

Synthesis, Characterization, Thermal and Antimicrobial Studies of Novel Schiff Base Ligand and its Ni(II) and Zn(II) Complexes

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Abstract

The novel Schiff base ligand has been synthesized by the condensation of 2-hydroxy-5-methoxybenzaldehyde and 2-amino-4-chloro-6-methylpyrimidine. The Schiff base and its Ni(II) and Zn(II) complexes were synthesized and characterized by FT-IR, ¹H-NMR and UV-Vis techniques for the ligands and FT-IR, UV-Vis, all reactions monitored by TLC, molar conductivity and magnetic susceptibility measurements for the corresponding complexes. The complex is paramagnetic. The results of the molar conductivity measurements indicated that all complexes are non-electrolytes in (DMSO). An octahedral geometry for all the complexes of. The ligands are bidentate, (L 8) through phenolic (OH) and azomethine nitrogen. The ligand and its complexes were screened for their antifungal and antibacterial activity against *Aspergillus niger*, *Penicillium chrysogenum*, *Fusarium moniliforme*, *Aspergillus flavus* and *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *B. subtilis*. The result indicated that the complexes exhibited good antifungal and antibacterial activities.

Keywords: Heterocyclic Schiff bases, 2-hydroxy-5-methoxybenzaldehyde and 2-amino-4-chloro-6-methylpyrimidine. Antimicrobial Activity.

1. INTRODUCTION

In medicinal chemistry to reduce the side effects of cisplatin in cancer treatment [1], bioinorganic researchers are interested to find more co-ordination complexes with biological activity by changing the metal as well as ligand [2]. But no significant activity was found and did not satisfy the needs of cancer treatment because it produce a number of side effects like thrombocytopenia, constipation, alopecia, peripheral neuropathy, edema and anemia [3]. Among the coordinating ligands, Schiff base ligands alone shows important role in various biological applications [4], and also its biological activity was increased when a transition metal was placed due to chelation properties [5]. Nitro functional group attached with aromatic or heteroaromatic coordinating ligands were well known from the beginning in medicinal chemistry. Nitro group easily attracts the electron and converted to localized electron deficient functionality sites. These localized electron deficient functionality interacts with nucleophile present in the biological systems like enzymes, nucleic acid, amino acids and protein by nucleophilic addition, electron transfer reduction or oxidation and normal molecular complexation [6].

Drugs containing nitro groups are used in antibacterial, antifungal, insecticides and herbicides [7]. Recently nitro functionality compounds have good anticancer activity was also identified [8]. Sudden growth of nanotechnology and their efficient biological activity due to larger surface area and biocompatibility, researchers inclined towards synthesizing a well stabilized and air stable metal nanoparticles [9]. But stabilization of metal nanoparticles and its synthetic route and conditions are unfavorable in all methods [10]. Number of methods are used to synthesize metal nanoparticles in different shape and size and best one is a two-step phase transfer process named Brust-Schiffrein technique [11]. The reactivity of Schiff-base ligand and its solubility in organic solvents take part important role in synthesizing Schiff base ligand stabilized metal nano particles. The functional group attached in the Schiff-base is responsible for their reactivity. Schiff-base with different functional group like amines, thiols, imine, amide, and hydroxyl compounds are used to stabilize the metal nano particles. Only few reports are available to synthesize Schiff base ligand stabilized metal nanoparticles. Already

our research team reported different functionalities of imine stabilized metal nanoparticles of Cu, Ni, Pt and Au with extreme biological activity including anticancer activity with less toxicity [12-15].

In recent years, numerous studies have reported the synthesis and structural analysis of metal complexes of pyrimidines with bidentate and tridentate ligands (ONO donors) with microbial activity. i.e., from donor ligands and complexes [16,17]. Pyrimidines are endowed with a wide range of biological activities [18-21]. Chelation of metal ions with pyrimidine rings increases their activity due to the ready availability of potential binding sites. Complexed metal ions provide information on their coordination properties and provide insights into understanding the role of metal ions in biological systems [22].

A literature survey indicates that no work has been done on Schiff base transition metal complexes derived from 2-hydroxy-5-methoxybenzaldehyde and 2-amino-4-chloro-6-methylpyrimidine. In this communication, we report the synthesis of a bidentate Schiff base formed by condensation of 2-hydroxy-5-methoxybenzaldehyde and 2-amino-4-chloro-6-methylpyrimidine (Figure 3). Solid complexes of these ligands with Ni(II) and Zn(II) were prepared and characterized using various physicochemical methods.

2. MATERIALS AND METHODS

2.1. Reagents and Solvents

2-amino-4-chloro-6-methylpyrimidine (Aldrich sigma), and 2-hydroxy-5-methoxybenzaldehyde, metal nitrate of AR grade was used for synthesis of ligand and metal complex.

2.2. Synthesis of Ligand

The ligand was prepared by a modification of the reported methods [23-25]. The Schiff base ligand has been synthesized by refluxing a mixture of 0.01 mol (1.2015g) of 2-hydroxy-5-methoxybenzaldehyde and 0.01 mol (1.2710 g) of 2-amino-4-chloro-6-methylpyrimidine in 50 ml super dry ethanol refluxed for about 4h. Schiff base thus formed was cooled to room temperature and collected by filtration, followed by recrystallization in ethanol and dried *in vacuo* over anhydrous calcium chloride (Yield:75%).

2.3 Synthesis of metal complexes:

To a hot ethanol solution (25ml) of the ligand (2 mol) and (25ml) of metal Nitrate (1mol) was added with constant stirring. The pH of reaction mixture was adjusted to 7-8 by adding 10% alcoholic ammonia solution and refluxed for about 3 h. The precipitated solid metal complex was filtered off in hot condition and washed with hot ethanol and dried over calcium chloride in vacuum desiccators. (Yield: 70%)

2.4. Physical Measurement

IR spectra were recorded on FTIR (ATR)-BRUKER -TENSOR37 spectrometer using KBr pellets in the range of 4000-400 cm^{-1} . ^1H NMR (Varian mercury 300MHZ) spectra of ligand were measured in CDCl_3 using TMS as internal standard. X-RD were recorded on BRUKER D8 Advance. TGA- DTA were recorded on Shimadzu. The carbon, hydrogen and nitrogen contents were determined on Elementar model vario EL-III. The UV-visible spectra of the complexes were recorded on model UV-1800, SHIMADZU spectrometer. Molar conductance of complexes was measured on Elico CM 180 conductivity meter using 10^{-4} M solution in DMSO. Magnetic susceptibility measurements of the metal chelates were done on a Guoy balance at room temperature using $\text{Hg}[\text{Co}(\text{SCN})_4]$ as a calibrant.

3. RESULTS AND DISCUSSION

Schiff bases of 2-amino-4-chloro-6-methylpyrimidine and its complexes have a variety of applications including biological, clinical and analytical. The coordinating possibility of 2-amino-4-chloro-6-methylpyrimidine has been improved by condensing with a variety of carbonyl compounds. An attempt has been made to synthesize Schiff bases from 2-amino-4-chloro-6-methylpyrimidine with 2-hydroxy-5-methoxybenzaldehyde. Physical characteristics, micro analytical, and molar conductance data of ligand and metal complexes are given in (Table 1 and 2).The analytical data of complexes reveals 2:1 molar ratio (ligand: metal) and corresponds well with the general formula $[\text{ML}(\text{H}_2\text{O})_2]$ (where M= Ni(II) & Zn (II)). The magnetic susceptibilities of Ni(II) & Zn(II) complexes at room temperature are consistent with high spin octahedral structure with two water molecules coordinated to metal ion. The

presence of two coordinated water molecules was confirmed by TGA-DTA analysis. The metal chelate solutions in DMSO show low conductance and supports their non-electrolyte nature.(Table 1)

Table1. Physical characterization, analytical and molar conductance data of compounds

Compound Molecular formula	Mol.Wt.	M.P. Decomp temp. °C	Colour	Molar Conduc. Mho Cm ² mol ⁻¹	μ _{eff} (BM)
L ₈	312.12	234	Reddish Brown	----	--
Ni- L ₈	554.10	>300	Yellowish Green	20.10	1.95
Zn- L ₈	560.10	>300	Faint pink	23.10	D

Table 2. Elemental Analysis of Ni(II) and Zn(II) Complex:-

Compound	Found (Calculated)			
	C	H	N	M
L ₈	55.71 (54.81)	5.57 (5.67)	14.49 (14.39)	--
Ni-L ₈	46.35 (45.23)	4.45 (4.33)	10.16 (10.03)	14.68 (14.63)
Zn-L ₈	56.32 (56.23)	5.40 (5.33)	15.19 (15.03)	11.44 (11.43)

3.1. ¹H-NMR Spectra of Ligand

The ¹H-NMR. Spectra of free ligand at room temperature show the following signals. 2.35 δ (s, 3H, Methyl hydrogen bonded to pyrimidine ring), 2.35 δ (s, 3H, Methyl hydrogen bonded to phenyl ring), 5.47 δ (s, 1H, Phenolic (OH) hydrogen of pyrimidine ring), 6.77 δ(s, 1H, Hydrogen bonded to pyrimidine ring), 7.84 δ (s, 1H, hydrogen bonded to azomethine carbon), 7.2-7.42 δ (D,4H, Aromatic Ha, Hb, protons of phenyl ring).

3.2. IR Spectra

The IR spectra of the complexes are compared with that of the ligand to determine the changes that might have taken place during the complexation The bands at 3363, 1678, 1516, 1309, and 1186 cm⁻¹ assignable to ν OH (intramolecular hydrogen bonded), ν C=C(aromatic), ν C=N (azomethine), ν C-N (aryl azomethine) and ν C-O (phenolic) stretching modes respectively[26-28] The absence of a weak broad band in the 3200-3400 cm⁻¹ region, in the spectra of the metal complexes suggests deprotonation of the intramolecular hydrogen bonded OH group on complexation and subsequent coordination of phenolic oxygen to the metal ion. This is further supported by downward shift in ν C-O (phenolic) [29] with respect to free ligand. On complexation, the ν (C=N)[30]band is shifted to lower wave number with respect to free ligand, denoting that the nitrogen of azomethine group is coordinated to the metal ion. The ν C-N band is shifted to lower wave number with respect to free ligand, The IR spectra of metal chelates showed new bands in between the 500-600 and 400-500 cm⁻¹ regions which can be assigned to ν M-O and M-N [31] vibrations respectively The IR spectra of Ni (II) show a strong band in the 3050-3600 cm⁻¹ region, suggesting the presence of coordinated water in these metal complexes. The presence of coordinated water is further confirmed by the appearance of non-ligand band in 830-840 cm⁻¹ region, assignable to the rocking mode of water. The presence of coordinated water is also established and supported by TGA/DTA analysis of these complexes. Hence it is concluded that the coordination takes place via phenolic oxygen and azomethine nitrogen of ligand molecule.

3.3. Thermogravimetric Analysis

The dynamic TGA with the percentage mass loss at different steps have been recorded. The simultaneous TGA/DTA analysis of Ni (II) was studied from ambient temperature to 1000 °C in nitrogen atmosphere using α-Al₂O₃ as reference. An analysis of the thermogram of the complexes indicated that Ni(II) complexes ligand L (Figure 1) show two step decomposition. The first weight loss 5.61 % , in between temp. 50-195°C could be correlated with the loss of two molecules of lattice water (calcd 6.50 %). The anhydrous compound does not remain stable at higher temperature, it undergoes rapid decomposition in the range 195-570°C, with 79.45 % mass loss corresponds to decomposition of the complex (calcd. 79.14 %) in second step. The decomposition is completed leading to the formation of stable residue of metal oxide NiO obs. 11.23 % (calcd. 14.35 %). kinetic and thermodynamic viz the energy of activation (E_a), frequency factor (Z), entropy change (-ΔS) and free energy change (ΔG) for the non-isothermal decomposition of complexes have been determined by

employing Horowitz-Metzger method [32] values are given in Table 3. The Calculated values of the given activation energy of the complexes are relatively low, indicating the autocatalysis effect of metal ion on the thermal decomposition of the complex. The negative value of activation entropy indicates that the activated complexes were more ordered than the reaction was slow. The more ordered nature may be due to the polarization of bonds in the activated state, which might occur through charge transfer transitions [33].

Table 3. The kinetic and thermodynamic parameters for decomposition of metal complexes

Complex	Step	Decomp.Temp. (°C)	n	Ea (kJmole ⁻¹)	Z (S ⁻¹)	ΔS (JK ⁻¹ mole ⁻¹)	ΔG (kJmole ⁻¹)	Correlation coefficient
Ni-L ₈	I	150	0.1	3.77	4.77 × 10 ⁴	-177.78	12.81	0.994
	II	490	0.8	12.26	1.48 × 10 ⁴	-172.88	28.12	0.987

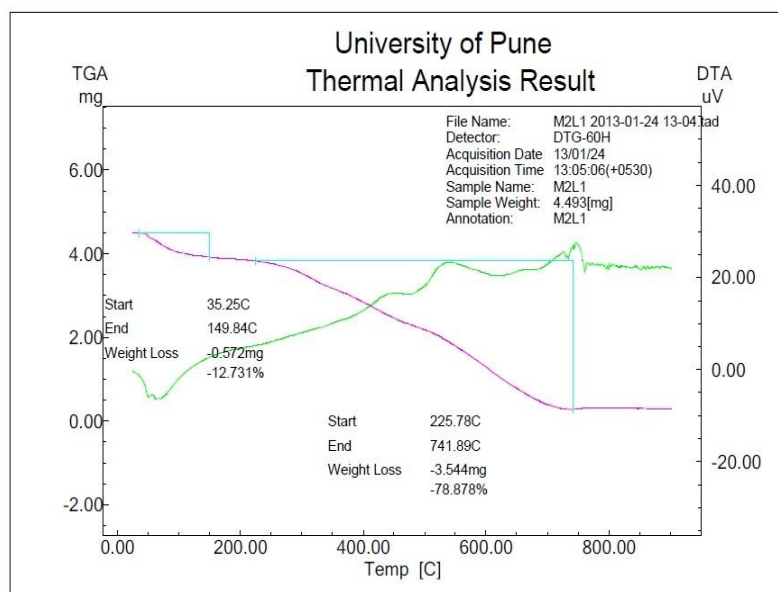


Figure 1. TGA-DTA Curve of Ni (II) Complex of Ligand L₈

3.4. Magnetic Measurements and Electronic Absorption Spectra

The electronic spectral studies of metal complexes of Cu (II) with Schiff bases were carried out in DMSO solution. The absorption spectrum of the Cu(II) complex shows bands at 13812 cm⁻¹ and 30030 cm⁻¹ are assigned to ²B_{1g} → ²A_{1g} and charge transfer respectively in an octahedral field [34]. The Ni(II) and Zn(II) complexes were Paramagnetic & diamagnetic in nature.

3.5. Molar Conductivity Measurements

The metal (II) complexes were dissolved in DMSO and the molar conductivity of 10⁻⁴M of their solution at room temperature was measured. The lower conductance values of the complexes support their non-electrolytic nature of the compounds.

3.6. Powder X-ray Diffraction

The x-ray diffractogram of Zn (II) complexes of L₈ was scanned in the range 20-80° at wavelength 1.543 Å (Figure 2). The diffractogram and associated data depict the 2θ value for each peak, relative intensity and inter-planar spacing (d-values) Table 4. The diffractogram of Zn(II) complex of L₈ had fifteen reflections with maxima at 2θ = 12.89° corresponding to d value 6.86Å. The x-ray diffraction pattern of these complexes with respect to major peaks of relative intensity greater than 10% has been indexed by using computer programmed [35] The above indexing method also yields Miller indices (hkl), unit cell parameters and unit cell volume. The unit cell of Cu(II) complex of L₈ yielded values of lattice constants, a= 9.76 Å, b=10.24 Å, c = 27.24 Å and unit cell volume V=2722.43096 Å³. In concurrence with these cell parameters, the condition such as a = b = c and α = β = γ = 90° required for

sample to be Monoclinic were tested and found to be satisfactory. Hence it can be concluded that Zn(II) complex has Orthorhombic crystal system. Hence it can be concluded Zn (II) complex of L₈ has monoclinic crystal system. The experimental density values of the complexes were determined by using specific gravity method [31] and found to be 0.8968 gcm⁻³ for Zn (II) complexes respectively. By using experimental density values, molecular weight of complexes, Avogadro's number and volume of the unit cell were calculated. Number of molecules per unit cell were calculated by using equation $\rho = nM/NV$ and was found Zn (II) complexes respectively. With these values, theoretical density were computed and found to be 0.8858 gcm⁻³ for respective complexes. Comparison of experimental and theoretical density shows good agreement within the limits of experimental error [33].

Table 4. Indexed X-ray Diffraction Data of Zn(II) Complex of Ligand L₈

Peak No.	2θ (observed)	2θ (calculated)	d (observed)	d (calculated)	Miller indices of Planes			Relative intensities (%)
					h	k	l	
1	6.65767	6.67146	6.63884	6.63044	1	0	2	100
2	8.979	8.9811	4.93263	4.93438	1	1	3	35.30
3	12.103	12.10039	3.67228	3.67465	2	2	1	29.22
4	13.96676	13.97132	3.19032	3.19049	0	1	6	8.90
5	14.83933	14.84833	3.00664	3.00591	1	1	6	10.09
6	16.75553	16.74528	2.67115	2.67356	2	3	4	17.53
7	20.80287	20.81213	2.16839	2.16799	1	5	5	6.83
8	23.52078	23.52943	1.92977	1.92951	2	6	3	15.40
9	24.82542	24.83049	1.83431	1.83433	4	1	6	1.75
10	26.39703	26.42041	1.73229	1.73119	0	2	11	5.58
11	29.21113	29.21608	1.57813	1.57814	1	8	4	1.96
12	32.71821	32.72699	1.42493	1.4248	6	2	3	1.63
13	34.00826	34.00059	1.37704	1.3775	0	2	14	2.02

Unit cell data and crystal lattice parameter

a (Å) = 8.97	Volume(V) = 2397.94124 (Å ³)
b (Å) = 13.57	Density(obs.) = 1.2554 gcm ⁻³
c (Å) = 19.70	Density(cal.) = 1.2444 gcm ⁻³
α = 90.00	Z = 3
β = 90.00	Crystal system = Orthorhombic
γ = 90.00	Standard deviation (%) = 0.038
	Porosity = 0.883%

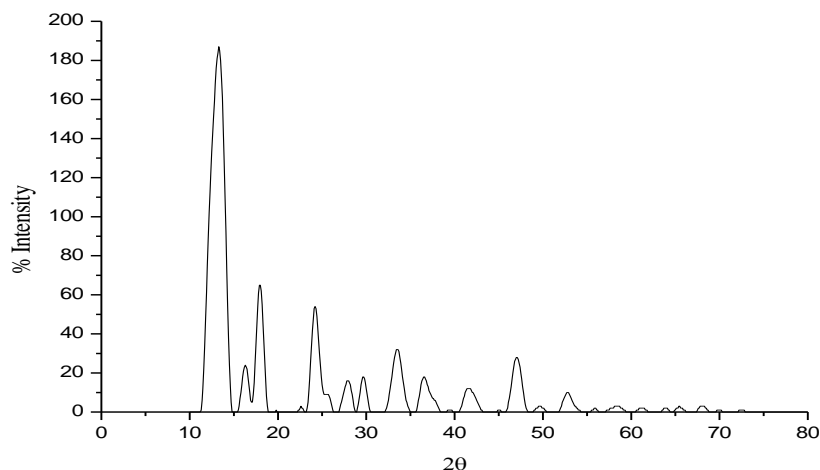


Figure 2. X-ray Diffractogram of Zn (II) complex of L₈

4. ANTIBACTERIAL ACTIVITY

Antifungal activity and Antibacterial activity of ligand and metal complexes were tested *in vitro* against fungal such as *Aspergillus niger*, *Penicillium chrysogenum*, *Fusarium moneliforme*, *Aspergillus flavus* and bacteria such as *E. Coli*, *B.Subtilis*, *S. Aurious* And *Bacillus subtilis* by paper disc plate method [36-39] The compounds were tested at the concentrations 1% and 2% in DMSO and compared with known antibiotics *viz* *Griseofulvin* and *Penicilin*. (Table 4 and 5)., it is found that the inhibition by metal chelates is higher than that of a ligand and results are in good agreement with previous findings with respect to comparative activity of free ligand and its complexes [40]

Table 4. Antifungal activity of ligands

Test Compound	Antifungal growth								
	Aspergillus niger		Penicillium chrysogenum		Fusarium moneliforme		Aspergillus flavus		
	1%	2%	1%	2%	1%	2%	1%	2%	
L ₈	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Ni- L ₈	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Zn- L ₈	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
+ve control	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
-ve control (Griseofulvin)	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve

Ligand & Metal: +ve – Growth (Antifungal Activity absent)

-ve - Growth (Antifungal Activity present)

RG - Reduced Growth (More than 50% reduction in growth observed)

Table 5. Antibacterial activity of ligands and their metal complexes

Test Compound	Diameter of inhibition zone (mm)							
	E. Coli		Salmonella typhi		Staphylococcus aureus		Bacillus subtilis	
	1%	2%	1%	2%	1%	2%	1%	2%
L ₈	14mm	18mm	16mm	18mm	20mm	25mm	19mm	22mm
Ni- L ₈	11mm	16mm	15mm	16mm	14mm	22mm	15mm	18mm
Zn- L ₈	14mm	16mm	13mm	16mm	17mm	22mm	11mm	18mm
DMSO	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Penicillin	14mm	14mm	17mm	17mm	30mm	30mm	19mm	19mm

Ligand & Metal: -ve

- No Antibacterial Activity

Zone of inhibition - -- mm

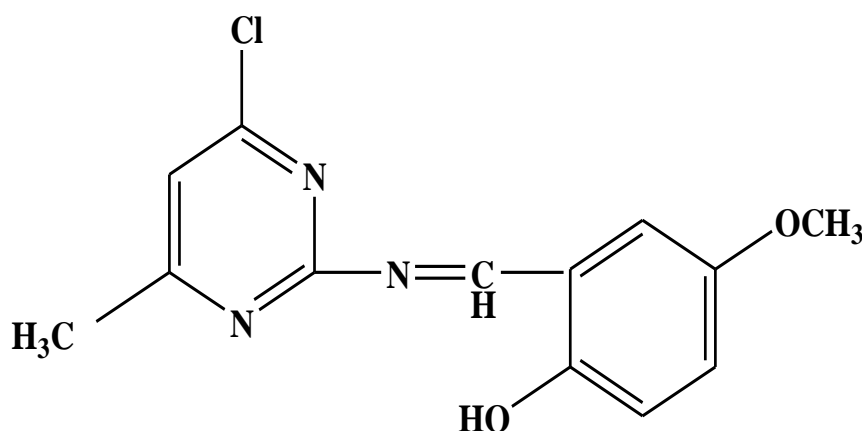


Figure 3. Structure of ligand

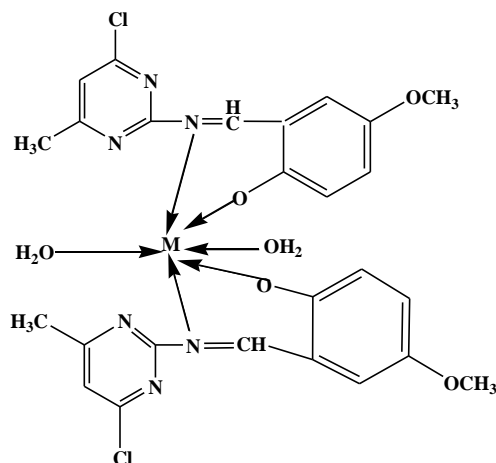


Figure 4. *The proposed Structure of the complexes*

When M= Ni (II) and Zn (II).

5. CONCLUSION

In the light of above discussion we have proposed octahedral geometry for Ni (II) and Zn (II) complexes. On the basis of the physico-chemical and spectral data discussed above, one can assume that the ligand behave as dibasic, NO bidentate, coordinating via phenolic oxygen and imino nitrogen as illustrated in Figure 4. The complexes are biologically active and show enhanced antimicrobial activities compared to free ligand. Thermal study reveals thermal stability of complexes. The X-ray study suggests orthorhombic crystal system for Ni (II) and Zn (II) complexes.

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AUTHOR CONTRIBUTIONS

D. T. Sakhare is the sole author. The author read and approved the final manuscript.

CONFLICTS OF INTEREST:

The authors declare no conflicts of interest.

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