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Synthesis, Characterisation and Antimicrobial Studies of 2-[Morpholin-4-yl(Pyridine-3-yl)Methyl] Hydrazinecarboxamide and its Transition Metal Complexes

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ABSTRACT

Co^{II}, Ni^{II}, Cu^{II} and Mn^{II} complexes of Mannich base, as ligand, was prepared by condensation of aqueous semicarbazide, morpholine and pyridine-3-carboxaldehyde. The structure of the newly synthesized Mannich base was investigated by UV-Vis, IR, ^{1}H-NMR, ^{13}C-NMR, molar conductance and magnetic susceptibility studies. The antimicrobial activities of the ligand and metals complexes have been screened in vitro against the organisms E.faecalis, Proteus mirabilis, Bacillus cereus, E.aerogens, ESBL E.coli, ESBL K.pneumoniae, by disc diffusion and well diffusion techniques. It is observed that the coordination of metal ions has pronounced effect on the microbial activities of the ligand. The metal complexes have higher antimicrobial effect than the free ligand.

KEYWORDS : Mannich base, Metal complexes, Disc and Well diffusion technique, Antimicrobial effect.

Introduction

The Mannich reaction is a powerful C-C bond formation process and has wide applications for the preparation of diverse amino alkyl derivatives. The Mannich reaction involves the condensation of a compound consisting of an active hydrogen atom with aldehyde and an amine (10 or 20). Literature survey shows that the compounds containing amide moiety have a strong ability to form metal complexes and show a wide range of biological activities. Metal ions are known to play very important roles in biological processes in the human body1,2. For example, copper(II) ion was the most abundant transition metal in humans. It was found either at the active sites or as structural components of a good number of enzymes3,4.. Mannich bases5 of heterocyclic molecules have been attracting the attention of the synthetic chemists for their wide range of antimicrobial properties6,7. Semicarbazides and thiosemicarbazides are found to be associated with antibacterial and antifungal activities8. The present study reports the synthesis and characterization of Mannich base, [(morpholin-4-yl) (pyridin-3-yl)methyl]hydrazinecarboxamide(MPH) and its metal Cobalt(II), Nickel(II), Copper(II) and Manganese(II) complexes , which contains an amide moiety. The antimicrobial activities of the ligand and metal complexes have been screened in vitro against the following microorganisms: E.faecalis, Proteus mirabilis, Bacillus cereus, E.aerogens, ESBL E.coli, ESBL K.pneumoniae by disc diffusion and well diffusion method9,10.

Experimental

Materials

All the reagents used, were of A.R. grade and the solvents used were highly purified compounds. The solvents were distilled according to the standard methods.

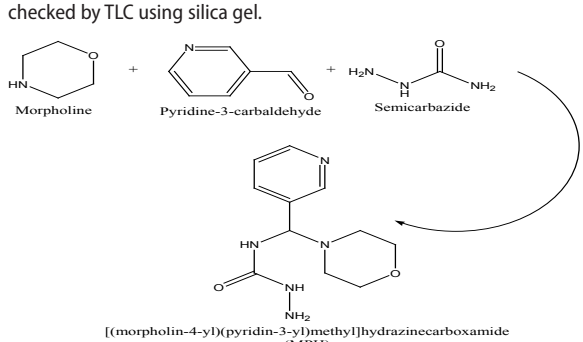
Physical measurements

By the use of elemental analyzer, the elements C, H and N were analysed. By previous literature procedures, the metal and anion contents of the complexes were estimated. Melting points were taken in an open capillaries and were uncorrected. IR spectra were recorded on a Shimadzu 8201 PC FTIR spectrophotometer and 1H-NMR spectra on a Bruker DRX-300 spectrometer(300MHz) using DMSO-d6 as solvent and TMS as an internal standard. Purity of the compounds was checked by TLC on Silica gel plates and was satisfactory. The solvent system employed was chloroform and the spots were identified by placing the plate in UV chamber(λmax 254 nm). Molar conductivity of the complexes was measured on a Systronic conductivity bridge with a tip type cell, using 10-3 M solution of the complexes in DMSO at room temperature. Magnetic susceptibility measurements of the complexes were done using a Gouy balance. Copper Sulphate was used as the calibrant. Antibacterial screening of newly synthesized compound was carried out against E.Faecalis, P.mirabilis, B.cereus and E.aerogens, ESBL E.coli and ESBL K. pneumonia. Muller-Hinton agar was used as the medium for the study of antimicrobial activity of the ligand and the complexes by employing well-diffusion and disc diffusion techniques. Rifampicin and Cefatoxime were used as standard for the antimicrobial studies.

Synthesis

Synthesis of [(morpholin-4-yl)(pyridin-3-yl)methyl]hydrazinecarboxamide(MPH):

Semicarbazide(2.6 g, 0.025 mol) was dissolved in water. To this solution, morpholine(2.2 mL, 0.025 mol) was added dropwise with constant stirring by keeping the reaction mixture on a magnetic stirrer. After 15 minutes, pyridine-3-carboxaldehyde(2.8 mL, 0.025 mol) was added in drops and the reaction mixture was kept in ice cold condition in an water bath over a magnetic stirrer and stirring was continued for an hour. The yellow coloured solid formed was filtered and then recrystallised from ethanol. The purity of the compound was checked by TLC using silica gel.



Synthesis of Co(II), Ni(II), Cu(II) and Mn(II) complexes of MPH

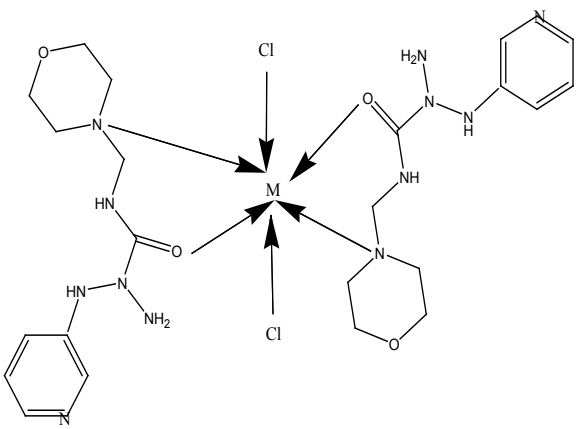
Cobalt(II) chloro complex was prepared by mixing ethanolic solution of cobalt(II) chloride with ligand dissolved in chloroform in 1:2 (metal:ligand) molecular ratio. The reaction mixture was stirred under ice bath maintained at 5-10°C for 2 h. The bluish green coloured precipitate obtained was filtered, washed with 1:1 ethanolic-acetone mixture and then dried in vacuo.

Nickel(II) chloro complex was prepared by mixing metal salt with MPH in 1:1 mol ratio. To the ligand in chloroform-ethanol(1:1), the metal salt in ethanol was added and stirred for 1 h, under ice cold condition on a water bath. The green coloured solid obtained was filtered,

washed with chloroform-ethanol mixture and dried in vacuo.

Copper(II) chloro complex was prepared by mixing the ligand and the metal salts in 1:1 mol ratio in ethanolic medium. The reaction mixture was stirred well in an ice bath using a magnetic stirrer. The brown coloured solid formed was filtered, washed with ethanol and dried in vacuo.

Manganese(II) chloro complex was obtained by adding ethanolic solution of MPH to the metal solution in 1:1 molecular ratio. The reaction mixture was stirred well and gets warm on a hot water bath. The resulting mild pink coloured solid was washed with ethanol and dried in vacuo.



M = Cu(II), Co(II), Ni(II), Mn(II)

Results and discussion

Characterisation of MPH:

The analytical and physical data of the ligand and the metal complexes are listed in Table 1. The analytical data are in good agreement with the general molecular formula proposed for all the complexes. The molar conductivities of the complexes are very low indicating the non-electrolyte nature. The complexes are very stable at room temperature in air.

The solubility of MPH was tested. It is soluble in methanol, ethanol, DMSO, chloroform and benzene. Melting point was determined using melting point apparatus and is about 202°C. The molecular mass of the ligand was determined by Rast method using biphenyl as the solvent.

Table 1: Physical characterization, Analytical, Molar Conductance Data

Compound Molecular Formula Molecular Weight	Found(Calculated) M	C	H	N	O	Cl	Molar conductance (Ω cm^{-2} mol^{-1})
MPH $C_{11}H_{17}N_5O_{2-251}$	--	52.58 (52.26)	6.82 (6.50)	27.87 (27.15)	12.73 (12.35)	--	--
$Cu(MPH)_2Cl_2$ $C_{22}H_{34}Cl_2N_{10}CuO_{4-637}$	8.76 (8.94)	43.06 (42.94)	6.39 (6.24)	23.18 (22.94)	8.83 (8.74)	9.78 (9.63)	24
$Co(MPH)_2Cl_2$ $C_{22}H_{34}Cl_2N_{10}CoO_{4-632}$	8.18 (8.36)	43.34 (42.95)	6.43 (6.35)	23.33 (23.28)	8.88 (8.74)	9.84 (9.74)	22
$Ni(MPH)_2Cl_2$ $C_{22}H_{34}Cl_2N_{10}NiO_{4-630}$	8.15 (8.28)	43.45 (43.32)	6.44 (6.28)	23.33 (23.28)	8.88 (8.78)	9.84 (9.76)	26
$Mn(MPH)_2Cl_2$ $C_{22}H_{34}Cl_2N_{10}MnO_{4-628}$	7.67 (7.84)	43.58 (43.38)	6.47 (6.35)	23.46 (23.35)	8.93 (8.78)	9.90 (9.78)	28

Infrared spectra

The IR spectrum of the free ligand was compared with those of the metal complexes. This is used to determine the coordination sites involved in the coordinates. The IR spectrum gives the details regarding the nature of the functional group attached to the metal ion. The IR spectrum of the compound showed bands in the region of 3407 cm-1 assigned to (O-H) and (N-H). The bands located in the regions of 2231 and 1924 cm-1 were attributed to the aromatic and aliphatic C-H stretching vibration. The absorption band in the region of 1669 cm-1 was assigned to (C=O). The split bands from 1426 to 1407 cm-1 were due to the mixed (N-H) and (C-N) vibration. The bands in the region of 1142 cm-1 was due to out of plane bending vibrations of aromatic C-H.

^{1}H-NMR spectra

The proton NMR spectrum of MPH was recorded using 300 MHz NMR spectrometer(BRUKER) by using DMSO as solvent. The spectrum showed the multiple peaks in the regions of 6.5 and 9.0 ppm were due to aromatic protons. A single peak appeared at 2.6 ppm was assigned to methyl proton. Splitting of signal appeared at 2.5 and 3.5 ppm was assigned to C-H and N-H protons.

Based on the above physical and spectral data, the structure of the synthesized compound was confirmed as [(morpholin-4-yl)(pyridine-3-yl)methyl]hydrazinecarboxamide.

The molar conductance of 10-3 solution of the complex measured. The molar conductance was showed to 32 ohm-1 cm mol-1, which indicates the non electrolytic behavior of the complex. That is the anions are present inside the coordination sphere.

The magnetic susceptibility of the complexes of [(morpholin-4-yl(pyridine-3-yl)methyl] hydrazinecarboxamide was determined using Gouy's balance. The magnetic susceptibility value was 3.52 B.M.

Antimicrobial Tests

The ligand and metal mixed-ligand complexes were tested for antimicrobial activities against six pathogens. The antimicrobial activities of the ligand and complexes were evaluated by the well-diffusion and disc diffusion techniques at the concenteration of 10 mg/mL and 50 mg/mL. Muller-Hinton agar was used as microbial growth medium. Rifampicin and Cefatoxime were used as reference antibiotic. The plates were inoculated at 37°±2° C for 24 h. Antimicrobial activity was evaluated by measuring the diameter of the inhibition zone(IZ) around the hole. Compounds were considered as active when the IZ was greater than 15 mm. The values are presented in Table 2.

Table 2. Antimicrobial activities of the ligand and metal mixed-ligand complexes

S. No.	Test Pathogen	Antimicrobial activity(IZ diameter in mm) $Cu(MPH)_2Cl_2$ B1	$Co(MPH)_2Cl_2$ B2	$Ni(MPH)_2Cl_2$ B3	$Mn(MPH)_2Cl_2$ B4	MPH A1	RIF
01	*E.Faecalis*	26	32	24	17	NI	16
02	*Proteus mirabilis*	NI	20	NI	13	NI	18
03	*Bacillus cereus*	27	32	27	19	16	20
04	*E.aerogens*	13	NI	15	NI	NI	16
05	*ESBL E.coli*	15	28	16	NI	NI	CTX 16
06	*ESBL K.pneumoniae*	15	26	12	NI	NI	16

IZ = Inhibition Zone, A1 = [(morpholin-4-yl)(pyridin-3-yl)methyl]hydrazinecarboxamide, B1 = Cu(MPH)2Cl2,B2 = Co(MPH)-2Cl2, B3 = Ni(MPH)2Cl2, B4 = Mn(MPH)2Cl2, RIF = Rifampicin, CTX = Cefatoxime, NI = No Inhibitory Effect

CONCLUSION

This paper describes the summary of Mannich reaction, mechanism, important properties and also describes about the metal coordination and importance of coordination compounds. The literature survey states that the coordination occurs through oxygen and nitrogen. The IR spectrum of the complex shows a negative shift in absorption band frequencies of C=O and C-N of pyridine which are suggesting the carbonyl oxygen and nitrogen of pyridine involved in the coordination. Experimental techniques employed in the synthesis and characterization of [(morpholin-4-yl)(pyridin-3-yl)methyl]hydrazinecarboxamide and its complexes were also discussed in detail. Based on the analytical and spectral studies, the structure of the ligand [(morpholin-4-yl)(pyridin-3-yl)methyl]hydrazinecarboxamide and its complexes were established. The electrolytic conductivity data of the complex indicates its non-electrolytic nature. The magnetic susceptibility value indicates the magnetic property of the complexes. Antimicrobial studies of these complexes against six pathogens shows that there is increased activity of the metal ions upon coordination to these ligand. The activity order is Co(MPH)2Cl2(B2)>Cu(MPH)2Cl2(B1)> Ni(MPH)2Cl2(B3) > Mn(MPH)2Cl2(B4). The metal complexes has been found to possess more activity than the free ligand.

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