ANTIBACTERIAL ACTIVITY STUDIES OF Co(II), Ni(II), Cu(II) AND Zn(II) COMPLEXES WITH MANNICH BASE LIGAND

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ABSTRACT. A Mannich base ligand (L) was prepared by reacting 2-mercaptobenzimidazole, diphenylamine and benzaldehyde. This ligand (L) was further used for the preparation of four metal complexes with Co(II), Ni(II), Cu(II) and Zn(II) ions. The reactions were monitored by TLC. The synthesized compounds were structurally characterized using FTIR spectroscopy, UV-Visible spectroscopy, ¹H- and ¹³C-NMR spectroscopy, ICP-OES/atomic absorption spectroscopy. It has been observed that the ligand (L) behaved in a monoanionic bidentate mode and the geometry of resulting complexes was tetrahedral. Conductivity analysis revealed their non-electrolytic nature. Antibacterial activity of the synthesized compounds was assessed through an agar well diffusion method against four strains of Gram-negative bacteria. L-Zn(II) complex showed best activity at all four concentrations against the four strains of Gram-negative bacteria.

KEY WORDS: Metal complexes, Mannich bases, 2-Mercaptobenzimidazole, Antibacterial activity

INTRODUCTION

Mannich base is a well known ligand obtained by the condensation of aldehydes, amines and active hydrogen substrates via Mannich reaction [1, 2]. The Mannich reactions are most versatile tools in organic synthesis, which provide a quantitative protocol for the construction of carbon–carbon bonds in organic scaffolds. Mannich bases of heterocyclic molecules have been grabbing the attention of the synthetic chemists for their multidirectional coordinating behavior and wide range of antimicrobial properties [3-5]. Such Mannich bases have also been well recognized as pharmaceutical agents for possessing a series of other biological activities like bactericidal [6, 7], fungicidal [8, 9], antimalarial [10], antitubercular, anti-inflammatory [11], analgesic [11, 12], anticancer [13] and anticonvulsant [14, 15].

Transition metals and their complexes played a vital role in the human health, are therefore of great interest as potential drugs [16-18]. The study of structural and binding features of various Mannich base complexes can play an important role in better understanding of the complex biological processes.

The present work includes the synthetic schemes for a Mannich base ligand (L) derived from 2-mercaptobenzimidazole and its transition metal complexes with Co(II), Ni(II), Cu(II) and Zn(II) ions. Structural elucidations as well as antibacterial activities of these compounds are part of this manuscript.

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**EXPERIMENTAL**

**Chemicals**

Diphenylamine, benzaldehyde and 2-mercaptobenzimidazole, zinc chloride (Riedel-de-Haën), hydrochloric acid 35%, copper chloride (Fluck Chemika), cobalt chloride and nickel chloride (Sigma Aldrich) were procured. The solvents methanol (MeOH), n-hexane, ethanol (EtOH) and tetrahydrofuran (THF) were distilled prior to use.

**Spectroscopic analysis**

The structure interpretation of ligand and its respective metal complexes was done by UV/Visible Spectrophotometer (Model T 90+), FT-IR Spectrophotometer (Agilent Micro Lab Carry 630 FTIR), Atomic absorption spectrophotometer (Model Perkin Elmer AA Analyst 100), ICP-OES (Perkin Elmer Optima 2100 DV), $^1$H-NMR and $^{13}$C-NMR (Avance 300-500). Conductance and melting points were recorded by Conductivity meter (Cyber scan 500 con) and (Gallen Kemp) apparatus, respectively.

**Synthesis of ligand (L)**

Equimolar solutions of 2-mercaptobenzimidazole (0.75 g, 5 mmol) and diphenylamine (0.84 g, 5 mmol) were dissolved in 40 mL of methanol in a round bottom flask. pH of the resulting solution was set to 3 by few drops of conc. hydrochloric acid. Later on, benzaldehyde (0.5 mL, 5 mmol) was added drop wise with continuous stirring to the above solution. The temperature of the reaction was elevated to 55 °C and kept for same up to 5 hours with vigorous stirring. Subsequently, precipitates were formed, which were collected by filtration, washed with small amount of methanol and n-hexane. Finally dried and stored in desiccator to prepare metal complexes.

![Scheme 1. Synthesis of ligand (L).](image-url)
Synthesis of metal complexes

The ligand (L) (0.40 g, 1 mmol) was dissolved in 20 mL of THF and pH of this solution was set to 8 by adding 2-3 drops of triethylamine in it. The resulting ligand solution was then added drop wise in the respective solution of metal salt (0.5 mmol) and stirred for 5-6 h followed by evaporation/concentration. The colored/colorless precipitates obtained were then filtered, washed with small amount of n-hexane, dried and stored in desiccator for further use. These quantities of each metal salts were used and pre-stirred; CuCl₂ (0.067 g, THF), ZnCl₂ (0.068 g, Methanol), CoCl₂ (0.064 g, ethanol) and NiCl₂ (0.064 g, ethanol).

Scheme 2. Proposed structures of metal complexes.

Scheme 3. Labeling of L for ¹H/¹³C-NMR interpretation.

Mannich base ligand (L). Molecular formula: C₂₆H₂₁N₃S; green solid; yield: 70%; melting point: 160–165 °C; IR (cm⁻¹): 1307 (C–N–C stretching), 3425 (N–H stretching), 1599 (CH bending); ¹H-NMR (DMSO-d₆, 400 MHz, δ): 5.35 (d, 1H, CH), 6.69–7.29 (m, 19H, ArH); 8.03 (d, 1H, NH); ¹³C-NMR (DMSO-d₆, 400 MHz, δ): 66.97 (s, C20), 144.7 (s, C7), 141.3–143.5 (s, C8, C14, C21), 116.3–135.5 (m, C1–6, C9–13, C15–19, C22–26).

L-Co(II) complex. Dirty green solid; yield: 60%; decomposition point: > 230 °C; M/L ratio: 1:2 (ML₂); Co(%), found: 5.9%, calculated: 6.6%; IR (cm⁻¹): 1308 (C–N–C stretching), 3391 (N–H stretching), 1596 (CH bending).
\textbf{L-Ni(II) complex.} Peach solid; yield: 65\%; decomposition point: > 170 °C; M/L ratio: 1:2 (ML$_2$); Ni(\%), found: 6.2\%, calculated: 6.6\%; IR (cm$^{-1}$): 1304 (C–N–C stretching), 3395 (N–H stretching), 1596 (CH bending).

\textbf{L-Cu(II) complex.} Brick red solid; yield: 85\%; decomposition point: >190 °C; M/L ratio: 1:2 (ML$_2$); Cu(\%), found: 7.5\%, calculated: 7.10\%; IR (cm$^{-1}$): 1314 (C–N–C stretching), 3515 (N–H stretching), 1594 (CH bending).

\textbf{L-Zn(II) complex.} White solid; yield: 82\%; decomposition point: >205 °C; M/L ratio: 1:2 (ML$_2$); Zn(\%), found: 9.81\%, calculated: 7.29\%; IR (cm$^{-1}$): 1308 (C–N–C stretching), 3344 (N–H stretching), 1596 (CH bending); $^1$H-NMR (CDCl$_3$, 300 MHz, $\delta$): 5.348 (s, 2H, CH), not observed (1H, NH), 6.48–7.24 (m, 38 H, ArH).

\textbf{Anti-bacterial activity}

Anti-bacterial activity of five compounds (L and its four metal complexes) was determined against four gram negative bacteria by agar well diffusion method (reference). Five different concentrations (100, 66, 33, 16.5, and 1.7 mg/mL) of each compound were prepared in DMSO. Four strains of gram negative bacteria, \textit{Klebsiella}, \textit{Proteus}, \textit{E. coli}, and \textit{Staphylococcus aureus} were swabbed on five sets of four L-agar plates. Using Pasteur pipette six wells were made in each plate having five wells of different dilutions ranging from 100 mg/mL to 1.7 mg/mL, and control well having DMSO only. After pouring 30 µL compound or control in each well, plates were incubated at 37 °C for 24 hours. Activity of each compound was determined by measuring inhibition zones in millimeters (mm) around each well [19].

\textbf{RESULTS AND DISCUSSION}

The ligand (L) and its metal complexes were prepared by the general outlines given above in experimental section (Schemes 1 and 2). All the metal complexes are stable solid towards air and moisture at room temperature; these are colored, except Zn(II) complex. Generally, the ligand and metal complexes are insoluble in most of the organic solvents, except DMSO and DMF. Physical properties of ligand and its respective metal complexes are given in experimental section.

\textbf{FT-IR spectra}

FTIR spectra of Ligand (L) and its four metal complexes were recorded in the range 4000–400 cm$^{-1}$. The presence of vibrational peaks in Ligand (L) at 3425 cm$^{-1}$ assigned to v(N–H) confirms that no reaction occurs at the –NH of 2-mercaptobenzimidazole instead it occurs from SH moiety. Another important feature of the FTIR spectrum of L is appearance of band at 1619 cm$^{-1}$, assigned to v(C=N) functional group present. The presence of C=N, NH functional groups in L and absence of stretching frequencies for C=S suggest that the Mannich reaction occurs through SH instead of NH moiety. These proposed structures are well supported by $^{13}$C-NMR, where chemical shift of C–S was observed at 167 ppm clarifying thiol (C–SH) moiety instead of C=S. $^{13}$C-NMR is a best tool to distinguish between C–SH and C=S, for C=S, chemical shift appears above 190 ppm [20]. The comparison of IR spectral data of ligand and metal complexes revealed the shifting of –NH and C–N–C peaks for the complexes. This suggests the bidentate coordinating behavior of ligand.
**Antibacterial activity studies of Co(II), Ni(II), Cu(II) and Zn(II) complexes**


### $^1$H-NMR spectra

The $^1$H-NMR of the Mannich base Ligand (L) was measured in DMSO-$d_6$. The $^1$H-NMR spectrum of L exhibits a multiplet in the range $\delta$ 6.69–7.29 ppm that can be attributed to the aromatic protons of benzimidazole and phenyl ring moieties. The peak for proton of –CH group that links the substrate 2-mercaptobenzimidazole and amino moiety has been observed as doublet at $\delta$ 5.35 ppm. Chemical shift at 8.03 ppm (L) represents the proton of –NH group of imidazole ring.

### $^{13}$C-NMR spectra

The $^{13}$C-NMR spectrum of L was measured and the chemical shifts of various carbons are given in experimental section. The main feature of this spectrum is the singlet at $\delta$ 144.7 and 66.97 ppm assigned to the carbons of C=S– and –CH groups. Disappearance of signal in higher chemical shift region (above 190 ppm for C=S moiety) [20] indicates and supports the reaction occurrence at sulfur of thiol group of 2-mercaptobenzimidazole.

### UV-Vis and magnetic moment data

UV-Visible and magnetic moment data is an important data to propose the geometry of metal complexes. In the electronic spectrum of the Co(II) complex, two bands at 11689 cm$^{-1}$, 17220 cm$^{-1}$ assignable to $^{4}A_2(F) \rightarrow ^{4}T_1(F)$ and $^{4}A_2(F) \rightarrow ^{4}T_1(P)$ transitions were observed. The appearances of these bands match well with the tetrahedral stereochemistry for Co(II) ion. This was further supported by its magnetic moment value at 4.17 B.M. [20]. The Ni(II) complex showed two bands at 11615 cm$^{-1}$, 15383 cm$^{-1}$ which can be attributed to $^{3}T_1(F) \rightarrow ^{3}A_2(F)$, $^{3}T_1(F) \rightarrow ^{3}T_1(P)$ transitions. These transitions together with magnetic moment value (3.09 B.M.) supports tetrahedral environment around the Ni(II) ion [21]. The Cu(II) complex exhibited a dingle broad band at 12946 cm$^{-1}$ that can be assigned to $^{3}T_2 \rightarrow ^{3}E$ transition of a tetrahedral geometry. The magnetic moment value of 2.19 B.M. of this complex further supported its tetrahedral geometry [20]. The Zn(II) complex was found to be diamagnetic.

### Thermal analysis study (TGA)

The thermal behavior of ligand (L) and its complexes was carried out via thermal analysis. Three steps decomposition was shown by all the metal complexes. The initial weight loss occurred between 30–220 °C attributed to removal of solvent molecules. Second decomposition occurred between 220–450 °C which has been attributed to loss of organic moiety and in third step, the curve becomes almost flat around 600 °C indicating decomposition of the organic part thus leaving behind respective metal oxide as residue.

### Antibacterial activity

Mannich bases are well recognized for their pharmaceutical properties including anti-bacterial, anti-inflammatory, anticancer, anti-tubercular, and anticonvulsant activities. In the present study, L-Zn(II) complex represent maximum inhibition among all the five compounds, at four different concentrations ranging from 100 mg/mL to 1.7 mg/mL against *Klebsiella*, *Proteus*, *E. coli*, and *Staphylococcus aureus*. Maximum activity was found at a concentration of 100 mg/mL as shown in the Figure 1. In addition, L-Ni(II) complex also represent remarkable antibacterial activity against *Klebsiella* at a concentration of 100 mg/mL.
Figure 1. Antibacterial activity of Manich base ligand (L) and its metal complexes against four different gram negative bacteria. Where Al-01 = L, Al-02 = L-Zn complex, Al-03 = L-Co complex and Al-05 = L-Cu complex.

Photo-physical studies

The fluorescence emission spectra of the ligand (L) and its Co(II), Ni(II), Cu(II) and Zn(II) complexes were recorded at excitation wavelength 350 nm at room temperature and are graphically represented in Figure 2. The metal complexes exhibited relatively broad bands assigned to charge transfer transitions. L-Zn(II) and L-Cu(II) complexes showed strong fluorescence emission with high intensity as compared to Ligand (L) and others metal complexes. The change in fluorescence intensity with formation of metal complexes is due to change in electron density on the ligand [22-25].

Figure 2. Fluorescence emission spectra of the ligand (L) and its metal complexes.
CONCLUSION

This research work includes the use of non-enolizable aromatic aldehyde (benzaldehyde) with 2-mercaptobenzimidazole and diphenyl amine in acid-catalyzed Mannich reaction to synthesize ligand (L). The resulting ligand was found to be a nice candidate for the coordination chemistry of Co(II), Ni(II), Cu(II) and Zn(II) resulting in tetrahedral geometry of these complexes. The ligand and metal complexes exhibited non-significant to appreciable antibacterial activity. L-Co(II) complex showed activity against *Staphylococcus aureus* and *Klebsiella* and no activity against *E.coli*, and *Proteus Fz-11*. L-Ni(II) complex also showed activity against *Klebsiella*.

Moreover, metal complexes particularly Cu(II)-L and Zn(II)-L exhibited an enhanced emission intensity compared to L and thus can be further investigated for the possible applications in light devices.

REFERENCES


