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Synthesis, Characterization and Biological Studies of Cu(II), Ni(II), Co(II) and VO(IV) Complexes of Tridentate ONO Donor with N'-[(E)-3-Bromo-5-chloro-2-hydroxybenzylidene]nicotinohydrazide

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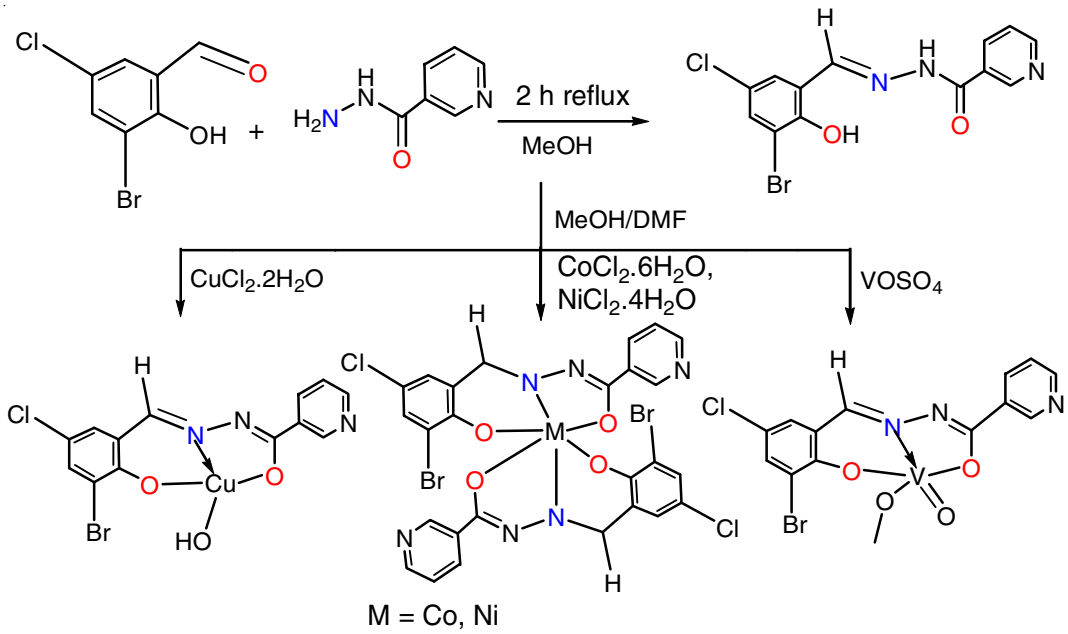
New Cu(II), Ni(II), Co(II) and VO(IV) complexes of the Schiff base, N'-[(E)-3-bromo-5-chloro-2-hydroxybenzylidene]nicotinohydrazide have been synthesized and characterized by NMR, MS, IR, electronic, magnetic measurements, EPR, redox properties and XRD. The ligand coordinates through one of the phenolic–O, azomethine–N and amide–O atoms to metal ions as shown by IR measurements. Electronic and magnetic measurements have been corroborative of a square-planar geometry for Cu(II) complex, octahedral geometry for Ni(II), Co(II) complexes and square pyramidal geometry for vanadium complex. The observed anisotropic 'g' values also confirm the presence of Cu(II) in a square planar environment and VO(IV) complex in a square pyramidal coordination geometry around the metal centre. The redox properties of the metal complexes have been investigated by cyclic voltammetry. Preliminary antibacterial screening shows the promising results against bacterial strains. The compounds have shown promising cytotoxic activity when screened using the *in vitro* method on human breast adenocarcinoma (MCF-7) cells.

Keywords: Tridentate hydrazone ligand, Schiff base, Transition metal complexes, Antibacterial activity, Cytotoxicity activity.

INTRODUCTION

Hydrazones play an important role in inorganic chemistry, as they easily form stable complexes with most of the transition metal ions [1]. Hydrazones possessing an azomethine -NHN=CH- proton constitutes an important class of compounds for new drug development. Therefore, many researchers have been synthesized these compounds as target structures and evaluated their biological activities [2,3]. Coordination compounds derived from aryl hydrazones have been reported to act as enzyme inhibitors were useful their pharmacological applications [4]. The important criteria for the development of metallodrugs as chemotherapeutic agents were showed the ability of the metallodrug to bring about DNA cleavage because of their redox properties, have been found to promote DNA cleavage. Schiff bases are important organic compounds in medicinal chemistry due to antibacterial and chemotherapeutic applications [5]. Cytotoxic activities of Schiff base complexes can be improved through coordinating to Cu(II) cation and it has been evaluated their potential antitumor activities on different cell lines [6]. Vanadium complexes of nicotinoyl hydrazone have promising anti cancer acitivity against HPV16 positive cervical cancers [7]. Hydrazones derived from isonicotinoyl hydrazide have been investigated for years as potential drugs for treatment of iron-overload associated diseases [8]. Some aroylhydrazones have shown DNA binding [9,10], antioxidant [10], cytotoxic [11] and anticancer activities [12,13].

In view of these facts that the nicotinic acid ring is an important for anticancer activity, we have synthesized and structural characterization of (E)-N'-(3-bromo-5-chloro-2-hydroxy-benzylidene)nicotinohydrazide have been reported in previous paper [14]. In present study (E)-N'-(3-bromo-5-chloro-2-hydroxybenzylidene)nicotinohydrazide (BCHBNH) and its Cu(II), Ni(II), Co(II) and VO(IV) complexes have been synthesized (**Scheme-I**), characterized and biological studies. The biological studies are done with the aim of anticancer agents, which will be active against human cancer cell lines. The study has been extended to screen the ligand and its metal complexes against MCF-7 human cancer cell lines.



Scheme-I: Synthetic routes of the ligand and their Cu(II), Ni(II), Co(II) and VO(IV) complexes

EXPERIMENTAL

The reagents and chemicals are obtained from commercial sources (Sigma-Aldrich, USA; Himedia, India; Merck, India; Genei, Bangaluru, India). Nicotinohydrazide, 3-bromo-5-chloro-2-hydroxybenzaldehyde, copper(II)chloride dihydrate, cobalt(II)chloride hexahydrate, nickel(II) chloride tetrahydrate, banadyl sulphate, 3-(4,5-dimethyl-2- thiaozolyl)-2,5-diphenyl-tetrazolium bromide (MTT) are used as received. Commercial solvents are distilled and then used for preparation of complexes.

Elemental analysis of the ligand and its complexes are carried out using a Vario EL III CHNS analyzer, Mass percentages of ligand are measured in Shimadzu QP-2010 coupled with GC-MS spectrometer, ^{1}H and ^{13}C NMR spectra are recorded on a Bruker 200/300 MHz spectrometer using TMS as an internal reference at 25 °C in DMSO. IR spectra are recorded in KBr disks with a Perkin Elmer FT-IR spectrophotometer, UV-visible spectra of solution are recorded on a Shimadzu 1700 PC series spectrometer, bio-analytical systems (BAS) model CV 50 electrochemical analyzer is used for cyclic voltammetric experiments, magnetic parameters are measured with Lakeshore VSM 7304 with a maximum field of 20 KOe. ESR spectra are recorded on a JEOL JES-TE 100 spectrometer in DMF solution at room temperature.

Preparation of N'-[(E)-3-bromo-5-chloro-2-hydroxybenzylidene]nicotinohydrazide (BCHBNH): Nicotinohydrazide (0.136 g; 0.1 mmol) with 10 mL of methanol is stirred in a round bottom flask followed by drop wise addition of methanolic solution of 3-bromo-5-chloro-2-hydroxybenzaldehyde (0.235 g; 0.1 mmol). The reaction mixture is then refluxed for 2 h and upon cooling to room temperature. A pale yellow crystalline solid precipitate is separated out from the mixture. Crystalline product is washed with ice-cold ethanol and dried in vacuum over anhydrous $CaCl_2$. Pale yellow, Yield: 80 %, m.p.: 266 °C. Anal. calc. for $C_{14}H_{10}N_2O_2BrCl$: C, 47.55; H, 2.85; N, 7.92. Found: C, 46.95; H, 2.72; N, 7.32 %. ESI-MS: *m/z* = 355 (m^+). ^{1}H NMR (ppm) (DMSO-d_6): 12.66 (1H, s, OH), 12.61 (1H, s, NH), 8.55 (1H, s, CH=N), 8.28-8.32 (1H, d, *J* = 7.8 Hz, H aromatic), 8.18-8.82 (1H, t, *J* = 1.2 Hz), 7.96-7.98 (2H, d, *J* = 7.2 Hz), 7.75 (s,2H) 7.59-7.63 (1H, q, *J* = 2.4 Hz, *J* = 7 Hz). ^{13}C NMR (ppm) 161.6 (C=O), 153.2 (C-O), 152.7 (C=N), 110.8, 117.6, 120.2, 123.3, 127.6, 129.2, 133.1, 135.5, 147.6, 148.6 (C aromatic).

Preparation of Cu(II), Ni(II), Co(II) and VO(IV) complexes: The copper(II) complex is isolated by adding (E)-N'-(3-bromo-5-chloro-2-hydroxybenzylidene)nicotinohydrazide (0.354 g, 1 mmol), which is deprotonated by treating with triethylamine (0.5 mmol) in methanol solution, to $CuSO_4{\cdot}H_2O$ (0.125 g, 0.5 mmol) in methanol (20 mL) and then stirring the solution at 40 °C for 20 min. The reaction mixture is than refluxed for 2 h. After cooling the solution to room temperature, the resulting precipitate is collected by suction filtration, washed with cold methanol and finally dried in vacuum over anhydrous $CaCl_2$.

The Co(II), Ni(II) and VO(IV) complexes are also prepared by the above procedure using $CoCl_2{\cdot}6H_2O$, $NiCl_2{\cdot}4H_2O$ and $VOSO_4$ instead of $CuSO_4{\cdot}5H_2O$, respectively.

[Cu(BCHBNH)·H_2O]: Dark green, yield: 74 %, m.p. 335.17 °C, Anal. calc. for $C_{13}H_9N_3O_3BrClCu$: C, 35.97; H, 2.09; N, 9.68. Found: C, 35.15; H, 1.98; N, 9.32 %. μ_{eff} (300 K): 1.83 μ_B.

[$Ni(BCHBNH)_2$]: Dark green, yield: 69 %, m.p. 351 °C, Anal. calc. for $C_{27}H_{19}N_6O_4Br_2Cl_2Ni$: C, 41.53; H, 2.45; N, 10.76. Found: C, 41.25; H, 2.42; N, 10.65 %. μ_{eff} (300 K): 3.12 μ_B.

[$Co(BCHBNH)_2$]: Dark red, yield: 81 %, m.p. 400 °C, Anal. Calc. for $C_{27}H_{19}N_6O_4Br_2Cl_2Co$: C, 41.52; H, 2.45; N, 10.76. Found: C, 41.37; H, 2.44; N, 10.42 %. μ_{eff} (300 K): 4.54 μ_B.

[VO(BCHBNH)·OCH_3]: Brown, yield: 76 %, m.p. 398 °C. Anal. Calc. for $C_{14}H_{10}N_3O_4BrClV$: C, 37.32; H, 2.24; N, 9.33. Found: C, 37.27; H, 2.17; N, 9.31 %. μ_{eff} (300 K): 1.72 μ_B.

X-ray crystallography: The single crystal of the ligand (BCHBNH) is grown in DMF/methanol mixture. Diffraction intensity data of the single crystal of BCHBNH is collected

on a Bruker Smart CCD X-ray single crystal diffractometer equipped with a graphite monochromated MoK_α radiation (λ = 0.71073 Å) by using a φ and ω scan mode at 295 K. The programs used for the collection and cell refinement are the SMART and SAINT programs [15]. Empirical absorption correction is applied using the SADABS programs [16]. Structure solution is performed with direct methods using SHELXL-97 and the structure refinement is done against F^2 using SHELXL-97 [17]. The crystallographic data of BCHBNH is summarized in Table-1.

TABLE-1
CRYSTALLOGRAPHIC DATA AND STRUCTURE REFINEMENT FOR BCHBNH

Empirical formula	$C_{13}H_9N_3O_2BrCl$
Formula weight	354.59
Temperature	295 K
Wavelength	0.71073 Å
Crystal system, space group	Monoclinic, $P2_1/c$
Unit cell dimensions	a = 18.2217 (5) Å, α = 90°
	b = 7.4666 (2) Å, β = 122.685°(1)
	c = 23.6916 (5) Å, γ = 90°
Volume	2712.93 (12) $Å^3$
Z, Calculated density	1.736 Mg/m^3
Absorption coefficient, F(000)	3.23 mm^{-1}, 1408
Crystal size	0.30 × 0.24 × 0.20 mm
θ range for data collection	θ = 2.3-27.8°
Limiting indices	-25 ≤ h ≤ 25; -10 ≤ k ≤ 10;
	-31 ≤ l ≤ 33
Reflections collected/unique	8116/4715 [R_{int} = 0.042]
Max. and min. transmission	0.564 and 0.444
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	8116/4/377
Final R indices[a,b] [I>2σ(I)]	R_1 = 0.047, wR_2 = 0.125
Largest diff. peak and hole	1.27 and -0.84 ($e.Å^{-3}$)

[a]$R_1 = \Sigma||F_o| - |Fc||\Sigma|F_o|$, [b]$R_2 = [\Sigma w(|F^2_o| - |F^2_c|)^2/\Sigma w|F^2_o|^2]^{1/2}$.

Antibacterial activity: The standardized disc agar diffusion method [18] is followed to determine the activity of the synthesized compounds against the sensitive organisms *Staphylococcus aureus* (ATCC 25923) and *Streptococcus pyogenes* (ATCC19615) as Gram-positive bacteria, *Pseudomonas fluorescens* (S97) and *Pseudomonas phaseolicola* (GSPB 2828) as Gram-negative bacteria. The antibiotics chloramphencol is used as standard reference in case of Gram-negative and Gram-positive bacteria, respectively. The tested compounds were dissolved in dimethyl sulphoxide, which have no inhibition activity to get concentrations of 2 and 1 µg/mL. This analysis is performed on medium potato dextrose agar (PDA). Uniform size filter paper disks are impregnated by equal volume (10 µL) from the specific concentration of dissolved tested compounds and carefully placed on inoculated agar surface. After incubation for 36 h at 27 °C, inhibition of the organisms, which is evidenced by clear zone surround each disk is measured and used to calculate mean of inhibition zones.

Cytotoxic studies: Cytotoxic studies are carried out on Human breast cancer cell line (MCF-7), which is obtained from National Centre for Cell Science (NCCS), Pune, India. Cell viability is carried out using the MTT assay method [19]. The MCF-7 cells are grown in minimum essential medium (MEM) containing 10 % fetal bovine serum (FBS). For screening experiment, the cells are seeded into 96-well plates in 100 mL of MEM containing 10 % FBS, at plating density of 10,000 cells/well and incubated at 37 °C, 5 % CO_2, 95 % air and 100 % relative humidity for 24 and 48 h prior to addition of complexes. The complexes at different concentration 0, 1, 5, 10, 20, 30, 40 and 50 µM are dissolved in DMSO and diluted in medium containing 1 % FBS. Triplicate is maintained and the medium containing without the complexes are served as control. After 24 and 48 h, 10 µL of MTT (5 mg mL^{-1}) in phosphate buffered saline (PBS) is added to each well and incubated at 37 °C for 4h. The medium with MTT is then flicked off and the formed formazan crystals are dissolved in 100 µL of DMSO and then measured the absorbance at 570 nm using Beckman Coulter AD340 absorbance detector (Beckman Coulter Inc, Fullerton, CA). The % of cell inhibition is determined using the following formula and chart is plotted between % of cell inhibition and concentration and from this IC_{50} value is calculated.

$$\text{Inhibition (\%)} = \frac{\text{Mean OD of untreated cells (control)}}{\text{Mean OD of treated cells (control)}} \times 100$$

RESULTS AND DISCUSSION

Crystal structure of (E)-N'-(3-bromo-5-chloro-2-hydroxybenzylidene)nicotinohydrazide: A pale yellow colour crystal of the compound, (E)-N'-(3-bromo-5-chloro-2-hydroxybenzylidene)nicotinohydrazide ($C_{13}H_9N_3O_2BrCl$) along with the atomic numbering scheme are given in Fig. 1. The selected bond lengths and bond angles are summarized in Table-2. The single crystal X-ray diffraction study reveals that the ligand crystallized with two molecules per asymmetric unit in to monoclinic crystal system with a space of $P2_1/c$.

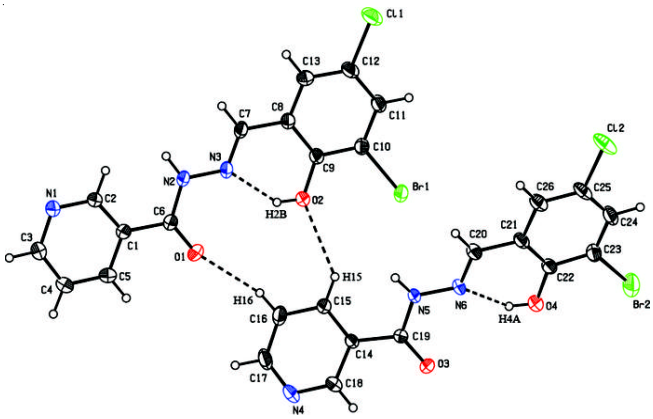


Fig. 1. ORTEP diagram of BCHBNH with the atom-numbering scheme, ellipsoids are drawn at the 30 % probability level

TABLE-2
BOND LENGTHS (Å), ANGLES [°] OF BCHBNH

O1—C6	1.205 (4)	N2–C6–O1	121.7 (3)
C6—N2	1.353(4)	N2–C6–C1	117.1 (2)
N2—H2A	0.853 (10)	C6—N2—N3	117.5 (2)
N2—N3	1.363 (3)	C7—N3—N2	119.8 (2)
N3—C7	1.261(4)	N3—C7—C8	119.3 (3)
N1—C2	1.331 (4)	C12—C13—C8	120.4 (3)
C7—C8	1.462(4)	C4—C5—C1	119.1 (3)
C8—C13	1.384(4)	O2—C9—C10	118.4 (3)
O2—C9	1.342 (3)	C9—C10—Br1	119.3 (2)
O2—H2B	0.814 (10)	C13—C12—Cl1	119.9 (3)

The molecule exists mainly in two planes. One plane corresponding to the phenyl ring and the other plane is benzene ring of the molecule. This twisting may be to relieve the steric interactions between the hydrogen atoms N2–H and C5–H. The three bond angles around C6 atom are not equal to 120° each. It is seen that the N2–C6–O1 (121.7 (3)°) bond angle is considerably greater than N2–C6–C1 angle (117.1(2)°). This observation is similar to the structures of hydrazones earlier reported [20], which is explained in order to decrease the repulsion between the lone pairs present in N2 and O1 atoms. The central part of the molecule adopts a completely extended double bonded conformation. It can be confirmed by the C6–O1 bond length (1.205(4) Å), which is considerably than the standard C=O bond length 1.21 Å and N2–C6 bond length (1.353(4) Å), which is shorter than standard N–C single bond length (1.47 Å). The N(3)–N(2) bond length of 1.3653(3) Å is delivered similar to the earlier reported benzoylhydrazone ligands [21].

In the crystal structure, the intermolecular N2-H2A···O3 and C2-H2···O3 hydrogen bonds (Table-3) generates a seven membered ring, with bifurcated R1 2(7) ring motif. The crystal packing (Fig. 2) is further stabilized due to weak intermolecular N5-H5A···N1, N2-H2A···O3, C15-H15···O2 and π···π [Cg4···Cg4 (2-x, 1-y, 1-z) = 3.614 (2) Å; Cg4 is the centroid of the ring (C21-C26)] interactions.

Infrared spectra: The important IR spectral data of the free ligand and their complexes are summarized in Table-4. The IR spectra of ligand, the stretching vibration bands of O–H, N–H and C=O are appeared at 3421, 3228 and 1612 cm^{-1}, respectively. These bands are disappears in formation of complexes. The loss of the phenolic OH proton is indicated by the absence of a band at about 3400 cm^{-1} in the complexes suggesting that the coordination to metal ion [22]. The stretching frequency of C=N slightly shifted to lower frequencies 1602–1581 cm^{-1} in the spectrum of corresponding metal complex and this value changes indicates the coordination of azomethine nitrogen to the metal ion [23]. A new band is appeared in the region 1239-1221 cm^{-1} in complexes and is assigned to the ν(C–O) mode, which is also an evidence for the coordination of ligand in the enolic form [24]. Copper(II) complex show a broad band at 3450 cm^{-1}. This is assigned to ν(OH) of coordinated water. The weak bands in the regions 553–527 cm^{-1} and 459-439 cm^{-1} can be assigned to the stretching modes of the metal to ligand bonds ν(M–O) and ν(M–N), respectively. Vanadium complex shows a strong band at 940 cm^{-1} due to the terminal ν(V=O) stretching [21,24].

TABLE-3
HYDROGEN BONDING BOND LENGTHS (Å), ANGLES (°) OF BCHBNH

D–H···A	D–H	H···A	D···A	D–H···A
O2—H2B···N3	0.81 (1)	1.89 (3)	2.576 (3)	142 (4)
O4—H2A···N6	0.82 (1)	1.83 (2)	2.569 (3)	149 (4)
C15—H15···O2	0.93	2.55	3.286 (4)	137
C16—H16···O1	0.93	2.41	3.185 (4)	141
N2—H2A···O3^{i}	0.85 (1)	2.06 (1)	2.906 (3)	171 (3)
C2—H2···O3^{i}	0.93	2.52	3.380 (4)	153
N5—H5A···N1^{ii}	0.86 (1)	2.16 (1)	3.009 (4)	170 (3)

Symmetry codes: (i) x, –y+3/2, z–1/2; (ii) –x+1, –y+2, –z.

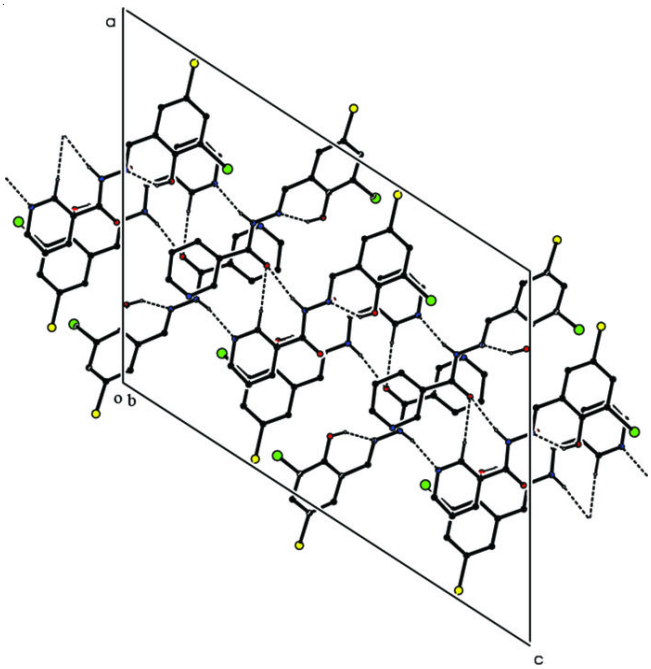


Fig. 2. Unit cell packing diagram of BCHBNH (the molecular structure viewed down b face in the unit cell)

Electronic spectra: The electronic spectrum of the ligand exhibits an absorption bands at 324 nm and 365 nm in DMF solution, which are attributed to n→π* and π→π* transition of the azomethine chromophore. The absorption regions, assignments and the proposed geometry of the complexes are given in Table-5. On complexation, this band is shifted to lower wavelength region, suggesting that the coordination of azomethine nitrogen with the metal ions. Cu(II) complex shows an absorption band at 602 nm, which may be assigned to $^{2}B_{1g}\rightarrow{}^{2}B_{2g}$ and $^{2}B_{1g}\rightarrow{}^{2}E_{g}$ transitions, in a square planar geometry [24]. The Ni(II) complex shows an absorption band at 572 nm. This peak corresponds to the transition $^{3}A_{2g}\rightarrow{}^{3}T_{1g}$ (ν_2) which indicates the octahedral environment of the ligand surrounding Ni(II) in the complex. The Co(II) complex shows absorption band at 637 nm. This is due to the octahedral environment of the ligand and the metal ion may be assigned to $^{4}T_{1g}(F)\rightarrow{}^{4}T_{2g}$ and $^{4}T_{1g}(F)\rightarrow{}^{4}T_{1g}(P)$ transition [25]. In VO(IV) complex exhibits a weak absorption band in the higher

TABLE-4
IR SPECTRAL DATA OF LIGAND AND ITS METAL COMPLEXES (cm^{-1})

Compound	ν(N-H)	ν(C=O)	ν(C=N)	ν(C-O)	ν(N-N)	ν(M-O)	ν(M-N)
BCHBNH	3228	1612	1591	1228	1027	–	–
[Cu(BCHBNH).H_2O]	–	–	1602	1221	1017	518	473
[Ni(BCHBNH)$_2$]	–	–	1582	1228	1020	527	439
[Mn(BCHBNH))$_2$]	–	–	1581	1227	1013	527	459
[VO(BCHBNH).OCH_3]	–	–	1597	1239	1012	544	456

TABLE-5
UV SPECTRAL DATA OF LIGAND AND ITS METAL COMPLEXES

Compound	Absorption (nm)	Band Assignment	Geometry
BCHBNH	324	INCT	–
	365	INCT	
[Cu(BCHBNH).H_2O]	347	INCT	Square planar
	408	$^2B_{1g}\rightarrow{}^2B_{2g}$,	
	602	$^2B_{1g}\rightarrow{}^2E_g$	
[Ni(BCHBNH)$_2$]	337	INCT	Octahedral
	386	INCT	
	572	$^3A_{2g}\rightarrow{}^3T_{1g}(\nu_2)$	
[Co(BCHBNH))$_2$]	372	INCT	Octahedral
	405	$^4T_{1g}(F)\rightarrow{}^4T_{2g}$,	
	637	$^4T_{1g}(F)\rightarrow{}^4T_{1g}(P)$	
[VO(BCHBNH).OCH_3]	324	INCT	Square pyramidal
	394	$^2B_{2g}\rightarrow{}^2E_g$,	
	654	$^2B_{2g}\rightarrow{}^2B_{1g}$	

frequency region 634 nm. This peak can be assigned to the transitions $^2B_{2g}\rightarrow{}^2E_g$ and $^2B_{2g}\rightarrow{}^2B_{1g}$ suggested a square pyramidal [26].

Magnetic properties: The linear fit of M *vs*. H values for all the systems under investigation indicate paramagnetic behaviour of the complexes [27]. The magnetic moment of Cu(II) complex is 1.83 μ_B indicating the presence of one unpaired electron. The electronic spectral results clearly indicated square planar geometry for the Cu(II) system, which should contain one unpaired electron, where μ_{eff} value would be in the range 1.8–2.1 μ_B. The observed magnetic moment of Co(II) complex is 4.54 μ_B respectively, indicating a high-spin character, excluding the oxidation to Co(III) complex. This is the expected value of 4.3-5.7 μ_B for the octahedral Co(II) complex, which is the sum of contributions due to spin only moments and spin-orbit coupling. The magnetic moment value of Ni(II) complex is 3.12 μ_B. This is the expected value of 2.9-3.3 μ_B for paramagnetic complex of Ni(II) with octahedral geometry. The magnetic moment of the VO(IV) complex is 1.72 μ_B. This value is consistent with an S = 1/2 ground state expected for the metal ion in oxovanadium(IV) complex [28,29].

EPR spectra: The EPR spectra are recorded in DMF solution and its spectral parameters are calculated for copper and vanadium complexes as given in Table-6 and also representative spectra are presented in Fig. 3. The $A_\parallel$ values are in the (136-156 × 10^{-4} cm^{-1}) range, indicative of an electron interacting with only one copper nucleus. The observed g values revealed that the unpaired electron lies predominantly in the ($d_{x^2-y^2}$) orbital with a $^2B_{1g}$ ground state [30]. The anisotropic spectrum also suggests a square planar geometry with higher covalency in the metal ligand bond. In vanadium complex, shows well-resolved axial anisotropy with two sets of eight line pattern with $g_\parallel < g_{4\%}$ and $A_\parallel >> A_{4\%}$. The characteristic of the one unpaired electron lies predominantly in the (d_{xy}) orbital with a $^2B_{2g}$ ground state, thus excluding the possibility of its direct interaction with the ligand [29].

Cyclic voltammetry studies: The electrochemical behaviours of the metal complexes are investigated using the cyclic voltammetric (CV) technique in DMF solution containing

TABLE-6
EPR SPECTRUM OF Cu(BCHBNH)·H_2O AND VO(BCHBNH)·OCH_3 COMPLEXES

Compound	$g_\parallel$	$g_\perp$	$A_\parallel$	$A_\perp$
[Cu(BCHBNH)·H_2O]	2.231	2.059	154.7	12.5
[VO(BCHBNH)·OCH_3]	1.951	1.989	164.7	56.5

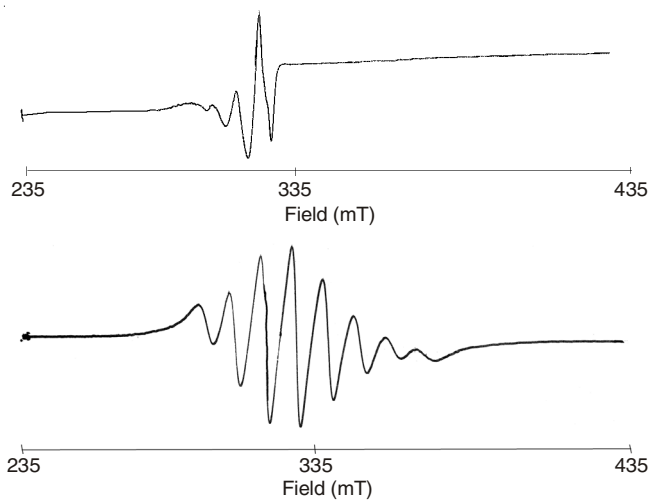


Fig. 3. EPR spectrum of Cu(BCHBNH)·H_2O and VO(BCHBNH)·OCH_3 complexes

5 mM TBAP as the supporting electrolyte. Cu(II) complex shows an anodic peak potential at E_{pa} = 0.57 V and a cathodic peak potential at E_{pc} = -0.12 V. These redox peaks are attributed to $Cu^{2+} + e^- \rightleftharpoons Cu^+$, exhibiting a reversible process. The half wave potential, located at $E_{1/2}$ = 0.22 V *versus* SCE with ΔE_p = 0.69 V, conformed a reversible single electron transfer process [31].

The Ni(II) complex shows an anodic peak potential at E_{pa} = 0.84 V and a cathodic peak potential at E_{pc} = -0.42 V. One irreversible redox peak couple at $E_{1/2}$ = 0.21 V *versus* SCE with ΔE_p = 126 mV, which suggests that the Ni(III) complex is unstable and undergone rapid decomposition. These redox peaks are assigned to $Ni^{3+} + e^- \rightleftharpoons Ni^{2+}$, that obtained for a Ni(II) complex [32]. The Co(II) complex shows an anodic peak without the corresponding cathodic peak and its anodic peak potential (E_{pa}) is found to be 0.42 V. As seen, the oxidation process is irreversible and has metal based character. The oxidation peak may be due to the reaction $Co^{2+} \rightleftharpoons Co^{3+} + e^-$ [32]. VO(IV) complex shows an anodic peak potential at E_{pa} = 0.67 V and a cathodic peak at the potential E_{pc} = -0.12 V. one irreversible redox peak couple at $E_{1/2}$ = 0.39 V *versus* SCE with ΔE_p = 55 mV, redox peaks can be attributed to $VO^{2+} + e^- \rightleftharpoons VO^+$, that obtained for a VO(IV) species [33].

Antibacterial activity: The antibacterial activity is highly influenced by the nature of metal ion (Table-7). The order for Gram-positive bacteria (*S. aureus*) is as follows: Cu(II) > VO(IV) > Ni(II) ≈ Co(II) >> BCHBNH and Gram-negative bacteria (*P. phaseolicola*) is as follows: Cu(II) > VO(IV) > Ni(II) ≈ Co(II) >> BCHBNH.

In this study, the activities of hydrazone complexes are related to the chelated phenolic ring. The chelation increases the lipophilic character of the metal chelate and favours its permeation through the lipoid layer of bacterial membranes [34].

TABLE-7
ANTIBACTERIAL ACTIVITIES OF BCHBNH AND ITS METAL COMPLEXES (µg/mL)

Compound	Diameter of inhibition zone (mm)			
	SA	SP	PF	PP
BCHBNH	1.8	0.8	0.4	0.7
[Cu(BCHBNH)·H_2O]	13.4	12.6	7.3	8.4
[Ni(BCHBNH)$_2$]	8.4	9.5	4.6	6.2
[Co(BCHBNH)$_2$]	7.3	8.4	4.7	5.6
[VO(BCHBNH)·OCH_3]	10.1	11.4	7.4	7.2
Chloramphencol	21.9	21.8	19.3	21.7

SA = *Staphylococcus aureus;* SP = *Streptococcus pyogenes;* PF = *Pseudomonas fluorescens;* PP = *Pseudomonas phaseolicola*

Also, the degree of inhibition is influenced by the type of the coordinated anion as well as the concentration. Finally, all the investigated complexes exhibited low to moderate activities against the studied organisms relative to the standard reference (control).

Cytotoxic studies: *in vitro* Cytotoxicity of our compounds (Table-8), experiments are carried out using human breast adenocarcinoma cancer cell line (MCF-7). These compounds are dissolved in DMSO and blank samples containing same volume of DMSO are taken as controls to identify the activity of solvent in this cytotoxicity experiment. Cisplatin is used as a standard to assess the cytotoxicity of compounds.

TABLE-8
in vitro CYTOTOXICITY OF COMPOUNDS TOWARDS MCF-7 CELLS

Compound	IC_{50} (µM)	
	24 h	48 h
BCHBNH	74.53 ± 0.10	68.52 ± 0.01
[Cu(BCHBNH)·H_2O]	11.44 ± 0.10	9.42 ± 0.01
[Ni(BCHBNH)$_2$]	19.58 ± 0.05	15.84 ± 0.06
[Co(BCHBNH)$_2$]	29.83 ± 0.07	27.45 ± 0.03
[VO(BCHBNH)·OCH_3]	31.13 ± 0.04	28.65 ± 0.50
Cisplatin	6.59 ± 0.02	4.83 ± 0.06

The cytotoxic effect of the complex may be interpretable as due to its amphiphilic nature and, hence, would penetrate the cell membrane easily, reduce the energy status in tumors and also alter hypoxia status in the cancer cell microenvironment, which are factors that would influence the antitumor acidity. Comparison with cisplatin suggested that Cu(II) complex should have potentially good inhibition activity to MCF-7 cells. The Cu(II)-hydrazone complex, which possess better cytotoxic activities than the other metal complexes. For reason that ligand may be attributed to the extended planar structure induced by $\pi \rightarrow \pi^*$ conjugation resulting from the chelating of metal ion with ligand [12,13,35].

Conclusion

Based on the structural analysis and the spectral data of N'-[(E)-3-bromo-5-chloro-2-hydroxybenzylidene]nicotinohydrazide (BCHBNH) and its metal complexes, the cation coordination is achieved through phenolic oxygen, amide oxygen and azomethine nitrogen. The investigated compounds are exhibited as dianionic ONO tridentate ligand. The results are clearly demonstrate, that Cu(II) complexes have square planar geometry, Ni(II), Co(II) complexes have octahedral geometry, VO(IV) complexes has square pyramidal geometry. The antibacterial studies suggested that the BCHBNH is found to be biologically active and their metal complexes show significantly enhanced antibacterial activity against microbial strains in comparison to the free ligand. Our results summarize that complex of [Cu(BCHBNH)·H_2O] enhances more potent effect by decreasing cell proliferation in MCF-7 cancer cells.

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