



A New Mannich Base Derived by Three Component Condensation of Benzamide, Benzaldehyde and Morpholine: Synthesis, Coordination Mode and Biological Activities of VO^{IV}, Mn^{II} and Fe^{II} Metal Chelates

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Abstract: Mannich bases play an active role for the formation of hydrazone derivatives. Mannich base was prepared by condensation of benzamide, benzaldehyde and morpholine. The synthesized base was reacted with VO^{IV}, Mn^{II} and Fe^{II} metal ions to form complexes. They were characterized by elemental analysis, IR, ¹H & ¹³C NMR, UV-Vis, EPR, mass spectral studies and magnetic measurements. The complexes are non-electrolytic in DMSO. The presence of coordinated water molecules in these complexes was indicated by IR spectra and TG analysis. It was found that, all the complexes exhibited octahedral geometry. In addition, they were screened for their antimicrobial activity against different bacterial and fungal strains.

Keywords: Anti tubercular activity, Stoichiometry, Electron paramagnetic resonance, Disc diffusion method.

1. Introduction

It is interesting to study heterocyclic compounds, because of their importance in organic and medicinal chemistry¹⁻³. The design, synthesis and production of these molecules having value as human therapeutic agents⁴ have attracted much attention recently. A number of protocols for the synthesis of heterocyclic compounds are available in the literature. For this, Mannich base has played a vital role for the formation of hydrazones⁵. Hydrazones are derivatives of benzamide with a carbonyl group in the 1-position as reported. They have several biological applications: antibacterial, antifungal, anti-inflammatory, antitumor, anti-HIV, anticonvulsant, antitubercular activities⁶⁻¹³. In the present study, we have prepared *N*-[Morpholino(phenyl)methyl] benzamide, which acts as a good antimicrobial agent. Benzamide and its derivatives are widely distributed in nature and have been known to exhibit broad spectrum biological activities. Based on the previous findings, it was thought worthwhile to synthesize a few bioactive benzamide containing hydrazone derivatives.

2. Materials and Methods

2.1. Experimental

All the reagents used for synthesizing the ligand and its complexes were of A.R. grade. The solvents used were commercial products of the highest available purity and were further purified by distillation.

Micro elemental data were obtained with Carlo Erba 1108 elemental analyzer. Metal contents were estimated by usual procedure¹⁴, after digesting the complexes with con.HNO₃. Conductance data were obtained in $\sim 10^{-3}$ M DMF solution of the complexes using digital conductivity meter. IR spectra were recorded using Perkin Elmer FT-IR spectrometer by using KBr pellets. Absorbance in UV-Visible region was recorded in DMF solution using UV-Visible spectrometer. The ¹H and ¹³C NMR of the ligand were recorded on a Bruker instrument employing TMS as internal reference and DMSO-d₆ as solvent. The FAB mass for the ligand was carried out using Mass spectrometer. The room temperature magnetic susceptibility measurements of the complexes were made by using a Gouy Magnetic Balance. The thermal analyses were recorded on universal V4.3A TA instrument. The antimicrobial activity was determined with the disc diffusion method.

2.2. Synthesis of the ligand

N-[Morpholino(phenyl)methyl]benzamide(MBB) was synthesized by employing the Mannich synthetic route¹⁵. Ethanolic solution of benzamide (12.1 g, 0.1 mol) was mixed with benzaldehyde (10 mL, 0.1 mol) followed by morpholine (9 mL, 0.1 mol) with constant stirring in an ice bath. After 15 days, the colourless solid obtained was washed with water and with acetone. The compound was dried at 60°C and recrystallised from ethanol. It was insoluble in water but soluble in organic solvents.

2.3. Synthesis of metal complexes

The methanolic solution of each of the metal salts(vanadyl sulphate, manganese sulphate and ferrous sulphate) was added slowly with constant stirring to the ethanolic solution of the ligand in 2:1 mol ratio at warm condition. The insoluble complex¹⁶ formed in each case was filtered, washed with ethanol and methanol to remove the unreacted metal and ligand, and then dried at 80°C.

2.4. Antimicrobial Screening

Antibacterial and antifungal activities¹⁷ of the ligand and 3 of its metal complexes were tested *in vitro* against 6 bacterial species viz. *E.coli*, *P.aeruginosa*, *S.typhi*, *B.subtilis*, *S.pyogenes*, *S.aureus* and 2 fungal species *A.niger* and *A.flavus* by disc diffusion method using agar nutrient as medium and gentamycin as control. The paper disc containing the compound (10, 20 and 30 µg/disc) was placed on the surface of the nutrient agar plate, previously spread with 0.1 mL of sterilized culture of microorganism. After incubating this at 37°C for 24 hrs, the diameter of inhibition zone around the paper disc was measured.

3. Results and Discussion

3.1. Structural Characterization of MBB

Molecular formula: C₁₈H₂₀N₂O₂, Yield: 96%, MP: 162-165°C, Mol.wt: 218, FT-IR KBr in cm⁻¹: 3292(NH), 1637(C=O), 3063, 3030(ν_{CH} aromatic), 2962, 2914, 2893(ν_{CH} alicyclic), 2852(ν_{CH} aliphatic), 1602($\nu_{C=C}$, ν_{C-N}), 1522(δ_{CH_2} morpholine ring), 1489, 1447(δ_{NH} secondary amide), 1354, 1330, 1310(ν_{CN} mixed with δ_{NH}), 1210(ν_{ring}), 1136, 1071(ν_{CNC}) 1111(C-N-C), 1024(ν_{C-O-C} as + ν_{C-O-C} sy morpholine), 1007, 948(δ_{CH^+} o.p.b morpholine), 915(π_{CH^+} δ_{CH^+} o.p.b ring), 747-897(δ_{NH} wagging and twisting), 747(δ_{CH} o.p.b benzene ring) 698(o.p.b of ring C=C-H benzene), 634(i.p.b of benzene), 409(o.p.b ring C=C). ¹H NMR (300MHz, DMSO-d₆): δ 8.82(s, NH), 5.93 & 5.90(d, CH), 7.94 - 7.27(m, CH benzene ring) 3.65 (s, O(CH₂)₂ of morpholine), 2.51(s, N(CH₂)₂ of morpholine). ¹³C NMR (300MHz, DMSO-d₆): 167.62 (s, C=O), δ 134.73-127.90(m, C phenyl ring), 66.74(s, O(CH₂)₂ of morpholine), 49.33(s, N(CH₂)₂ of morpholine). UV-Vis(DMF): 274($n \rightarrow \pi^*$), 236($\pi \rightarrow \pi^*$). FABMS: m/z = 296(C₁₈H₂₀N₂O₂), m/z = 120(C₇H₆NO⁺), m/z = 105(C₇H₅O⁺), m/z = 70 (C₆H₅⁺). Calculated: C 72.97%, H 6.76% and N 9.46%. Found: C 73.05%, H 6.80% and N 9.53%.

Based on the data obtained from various physical and chemical studies, the molecular structure of MBB is shown in Fig. 1.

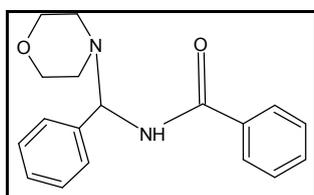


Fig.1: *N*-[Morpholino(phenyl)methyl]benzamide

3.2. Characterization of VO^{IV}, Mn^{II} and Fe^{II} Complexes of MBB

All the complexes were stable and non-hygroscopic in nature. They were insoluble in water, ethanol, methanol, acetone, chloroform and were soluble in DMF and DMSO.

Bonding Atoms and Stereochemistry

Comparison of IR Spectra of the metal complexes with that of the ligand suggests the coordination sites of the ligand, the mode of coordination of sulphato group to the metal centre. The magnetic moment, electronic and electron paramagnetic resonance measurements of the complexes have also been carried out. From these studies, an attempt is made to assign the stereochemistry around the metal ions.

Microanalysis

To find out the stoichiometry¹⁸ of the complexes, the percentage of the metal ions, anions and CHN were determined. The CHN analyses were in good agreement with the calculated values. From the data obtained, the molar ratio was confirmed. This was in agreement with the ratio found, according to elemental analysis (Table 1).

Molar Conductance Measurements

The electrical conductance measurements¹⁹ of the complexes in $\sim 10^{-3}$ M DMF solutions at room temperature were done, in order to ascertain whether the anion was within or outside the coordination sphere of the complex. The molar conductance values " Λ_M " confirmed that, all the complexes were non-electrolytes.

Table 1 Analytical and Conductance Data for VO^{IV}, Mn^{II} and Fe^{II} Complexes of MBB

Complex	% C Obs (Cal.)	% H Obs. (Cal.)	% N Obs. (Cal.)	%Metal Obs. (Cal.)	%Anion Obs. (Cal.)	Λ_M ohm ⁻¹ cm ² mol ⁻¹
VO ₄ .MBB. H ₂ O	37.69 (38.09)	3.10 (3.53)	5.21 (4.94)	8.56 (8.98)	17.15 (16.93)	05.17
MnSO ₄ .2MBB	41.90 (41.62)	4.00 (3.85)	5.18 (5.39)	10.64 (10.59)	18.77 (18.50)	11.08
FeSO ₄ .MBB. 2H ₂ O	35.89 (35.41)	3.01 (3.28)	4.33 (4.59)	8.95 (9.16)	16.16 (15.74)	39.65

Table 2 Important IR Absorption Bands (cm⁻¹) of MBB and of VO^{IV}, Mn^{II} and Fe^{II} Complexes

Compound	ν_{NH}	$\nu_{C=O}$	ν_{CNC}	ν_3	ν_4	ν_1	ν_2
MBB	3292	1637	1111	-	-	-	-
VO ₄ .MBB.H ₂ O	3402	1543	1101	1101, 1041, 993	709, 680, 653	818	529
MnSO ₄ .2MBB	3369	1492	1018	1123, 1018	653, 626, 607	823	532
FeSO ₄ .MBB.2H ₂ O	3389	1621	1017	1091, 1017	665, 624, 604	827	542

Table 3 Colour, Electronic Spectral Bands, Transition Assignments, Metal Environment and Magnetic Moment Values of VO^{IV}, Mn^{II} and Fe^{II} Complexes of MBB

Complex	Colour (μ_{eff} , B.M)	Environment	Absorption maxima (cm ⁻¹)	Transition Assignment
VO ₄ .MBB.H ₂ O	Dark Green (1.68)	N, 5O	17931 23458 31760,39325	² B ₂ → ² B ₁ ² B ₂ → ² A ₁ CT
MnSO ₄ .2MBB	Colourless (4.99)	2N, 4O	18362 22457 31293	⁶ A _{1g} → ⁴ T _{1g} (G) ⁶ A _{1g} → ⁴ T _{2g} (G) CT
FeSO ₄ .MBB.2H ₂ O	Brown (5.82)	N, 5O	13544 22210	⁶ A _{1g} → ⁴ T _{1g} (G) ⁶ A _{1g} → ⁴ T _{2g} (G)

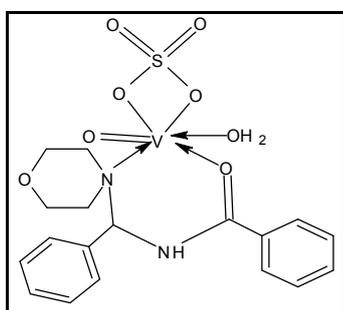
Analytical and conductance data (Table 1) indicate the stoichiometry of the complexes as VO₄.MBB.H₂O, MnSO₄.2MBB and FeSO₄.MBB.2H₂O. The IR spectrum²⁰ of the complexes registered lower frequency values for the C=O by about 94, 145 and 16 cm⁻¹ respectively. The CNC of the ligand shifted to lower frequency of 10, 93 and 94 cm⁻¹ respectively, which suggests bidentate coordination of the ligand (Table 2). The complex display a strong V=O stretching frequency at 993 cm⁻¹.

The VO^{IV}, Mn^{II} and Fe^{II} complexes exhibit absorption band at 1101, 1041 & 993 and 1123 & 1018 and 1091 & 1017 cm⁻¹ corresponding to ν_3 of sulphato group. The ν_4 appears at 709, 680 & 653 and 653, 626 & 607 and 665, 624 & 604 cm⁻¹ respectively. The ν_1 and ν_2 mode of coordinated sulphato group occurs at 818, 823 & 827 and 529, 532 & 542 cm⁻¹ which indicate the bidentate chelating sulphato group²¹.

The complexes of VO^{IV} and Fe^{II} sulphates show bands at 3402, 1647, 870, 653 and 529 cm⁻¹ and 3779, 1621, 827, 665 and 542 cm⁻¹ which are assigned as ν_{OH} , δ_{HOH} , ρ_{rHOH} , ρ_{wHOH} and $\nu_{\text{M-O}}$ modes of coordinated water²².

The UV-Visible spectrum²³ of VO^{IV} sulphato complex in Table 3 shows the absorption bands at 17931 cm⁻¹, which is ascribed to ²B₂→²B₁ transition. The band at 23458 cm⁻¹ corresponds to ²B₂→²A₁ transition. The electronic bands at 31760 and 39325 cm⁻¹ are assigned to charge transfer transitions.

EPR spectrum²⁴ of the oxovanadium(IV) complex does not show any resolution at g_{\parallel} region; so it can be assumed that g_x and g_y are same or nearly the same. The g value of 1.9013 for the complex indicates strong interaction between the ligand and the metal ion. Hence distorted octahedral geometry is proposed. The room temperature magnetic moment (μ_{eff}) value is 1.68 B.M. which suggests a hexa coordinate geometry (Fig.2) around VO^{IV} ion.

**Fig. 2: VO₄.MBB.H₂O**

The UV-Visible spectrum of manganese(II)sulphato complex shows three absorption bands: at 18362 cm⁻¹ due to ⁶A_{1g}→⁴T_{1g}(G) transition, at 22457 cm⁻¹ due to ⁶A_{1g}→⁴T_{2g}(G) transition and at 31293 cm⁻¹ due to the charge transfer transitions. The room temperature magnetic moment value was found to be 4.99 B.M. These observations suggest a high spin octahedral geometry (Fig.3) of the complex²⁵.

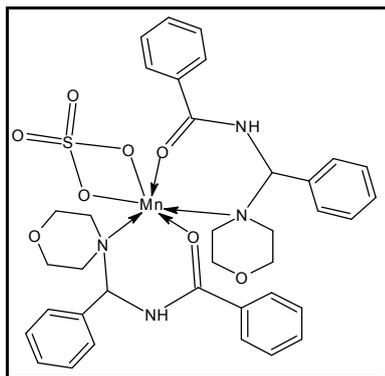


Fig.3: MnSO₄.2MBB

The UV-Visible spectrum of iron(II)sulphate complex exhibits electronic transition bands at 13544 and 22210 cm^{-1} due to ${}^6A_{1g} \rightarrow {}^4T_{1g}$ and ${}^6A_{1g} \rightarrow {}^4T_{2g}(G)$ transitions respectively. The μ_{eff} value of 3.54 B.M. indicates high spin octahedral geometry²⁶ around Fe^{II} ion(Fig.4).

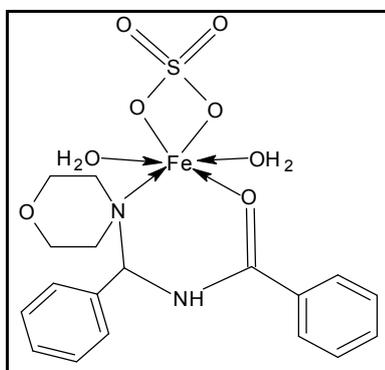


Fig.4: FeSO₄.MBB.2H₂O

Thermal Analysis

At different steps, the percentage mass loss has been recorded by using dynamic TG analysis. In the first step, the elimination of coordinated water²⁷ molecules takes place. The VO^{IV} and Fe^{II} complexes lose their weight in the temperature range $\sim 130\text{-}240^\circ\text{C}$ and $120\text{-}260^\circ\text{C}$ respectively corresponding to coordinated water molecules. After the total loss of water, the organic moiety decomposes on further increase in temperature. The complete decomposition of the organic moiety occurs at $\sim 430\text{-}600^\circ\text{C}$ and the observed residue corresponds to respective metal oxide²⁸. The occurrence of endothermic peak and elimination of the water molecules at comparatively higher temperature confirm our earlier observation based upon the IR spectrum that the water molecules are coordinated in the metal complexes.

4. Antimicrobial Screening

4.1. Antibacterial Activity

Among the various complexes, the $\text{VO}(\text{IV})$ sulphato complex was found to be the most active against all the species. This pronounced activity may be explained as follows. The lipids and polysaccharides are important constituents of the cell wall and membrane, which are very much preferred for the metal ion interaction. The cell also contains many aminophosphates and carbonyl and cysteinyl ligands, which maintain the integrity of the membrane by acting as a diffusion barrier and also provide suitable sites for binding. Since the $\text{VO}(\text{IV})$ sulphato complex has one labile water molecule and coordinately unsaturated, the metal center in this complex can exchange water molecules for biological binding sites²⁹. As a result of chelation, the polarity of the metal ion is reduced. The increased lipophilic character favors the interaction of metal complexes of the cell, resulting in interference with the normal cell processes.

Table 4 Antibacterial activities of ligand and its complexes

Compound	<i>E. coli</i>			<i>P. aer.</i>			<i>S. typhi.</i>			<i>B. sub</i>			<i>S. pyo</i>			<i>S. aur</i>		
	10	20	30	10	20	30	10	20	30	10	20	30	10	20	30	10	20	30
Conc.	10	20	30	10	20	30	10	20	30	10	20	30	10	20	30	10	20	30
MBB	16	18	20	14	16	18	14	16	20	15	18	21	15	17	20	16	18	24
Standard	12	15	18	10	13	16	11	13	18	12	14	16	14	14	17	13	15	19
VO ₄ .MBB.H ₂ O	23	32	46	20	28	39	21	28	40	30	41	56	24	38	48	25	35	47
MnSO ₄ .2MBB	16	20	22	14	18	20	15	18	22	19	20	26	18	18	24	14	20	26
FeSO ₄ .MBB.2H ₂ O	21	29	37	19	24	35	18	23	33	26	30	41	22	29	39	19	31	38

4.2. Antifungal Activity

It is interesting to note that, the fungitoxicity of free ligand is less severe than that of metal chelates. Among the complexes screened, the order of activity was found to be VO(IV) complex > Fe(II) complex > Mn(II) complex > MBB. A possible mechanism of toxicity may be speculated in the light of chelation theory³⁰. Chelation considerably reduces the polarity of the metal ion mainly due to partial sharing of its positive charge with donor groups and possible -delocalization over the chelate ring. This increases the lipophilic character of the neutral chelate, which favors its permeation through lipid layers of fungus membranes. Furthermore, the mechanism of action of the compounds may involve the formation of a hydrogen bond through the uncoordinated O, S, and N hetero atoms with the active centers of the cell constituents. This results in interference with the normal cell process. The presence of lipophilic and polar substituents like C=O, C=S, SH, and NH are expected to enhance the fungitoxicity.

Table 4 Antifungal activities ligand and its complexes

Compound	<i>A. niger</i>			<i>A. flavus</i>		
	10	20	30	10	20	30
Conc.	10	20	30	10	20	30
MBB	11	13	17	14	19	23
Standard	09	11	14	12	16	17
VO ₄ .MBB.H ₂ O	26	31	40	22	27	34
MnSO ₄ .2MBB	18	22	25	16	21	25
FeSO ₄ .MBB.2H ₂ O	21	28	31	19	22	29

Conclusion

A new Mannich base ligand MBB and the corresponding VO^{IV}, Mn^{II} and Fe^{II} complexes were synthesized. The structures of the compounds were confirmed by elemental analyses and spectral studies. The spectral data revealed that the ligand acted as bidentate to the metal ion through the azomethine nitrogen and carbonyl oxygen atoms. Based on the electronic spectral data and the magnetic susceptibility measurements, all the complexes exhibited octahedral geometry. The ligand and the complexes were screened for their antibiological activities. All the antimicrobial studies showed that the VO(IV) sulphato complex was more active than the rest.

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