Research Article

Synthesis and Spectral Studies of Copper (II) and Manganese (II) Complexes Using 2-Hydroxybenzyl-(pyridine-2-carbo)iminohydrazone and 2-Furyl(pyridine-2-carbo)iminohydrazone: Evaluation of Their Magnetic, and Antimicrobial Properties

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Three new coordination complexes \([\text{Cu(HPBH)}_2]\text{Cl}_2\) (1), \([\text{Cu(FPFH)}_2]\text{Cl}_2\) (2), and \([\text{Mn(HPBH)}_2]\text{Cl}_2\) (3) (where HPBH=2-Hydroxybenzyl(pyridine-2-carbo)iminohydrazone, and FPFH=2-furyl(pyridine-2-carbo)iminohydrazone) have been synthesized using two different tridentate hydrazones ligand. The ligands were prepared by condensation of Pyridine-2-acetylchloride, 2-Hydroxybenzaldehyde and Furan-2-carbaldehyde with hydrazine, respectively, in spite of varying the carbonyl functionality attached to the pyridine moiety present in the hydrazones ligand. In both the Schiff bases, we obtained three mononuclear complexes 1, 2, and 3 which were clearly characterized from physicochemical studies. Spectroscopic investigations like \(^1H \text{ and } ^{13}C \text{ NMR, mass spectrometric, FTIR, and UV/Vis}\) have been carried out for new compounds. For complexes 2 cyclic voltammetry, magnetic and EPR properties have also been recorded. Antimicrobial studies have also been performed for these compounds with different antimicrobial species.

1. Introduction

Hydrazones are important class of ligands with interesting ligation properties due to the presence of several coordination sites [1] and are widely applied in the field of insecticides, medicines, and analytical reagents due to their excellent bioactivity [2]. Various important properties of carboxylic acid hydrazides along with their applications in medicine and analytical chemistry have led to increased interest in their complexation characteristics with transition metal ions [3]. In this respect, the formation of metal complexes plays an important role to enhance their biological activity [4]. Studies have also shown that the azomethine nitrogen which has a lone pair of electrons in an sp\(^3\) hybridised orbital has considerable biological importance. Hydrazones are found to possess antimicrobial, antitubercular, anticonvulsant, and antiinflammatory activities [5–11]. The problem of resistance to antimicrobial activity is being addressed by medicinal chemists, and various strategies have been devised and attempted in order to enhance the activity or broaden the spectrum of drugs [12]. It has been demonstrated that transition elements play a very important role in various medicinal compounds. Coordination chemistry of manganese has been studied extensively [13, 14] due to its occurrence in the active site of several enzymes involved in the chemistry of reactive oxygen species [15]. EXAFS studies for the oxygen-evolving complex (OEC) [16] and crystal structure of the manganese peroxidase (isolated from \textit{Phanerochaete chrysosporium}) [17] show that manganese center is surrounded by O- or N-donor ligands. Manganese complexes having tetradentate ONNO donor ligands are found to be artificial mimics of some of these enzymes. In addition, they also act as catalysts for important reactions [18]. In this paper we synthesized copper(II) and manganese(II) complexes with
NCOCl + H₂N-NH₂ + CHO → MeOH

Scheme 1: Route for the synthesis of the 2-Hydroxybenzyl-(pyridine-2-carbo)-iminohydrazone.

Table 1: Synthesis of the 2-Hydroxybenzyl-(pyridine-2-carbo)-iminohydrazone and 2-furyl-(pyridine-2-carbo)-iminohydrazone Ligand.

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<th>Substrate (2 + 3)</th>
<th>Product</th>
<th>Time (hrs.)</th>
<th>Yield (%)</th>
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2. Experimental

All the chemicals used in the present investigation were of the analytical reagent grade (AR). Pyridine-2-acetyl chloride, Furan-2-carbaldehyde, 2-Hydroxybenzaldehyde (Fisher Scientific), Hydrazine (Chemical Drug House, India), all metal salts and solvents (Qualigens Fine Chemicals, India) were purchased and used as received. The elemental analysis (C, H, N) was done at the Regional Sophisticated Instrumentation Centre, Central Drug Research Institute, Lucknow. ¹H NMR and ¹³C NMR spectra of the samples were measured in DMSO-d₄ at IIT Delhi, India. The IR spectra were recorded as KBr pellets using a Perkin-Elmer 783 spectrophotometer in the range 4000–400. UV/vis spectra of the complexes were recorded on a Shimadzu UV-1601 spectrophotometer. The EPR spectra of the complexes were recorded as polycrystalline sample on a Varian E-4 EPR Spectrometer.

2.1. Synthesis of Ligand

2.1.1. Ligand 1. In a round bottom flask (100 mL), a methanolic solution (10.0 mL) of Pyridine-2-acetyl chloride (0.001 mmol, 0.141 mL) in an aqueous methanolic solution (10 mL) of hydrazine (0.001 mmol, 0.154 g) and 2-Hydroxybenzaldehyde (0.001 mmol, 0.102 mL) in methanol (5 mL) were taken and continued to stir at room temperature ∼4 h. A yellowish crystalline solid was obtained as shown as Scheme 1. After the completion of this reaction, the product was monitored by thin layer chromatography and was taken in methanol and washed with hot water (3 × 20 mL) and then with brine solution (3 × 20 mL), and then the solvent under reduced pressure was evaporated to afford the product (Table 1).

2.1.2. Ligand 2. In a round bottom flask (100 mL), 0.001 mmol Pyridine-2-acetyl chloride (0.141 mL), in an aqueous methanolic solution (10 mL) of hydrazine (1.7 mmol, 0.154 g) and 0.001 mmol 2-Furanecarboxaldehyde (0.960 g) in water (5 mL) were taken and continued to stir at room temperature 1/2 h and then reflux the reaction mixture for 2 h. After the completion of this reaction, the reaction mixture was monitored by thin layer chromatography. Then, the reaction mixture was taken in methanol and washed with hot water (3 × 20 mL) (thrice) and then with brine solution (3 × 20 mL) (thrice), and then the solvent under reduced pressure was evaporated to afford the product; orange crystalline powder was obtained (Scheme 2) (Table 1).

2.1.3. Analytical Data of the Ligand HPBH and FPFH. Ligand (C₁₃H₁₀N₅O₂): Yield: 52%; M.P. 230° C, Mol. wt. 241; color: yellowish; analytical data for HPBH found (calc.): C, 63.73 (63.11); H, 4.56 (4.21); N, 17.42 (16.97). IR (KBr, cm⁻¹):
2.2. Synthesis of Metal Complexes

2.2.1. Complex 1. A quantity of (0.002 mmol, 0.282 g) of 2-Hydroxybenzyl (pyridine-2-carbo) iminohydrazone ligand was dissolved in 100 mL methanol, and a solution of copper chloride (0.001 mmol, 0.170 g) in 25 mL methanol was added dropwise with continuous stirring; dark bluish product appeared after one night standing. The mixture was stirred for 4 h at 35°C. The resulting precipitates were filtered and washed with acetone and dried over anhydrous calcium chloride in a vacuum desiccator (Scheme 3).

2.2.2. Complex 2. A hot ~70°C aqueous ethanolic solution (20 mL, 1:1 v/v) of the copper chloride metal salt (0.001 mmol, 0.170 g) and a hot ethanolic solution (20 mL) of the 2-furyl (pyridine-2-carbo) iminohydrazone (0.001 mmol, 0.216 g) were mixed in the molar ratio (1:2). The mixture was refluxed for about 8 h at a temperature of ~78°C. On cooling the contents to a temperature ~5°C the compounds were separated. After this the compound was taken in hot methanol and washed with hot water, and then the solvent under reduced pressure was evaporated to afford the product; bluish solid was obtained (Scheme 4).

2.2.3. Complex 3. An ethanolic solution of 2-Hydroxybenzyl (pyridine-2-carbo) iminohydrazone ligand (0.282 g, 0.002 mmol) was added dropwise to a 0.001 M mol solution of manganese chloride (0.197 g) in methanol solution with continuous refluxed at 55°C for 12 h. One night standing, the
reaction mixture was monitored by thin layer chromatography. The organic layers were collected; then crude products were taken in hot methanol and washed with water was evaporated and then the solvent under reduced pressure to afford the product; a dirty brownish solid was obtained (Scheme 5).

2.3. Analytical Data of the Metal Complexes 1, 2 and 3

2.3.1. Complex 1. Yield: 26%; MP: 256°C; Mol. wt: 722; color: bluish; analytical data for [CuC\(_6\)H\(_5\)N\(_2\)O\(_4\)]Cl\(_2\) found (calc.): C, 43.21 (42.15); H, 2.77 (2.11); N, 11.63 (11.17); IR (KBr, cm\(^{-1}\)): 3216 \(\nu\)HN, 1700 \(\nu\)C=O, 1602 \(\nu\)C=N, 3415 \(\nu\)OH, \(^1\)H NMR (DMSO-\(d_6\)) \(\delta\) ppm: 1.601 (s, 1H, OH-Ar), 7.158–7.953 (m, 8H, Ar), 8.92 (s, 1H, CH=N). 

2.3.2. Complex 2. Yield: 25%; MP: 268°C; Mol. wt: 672; color: bluish; analytical data for [CoC\(_6\)H\(_5\)N\(_2\)O\(_4\)]Cl\(_2\) found (calc.): C, 41.65 (40.68); H, 2.67 (2.11); N, 11.21 (11.17); IR (KBr, cm\(^{-1}\)): 3440 \(\nu\)NH, 1700 \(\nu\)C=O, 1608 \(\nu\)C=N, 3415 \(\nu\)OH, \(^1\)C NMR (DMSO-\(d_6\)) \(\delta\) ppm: 117.53–121.07 (11C, CH-Ar), 153.65 (1C, C=O), 164.95 (1C, CH=N).

2.3.3. Complex 3. Yield: 47%; MP: 276°C; Mol. wt: 749; color: dirty brownish; analytical data for [MnC\(_6\)H\(_5\)N\(_2\)O\(_4\)]Cl\(_2\) found (calc.): C, 40.68 (40.68); H, 2.67 (2.11); N, 11.21 (11.17); IR (KBr, cm\(^{-1}\)): 3216 \(\nu\)HN, 1700 \(\nu\)C=O, 1602 \(\nu\)C=N, 3415 \(\nu\)OH, \(^1\)H NMR (DMSO-\(d_6\)) \(\delta\) ppm: 1.601 (s, 1H, OH-Ar), 7.158–7.953 (m, 8H, Ar), 8.92 (s, 1H, CH=N). 

3. Results and Discussion

The IR spectra of 1, 2, and 3 were analyzed in comparison with that of their respective free ligand (HPBH and FPFH) in the region 4,000–400 cm\(^{-1}\). The IR spectra of the hydrazones contain a strong C-O absorption band at 1,651–1,659 cm\(^{-1}\) and N-H absorption band at 3,187–3,250 cm\(^{-1}\). Both of these bands disappear on complexation of two, and a new C-O absorption band appears at 1,040–1,089 cm\(^{-1}\) in this complex, indicating that the hydrazones ligands have undergone deprotonation on complexation. These data give evidence for the coordination of the ligand (HPBH and FPFH) to the respective metal ions (Cu (II) and Mn (II)) via two nitrogen atoms and oxygen atom. The infrared spectra of 1, 2, and 3 display IR absorption bands at 1,637 cm\(^{-1}\), 1,583 cm\(^{-1}\), and 1,587 cm\(^{-1}\) which can be assigned to the C=N stretching frequencies of the coordinated ligands (HPBH and FPFH), whereas for the free ligand (HPBH and FPFH) the same bands are observed at 1,682 cm\(^{-1}\) and 1,685 cm\(^{-1}\), respectively. The shift of these bands on complexation towards lower wave number indicates coordination of the azomethane nitrogen to the metal centre. The ligand coordination to the metal centre is substantiated by two bands appearing at 412 cm\(^{-1}\), 414 cm\(^{-1}\), 416 cm\(^{-1}\) for 1, 2, and 3, respectively, which are mainly attributed to \(\nu\) (M-N) [19] and at 380 cm\(^{-1}\) for 2 which are mainly attributed to \(\nu\) (M-O) group [20]. The low energy pyridine ring in plane and out of plane vibrations was observed in the spectrum of the two ligands 625 and 635 cm\(^{-1}\), respectively, whereas the corresponding bands for the complexes were shifted to higher frequencies 631–608 cm\(^{-1}\), 662–682 cm\(^{-1}\), and 690–714 cm\(^{-1}\) for 1, 2, and 3, respectively, which is a good indication of the coordination of the heterocyclic nitrogen. The EPR spectra of the Co (II) complexes were recorded as polycrystalline sample at LNT since the rapid spin lattice relaxation of Cu (II) broadens the lines at higher temperature. The g values lie in the range \(g_{II} 2.912–2.914\) and \(g_{II} 2.504–2.514\) [21]. The copper complex parameters are consistent with tetragonally elongated octahedral geometry (\(g_{II} > g_{\parallel} > g_{\perp} > 2.0\)), and the unpaired electron occupies predominantly the \(dx^2 - y^2\) orbital for the Cu (II) chelates. The value is consistent with the magnetically dilute high spin \(d_5\) Mn (II) complex. EPR study of 3 The X-band EPR spectrum from a solution of \([\text{Mn(HPBH)}_2]\) (3) in DMSO at 10 K is shown in Scheme 5. The spectrum was recorded with the external magnetic field oriented either perpendicular or parallel to the microwave magnetic field. In perpendicular mode, signals are observed in a wide spectral region. A sextet of lines is observed at \(g = 2.0\) which is attributed to Mn (II) (\(S = 5/2\)) system. In parallel mode, a broad signal is observed with a valley at \(g = 5.45\). The spectrum is consistent with a Mn (II) (\(S = 5/2\)) monomer. The X band EPR signals show similarities with signals of other Mn (II) (\(S = 5/2\)) monomers. El mass
spectrum of the ligands showed molecular ion peak at m/z 241 and 216 amu (C_{13}H_{10}N_{3}O_{2} and C_{11}H_{10}N_{3}O_{4} calculated atomic mass 240 and 215 amu), and other peaks at 170, 171, 181, 184, and 188 may be due to different fragments. The weak peak described at 135 amu is assigned to the fragment [C_{6}H_{6}N_{2}]^{+}, corresponding to the loss of CO group. A very weak peak at 119 amu is assigned to the fragment [C_{6}H_{6}N_{2}]^{+}, corresponding to the loss of CONH_{2} group. The most intense peak at 91 corresponds to the fragment [CH_{3}N]^{+}. Other peaks at 88, 78, 60, and 44 correspond to fragments [CH_{3}N]^{+}, [C_{6}H_{6}N]^{+}, [CONH_{2}]^{+}, and [CO]^{+}, respectively. The intensity of these peaks gives an idea of the stability of these fragments.

In the spectra of UV-Vis spectroscopy with copper (II) salts in ethanol result in the formation of blue-colored compounds, the unsubstituted hydrazones complexes forming [Cu(HPBH/FPFH)]_{X} type of compound. Because of their very little solubility in noncoordinating solvents, they could not be recrystallized following their preparation. However, these indicate that the compounds obtained were quite pure and needed no further purification. The complexes were fairly stable, and the compound dissolves in coordinating solvents such as pyridine forming. The insolubility of these compounds has further precluded the possibility of determining their geometry in solution. The assignment of gross stereochemical features to copper (II) complexes from spectral data is made exceedingly difficult because of broadness of the major absorption maxima in some cases, arising from as many as three closely spaced electronic transitions, Jahn-Teller distortion and spin-orbital coupling. The electronic spectra of the copper (II) complex display a broad band at 14920 cm^{-1} due to 2B_{1g} → 2E_{g} and two bands at 16390 and 27250 cm^{-1} assigned to d-d transitions and a charge transfer band, respectively, of an octahedral environment [22]. The first absorption band, in octahedral site symmetry, is taken to be equal to 10Dq. The spectra of this complex yield, by first absorption band, in octahedral site symmetry, is taken to be equal to 10Dq. The spectra of this complex yield, by first absorption band, in octahedral site symmetry, is taken to be equal to 10Dq.

4. Cyclic Voltammogram

From the cyclic voltammetric analysis, we determine the maximum current and potential in which charge transfer complexes were stable and beyond that the charge transfer complexes get decomposed. Cyclic voltammetry is a dynamic electrochemical method, where variation in current is recorded at well-defined applied potential depending on the scan rate; the current-potential curves are drawn. The measured oxidation potential of an electroactive substance correlates directly with the ionization potential \( I_a \) and the reduction potential with the electron affinity \( E_a \). Since the vacuum level potentials of the common reference electrodes can be estimated, the band edge positions of electroactive materials can be approximated. Ideally, one peak couple of oxidation and reduction potential should appear in the cyclic voltammogram measurement for the valence band (VB) and the conduction band (CB), respectively. Complex is first oxidized at the surface of the layer, and then the oxidized valence bands are filled up by electrons in the inner nonoxidized region of the layer, and reduction takes place. Voltammogram of these complexes displays a reduction peak at \( E_{pc} = -1.4 \text{ V} \) with an associated oxidation peak at \( E_{pa} = 0.6 \text{ V} \) at a scan rate of 50 mV/s. The peak separation of this couple (\( \Delta E_p \)) is 0.8 V and increases with scan rate. The \( \Delta E_p \) is 1.1 and 1.4 at scan rates 100 mV/s and 200 mV/s, respectively. Thus, the analyses of cyclic voltammetric responses at different scan rate give the evidence for quasi-reversible one electron reduction. The most significant feature of the copper(II) complex is the Cu(II)/Cu(I) couple. The ratio of cathodic to anodic peak height was less than one. However, the peak current increases with the increase of the square root of the scan rates. This establishes the electrode process as diffusion controlled.

5. Thermogravimetric Analysis (TG/DTA)

The thermal analysis (TG/DTA) of the ligand and its metal complexes was recorded under nitrogen atmosphere at the heating rate of 10°C/min. The ligand is stable up to 215°C and shows a continuous weight loss up to 380°C. Therefore the whole ligand gets decomposed in a single step (Figure 1(a)). The DTA of the ligand shows two endothermic peaks, one broad endothermic peak at 228°C with a shoulder at 210°C, which corresponds to the melting and the first inflexion point. The second inflexion on the DTA curve occurs at 351°C which represents a small weight loss step from 360°C to
380°C. The thermal gravimetric (TG) analysis was used as a probe to prove the associated water or solvent molecules to be in coordination sphere or in crystalline form [24].

The thermogram of copper(II) and manganese (II) complexes is more stable than the hydrazones ligand and does not decompose up to 255°C, 253°C, and 250°C, respectively (Figure 1(b)). It shows a major step of decomposition from 255°C to 330°C which is detected by DTA at 320°C; this corresponds to the loss of two pyridine ring and two imines moieties (observed weight 75.5%, theoretical weight 72.85%). It is very interesting to note that the complexes gain some 7% weight from 335°C to 410°C and do not decompose further. This weight gain of complexes may be attributed to the migration of the metal (Mlayer) to the new vacant sites produced by the partial reduction of M²⁺ to M¹⁺ in case of Copper and M⁴⁺ to M³⁺ in case of cobalt and nickel, then subsequent oxidation of M¹⁺, M²⁺ in copper, cobalt, and nickel complexes, respectively [25].

6. Microbiology Properties

In vitro antibacterial activity studies were carried out by using the standardized disc-agar diffusion method [23, 25, 26] to investigate the inhibitory effect of the synthesized ligands and complexes against S. aureus ATCC 29253, S. aureus ATCC 3160 antibacterial, and S. cerevisiae MTCC 316, Candida albicans (227) as a kind of fungi. For the antibacterial and antifungal assays, the compounds were dissolved in dimethylformamide. Further dilutions of the compounds and standard drugs in the test medium were prepared at the required quantities of 500 and 1000 ppm concentrations with dextrose broth. The minimum inhibitory concentrations (MICs) were determined using the twofold serial dilution technique. A control test was also performed containing inoculated broth supplemented at the same dilutions used in our experiments and found inactive in the culture medium. Gentamycin and Amphotericin B were used as control drugs.

The data on the antimicrobial activity of the compounds and the control drugs as MIC values are given in Table 2. The cultures were obtained from SRL broth for all the bacterial strains after 24 h of incubation at 37°C. C. albicans were maintained in dextrose broth after incubation for 24 h at 25°C. Testing was carried out in dextrose broth at pH 7.4 and the two fold serial dilution technique was applied. A set of tubes containing only inoculated broth was used as controls. For the antibacterial assay after incubation for 24 h at 37°C and after incubation for 48 h at 25°C for the antifungal assay, the last tube with no growth of microorganism and/or yeast was recorded to represent the MIC expressed in ppm. Every experiment in the antibacterial and antifungal assays was replicated twice, and the data is given in Table 2.

The observation on the biological assay indicates that the antibacterial action due to all compounds has N, O groups which is of considerable chemotherapeutic interest. From Figure 2 and Table 2, it is evident that among all the newly synthesized compounds 1, 2, and 3 tested for their antibacterial and antifungal activities against Staphylococcus aureus (ATCC 25923), Staphylococcus aureus (ATCC 3160), Candida albicans (227), and Staphylococcus cerevisiae (361) were determined as MIC values. All the investigated compounds showed good activity against S. aureus. The zone of inhibition in mL of the test compounds against the microorganism Staphylococcus aureus (ATCC 25923), Staphylococcus aureus (ATCC 3160), Candida albicans (227), and Staphylococcus cerevisiae (361). These data indicate that among the bacteria employed, S. aureus is found to be more sensitive to these compounds whereas the gram-negative bacteria show resistance to most of the compounds. The zone of inhibition tabulated reveals that the antibacterial activity of the compounds is specific to the microorganism examined. Analysis of the data showed that generally the fungi C. albicans (227) was more susceptible to the irreversible toxic effects of screened compounds than S. cerevisiae (361). Variation in the response of fungi studies to chemical screened may be attributed to the tolerance of them by test fungi. The effects
Table 2: Antimicrobial activity of hydrazone ligand and their metal complexes.

<table>
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<tr>
<th>Compounds</th>
<th>Time hrs</th>
<th>S. aureus MTCC 3160 100 µg</th>
<th>S. aureus MTCC 25923 100 µg</th>
<th>C. albican MTCC 227 100 µg</th>
<th>S. cerevisiae MTCC 361 100 µg</th>
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<td></td>
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Figure 2: Showing the bar graph (a) and (b) of antibacterial and antifungal activities at 1000 ppm concentration after 48.

of assayed chemicals on test fungi differed in accordance with the concentrations used. Generally fungi toxicity enhanced with the increase in the dose of compounds. The higher the concentration, the longer was the persistence of chemicals.

7. Conclusion

This research work has achieved the synthesis of hydrazone tetradentate nitrogen and oxygen donor ligands. Using these ligands we synthesize the two Cu(II) and one Mn(II) metal complexes and screened them for their antimicrobial property by performing minimum inhibitory concentration (MIC). Antimicrobial results showed that metal complexes have high killing activity compared to the ligand. MIC values decreased almost stoichiometrically with multiplication of structure. A whole line of current antifungals target ergosterol biosynthesis pathway or its end product which is unique to fungi. At respective MIC values Cu(II) complexes lead to enormous reduction in ergosterol content followed by Cu(II) complex, Mn(II) complex, and the ligand, respectively.

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References


