

Research Article

Aminomethylation, Structure and Biological activity of the new Mannich base and its Transition metal Complexes Derived from Isoindole-1, 3 (2H)-dione

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A new Mannich base N-(phenothiazinomethyl)phthalimide (PMP) was synthesised by Mannich condensation of phenothiazine, formaldehyde and phthalimide. The transition metal complexes with this ligand were synthesized using copper, cobalt and nickel halides in non-aqueous media. The ligand and metal complexes were characterized on the basis of elemental analysis, UV, IR and NMR spectral studies. The bidentate chelation of PMP, bonding through carbonyl oxygen of the imide moiety and C-N-C of the amine ring is suggested. Based on the IR, ¹H NMR studies octahedral geometry is assigned for all the metal complexes. The ligand and the three metal complexes were screened for their in vitro antibacterial and activities employing agar well diffusion technique. The complexes are found to be more active than the ligand.

Keywords: Mannich base, Phthalimide, transition metal complex, antibacterial activity

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Introduction

Mannich base complexes have remained an important and popular area of research due to their simple synthesis, adaptability and diverse range of applications. Many of the mannich base compounds synthesized by the condensation of a secondary amine and cyclic imide have been recorded. Isoindole -1, 3 (2H)-dione [1], phthalimide, is an aromatic imide, containing isoindole moiety, which is motif in nature. In the combined form with succinimides and maleimides, isoindole -1,3(2H)- diones used as plastic modifiers to improve resistance to heat, antioxidant and anti-foulant properties. Isoindole -1,3(2H) – dione and its derivatives have received much attention owing to the varied biological and pharmaceutical activities including antimicrobial [2, 3], antihypertensive [4, 5], anti-viral [6], antitumor [7], anti-inflammatory agents [8, 9], as a inhibitors of HIV-I integrase [10] and serve as ligands to form bioactive metal complexes [11-14]. They also exhibit liquid phase crystalline properties [15-18] with good antibacterial activities.

Materials and Methods**General**

All the reagents used for synthesis of the ligand and the metal complexes were of A.R. grade. The solvents used were commercial products of the available purity and were further purified by distillation. 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 NMR of the ligand was recorded on a Bruker instrument employing TMS as internal reference and DMSO-d₆ as solvent.

Synthesis of the ligand Phenothiazino Methyl Phthalimide (PMP)

0.05 mol of Phthalimide, and 0.05 mol of Formaldehyde are dissolved in 100 mL of ethanol and taken in a 250mL RB flask. Then 0.05 mol of the Phenothiazine dissolved in 50 mL ethanol added in small aliquots to the reaction mixture kept in ice bath and the stirring was continued for about 3hrs. Then it is cooled in refrigerator for overnight. After cooling it for a night, the contents are refluxed for about 5 hrs. Then, the solvent was recovered from the mixture by distillation. Mannich base separates out as pale yellow coloured solid. It is filtered and washed with hot water, recrystallised in alcohol and dried in air-oven at 60°C. The yield is found out to be about 72%.

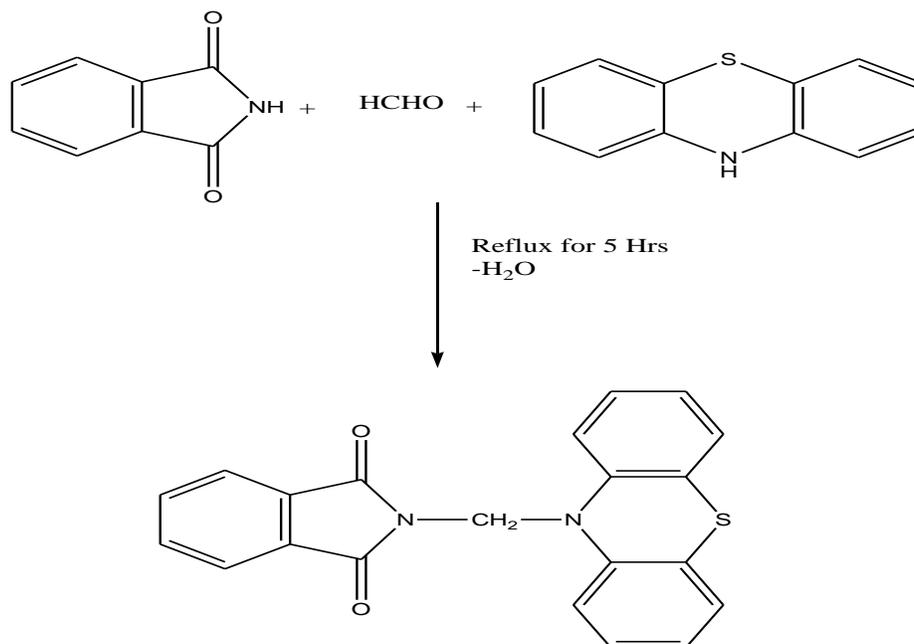


Figure 1 Reaction Scheme for the synthesis of the ligand

Synthesis of Metal complexes

0.01 mol of the ligand is dissolved in 10mL of ethanol taken in a 50mL RB flask and with this 0.01 mol of the metal chloride dissolved in 10mL of distilled water was added. Then the contents of the flask were refluxed for about 3 hrs. A characteristic coloured precipitate settles at the bottom. It was filtered, dried in air oven at 60°C and recrystallised from alcohol.

Table 1 Physical data of the ligand and the complexes

Compound	Yield (%)	Colour	Mp (°C)
PMP -Ligand	72	Pale yellow	240
PMP -Co	76	Pale pink	215
PMP-Ni	70	Pale green	242
PMP -Cu	78	Pale blue	224

Antibacterial activity

For the antibacterial study, nutrient agar was used as the medium. The ligand as well as the complexes were screened for antibacterial activity against certain pathogenic bacteria by disc diffusion method at concentration of 10µg / ml in DMSO using *Bascillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosaa*. 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.

The zone of inhibition was measured in mm and the activity was compared with Gentamycin in 1 µg / disc. A comparison of the diameters of inhibition zones of the compounds investigated shows that Cu(II) complex exhibit highest antibacterial activity against all the bacterial species studied. The results are tabulated in **Table 2**.

Table 2 Antibacterial activity

Samples	<i>Escherichia coli</i> (mm)	<i>Staphylococcus aureus</i> (mm)	<i>Bacillus subtilis</i> (mm)	<i>Pseudomonas aeruginosaa</i> (mm)
Ligand (50µl)	1.60±0.09	1.80±0.06	0.80±0.07	1.35±0.07
Co Complex (50µl)	2.60±0.17	1.40±0.11	1.50±0.07	2.20±0.07
Ni Complex (50µl)	2.10±0.05	0.80±0.01	0.90±0.01	1.40±0.02
Cu Complex (50µl)	5.30±0.40	4.10±0.37	3.70±0.23	4.70±0.28
Standard (30µl)	9.60±0.65	8.80±0.61	8.40±0.58	8.70±0.60
Control (Solvent)(30µl)	0	0	0	0

Results and Discussion

¹H NMR spectra

The peaks observed in the ¹H NMR spectra of the Mannich base under study are shown in **Figure 2**. The signals around 6.9- ppm corresponds to the hydrogens of the phenothiazine ring, and the signals around 7.0 -8 ppm are due to the phenyl protons. The appearance of peak at 5.7 ppm indicates the methylene hydrogens attached to the nitrogen. The aromatic protons gives a sharp peak at 7.8ppm. Further, the formation of the ligand is ascertained by the disappearance of a signal corresponding to the -NH proton of secondary amine as it was eliminated in the Mannich reaction.

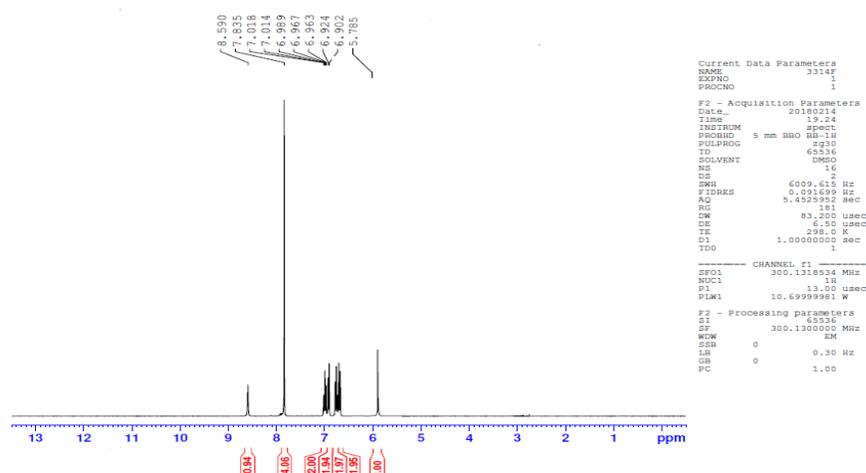


Figure 2 ¹H NMR spectra of the ligand

IR Spectra

The notable signal is the presence of an intense band at $\sim 1655\text{cm}^{-1}$ which is due to $\nu\text{C}=\text{O}$ carbonyl group. The most observable change in the IR spectra is the disappearance of the -NH stretching vibration and appearance of an intense band at 1188cm^{-1} due to $\nu\text{C}-\text{N}-\text{C}$ stretching which is formed due to the aminomethylation. The absence of band at 3300cm^{-1} due to amino -NH disappears implying its condensation after deprotonation. These results confirms the formation of the Mannich base.

In all the complexes, band due to $\nu\text{C}=\text{O}$ and $\nu\text{C}-\text{N}$ shifted towards lower frequency viz. 1596cm^{-1} in the case of carbonyl and 1101cm^{-1} for the -CNC clearly indicating the imido nitrogen and carbonyl oxygen are involved in coordination with metal ions. These changes confirm the formation of metal complexes.

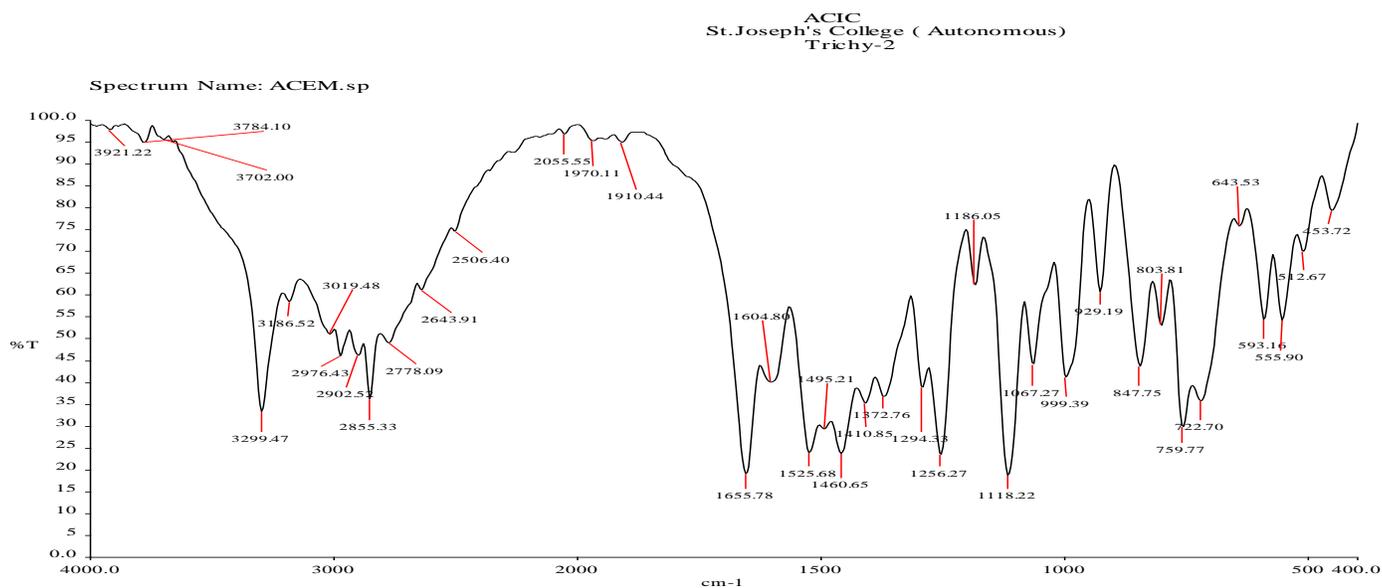


Figure 3 IR Spectra of the ligand

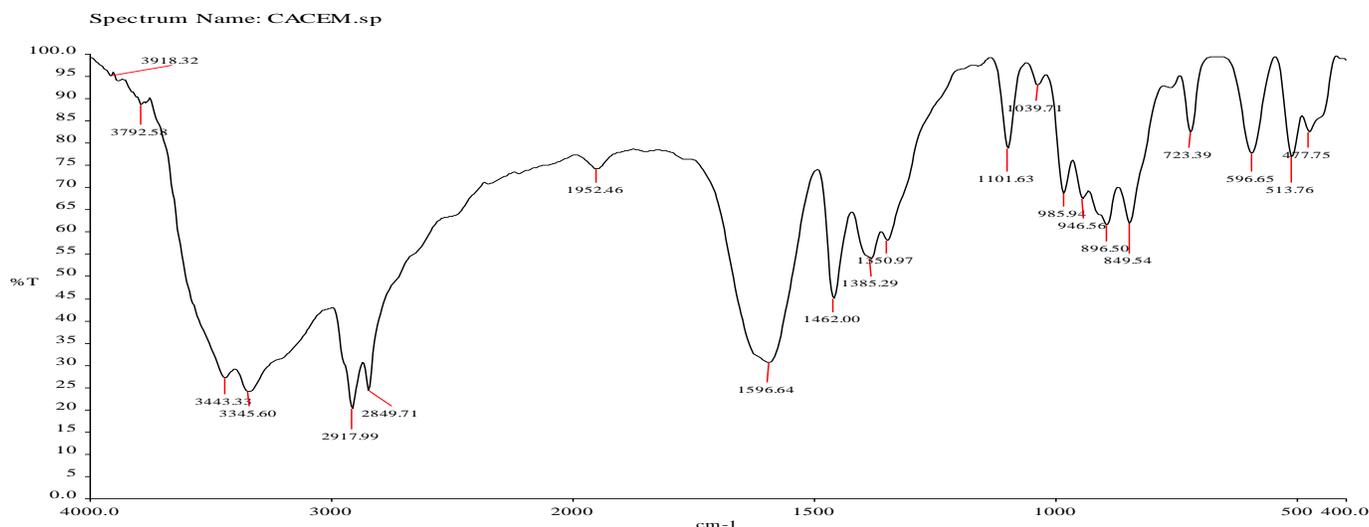


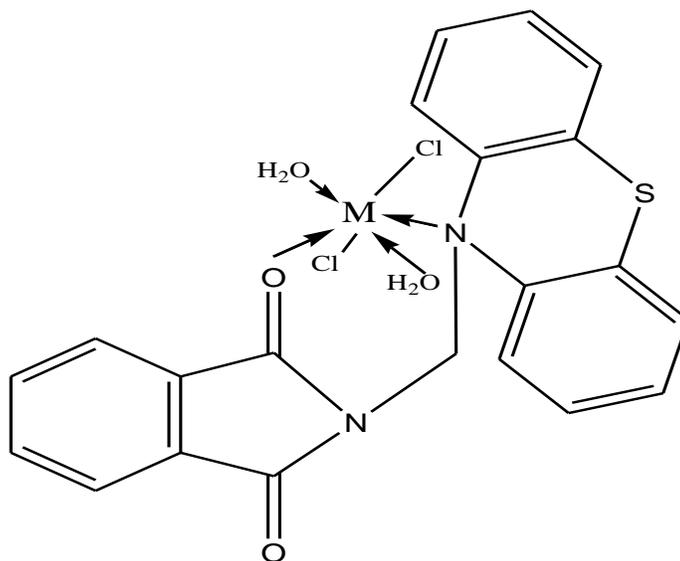
Figure 4 IR Spectra of the metal complex

Table 3 IR Spectral data of the ligand and the complexes

IR Signals	Interpretation
1655cm^{-1}	$>\text{C}=\text{O}$ bond
1186cm^{-1}	$-\text{C}-\text{N}-\text{C}$ stretching
1460cm^{-1}	Angular deformation of C-H

UV-Visible spectra

The absorption spectra of the ligand and metal complexes in methanol were recorded over the wavelength range of 200 to 600 nm. The UV spectrum of the Mannich base ligand showed sharp absorption maxima and the three main absorption bands at 202, 245 and 291 nm. The first band due to $\pi \rightarrow \pi^*$ transitions of the aromatic rings, underwent blue shift in the complexes indicating the metal complex formation. The second band ca. 245 nm is assigned to the carbonyl group of ligand chromophore. Further weak $d \rightarrow d$ transitions were observed owing to the transition metals being Laporte forbidden and hence these spectra were recorded at 100 ppm. Based on the foregoing observations and from the NMR spectral data and the IR spectral data of both the ligand and the metal complexes, the structure of the so formed complexes can be concluded as given below.



Where M- Cu, Co or Ni

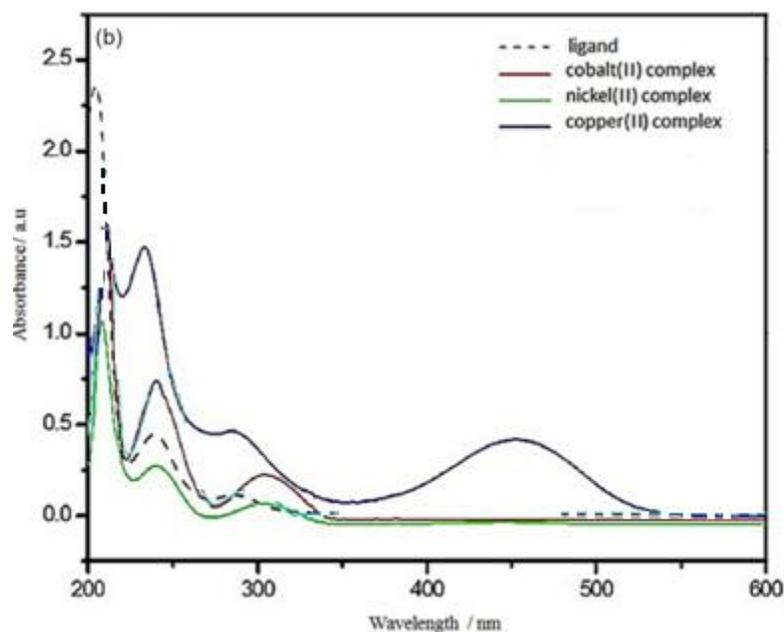


Figure 5 UV Spectra of the ligand and the metal complexes

Antimicrobial activity

The ligand as well as the complexes were screened for antibacterial activity against certain pathogenic bacteria by disc diffusion method at concentration of $10\mu\text{g} / \text{ml}$ in DMSO using *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. The zone of inhibition was measured in mm and the activity was compared with Gentamycin in $1\mu\text{g} / \text{disc}$. The results showed that the chelating tends to make the ligand act as more potent bactericidal agents, thus destroying more bacteria than the free ligand. Such increased activity of metal chelates can be explained on the basis of overtone concept and chelation theory. According to the overtone concept of cell permeability, the lipid membrane that surrounds the cell favours the passage of only lipid-soluble materials in which liposolubility is an important factor that controls the anti microbial activity. On chelation it increases the delocalization of π -electrons over the whole chelate ring and enhances the lipophilicity of complexes. This lipophilicity enhances the penetration of complexes into the lipid membranes and blocks the metal binding sites in enzymes of microorganisms. These complexes also disturb the respiration process of the cell and thus block the synthesis of proteins, which restricts further growth of the organism.

Conclusion

A new Mannich base ligand has been synthesized using phthalimide and made into complexation with three transition metals viz., copper, cobalt and nickel. All the four compounds, were characterized by physicochemical and spectral methods. It has been observed that the ligand behaves as a neutral bidentate chelating agent through the N and O donor sites through the imide and carbonyl moieties respectively. The spectroscopic data is in support of our expected structure and all the three complexes were assigned with octahedral geometry. The antibacterial property of the ligand and the complexes were studied using disc diffusion method and found that complexes possess better antibacterial activity than that of the free ligand.

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