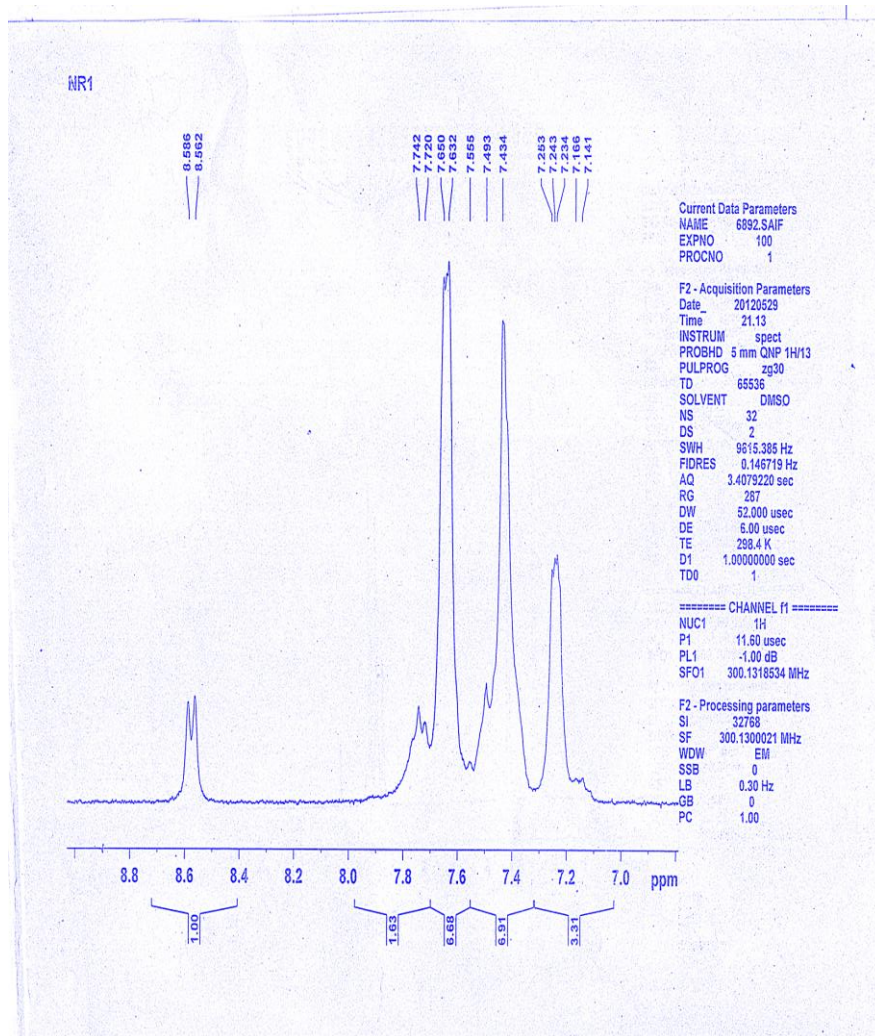


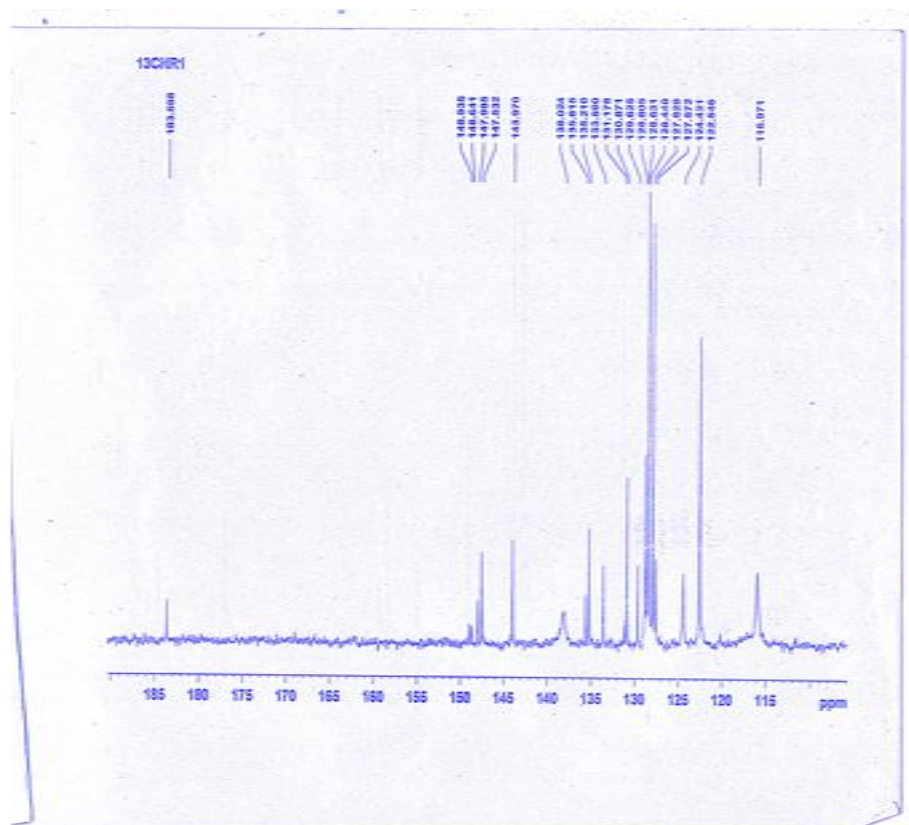
The proton NMR spectra of Hbhe shows complex spectrum of molecule due to cis-trans orientation of =N-OH group with respect to benzimidazole part of molecule as shown below.



The CH₃ proton signals were observed at δ =2.35, 2.40, 2.53, 2.61 and 3.52 ppm. The phenyl ring (C-H) proton signals were observed as multiplets between 7.165-7.742 ppm (Fig HNMR-3). The ring N-H proton signal was located at 1182 ppm whereas (N-OH) proton signal was observed at δ =12.294 and 12.541 ppm. The ¹HNMR spectra of Hbhpmm shows (Fig-NR-1) phenyl ring proton signals at multiplets between 7.141 - 7.742 ppm. The NH proton signals were located at 8.586 while (N-OH) proton signals were located at 12.829 and 12.612 ppm. The doublet of (N-OH) proton signal suggested the cis-trans orientation of oxime (=N-OH) group above benzimidazole ring.

The ¹³CNMR spectrum also shows ¹³C twelve signals for ring carbon atom. The aldimine (C=N) carbon signal is assigned to NMR band at δ = 149.21ppm. ¹³CNMR signals at 18.36, 38.67, 38.95, 39.23 to 40.34 ppm were assigned to methyl carbon and DMSO carbon of solvent. The ¹³CNMR signals of benzimidazole ring were observed at δ = 111.64, 113.00, 119.15, 121.47, 123.26, 134.70+41.62, 143.09, 143.59, 143.97, 147.85 and 149.21 ppm. The ¹³C signal at 149.21 is assigned to C=N-OH carbon atom. The Mass spectra of ligands were recorded. The m/z value of Hbhe was 175(30%) 159(15%) 145(16%) 118(100%), 104(8%) 92(45%), 58(9%) and 57(17%). The mass spectrum of Hbhpmm show m/z ratio 227(M⁺ =55%), 228(15%), 212(15%), 201(15%), 118(100%base peak), 91(40%), 77(55%) and 57(45%). The mass results confirm the formation of proposed products. The ¹HNMR as well as ¹³C NMR spectra of ligands and one Cd complex were recorded in CDCl₃ or DMSO. The proton N-OH signals of ligands were absent in [CdL₂](HL=Hbhe or Hbhpmm) indicating deprotonation of NOH proton. The other proton NMR signals were found slightly shifts to higher ppm value.





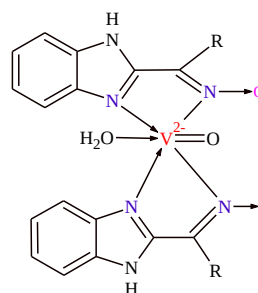
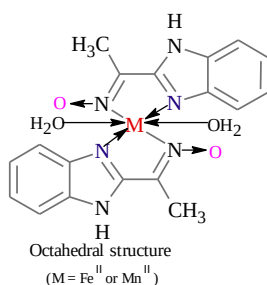
IV. Anti-amoebic activity

In-vitro anti-amoebic activity of ligands and neutral bis chelates were assed against HM-1/1 MSS strain of *E. Histolytica*. The trophozoites were maintained axenically in PEHPS medium by serial culture²⁶. Trophozoites were used for 3-days (72 hours) axenic culture, grown in 16×125 mm screw capped tubes containing 11ml of PEHPS were incubated with 1000 trophozoites mL⁻¹ and incubated at 36⁰c, the sub- cultivation frequency was every four days. The drug potency test was performed by reported procedure. Borosilicate screw capped culture tubes containing 5.5mL of fresh sterile PEPHS medium were added with 50mL of different concentration of samples of Metronidazole. In all determination the Metronidazole and sample concentration were maintained in the range of 0.0-3.0 and 0.0-50.0 μ M/mL respectively. Metronidazole was dissolved in phosphate buffer saline (PBS), all the compound tested were dissolved in DMF. The tubes were incubated for 72 hours at 36⁰c and then cooled on ice water for 20 minutes and the numbers of trophozoites mL⁻¹ in each tube were counted with haemocytometer. The results were evaluated as the % of growth inhibition compared with the untreated controls and plotted as prohibit value as a function of drug concentration. The IC₅₀ and 95% confidence limits were inter-plotted in the corresponding responses curve. For each response three experiments were performed for each compound tested to assess the accuracy of the results. The *in-vitro* inhibitory effect of compound (1-6 in Table-IV) screened against *E.Histolytica* upon the growth of axenic culture of HM1/1MSS strain are summerised in Table-IV. The anti- amoebic effect was compared with Metronidazole. Metronidazole has almost 50% inhibition concentration (IC₅₀) of 2.28 μ M in present experiment which occur within the range of IC₅₀ value of Metronidazole (2.92 μ M) as reported previously³² from the results observed from Table-III, it appeared that compounds screened for anti-amoebic activity in-vitro for have IC₅₀ value ~10 μ M mL and two of these had IC₅₀ less than 4 μ M. Marshall and Russell et al have showed that compound showing IC₅₀ value less than 20 μ M can be considered as having significant amoebicidal agents. It has been observed that VO(II) complexes with Hbhpm ligand having phenyl substituent show larger anti-amoebic activity than free ligands and other metal complexes from the observed potency it appears that some new benzimidazole derivatives may be highly active anti-amoebicidal agent.

Table-IV In-Vitro Anti-amoebic activity of benzimidazole derivatives and their metal complexes.

SL.NO	Compounds	IC ₅₀ (μ M/mL)	S. D.
1.	Hbhe=HL	13.26	1.60
2.	Hbhpm=HL	9.58	1.50
3.	VO(Hbhe) ₂ SO ₄	4.50	0.65
4.	VO(hbe) ₂ H ₂ O	4.00	0.69
5.	VO(bhpm) ₂ H ₂ O	3.80	0.56
6.	Fe(bhe) ₂ (H ₂ O) ₂	6.53	1.34
7.	Fe(bhpm) ₂ (H ₂ O) ₂	5.4	1.42
8.	Mn(bhe) ₂ (H ₂ O) ₂	6.21	1.48
9.	Mn(bhpm) ₂ (H ₂ O) ₂	7.34	1.63

The probable structure of $ML_2(H_2O)_2$ and $VO(L)_2H_2O$ ($HL = Hbhe$ or $Hbhpm$ and $M = Fe^{II}$ or Mn^{II}) are shown below.



V. Conclusion

The ligands coordinates as (N,N) donor chelate molecule. In neutral or weakly acid medium coordinate as neutral and mono-anionic bidentate coordinating molecule in basic medium. The anti-amoebic activity of metal complexes were much larger than free benzimidazole derivatives. The VO(II) complexes can be tested for their use as anti- amoebic activity.

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