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Synthesis, Characterization and Antimicrobial Studies on a New Mannich Base N-(Morpholinomethyl)phthalimide and its Zinc(II), Cadmium(II) and Mercury(II) Complexes

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ABSTRACT

The new Mannich base N-(morpholinomethyl)phthalimide (MMP) was prepared by Mannich condensation of morpholine, formaldehyde and phthalimide. The d_{10} transition metal complexes with this ligand were synthesized in non-aqueous media. The ligand and metal complexes were characterized on the basis of elemental analysis, molar conductance, UV, IR and NMR spectral studies. The bidentate chelation of MMP, bonding through carbonyl oxygen of the imide moiety and C-N-C of morpholine ring is suggested. Based on the IR, ^{1}H NMR studies octahedral geometry is assigned for all the metal complexes. The ligand and the Zn^{II} and Cd^{II} complexes were screened for their in vitro antibacterial and antifungal activities employing agar well diffusion technique. The complexes are more active than the ligand.

Keywords: Mannich base, d_{10} metal complexes, bidentate chelation, antimicrobial activity, octahedral geometry

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INTRODUCTION

Synthetic cyclic imides possess structural features, which confer potential biological activity and pharmaceutical use [1] on them. Their molecules contain an imide ring, so that they are hydrophobic and neutral, and can therefore cross biological membranes in vivo. Metal complexes of Mannich bases have been studied extensively due to the selectivity and sensitivity of the Mannich base ligands towards various metal ions. Metal complexes of Mannich bases are known to behave as antimicrobial, antimalarial, antidepressant, antipyretic, analgesic and anti-inflammatory drugs [2-5]. Recently, a new Mannich base ligand, N-(morpholinomethyl)phthalimide has been prepared by us by the condensation of morpholine, formaldehyde and phthalimide and its Zn^{II}, Cd^{II} and Hg^{II} complexes have been synthesized and characterized

MATERIAL AND METHODS

All the reagents used were of A.R. grade and the solvents used were commercial products of the highest available purity. Elemental analysis (C, H and N) was performed using Carlo Erba 1108 elemental analyzer. The conductance data of the complexes were obtained in ~10^{-3} M DMF solutions at room temperature using a digital conductivity bridge, Systronics Direct Reading Conductivity Meter 304 with a dip type conductivity cell. Infrared spectral measurements were made for the free ligand and its metal complexes as KBr pellets using Perkin Elmer Spectrum-1 FT IR Spectrometer. The far IR spectra were taken using Ratio Recording Bruker 3000 Hyperion Microscope with Vertex 80 FTIR spectrometer. The mass spectral study of the ligand was carried out using JEOL-GC MATE II GC-

MS (EI/CI) mass spectrometer. The ^{1}H NMR spectra of the ligand and the complexes were recorded on JEOL GSX-400 FT NMR spectrometer (400 MHz) employing TMS as internal reference and DMSO-d6 as solvent at ambient temperature. The ^{13}C NMR spectrum of the ligand was recorded using JEOL GSK-400 spectrometer in $CDCl_3$ solution at ambient temperature. The metal and anion contents were estimated by standard literature procedures [6].

RESULTS AND DISCUSSION

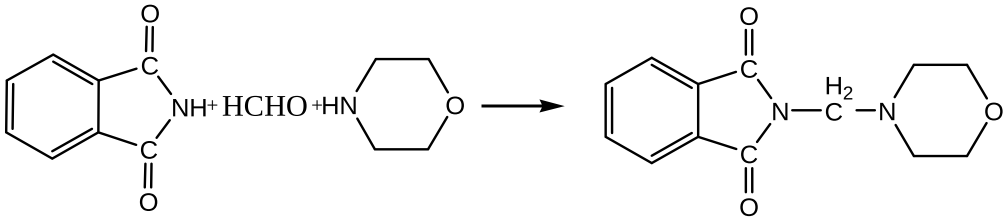
Synthesis of the ligand

N-(Morpholinomethyl)phthalimide (MMP) was synthesized by Mannich condensation reaction [7] of morpholine, formaldehyde and phthalimide in 1:1:1 mol ratio. Phthalimide (29.42 g, 0.1 mol) was mixed with formaldehyde (15 mL, 0.1 mol) followed by adding morpholine (17.6 mL, 0.1 mol) into the mixture at room temperature with constant stirring. After 2 days the colourless solid mass obtained was filtered, washed with water and dried at 100°C in an air oven and recrystallised from ethanol. The percentage yield of the compound was 85 (M.Pt: 114°C).

The analytical and spectral data obtained for the ligand are summarized below:

Analysis: Calculated for $C_{13}H_{14}N_2O_3$: C 63.41, H 5.69, N 11.38 %, Found: C 63.55, H 5.65, N 11.50 %; FT-IR (KBr, cm^{-1}): 2962(aromatic C-H), 2853(aliphatic C-H), 1771, 1704, 1640(C=O), 1640-1413(aromatic C = C), 1267, 1152(C-N-C), 1112 (C-O-C);^{1}H NMR (DMSO-d$_6$, δppm): 7.85(4H, aromatic), 4.43(2H, N-CH$_2$-N), 2.50(4H, N(CH$_2$)$_2$), 3.54(4H, O(CH$_2$)$_2$) (Fig.1); ^{13}C-NMR (DMSO-d$_6$, δppm): 134.50– 123.13(aromatic carbons), 134.50(Bridgehead carbons) 168.68(C=O carbons), 65.94(O(CH$_2$)$_2$ carbons), 50.34(N(CH$_2$)$_2$ carbons) (Fig.2); Mass (m/z): 246 (M+ peak), 202, 160, 77 (Fig.3) the fragmentation pattern of the ligand [8] is shown in **Scheme-2**.

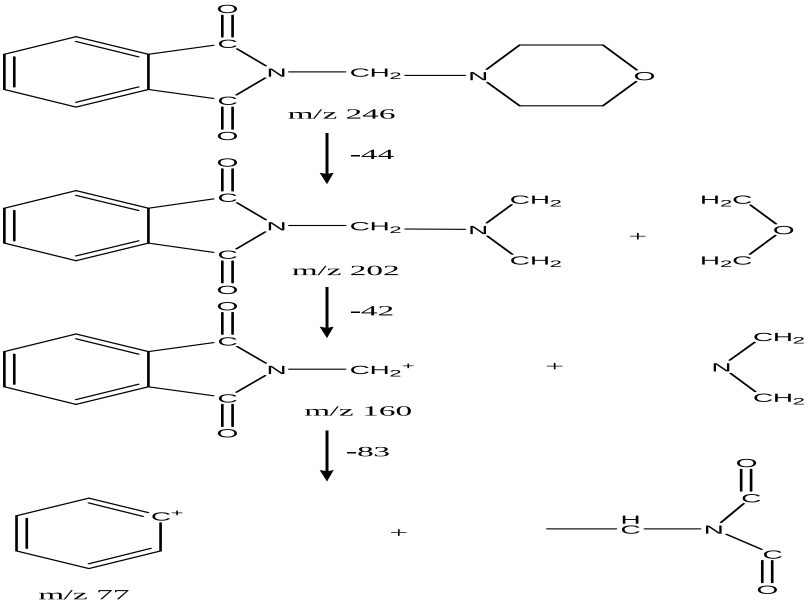
Thus based on elemental analysis, UV, IR, ^{1}H NMR, ^{13}C NMR and mass spectra data, MMP is assigned the molecular structure as provided in Scheme-1. Its crystal structure determined by single crystal X-ray diffraction has already been reported by us [9].



Scheme-1

Synthesis of the metal complexes

All the complexes of MMP were isolated from non-aqueous medium using ethanol [10]. In each case, the hot ethanolic solution of the metal salt was added slowly with constant stirring to the hot ethanolic solution of the ligand in 1:1 mol ratio. The insoluble complex formed was filtered, washed with ethanol to remove the unreacted metal salt or ligand, and then dried in an air oven at 85°C.



Scheme-2 Mass spectral fragmentation pattern of MMP

Characterization of metal complexes

Chemical and microelemental analysis

To find out the stoichiometry of the metal complexes, the weight percentages of the metal ions, anions and C, H, N were determined. The observed analytical data (Table 1) are in good agreement with the calculated values. The molar conductance values (Table 1) measured in ~ 10^{-3} M DMF solutions show that all the complexes are non-electrolytes [11].

Bonding atoms and stereochemistry

A comparison of IR and ^{1}H NMR spectra of the metal complexes with those of the ligand, suggests the coordination sites of the ligand and the modes of coordination of anions to the metal centre. Far IR spectra have been taken only for Zn^{II}, Cd^{II} and Hg^{II} chloro complexes. From these studies an attempt is made to fix the modes of coordination of the ligand and to assign the stereochemistry around the metal ion in a complex. The structurally important IR and far IR absorption frequencies of the Zn^{II}, Cd^{II} and Hg^{II} complexes are presented in Table 2.

Table 1. Analytical and Conductance Data for MMP and its Zn^{II}, Cd^{II} and Hg^{II} Complexes

Compound	Contents (found / Calcd) %										Λ_M Ohm^{-1} cm^2 mol^{-1}
	C		H		N		Metal		Anion		
MMP ($C_{13}H_{14}N_2O_3$)	63.55	63.41	5.65	5.69	11.50	11.38	-		-		-
$ZnCl_2$.MMP.$2H_2O$	3.67	3.66	7.34	7.32	17.14	17.10	18.62	18.57	22.61	22.10	14.35
$ZnSO_4$.MMP.$2H_2O$	3.44	3.44	6.89	6.87	16.08	16.05	23.61	23.57	30.51	30.92	27.12
$CdCl_2$.MMP.$2H_2O$	3.27	3.26	6.54	6.52	26.24	26.18	16.58	16.53	46.68	46.04	32.45
$CdSO_4$.MMP.$2H_2O$	3.08	3.08	6.17	6.16	24.75	24.74	21.14	21.13	15.20	15.88	10.09
$HgCl_2$.MMP.$2H_2O$	2.71	2.70	5.42	5.41	38.82	38.75	13.74	13.72	38.76	38.25	12.38

IR spectral analysis

The carbonyl stretching vibrations [12] of the free MMP ligand are appearing at 1771(sh), 1704(s) and 1640(m) cm^{-1}. But in the chloro complexes of Zn^{II}, Cd^{II} and Hg^{II} the carbonyl absorption bands have shifted to two regions at about 1700 and 1610 cm^{-1} indicating the binding of one of the two carbonyl oxygen atoms to the metal ion [13,14]. It is consistent with the observations of Adams et al and Ian Fairlamb et al. The C-N-C stretching vibrations of the free MMP ligand appear at 1267 and 1152 cm-1. In the chloro complexes of Zn^{II}, Cd^{II} and Hg^{II} the absorption bands have lowered to the region of 1153-1051 cm^{-1} suggesting the coordination of the tertiary amino nitrogen atom of the morpholine moiety of MMP. In the case of the sulphato complexes of Zn^{II} and Cd^{II} the carbonyl stretching vibrations are found to appear in two different regions of 1771-1704 and 1640-1610 cm^{-1} suggesting the involvement of only one carbonyl oxygen in coordination to the metal ion. The C-N-C vibrations of the Mannich base ligand in the sulphato complexes have also lowered to 1151 and 1112 cm^{-1} pointing to the binding of the morpholine ring nitrogen atom to the metal ion. The presence of coordinated water molecules [15] in all the five complexes prepared is supported by the appearance of absorption bands at about 3450, 1610, 850 and 630 cm^{-1} assigned to ν_{OH}, δ_{HOH}, ρr_{HOH} and ρw_{HOH} vibrations respectively. The far IR spectra [16] of the Zn^{II} complexes indicate the vibrations due to Zn-O at ~530 cm^{-1}, Zn-N at ~ 460 cm^{-1} and Zn-Cl at 352 cm^{-1}. Similarly the far IR spectra of Cd^{II} complexes show ν_{Cd-O} at ~450, ν_{Cd-N} at ~ 390 and ν_{Cd-Cl} at ~330 cm^{-1}. Similarly the Hg^{II} complexes have vibrational frequencies at 405 (νHg-O), 303 (ν_{Hg-N}) and 280 (ν_{Hg-C}l) cm^{-1}. The ν_3 vibrations of the sulphato complexes appear at 1003, 968 and 898 cm^{-1} while ν_4 vibrations appear at 773-736, 737-710 and 710-675 cm^{-1}. The ν_1 vibrations are seen at 850 cm^{-1} and ν_2 vibrations are seen at 526 cm^{-1}. These observed vibrations of the sulphato group are consistent with those associated with the chelating bidentate sulphato group.

^{1}H NMR spectral analysis

The ^{1}H NMR resonance signals of the MMP complexes with Zn^{II}, Cd^{II} and Hg^{II} are presented in Table 3 and the spectra of the complexes are shown in Figure 4. The proton signal of $N(CH_2)_2$ group of morpholine ring in MMP has experienced a downfield shift from δ2.49 ppm to δ2.51 ppm in the metal complexes. The downfield shifting of the $N(CH_2)_2$ proton signal points to the involvement of morpholine ring nitrogen in bonding with the metal atoms [17]. The aromatic protons in the metal complexes have also experienced a small downfield shift. This may indicate the coordination of one of the carbonyl oxygen atoms to the metal centre. The drift of electron density from the carbonyl oxygen to the metal centre may cause some depletion of electron density in the aromatic ring. In all the complexes signals due to protons of the coordinated water molecules [18] appear at about δ3.35 - 3.83 ppm.

Such a peak is not seen in the spectrum of the free MMP ligand. Hence it is concluded that ^{1}H NMR spectra of all the complexes studied support the modes of coordination suggested by IR data. i.e. coordination of a carbonyl oxygen, morpholine ring nitrogen and aqua ligand.

Based on the results of analytical, conductance, IR and ^{1}H NMR data, a hexacoordinated geometry is assigned for the Zn^{II}, Cd^{II} and Hg^{II} complexes of MMP as shown in Figure 5 and 6.

In vitro Antimicrobial Activities

Based on the values of the measured zones of inhibition, (Table 4 and 5) all the microorganisms tested are found to be sensitive to the test compounds. It has been observed that the increase in concentration of the test compound increases the zone of inhibition. It is seen that the metal chloro and sulphato complexes of MMP exhibit higher activity than the free MMP ligand against both the bacterial organisms *E.coli* and *S.aureus*. The enhanced activity of the metal complexes can be explained on the basis of chelation theory [19]. Chelation reduces the polarity of the metal ion and increases lipophilic or hydrophobic character of the metal chelate favouring the diffusion of the metal chelate through the cell membrane. In other words the chelation of the metal ion leads to the breakdown of permeability barrier of the cell wall. Hence the permeation mechanism through the lipid layer of the microorganism becomes more effective, causing an increase in the antibacterial activity of the complexes [20].

The metal chloro and sulphato complexes of MMP have been screened for their fungitoxity by employing *A.niger* and *C.albicans*. Metal complexes are invariably found to be more toxic to the fungal species than the free ligand. The increased activity of the metal chloro and sulphato complexes against fungal species screened can also be explained on the basis of chelation theory. Further the presence of uncoordinated heteroatoms such as O and N in the chelate molecule will involve in the formation of hydrogen bonds with the active centres of the cell constituents resulting in the interference of the normal cell processes. Thus the presence of polar groups like C=O, C-N, C-O etc. in the chelate molecules are expected to enhance the fungitoxity [21]. Thus the molecules tested have greater chance of interaction with the nucleotide bases present in the cells of fungal species. The metal complexes at a higher concentration of 100 µg/mL show comparable activity to that of the standard drugs used.

Table 2. Important IR Absorption Bands (cm^{-1}) of MMP and its Zn^{II}, Cd^{II} and Hg^{II} Complexes

Compound	$\nu_{C=O}$	ν_{CNC}	ν_{M-O}	ν_{M-N}	ν_{M-X}	ν_{OH}	δ_{HOH}	ρ_{rHOH}	ρ_{wHOH}
MMP	1771 1704 1640	1267 1152	-	-	-	-	-	-	-
$ZnCl_2.MMP.2H_2O$	1699 1608	1128 1051	530	465	352	3434 3267	1608	865	650
$ZnSO_4.MMP.2H_2O$	1772 1706 1610	1150 1112	526	460	-	3450	1610	852	612
$CdCl_2.MMP.2H_2O$	1769 1702 1611	1152 1107	461	392	329	3459 3518	1611	840	660 640
$CdSO_4.MMP.2H_2O$	1772 1705 1609	1151 1112	440	360	-	3459	1609	853	611
$HgCl_2.MMP.2H_2O$	1770 1711 1612	1153 1106	405	303	280	3463	1612	859	632

Table 3. ^{1}H NMR Resonance Signals (δppm) for MMP and its Zn^{II}, Cd^{II} and Hg^{II}Complexes

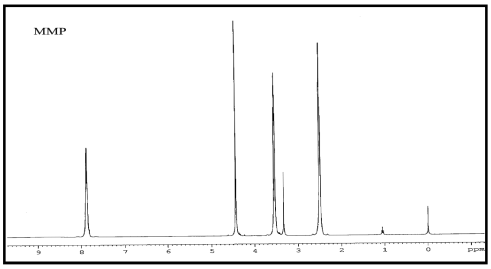
Compound	$O(CH_2)_2$ of morpholine ring	$N(CH_2)_2$ of morpholine ring	Methylene $N\text{-}CH_2\text{-}N$	Aromatic protons	Water
MMP	3.54	2.50	4.43	7.85	-
$ZnCl_2.MMP.2H_2O$	3.56	2.51	4.44	7.90 to 7.83	3.72
$ZnSO_4.MMP.2H_2O$	3.55	2.51	4.44	7.91 to 7.86	3.61
$CdCl_2.MMP.2H_2O$	3.56	2.51	4.44	7.90 to 7.83	3.83
$CdSO_4.MMP.2H_2O$	3.54	2.50	4.44	7.90 to 7.85	3.35
$HgCl_2.MMP.2H_2O$	3.56	2.51	4.44	7.91 to 7.86	3.36

Table 4. Antibacterial Activity of MMP and its Metal Complexes

Compound	**Zone of Inhibition (mm)**							
	E.coli				***S.aureus***			
	Conc. of Compound (μg/mL)				**Conc. of Compound (μg/mL)**			
	25	**50**	**75**	**100**	**25**	**50**	**75**	**100**
MMP	6	7	10	12	5	7	10	11
$ZnCl_2$.MMP.$2H_2O$	20	21	22	24	8	10	12	13
$ZnSO_4$.MMP.$2H_2O$	14	16	19	23	11	12	15	16
$CdCl_2$.MMP.$2H_2O$	14	15	17	19	8	9	10	11
$CdSO_4$.MMP.$2H_2O$	10	12	13	15	9	10	12	13
Kanamycin	15	-	-	-	-	-	-	-
Tetracyclin	-	-	-	-	13	-	-	-

Table 5. Antifungal Activity of MMP and its Metal Complexes

Compound	**Zone of Inhibition (mm)**							
	A. niger				***C. albicans***			
	Conc. of Compound (μg/mL)				**Conc. of Compound (μg/mL)**			
	25	**50**	**75**	**100**	**25**	**50**	**75**	**100**
MMP	5	6	7	8	6	8	9	10
$ZnCl_2$.MMP.$2H_2O$	8	10	12	13	8	9	10	12
$ZnSO_4$.MMP.$2H_2O$	11	12	13	15	10	12	13	16
$CdCl_2$.MMP.$2H_2O$	8	9	11	12	7	8	9	10
$CdSO_4$.MMP.$2H_2O$	9	10	12	13	9	11	12	14
Amphotericin	12	-	-	-	-	-	-	-
Nystatin	-	-	-	-	14	-	-	-



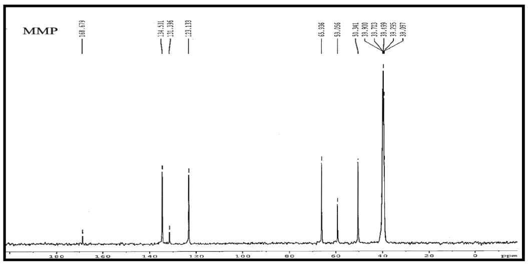


Fig. 1. ^{1}H NMR Spectrum of MMP **Fig. 2. ^{13}C NMR Spectrum of MMP**

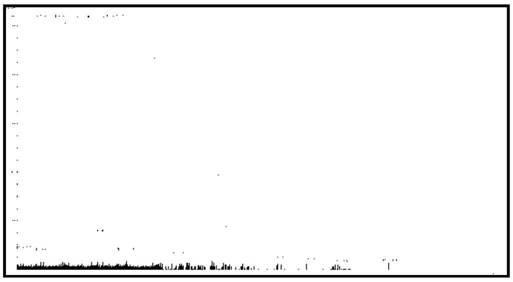


Fig. 3 Mass Spectrum of MMP

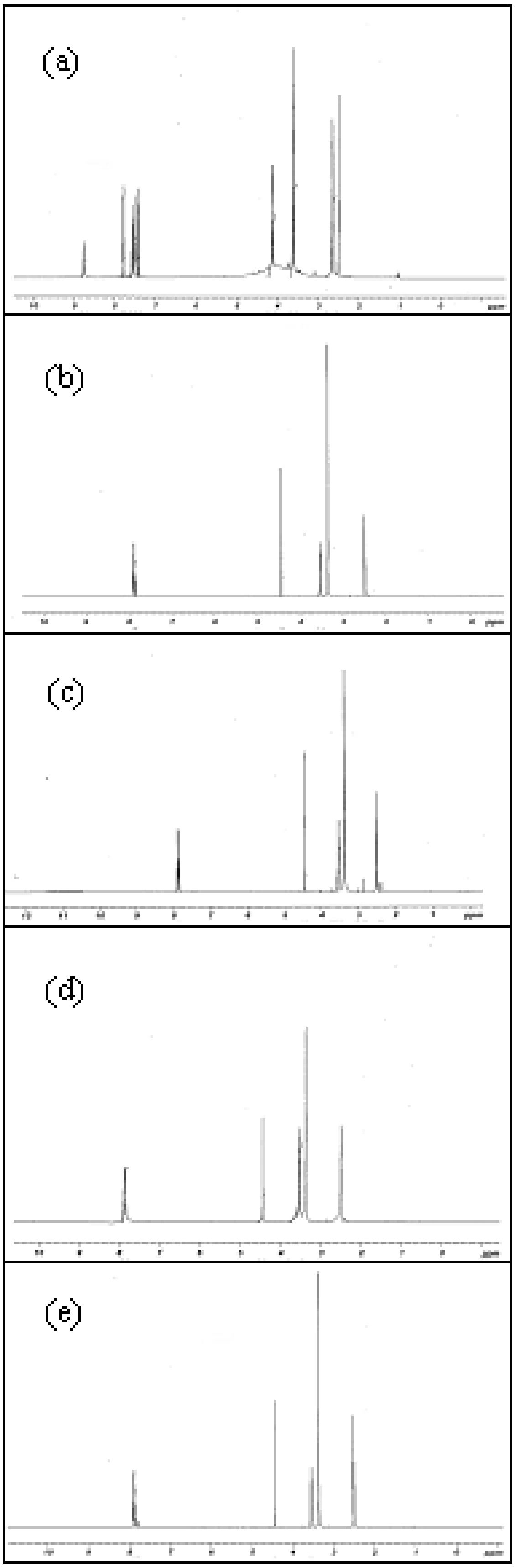


Fig. 4. ^{1}H NMR Spectrum of (a) ZnCl2.MMP.2H2O, (b) ZnSO4.MMP.2H2O, (c) CdCl2.MMP.2H2O, (d) CdSO4.MMP.2H2O, (e) HgCl2.MMP.2H2O

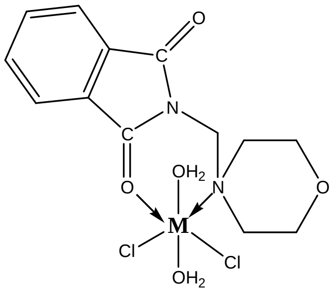


Fig. 5. Structure of M Cl_2.MMP.$2H_2O$
(M= Zn^{II}, Cd^{II} and Hg^{II})

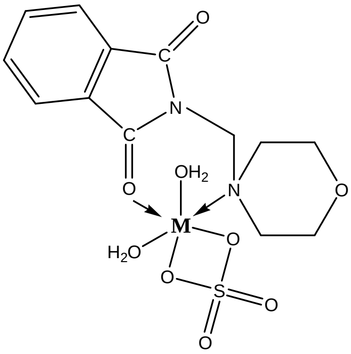


Fig. 6. Structure of M SO_4.MMP.$2H_2O$
(M= Zn^{II}, Cd^{II} and Hg^{II})

CONCLUSION

The new Mannich base N-(morpholinomethyl)phthalimide (MMP) was prepared and characterized on the basis of elemental analysis, IR, NMR and mass spectral data. The bidentate chelation of MMP to Zn^{II}, Cd^{II} and Hg^{II} ions through one carbonyl oxygen of the imide moiety and C-N-C of the morpholine ring has been suggested. All the complexes studied are non-electrolytes and are assigned an octahedral geometry. Both the MMP ligand and the Zn^{II} and Cd^{II} complexes screened for in vitro antimicrobial activity are found to be toxic to the bacterial and fungal species tested.

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