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Novel Hydroxy Pyrimidine based Chelating Ligand for Microbial Evaluation Studies

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ABSTRACT

Schiff base metal(II) complexes derived from 2,4-diamino-6-hydroxy pyrimidine with *O*-phthalaldehyde and their Zn(II), Cu(II) and Co(II) metal complexes have been synthesized using ethanol under reflux condition for 2 hours. The synthesized ligand and its metal complexes were characterized by UV-Vis, FT-IR and ¹H-NMR spectral techniques. The elemental analysis suggests the 1:1 (metal: ligand) as the stoichiometric ratio. FT-IR and ¹H-NMR confirm the formation of ligand and its corresponding metal complexes. HRMS data supports the formation of the compounds and its coordination towards metal complexes. Using well diffusion method, antimicrobial effects of the synthesized compounds and metal complexes were tested against four bacterial species. Metal complexes show significant biological activity than the Ligand.

KEYWORDS: Phthalaldehyde, Schiff base metal complexes, antimicrobial effects.

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INTRODUCTION

The major threat to the global health care is infectious diseases caused by the microorganisms. Recently, most of hospital deaths are caused by infectious pathogens. Antibiotics are commonly used to treat bacterial infections, which are known to target the growth process of the bacteria.

Schiff based metal complexes show excellent biological applications as antibacterial agents¹. Already, it has been reported that Schiff based complexes have remarkable antimicrobial activity^{2,3}. Phthalaldehyde derivatives have received great attention due to their potent biological activity as antimicrobial⁴⁻⁹. Thus, Our aim is to synthesis Schiff base metal complexes derived from 2,4-diamino-6-hydroxy pyrimidine with *o*-phthalaldehyde and their Zn(II), Cu(II) and Co(II) complexes and to assess the antibacterial properties of zinc(II) complex comparing with free ligand 2,2'-((6-hydroxypyrimidine-2,4-diyl) bis (azanylylidene)) bis (methanylylidene) dibenzaldehyde (HPADBA)¹⁰.

We have been investigated the biological activities of the ligand and its metal complexes. The antimicrobial activity of complexes and ligand were evaluated against four bacteria strains (*C.Tropicalis*, *S.Aureus*, *P.Aeruginosa* and *P.Mirabilis*). The solid Zn(II) complex and the ligand (HPADBA) are characterized by UV-Vis, IR, HR-LCMS and ¹H-NMR studies¹¹.

EXPERIMENTAL

Materials and Method

All the needed materials were purchased from Merck, India as AR grade. Ethanol was distilled and used. IR spectra were recorded from the range 4000 – 400 cm⁻¹ with KBr. The NMR spectra were carried out using a Bruker 400 MHz using TMS as an internal standard, DMSO is taken as the solvent. The mass spectra were obtained using acetonitrile as the solvent. The UV-Vis absorption and the Fluorescent emission spectra were recorded by using Shimadzu UV-(V-530) spectrophotometer and FP-6200 spectrofluorophotometer respectively.

Synthesis of 2,2'-((6-hydroxypyrimidine-2,4-diyl)bis(azanylylidene)) bis(methanylylidene) dibenzaldehyde (HPADBA) :

2,4- diamino-6-hydroxy pyrimidine (1.26 g) (1mmol) was mixed with *O*- phthalaldehyde (2.49 g) (2mmol) in ethanol and refluxed for 6hrs. A dark green colour precipitate starts separating out. It was filtered and dried and recrystallized using ethanol. (**Scheme 1**). Yield 60-65%.

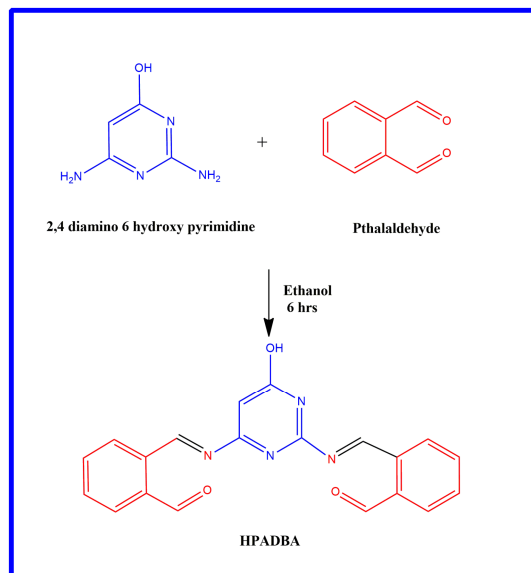


Fig.1: Synthesis of HPADBA (Scheme 1)

Synthesis of Zn(II) complex:

The metal(II) complex was synthesized by addition of solution of zinc(II) chloride (1mmol) to the solution of ligand (1mmol). The resulting mixture was stirred for 2 hrs and the complex was precipitated. The precipitate was filtered and washed with ethanol. (**Scheme.2**). Yield 67-75%.

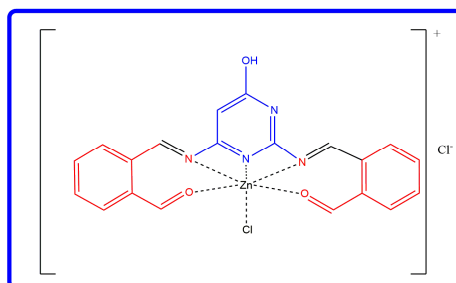


Fig. 2: zinc(II) complex of the ligand (HPADBA)- (Scheme 2)

Synthesis of Cu(II) complex:

The metal(II) complex was synthesized by addition of solution of copper(II) chloride (1mmol) to the solution of ligand (1mmol). The resulting mixture was stirred for 2 hrs and the complex was precipitated. The precipitate was filtered and washed with ethanol. (**Scheme.3**). Yield 70-71%.

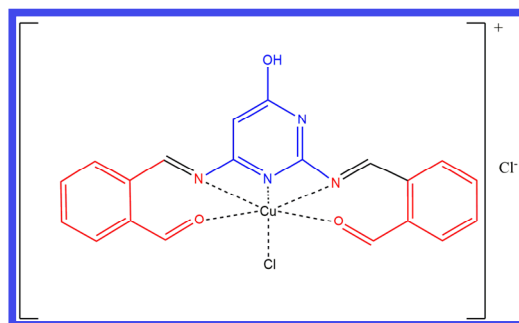


Fig. 3: Copper (II) complex of the ligand (HPADBA) - (Scheme 3)

Synthesis of Co(II) complex:

The metal(II) complex was synthesized by addition of solution of cobalt(II) chloride (1mmol) to the solution of ligand (1mmol). The resulting mixture was stirred for 2 hrs and the complex was precipitated. The precipitate was filtered and washed with ethanol. (Scheme.4). Yield 50-60%.

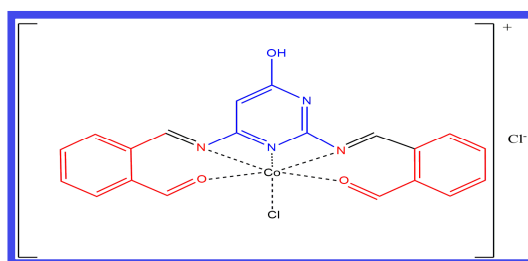


Fig. 4: Cobalt(II) complex of the ligand (HPADBA) - (Scheme 4)

RESULTS AND DISCUSSION

The metal(II) complexes of $[ML.Cl]Cl$ type were obtained through the reaction of (HPADBA) with the various metal salt in good yield. The HPADBA ligand and its Zinc, Cobalt and Nickel complexes were characterized through various spectral studies. **Table 1** depicted the analytical data and physical properties of the ligand and its complexes. The molecular formula of $[ML.Cl]Cl$ (stoichiometry 1:1) for copper(II), zinc(II) and cobalt(II) complexes is implied from Elemental analysis and Spectral data.

Table.1. Physical characterization, analytical data of the ligand (HPADBA) and its metal (II) complexes

Compounds	F.W. (g/mol)	Color	Found Calculated (%)				
			C	H	N	O	M
HPADBA	358.35	Green	67.03	3.94	15.63	13.39	-
			66.97	3.43	15.28	13.10	-
[Cu-HPADBA-Cl ₂]	474.91	Brown	50.38	2.96	12.74	6.71	13.33
			50.51	2.43	12.70	6.70	13.24
[Zn-HPADBA- Cl ₂]	475.9	Yellow	50.1	2.95	11.71	6.69	13.66
			50.2	2.86	11.51	6.54	13.43
[Co-HPADBA- Cl ₂]	470.98	Pale blue	50.87	2.99	11.48	6.78	12.48
			50.51	2.86	11.34	9.86	12.24

CHARACTERIZATION OF LIGAND (HPADBA):

The ligand(HPADBA) was characterized using ¹H-NMR data (Fig.5). ¹H-NMR was recorded using DMSO as the solvent.(N-CH) has a characteristic peak at 7.27 (δ).The aromatic protons has resonated in the range of (7.0-7.8)(δ).

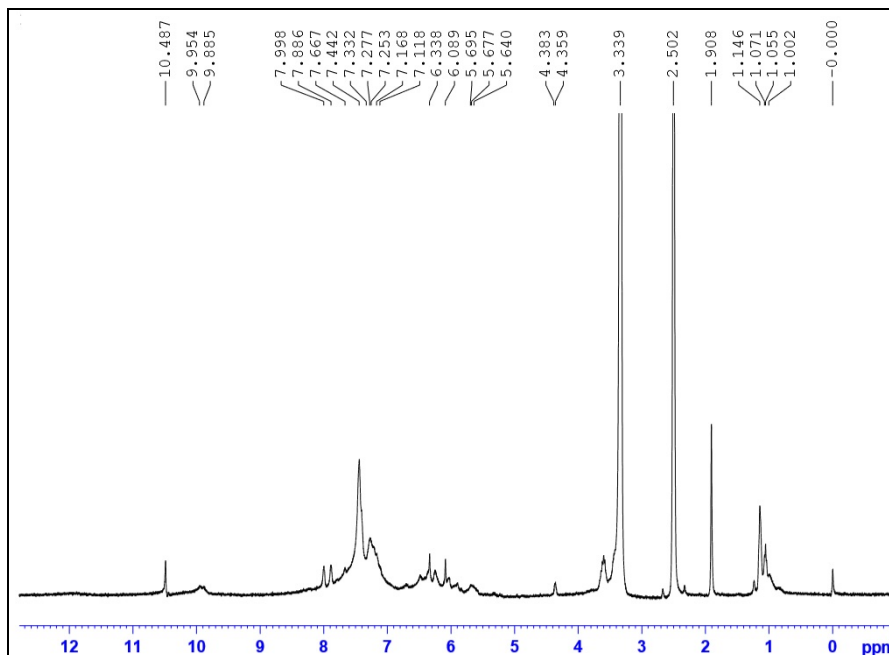


Fig.5 NMR Spectrum of ligand (HPADBA)

ABSORBANCE STUDIES:

The absorbance property of the (HPADBA) and its Co²⁺, Cu²⁺ and Zn²⁺ metal complexes were studied in ethanol. A new peak at 420 nm other than that of the ligand was observed for the metal complexes. Cobalt has absorbance at 518 nm. Zinc has absorbance at 520 nm and 695 nm. Copper has a absorbance at 410 nm and 752nm. The presence of absorption bands above 450 nm shows a bathochromic shift which clearly confirms the formation of corresponding metal complexes.

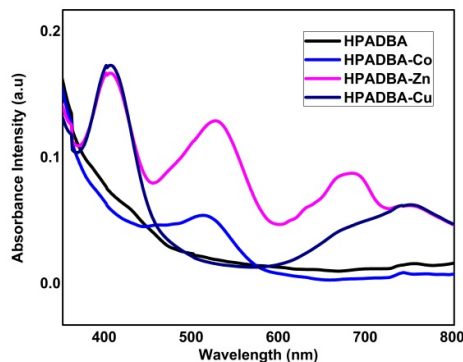


Fig.6. UV-Visible spectra of [HPADBA] and its Metal complexes

The IR spectra of the free ligand and metal complexes were recorded in the range 4000-400 cm^{-1} and the spectral data are listed in **Table.2**. A broad band centred at 3320 cm^{-1} is the characteristic of ν (OH). The IR spectrum of the ligand shows a band at 709,720, 713 and 718 due to the bonding of aldehydes oxygen with the metals, ν (M-O) stretching vibration. The (C-H) Bending peak is arrived at 810 cm^{-1} . The bands at 1620 cm^{-1} , 3220 cm^{-1} are due to ν (C=N) stretching, phenolic OH respectively. The FT-IR spectra are depicted in **Fig.7, Fig.8, Fig.9 and Fig.10**. IR spectrum of the Metal(II) complexes show significant variations and lower shifts compared with the free ligand confirms the complex formation¹²⁻¹⁴.

Table.2. IR spectral data of ligand (HPADBA) and its metal (II) complexes (cm^{-1})

Compounds	ν (C-H)	ν (C=N)	ν (-M-O)	ν (-OH)
HPADBA	810	1620	709	3320
Co-[HPADBA Cl]Cl	890	1590	720	3320
Cu-[HPADBA Cl]Cl	900	1588	718	3315
Zn-[HPADBA Cl]Cl	795	1560	713	3140

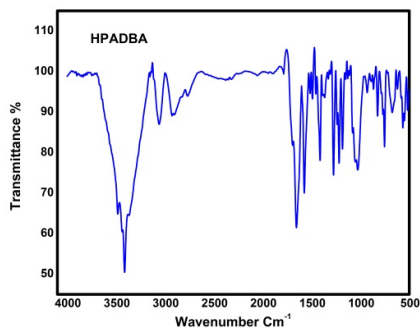


Fig.7. IR Spectra of ligand [HPADBA]

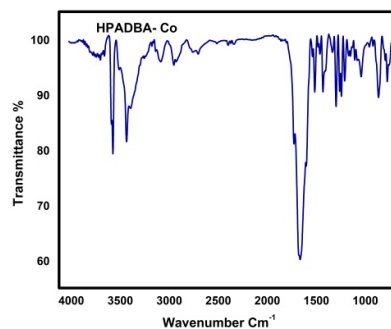


Fig.8. IR Spectra of Co(II)-[HPADBA] Complex

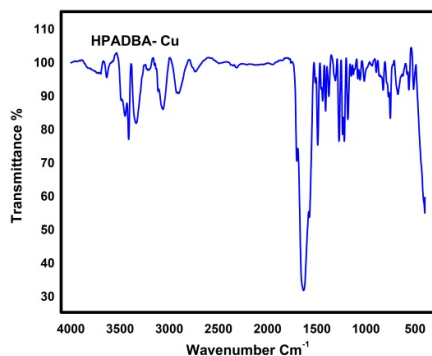


Fig.9. IR Spectra of Cu(II)-[HPADBA] Complex

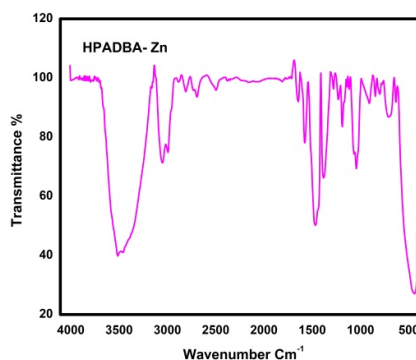


Fig.10. IR Spectra of Zn(II)-[HPADBA] Complex

CYCLIC VOLTAMMETRIC STUDIES:

Cyclic voltammetry is an important tool to measure the formal electrode potential of electron transfer reactions to follow unravel mechanisms of electrochemical oxidation and reduction processes. The redox behaviour of copper(II), zinc(II) and cobalt(II) complexes has been investigated by cyclic voltammetry using ethanol as solvent and Potassium Chloride (KCl) as supporting electrolyte. The cyclic voltammogram of all the complexes has both reduction and oxidation peaks which distinguishes itself from that of the ligand. Copper has one reduction peak at -0.12V in cathodic side and oxidation peak at 0.11V in anodic side. Cobalt and zinc has reduction peak at -1.8V and oxidation peak at 0.05V and -0.8V and 0.1V. The peak separation, $\Delta E_p = 0.75V$ which is greater than required for reversible process (59 mV) indicates that the redox couple is irreversible. It is shown in **Fig. 11**¹⁵⁻¹⁶.

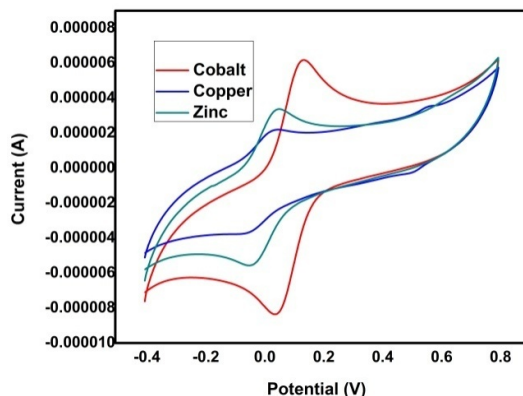


Fig.11. Cyclic voltammetric Spectra of Zn(II),Cu(II) and Co(II)-[HPADBA] Complex

MASS SPECTRAL STUDIES:

Mass spectrometry displays the spectra of the masses of the molecules comprising a sample of material. It is used for elucidating the chemical structures of molecules. The mass spectra of the **Zn(II)(HPADBA)** shows a peak at m/z 529 which is due to $(M+H+38)$ confirmed the 1:1 stoichiometric composition of the metal(II) complexes of $[M(HPADBA)Cl]Cl$ type as shown in **Fig.12**. The adduct formation is due to K^+ ions in it. The peak around 239.2 is due to the fragmentation of ligand HPADBA¹⁷⁻¹⁸.

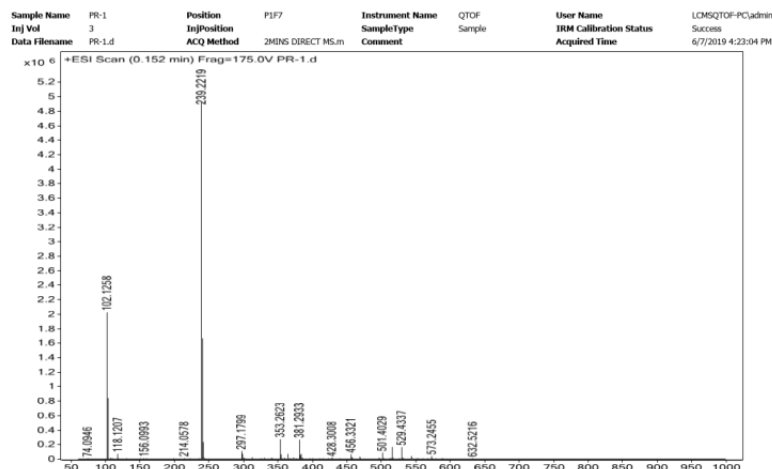


Fig 12. HR-LCMS mass spectrum of [Zn(HPADBA)Cl]Cl

BIOLOGICAL STUDIES

HPADBA and its complexes were tested against the bacteria *C.Tropicalis*, *S.Aureus*, *P.Aeruginosa* and *P.Mirabilis* by well diffusion method using *in vitro* method. Amikacin was taken as the standard for antibacterial studies. Every bacterial strain was incubated in Nutrient Broth (NB) at 37⁰C for 24 hours. The wells each of 5 mm were made in Muller- Hinton agar using cork borer. The stock solution was prepared in 10⁻³ mL⁻¹ concentration (DMSO) and then 100µl of the solution was transferred into each well. The plates were incubated for 24 hours and examined for clear inhibition of the zone around the well at 37⁰C. The inhibition zone was developed and measured. The antimicrobial studies reveal that *P.Aeruginosa* shows better activity with other organisms.

All the compounds showed a significant biological activity against four bacteria species. On comparing the biological activity of the ligand and its metal(II) complexes with the standard, the zinc complex shows potential antibacterial activity against all the bacterial strains.

Table.3. Antimicrobial activity data of the ligand and metal (II) complex(Zone of inhibition in nm)*

Compounds	<i>C.Tropicalis</i>	<i>S.Aureus</i>	<i>P.Aeruginosa</i>	<i>P.Mirabilis</i>	Amikacin
HPADBA	10	12	13	13	18
[Co(HPADBA)Cl]Cl	12	14	16	10	18
[Cu(HPADBA)Cl]Cl	15	15	12	9	18
[Zn(HPADBA)Cl]Cl	20	15	19	13	18

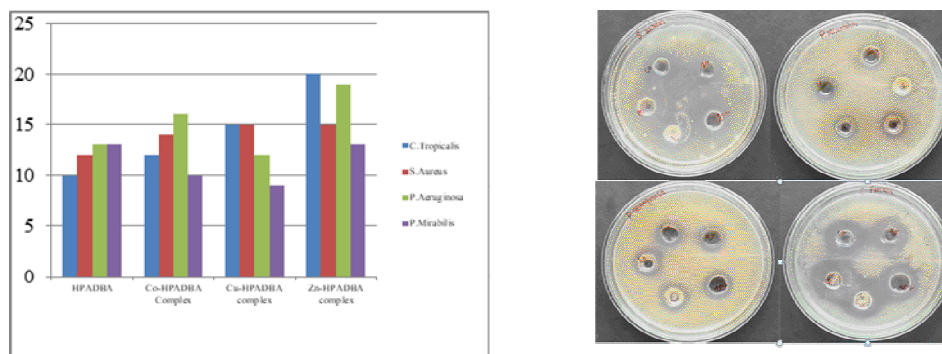


Fig.9. Inhibition Zones formed for (a)*C.Tropicalis* (b). *S.Aureus*,(c). *P.Aeruginosa* and (d)*P.Mirabilis*

CONCLUSION

In our research work, a novel *o*-phthalaldehyde based Schiff based ligand and complexes were synthesized and analysed. Biological activity of the ligand and complexes were evaluated with four different bacteria and the zinc(II) complex is found to have excellent antibacterial activity.

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