Synthesis, characterization and antimicrobial studies of (morpholine-4-yl-pyridin-2-yl-methyl)-urea and its metal (II) complexes

M. Syed Ali Padusha	T. Chandrasekaran	M. Suresh
P. G. & Research Department of Chemistry, Jamal Mohamed College Trichy-20	P. G. & Research Department of Chemistry, Jamal Mohamed College Trichy-20	P. G. & Research Department of Chemistry, Jamal Mohamed College Trichy-20

ABSTRACT *Morpholine, pyridine-2-carbaldehyde and urea reacted yielding a new N-terminal Mannich base, (morpholine-4-yl-pyridin-2-yl-methyl)-urea (MPU). Cobalt (II), Nickel (II) and Copper (II) complexes of MPU were synthesized. The structure of MPU was established by analytical methods such as elemental analysis, melting point, TLC and spectral methods such as IR, 1H NMR, 13C NMR and Mass spectral studies. The elemental analysis, molar conductance, magnetic susceptibility study, UV-vis, IR, 1H NMR, 13C NMR and ESR studies were carried out to establish the structure of the complexes. The antimicrobial activity of the ligand and its complexes was extensively studied by disc diffusion method. The results of antimicrobial studies of the compounds revealed that they possess inferior potency when compared to the standard employed.*

1. Introduction

Antimicrobial agents have been developed to combat the severity and spread of many of the infectious disease caused by bacteria and fungi. The emergence of resistance to such drugs is a natural biological phenomenon. Infections caused by resistant microbes often fail to respond to treatment, resulting in prolonged illness and greater risk of death. This provides greater opportunities for the resistant strain to spread to other people[1]. The experts agree that we need to improve surveillance for emerging antimicrobial resistant (AR) problems, to prolong the useful life of antimicrobial drugs, to develop new drugs, and to utilize other measures, e.g., improved vaccines, diagnostics, and infection control measures to prevent and control AR. Several Mannich bases have been reported as antimicrobial agents[2]. A number of morpholine derivatives have been shown to possess strong bactericidal properties. Morpholine hydroperiodide has been used as a water disinfectant. Morpholine derivatives are widely used in the control of fungi[3].

Metal complexes of Mannich bases reported as many antimicrobial agents[4]. Efficacy and drug action of therapeutic agents has been accelerated by the metal ion present in their analogs[5]. The pharmacological potency of metal complexes depends on the nature of the metal ions and the ligand. Increased drug action has been noticed in some metal complexes when compared with the ligand[6,7]. Owing these considerations an attempt has been made to synthesis a Mannich base using morpholine, urea and pyridine-2-carboldehyde. Further metal complexes have been synthesized using Co(II), Ni(II) and Cu(II) chlorides.

2. Experimental

2.1. Materials and Methods

All the chemicals used were of Merck and Sigma Aldrich products, available commercially. The purchased chemicals used without further any purification. The homogeneity of the compounds was monitored by thin layer chromatography using silica-G coated glass plates and visualized by iodine vapour. Melting points were determined on an open capillary and are uncorrected. The elemental analysis was performed using CHON analyzer. NMR spectra were recorded on 300 MHz Bruker spectrometer, using DMSO-d$_6$ as the solvent and TMS as a internal standard, chemicals shifts are expressed in ppm. IR spectra were recorded on Shimadzu FT-IR spectrometer in the 4000-400 cm^{-1} region. UV spectrum was recorded using UV-Visible Perkin Elmer model Arithmetic ranged from

200-1100 nm. Biological studies of the synthesized compounds were screened by disc diffusion method at EUMIC Laboratory Tiruchirappalli.

2.3. Synthesis of (Morpholine-4-yl-pyridin-2-yl-methyl)-urea. (MPU)

To the ethanolic solution of urea (1.5 g, 0.025 mol) taken in a round bottom flask, morpholine (2.1 mL, 0.025 mol) and pyridine-2-carboldehyde (2.6 mL, 0.025 mol) were added. The reaction mixture was kept over a magnetic stirrer and stirred well in an ice cold condition for 3 hr. The colourless solid formed was filtered and washed several times with petroleum ether (40-60%). The crude solid obtained was dried and recrystallized using absolute alcohol. The recrystallized product was dried over vacuum.

2.4. Synthesis of metal (II) complexes

To the methanolic solution of MPU (1.18 g, 0.005 M), copper (II) chloride (0.85 g, 0.005 M) was added. The reaction mixture was taken in around bottom flask and kept over a magnetic stirrer cum hot plate and stirred under hot condition. After 2 hr the product separated as a pale green solid was washed, filtered and dried over vacuum. The same procedure was followed to prepare rest of the complexes.

Scheme

(Morpholin-4-yl-pyridin-2-yl-methyl)-urea
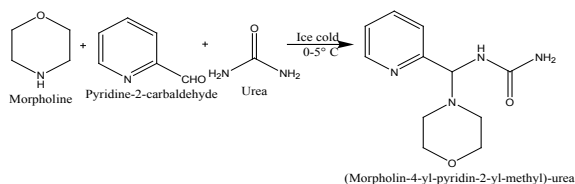
Structure of Metal Complexes
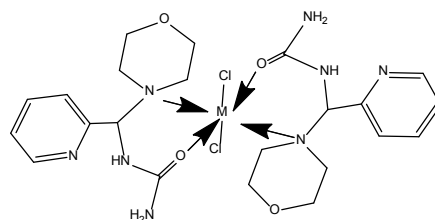
M = Co (II), Ni(II) and Cu(II)

3. Result and discussion

The complexes are freely soluble in DMF, DMSO and insoluble in alcohols such as methanol and ethanol. The molar conductance of the soluble complexes is lie in the range λ = 13–27 10^{-3} Ω^{-1} $mol^{-1}cm^2$ in DMSO, indicating their non-electrolytic nature. The results of elemental analyses data of the MPU and its complexes are presented in Table 1, are in agreement with the proposed structures and the complexes are of [1 : 2] [M : L] ratio.

Table 1. Molecular weight, Yield, Melting point, Elemental analysis and Molar conductivity of the MPU and its metal complexes.

S. no	Compounds, mol. Formula and molar weight	Colour and (%) yield	M.P. (⁰C)	[Found% (Calcd.%)]					Molar Conductance (Ω^{-1} $mol^{-1}cm^2$)
				C	H	N	O	Cl	
1	MPU $C_{11}H_{16}N_4O_2$ [236]	White (71)	147	55.92 (55.26)	6.83 (6.50)	23.71 (23.02)	13.54 (13.46)	—	—
2	$Co(MPU)_2Cl_2$ $C_{22}H_{32}Cl_2N_8CoO_4$ (601)	Brown (75)	236	43.87 (43.52)	5.35 (5.29)	18.60 (18.38)	10.62 (10.08)	11.77 (11.50)	27
3	$Ni(MPU)_2Cl_2$ $C_{22}H_{32}Cl_2N_8CoO_4$ (601)	Green (80)	274	43.88 (43.50)	5.36 (5.30)	18.67 (18.50)	10.63 (10.52)	11.78 (11.50)	24
4	$Cu(MPU)_2Cl_2$ $C_{22}H_{32}Cl_2CuN_8O_4$ (606)	Green (80)	254	45.53 (45.44)	5.31 (5.22)	18.46 (18.44)	10.54 (10.33)	11.68 (11.58)	23

3.1. Infra Red Spectra

The IR spectrum of ligand showed characteristic band at 3427 cm^{-1} can be attributed to the ν (-NH_2) group. A sharp band appeared at 1647 cm^{-1} can be attributed to the ν (C=O) group of the ligand has been found shifted to 1630-1620 cm^{-1} in the spectra of the complexes indicate the involvement of oxygen atom of carbonyl in binding with metal ions[8]. The band appeared at 1112 cm^{-1} due to ν (CNC) of morpholine moiety of ligand is shifted to lower frequency by 10-20 cm^{-1} in the complexes suggest that the coordination is through N atom of morpholine[9]. Hence it is concluded that the compound MPU is a neutral bidentate ligand. The characteristics frequencies of ligand and its complexes are presented in Table 2.

Table 2. Characteristic IR bands of the MPU and its metal complexes

Compounds	ν(NH_2)	ν(NH)	ν(C=O)	ν(CNC)	ν(C-O)	ν(CH)	ν(M-N)	ν(M-O)
MPU	3426	3311	1647	1112	1543	2930	-	-
$Co(MPU)_2Cl_2$	3420	3308	1630	1086	1540	2936	496	442
$Ni(MPU)_2Cl_2$	3424	3306	1634	1092	1530	2934	501	430
$Cu(MPU)_2Cl_2$	3426	3310	1626	1070	1528	2937	487	470

3.3. UV–vis spectra

The UV–vis spectra of the ligand and its metal (II) complexes are recorded in DMSO solution in the wavelength range 250–1100 nm and their corresponding data are given in Table 4. The UV–vis spectra of the free ligand (MPU) showed three absorption bands in the ultraviolet region (259 and 308 nm), the first high intensity bands observed at 259 nm. The electronic spectrum of Co(II) complexes showed three transitions at 10690, 15821 and 21752 cm^{-1} are assignable to $^4T_1g{\rightarrow}^4T_2g(F)$, $^4T_1g(F){\rightarrow}^4A_2g(F)$ and $^4T_1g(F){\rightarrow}^4T_2g(P)$ transitions respectively, are in conformity with octahedral arrangements for Co(II) ion[11,12]. The appearance of two bands, one at 14920 cm^{-1} and another at 23807 cm^{-1} due to $^3A_2g(F){\rightarrow}^3T_1g(F)$ and $^3A_2g(F){\rightarrow}^3T_1g(P)$ transitions, favour an octahedral geometry for the Ni(II) complex. The absence of any band below 10,000 cm^{-1} eliminates the possibility of a tetrahedral environment in this complex[13,14]. The Cu(II) complexes display two prominent bands. Low intensity broad band at 17124 cm^{-1} is assigned as 10 Dq band corresponding to $^2Eg{\rightarrow}^2T_2g$ transition. In addition, there was a high intensity band at 23604 cm^{-1} is due to symmetry forbidden ligand → metal (L→M (CT)) charge transfer transition. Therefore distorted octahedral geometry around Cu (II) ion is assigned on the basis of electronic spectral data[11,15,16]. This is further confirmed by its magnetic susceptibility value.

3.2. ¹HNMR and ¹³CNMR spectra

¹H NMR spectra of the free ligand and its metal complexes were recorded in DMSO-d_6. Ligand shows a multiplet between δ 8.50- 7.50 is due to aromatic protons. A sharp absorption peak around δ 5.80 is due to the -$CONH_2$ proton. Ligand shows two signals, one at δ 5.50 and the other at δ 5.40 are assigned to the -NH and -CH proton respectively. The signals appeared in the region of δ 3.5 and δ 2.4 are due to -O-CH_2 proton and -N-CH_2 proton of morpholine moiety respectively. The aromatic protons appeared at δ 8.50-7.50 in the spectrum of ligand has been found shifted to δ 8.62-7.53 in the spectra of metal complexes. The signal due to -$CONH_2$ proton appeared at δ 5.8 in the spectrum of ligand has been found shifted to δ 6.0 in the spectra of the complexes. These shifting further support the involvement of oxygen atom of carbonyl with metal ion. The signal due to -$N(CH_2)_2$ proton of morpholine appeared at δ 2.4 in the spectrum of the ligand has been found shifted to δ 2.45-2.62 in the spectrum of metal complexes. This supports the participation of N atom of morpholine of the ligand in the complex formation. The absences of signal in the region of δ 10-11 confirm the absence of keto-enol form[10]. The ¹H NMR spectral data are presented in Table 3.

Table: 3. ^{1}H NMR and ^{13}C NMR spectral data of the MPU and its metal complexes (Solvent-DMSO-d$_6$)

Compounds	Proposed assignment of protons (ppm)
MPU	^{1}H-NMR : (300 MHz, DMSO-d$_6$, δ ppm) 8.5-7.50 (m, 4H, pyridine ring), 5.80 (s, 2H, CONH$_2$), 5.50 (d, 1H, NH), 5.4 (d, 1H, CH) 3.50 (m, 4H, CH$_2$-O-CH$_2$), 2.4 (m, 4H, CH$_2$-O-CH$_2$) 2.4 (m, 4H, CH$_2$-O-CH$_2$). ^{13}C-NMR: (100 MHz, DMSO-d$_6$, δ ppm) 163.20(CO), 158.41, 148.92, 136, 123.34(pyridine ring), 72.22(CH), 71.60 (CH$_2$-O-CH$_2$), 52 (CH$_2$-N-CH$_2$).
Co(MPU)$_2$Cl$_2$	^{1}H-NMR : (300 MHz, DMSO-d$_6$, δ ppm) 8.58-7.55 (m, 4H, pyridine ring), 5.85 (s, 2H, CONH$_2$), 5.52 (d, 1H, NH), 5.41 (d, 1H, CH) 3.56 (m, 4H, CH$_2$-O-CH$_2$), 2.45 (m, 4H, CH$_2$-O-CH$_2$). ^{13}C-NMR: (100 MHz, DMSO-d$_6$, δ ppm) 163.22 (CO), 158.40, 148.92, 136, 123.40 (pyridine ring), 71.30 (CH), 71.62 (CH$_2$-O-CH$_2$), 52.11 (CH$_2$-N-CH$_2$).
Ni(MPU)$_2$Cl$_2$	^{1}H-NMR : (300 MHz, DMSO-d$_6$, δ ppm) 8.62-7.53 (m, 4H, pyridine ring), 5.86 (s, 2H, CONH$_2$), 5.60 (d, 1H, NH), 5.46 (d, 1H, CH) 3.89, (m, 4H, CH$_2$-O-CH$_2$), 2.62 (m, 4H, CH$_2$-O-CH$_2$). ^{13}C-NMR: (100 MHz, DMSO-d$_6$, δ ppm) 163.21 (CO), 158.39, 148.93, 136, 123.31 (pyridine ring), 72.20 (CH), 71.6 1(CH$_2$-O-CH$_2$), 52.08 (CH$_2$-N-CH$_2$).
Cu(MPU)$_2$Cl$_2$	^{1}H-NMR : (300 MHz, DMSO-d$_6$, δ ppm) 8.61-7.60 (m, 4H, pyridine ring), 5.82 (s, 2H, CONH$_2$), 5.56 (d, 1H, NH), 5.45 (d, 1H, CH), 3.50 (m, 4H, CH$_2$-O-CH$_2$), 2.4 (m, 4H, CH$_2$-O-CH$_2$). ^{13}C-NMR: (100 MHz, DMSO-d$_6$, δ ppm) 164.0 (CO), 158.50, 147.9, 136.10, 123.23 (pyridine ring), 71.81 (CH), 71.22(CH$_2$-O-CH$_2$), 53.02(CH$_2$-N-CH$_2$).

3.4. Magnetic Moments:

The magnetic moment study was carried out at room temperature for all the metal complexes and the results are given in Table 4. The magnetic moment of Co (II) complex is 4.81 BM suggests the high spin nature of six-coordinated octahedral complexes[8]. For Ni (II) and Cu(II) complexes, the values are 2.82 BM and 1.89 BM respectively. These values corroborating spin-free octahedral configuration for Ni(II) complex and distorted octahedral geometry for Cu(II) complex[16,17].

Table 4. UV absorption of MPU and its metal complexes

Compounds	Colour	Magnetic Moment B.M	Absorption Maxima ν (cm^{-1})	Transition Assignment
MPU	Colourless	-	38610(259 nm)	π→π*
2. Co(MPU)$_2$Cl$_2$	Brown	3.94	10690 15821 21752	$^4T_1g→^4T_2g(F)$ $^4T_1g(F)→^4A_2g(F)$ $^4T_1g(F)→^4T_2g(P)$
3. Ni(MPU)$_2$Cl$_2$	Green	2.78	14920 23807	$^3A_2g(F)→^3T_1g(F)$ $^3A_2g(F)→^3T_1g(P)$
4. Cu(MPU)$_2$Cl$_2$	Blue	1.90	16607 23604	$^2Eg→^2T_2g$ L→M(CT)

3.7. ESR spectra

ESR spectral studies of Cu(MPU)$_2$Cl$_2$ was recorded at room temperature (300 K) and the spectrum is presented in Fig 2. The ESR spectral analysis of spectra showed that g|| = 2.21 and g⊥ = 2.01. Thus g|| > g⊥ > 2.0023. These observations indicate that the unpaired electron is localized in d_x^2-d_y^2 orbital of the Cu(II) ion and hence the system is axially symmetric. Thus the tetragonally elongated octahedron geometry is confirmed for the aforesaid complex[16,18].

3.8. Antimicrobial studies

The potency of the synthesized compounds as antimicrobial was tested against pathogenic strains by disc diffusion method[19]. The synthesized compounds were dissolved in DMSO at a concentration of 25, 50, 100, and 200 mg/L. the respective microbial culture was swabbed into the nutrient agar plates for uniform distribution of colonies. All synthesized compounds were poured into each well using a sterile micro pipette and streptomycin was used as standard. The plates were incubated for 72 hr. The zone of inhibition was measured after the incubation. The observed values of antimicrobial data are tabulated in 7 and 8. It is observed that the metal chelates have higher activity than the free ligand, which can be explained on the basis of overtone's concept and chelation theory[20,21]. According to overtone's concept of cell permeability the lipid membrane that surrounds the cell favors the passage of only lipid soluble material. Thus the liposolubility is an important factor that controls the antimicrobial activity. On chelation the polarity of the metal ion is reduced to a greater extent due to overlap of the ligand ion with donor groups. Further it increases the delocalization of electrons over the whole chelate ring and enhances the lipophilicity of the complex. This increased lipophilicity enhances the penetration of the complexes into the lipid membrane and blocking of metal binding sites on the enzymes on the microorganism.

Table 7. Disc diffusion method results of MPU, metal complexes and standard drugs (diameter of the zone of inhibition (mm)) at 25, 50, 100 and 200 μg/mL

S.No	Gram positive bacteria (Staphylococcus aureus) Concentration μg/mL					Gram negative bacteria (Escherichia Coli) Concentration μg/mL				
	25	50	100	200	Average	25	50	100	200	Average
MPU	20	20	20	20	20.00	22	22	23	27	23.50
Co(II)	25	25	26	26	25.50	23	24	26	28	25.25
Ni(II)	21	22	22	23	22.00	22	23	24	25	23.50
Cu(II)	23	23	24	25	23.75	27	28	32	35	30.50
Standard	24	25	25	27	25.25	25	28	29	30	28.00

Table 8. Disc diffusion method results of MPU, metal complexes and standard drugs (diameter of the zone of inhibition (mm)) at 25, 50, 100 and 200 μg/mL

S.No	Candida Concentration μg/mL					Aspergillus Niger Concentration μg/mL				
	25	50	100	200	Average	25	50	100	200	Average

MPU	17	18	18	18	18.00	16	17	17	18	17.00
Co(II)	23	23	24	23	23.25	24	24	25	28	25.25
Ni(II)	20	20	19	19	19.50	22	22	22	23	22.25
Cu(II)	21	21	21	21	21.00	20	20	21	21	20.50
Standard	25	25	26	26	25.50	24	25	26	28	25.75

3.9. Conclusion

The ligand, MPU and its metal complexes have been synthesized and characterized by elemental analysis, IR, NMR, UV, ESR and magnetic measurements. It is revealed from the IR studies that the ligand coordinated to metal as a neutral bidentate ligand. The results of UV spectral studies and magnetic susceptibility studies conforms octahedral geometry of the metal complexes. Antimicrobial screening of ligand and its metal complexes showed their excellent activity. The zone of inhibition of metal complexes are comparably high than the free ligand.

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