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Research Article

Chemistry for New drug discovery

Synthesis, Spectro Chemical Investigation of 4-Methyl Benzylamine Schiff Base Metal Complexes and Its Biological Studies

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Abstract: Schiff bases and their complexes are versatile compounds synthesized from the condensation of an amino compound with carbonyl compounds and widely used for industrial purposes and also exhibit a broad range of biological activities including antifungal, antibacterial, anti-malarial, anti-proliferative, anti-inflammatory, antiviral, and antipyretic properties. Many Schiff base complexes show excellent catalytic activity in various reactions in the presence of moisture. Over the past few years, there have been many reports on their applications in homogeneous and heterogeneous catalysis. The influence of certain metals on the biological activity of these compounds and their intrinsic chemical interest as multidentate ligands has prompted a considerable increase in the study of their coordination behaviour. Development of a new chemotherapeutic Schiff bases and their metal complexes is now attracting the attention of medicinal chemists. Primary benzylamines are highly important building blocks in the pharmaceutical and polymer industry. The present paper deals with the synthesis and characterization of metal complexes of new Schiff bases derived from 4-Methyl Benzylamine. The bidentate ligand is derived from the inserted condensation of 4-Methyl Benzylamine with 2-hydroxybenzaldehyde (OHB) in a 1:1 molar ratio. Using this bidentate ligand, complexes of Zn (II), Cd (II) with general formula ML_2 have been synthesized. The synthesized complexes were characterized by several techniques using IR, ^{1}HNMR and Mass analysis. Infrared spectral data indicate the coordination between ligand and central metal ion through deprotonated phenolic oxygen and azomethine nitrogen. In addition, the authors have screened the compounds for biological activity. It was found that the compounds have shown activity against the organisms like *Candida albicans*, *Bacillus subtilis* and *Salmonella typhi*.

Keywords: Synthesis, Characterization, Schiff base (OHB), 4-Methyl Benzylamine, Biological activity.

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1. INTRODUCTION

Schiff bases are versatile organic compounds which are widely used and synthesized by condensation reaction of different amino compounds with aldehydes or ketones known as imine. Schiff base ligands are considered as privileged ligands as they are simply synthesized by condensation. They show broad range of application in medicine, pharmacy, coordination chemistry, biological activities, industries, food packages, dyes, and polymer and also used as an O_2 detector. Coordination complexes are gaining importance in recent years especially in the designing of long-acting drugs in metabolism. The metal complexes from bidentate ligands have often been studied recently because of their technical applications [1,2] and applications in enhancement of drug action [3,4]. Transition metals are essential for normal functioning of living organisms, therefore of great interest as potential drugs[5]. The coordination chemistry of nitrogen donor ligands is an active area of research. A great deal of attention in this area has been focused on the complexes formed by metals with bidentate ligands using both oxygen and nitrogen[6,7]. The Schiff bases are an important class of ligands in coordination chemistry. The study of structural and binding features of various Schiff base complexes can play an important role in better understanding of the complex biological process. Schiff bases derived from 2-hydroxybenzaldehyde (OHB) are well known for their interesting ligation properties and exclusive applications in different fields[8,9,10]. It is well known from the literature that Schiff bases derived from 4-Methyl Benzylamine have a strong ability to form metal complexes[11]. The interaction of these donor ligands and metal ions gives complexes of different geometries, and literature survey reveals that these complexes are potentially more biologically active. Thus, in recent years Schiff bases and their metal complexes have attained much attraction because of their extensive biological activities[12,13]. Keeping the above fact in our mind and in continuation of our earlier work on transition metal complexes with Schiff bases[14,15], the ligand OHBMBA Schiff base(L) has been synthesized. In the present paper, the synthesis and characterization of the ligand and its complexes with Zn (II), Cd (II) are being reported.

2. MATERIALS AND METHODS

2.1 *Instrumentation*

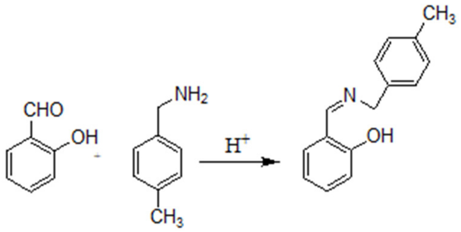
The Infrared spectra were recorded as potassium bromide (KBr) discs using a JASCO FT/IR-5300.The ^{1}H NMR spectra (400 MHz) were recorded using the ACF200 Broker Germany Spectrometer. Melting points were measured on an unmelt capillary melting point apparatus. All materials used in this investigation were purchased from Sigma/Aldrich and AR (Merck). Solvents used were of reagent grade and purified before use by the standard methods.

2.2 *Preparation of the ligand and its metal complexes: (4-Methyl Benzylamine and 2-hydroxybenzaldehyde Schiff base (OHBMBA)*

4-Methyl Benzylamine 4.22g (0.02 mol) and 2-hydroxybenzaldehyde 2.44g (0.02 mol) were dissolved in 25ml of methanol were taken in 250 mL borosil reflection flask and 1 mL of Acid. The mixture was refluxed for 3 hour on water bath and then cooled to room temperature, white coloured sharp needles were separated out and washed with methanol and dried in vacuum desiccators over $CaCl_2$ anhydrous.

2.3 *Preparation of 4-Methyl Benzylamine and 2-hydroxybenzaldehyde Schiff base (OHBMBA) metal complexes*

For the Preparation of Zn (II) and Cd (II) metal chloride salts were used. Dissolve 3.2942 g (0.01 mol) of newly synthesized ligand in adequate methanol. To this solution, aqueous solution of 1.3434 g (0.01mol) and1.718 g (0.01mol)[16-17] metal chlorides, and 1 mL of Sodium acetate. The mixture was refluxed for 6 hours in a water bath and then cooled to room temperature; Colourless sharp needles were separated out. The colourless metal complexes were washed with methanol and recrystallized from ether and dried in vacuum desiccator over $CaCl_2$ anhydrous. The prepared metal complexes were in 1:2 ratios.



Synthesis of Ligand

3. RESULTS AND DISCUSSION

3.1 *Infrared spectral analysis*

Infrared spectra were recorded with a JASCO FT/IR-5300 Spectrometer (4000-400 cm^{-1}) using KBr pellets. By utilizing this spectroscopy, the presence of important functional groups in the compound can be identified. Table 1 through light on the observation made in analysing IR spectra of ligand and metal complexes. The typical IR spectra are presented in Fig.1, 2 and 3.

3.2 *Interpretation of OHBMBA and Zn (II) and Cd (II) complexes*

The Infrared spectrum of the ligand was compared with the spectra of Zn(II) and Cd(II) complexes. The data was summarized in a table along their assignment. The typical IR spectra were shown in Fig.1, 2 and 3. The IR spectrum of the ligand has shown a broad band at 1643 cm^{-1} [14], which was assigned due to υC=N stretching of azomethine group. In complexes this band was shifted to lower regions, 1640 cm^{-}1 and 1639 cm^{-1} [18] for Zn(II) and Cd(II) complexes respectively, suggesting the involvement of azomethine group (>C=N) group in complexation. This was due to the reduction of electron density on nitrogen. There by indicating the coordination of the metal in through the nitrogen atoms. The IR spectrum of metal chelates shows the disappearance of the υ(OH)[19] bond at 3410 cm^{-1}. It indicates the proton displacement from the phenolic (OH) group on complexation. Thus, bonding of the metal ions to the ligands under investigation takes place through a covalent link with oxygen of the phenolic group. New bands were observed in the complexes, which were not observed in ligands. The bands at 749.80 cm^{-1} and 734.89 cm^{-1} were assigned to stretching frequencies of (M-O), the band at 687.47cm^{-1} and 665.45cm^{-1} were assigned to the stretching frequencies (M-N) respectively[20,21].

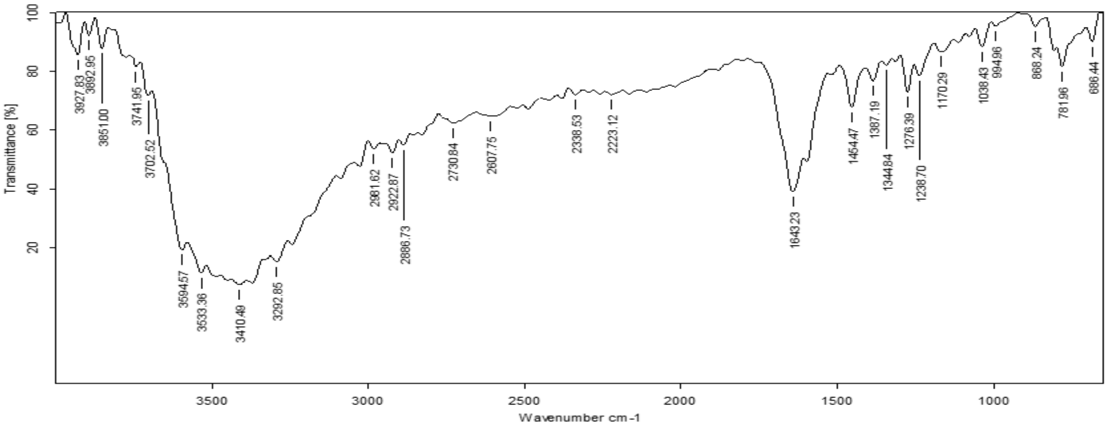


Fig:1 IR Spectrum of OHBMBA Ligand

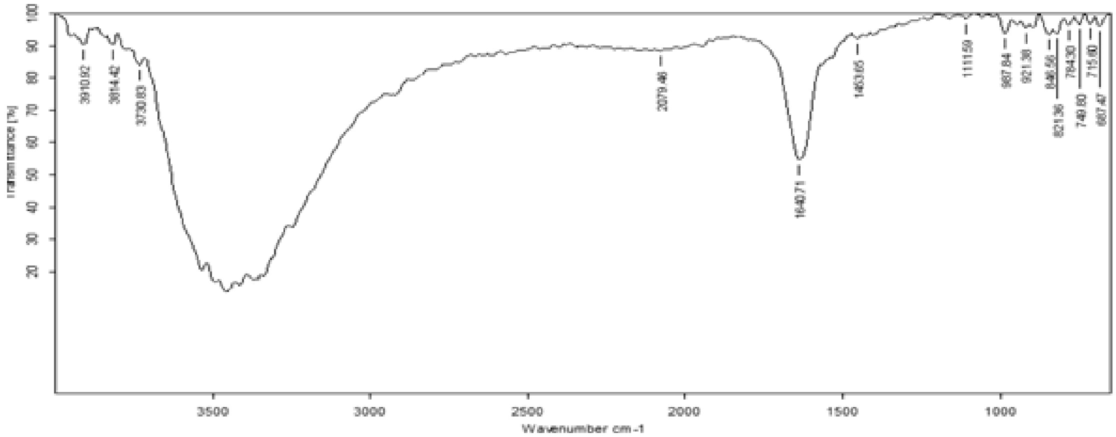


Fig:2 IR Spectrum of $[Zn(OHBMBA)_2]$ Complex

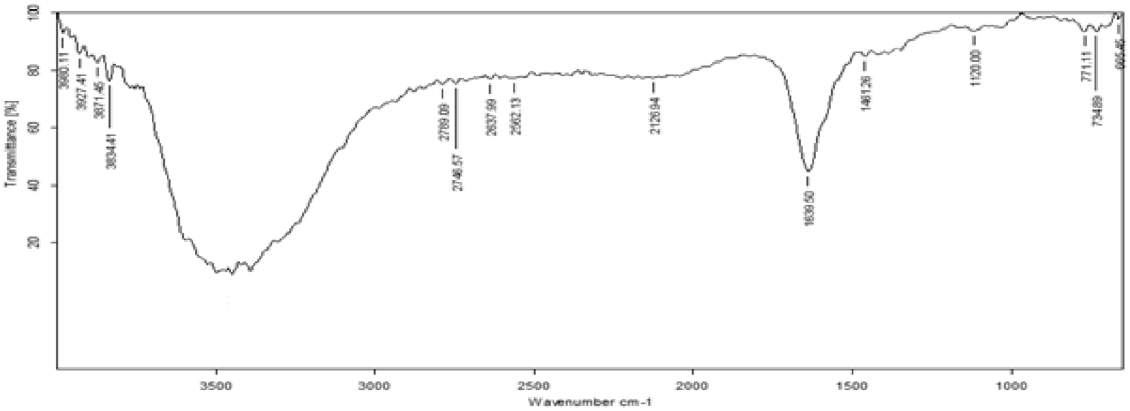


Fig:3 IR Spectrum of $[Cd(OHBMBA)_2]$ Complex

Table-1: The important IR bands of the Ligand and Their Metal Complexes

Compound	υ OH (Phenolic)	υ C=N	υ M-O	υ M-N
OHBMBA	3410	1643.23	-	-
OHBMBA-Zn	-	1640.71	749.80	687.47
OHBMBA-Cd	-	1639.50	734.89	665.45

3.3 Mass Spectral Studies

The mass spectra of azo Schiff base and its complexes were recorded at room temperature, and they are used to compare their stoichiometry composition. The azo Schiff base shows Base peak at 225 *m/z*. The base peak for the Zinc (II) complex observed at 584*m/z* confirms the stoichiometry of metal chelates as $[ML_2Cl_2]^{23}$ type. It is also supported by the mass spectra of the other complexes, whereas the Cadmium(II) complex shows base peak at 632 *m/z* confirming $[ML_2Cl_2]$ type. The mass spectral data support the structures of mononuclear transition metal complexes. The isotopic peaks from chlorine atoms are identified at the mass spectra of all compounds. The mass spectra of the ligand were shown in Figure 4.

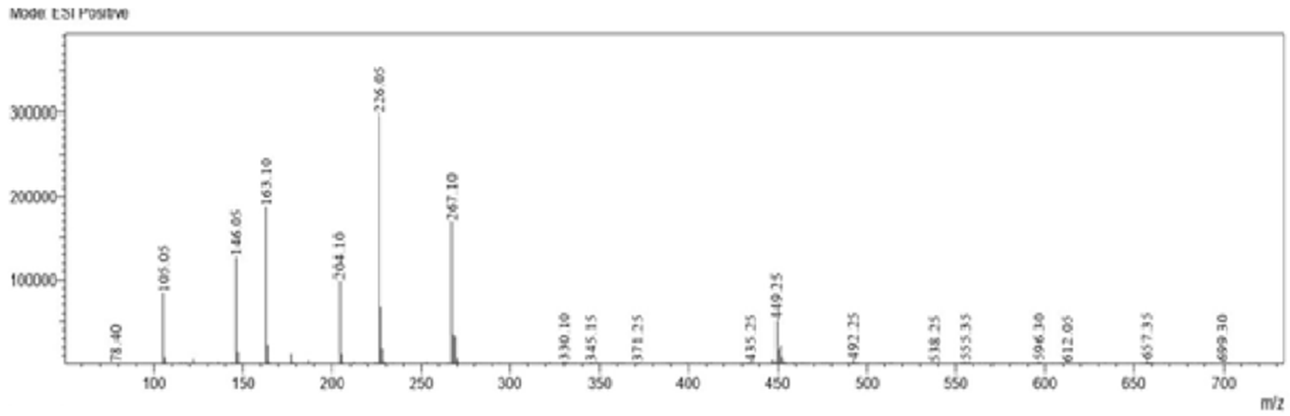


Fig:4 Mass Spectrum of (OHBMBA) Ligand

3.4 NMR Spectrum of OHBMBA Ligand and its Metal complexes

The ^1H NMR spectra of ligand and metal complexes in DMSO-d6 as solvent were used. An observed at 4.76 ppm [21] for ^1H NMR spectrum of the OHBMBA ligand is assigned to the methyl protons attached to azomethine (C=N) group. A singlet proton present on the azomethine group observed at 6.41 ppm. Another singlet methyl protons on the benzyl ring is observed at 2.34 ppm. Another multiplets observed at 6.3-7.66ppm for ^1H NMR spectrum of the = C-H proton of the phenyl ring. A singlet is observed in the region 9.0 due to the aromatic –OH proton[22]. In the ^1H NMR spectrum of the OHBMBA– Zn complex, a signal appeared due to methyl protons attached to azomethine group has been shifted to 4.76 ppm compared to 5.1 ppm in the case of ligand. This downfield shift indicates the deshielding of azomethine proton on coordination through the nitrogen atom of azomethine group. The multiplet observed in the region 6.6-6.9 ppm due to aromatic two C-H protons of phenyl ring for (Zn) complex may be due to the drifting of the ring of electrons towards the metal ion. Another multiplet observed in the region of 7.9-8.2 ppm[24] indicates two C-H protons of phenyl ring. In the ^1H NMR spectrum of the OHBMBA– Cd complex, a signal appeared due to methyl protons attached to azomethine group at 5.2 ppm. The multiplet observed in the region 7.8 ppm due to aromatic two C-H protons of phenyl ring for (Cd) complex. The multiplet observed in the region 6.6-6.9 ppm due to aromatic two C-H protons of phenyl ring for (Cd) complex may be due to the drifting of the ring of electrons towards the metal ion. In both the complexes, the O-H proton (9.0) was not observed indicating the deprotonation.

3.5 Biological activity

3.5.1 Method Employed

To prove the antibacterial activity of the ligand and metal complexes on bacteria, paper disc diffusion procedure was employed.

3.5.2 Medium

The nutrient agar medium was employed in the testing. Peptone (6g) was dissolved in a litre of distilled water. Beef extract (6g) was added to the peptone solution and agar (18g) was mixed. The contents were mixed thoroughly and the P^H was adjusted to 7.4–7.6. The solution was sterilized in the autoclave for 12–18 minutes at 15 lbs per sq. inch pressure, to prove the same.

3.5.3 Testing Equipment

Petri dishes, hot air oven, autoclave, sterilized pipettes, spreader, suitable Whatman filter paper and incubator.

3.5.4 Organisms selected for testing

Candida albicans, Bacillus subtilis and Salmonella typhi

3.5.5 Maintenance and Sterility

All the required apparatus was sterilized before use and every reasonable precaution was taken to avoid contamination throughout the operation.

3.5.6 Procedure of Testing

The solutions of the synthesized compounds were prepared at different concentrations viz 50ppm, 100ppm concentrations DMF solvent. Few sterilized filter paper (Whatman) discs soaked in the solvent were used for preparing the test solution. These sterilized paper discs were soaked in the 5ml of the solution of known concentration of the corresponding test samples, for 2–3 minutes[25]. Sufficient time was allowed for the solvent to evaporate from the paper discs. Sterilized nutrient agar 15–20 mL was poured into Petri dishes under aseptic conditions. The bacterial culture was poured over the solidified surface of the nutrient agar in Petri dishes and spread evenly for uniform distribution with a spreader. Paper discs soaked with the solution of known concentration of the desired test samples were placed under aseptic conditions at a distance in each Petri plate, containing known bacterial suspension. These Petri dishes were labelled with the compound number and incubated for 24 hours at 25–30°C in the incubator. After 24 hours of incubation each Petri plate was observed for bacterial growth. Later the zone of inhibition of bacterial growth in each Petri plate was measured. The discs soaked with the respective solvents of the test solution were used as controls. The bacterial growth in the test Petri plates was compared with the growth in the controls. The zone of inhibition of bacterial growth in the Petri plates under examination was measured.

3.6 Antibacterial Activity

The present investigation was an attempt to find out the antibacterial activity of ligands and their metal complexes against *Candida albicans, Bacillus subtilis* and *Salmonella typhi* in the range 50–200 urn/mL[26]. Choosing serial paper disc diffusion method 10-12. The Antibacterial activity results were given in the Table-2. The results of the biological activity of the metal complexes indicated the following facts.

Table 2: Antibacterial Activity of the Metal complexes Total Area of Zone of clearance in mm

S. No.	Compound	*Candida albicans*	*Bacillus Subtilis*	*Salmonella typhi*
1	OHBMBA	10	9	10
2	$Zn(OHBMBA)_2$	12	12	13
3	$Cd(OHBMBA)_2$	11	13	14

The high antimicrobial activities of all the newly synthesized metal complexes surmounting that of ligands showed that complexation of the organic moiety to the metal ions substantially enhanced their activities such increased activity of metal chelates[27, 28, 29, and 30]had been explained by Overtones concept and the Tweedy's chelation theory[13]. On chelation the polarity of the metal ion is reduced to a greater extent due to the overlap of the ligand orbital and partial sharing of positive charge of metal ion with donor groups. It was further noted that the delocalization of El–electrons over the whole chelate ring enhanced the lipophilicity of the complexes. This increased lipophilicity enhanced the penetration of the complexes into lipid membrane and blocking the metal binding sites on enzymes of microorganism thus retards the normal cell processes.

3.6 Anti-Bacterial Activity of the Complexes

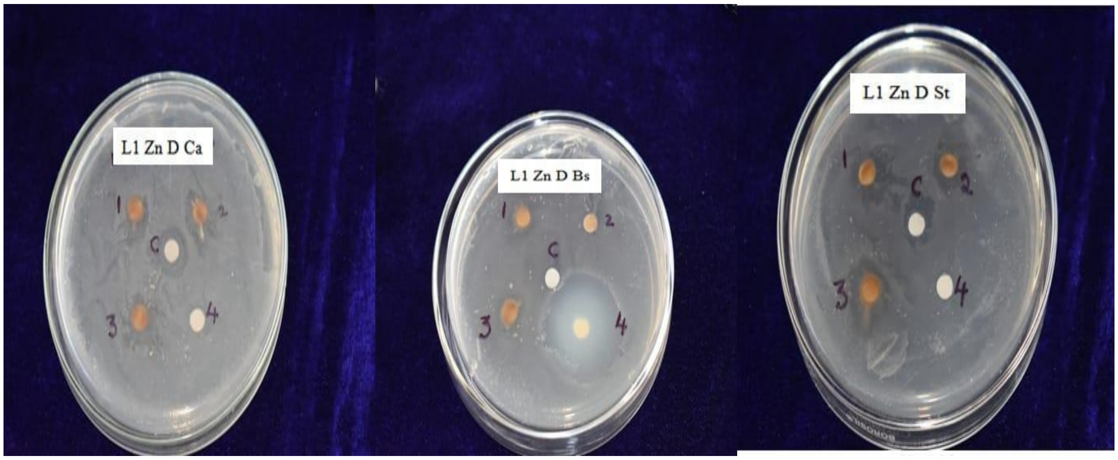


Fig: 5 [Zn $(OHBMBA)_2$] *Candida albicans,* [$Zn(OHBMBA)_2$] *Bacillus subtilis,* [$Zn(OHBMBA)_2$]*Salmonella typhi*

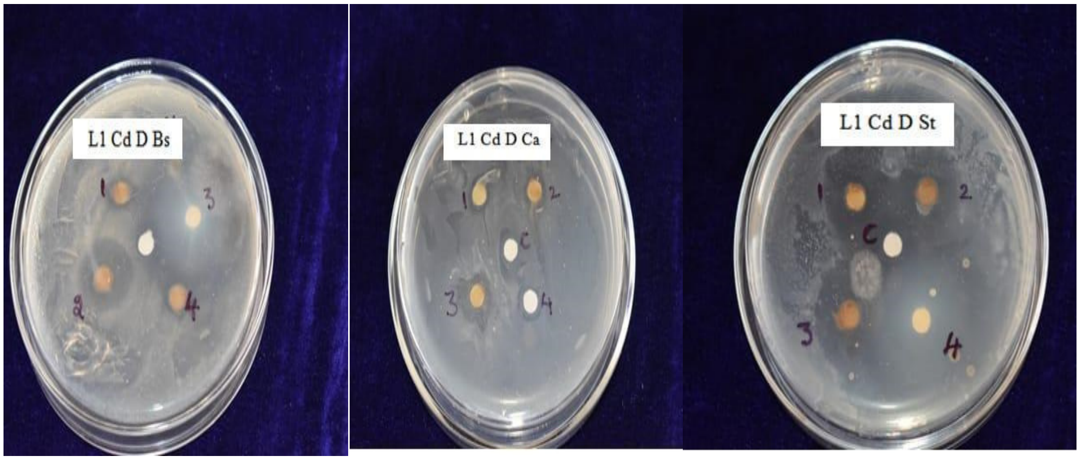


Fig: 6 [Cd (OHBMBA)$_2$] *Candida albicans, [*Cd (OHBMBA)$_2$] *Bacillus subtilis*, [Cd (OHBMBA)$_2$] *Salmonella typhi*

4. CONCLUSION

The outcome of the above results confirms IR spectra suggest that the ligand behaves as bidentate and coordinates to the central metal ion through azomethine nitrogen and phenolic –OH group. This has been further confirmed on the basis of NMR spectral studies. Thus, on the basis of above physicochemical and spectral studies the complexes are found to have higher biological activities as compared to the respective ligand and the parent drug that somehow justifies the purpose of the research work. The present work will be further extended to the synthesis of metal complexes using other biologically active metals and evaluation of their biological activities.

5. AUTHOR CONTRIBUTION STATEMENT

Gose Ramamohan and A.Jayaraju conceptualized and gathered the data with regard to this work. Prof. J. Sreeramulu, and N. Nagaraju analysed these data, Y.C. Pullanna gathered the data of Biological activity and necessary inputs were given towards the designing of the manuscript. All authors discussed the methodology and results and contributed to the final manuscript.

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7. CONFLICT OF INTEREST

Conflict of interest declared none

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