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Synthesis, Characterisation, Docking Studies And Biological Activity Of Metal(II) Complexes Of Schiff Base Ligand Derived From 4-Chloro-2-Benzothiazolamine And Imidazole-2-Carboxaldehyde

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Abstract: *A new Schiff base ligand derived from 4-chloro-2-benzothiazolamine and imidazole-2-carboxaldehyde and its Cu (II), Co(II), Ni(II) and Zn(II) complexes were synthesized. These compounds were characterised by FT-IR, Elemental analysis, Mass, ^{1}H NMR, TGA, Molar conductance, Electronic spectra, Magnetic moment measurements and SEM. The analytical data has shown the metal to ligand ratio is 1:1. IR spectral studies revealed that binding sites of ligand with metal ions through the azomethine nitrogen and imidazole nitrogen atoms. Thermal analysis confirms there are no coordinated water molecules present in the metal complexes. Schiff base and its metal complexes were screened against the bacterial strains such as gram positive and gram negative to know the anti microbial activity of these compounds, in which metal complexes have shown greater activity than free ligand. The complete cleavage of DNA with metal complexes have also studied by using agarose gel electrophoresis technique, and anticancer activity of ligand and metal complexes were confirmed through the $IC_{(50)}$ values against the HeLa and MCF-7 cancer cell lines. Further, docking scores were also matches with these findings.*

Keywords: *Schiff base, Metal complexes, Antimicrobial activity, Anticancer activity and Docking studies.*

I. Introduction

There are numerous Schiff base ligands [1-3] have been reported by virtue of their potential applications in industry, pharma, drug and catalysis. Subsequently, considerable importance has been given to these ligands. Metal complexes containing sulphur, nitrogen chelating ligands have gained much attention because of their compelling physico-chemical properties, distinct biological activities [4-6] and models of metallo enzyme actve sites. Metal complexes of Schiff base ligands have a wide variety of biological applications such as anti microbial [7], antitumour [8] and antioxidant [9] activities. These complexes are also used as catalysts[10]. Imidazole moiety played a vital role in a variety of metallo proteins, especially heme proteins because of its biological significance. Schiff bases based on benzothiazoles are bicyclic ring systems with nitrogen and sulphur heterocycles[11] play an important role in life science and in the synthesis of speciality and fine chemicals. According to the cell biologists for most of the anti cancer and anti viral therapies DNA is the primary target molecule. It is important, to design the new types of pharmaceutical molecules the interaction of DNA with small molecules. There are reports which states that some metal complexes interacted with DNA could induce breakage of DNA strands by appropriate methods [12,13]**.**An intensive study on 2-amino benzothiazole was done in 1950s.It was concluded that 2-amino benzothiazole scaffold is one of the previlaged structure in medicinal chemistry and cytotoxic on cancer cells. [14,15].Rupinder kaurgill and Dr. P. M. S Bedi synthesised 2-amino benzothiazole derivatives and evaluate In Vitro cytotoxic activity against different human cancer cell lines namely Lung A-549, Prostate PC-3 and lukemia [16]. It is a well known approach to design new drug like molecules which are combination of 2-amino benzothiazole with other heterocyclic compounds allows achieving new pharmacological profile, action, toxicity lowering. Accordingly, in the present work a new Schiff base ligand derived from the condensation of 4-chloro- 2-benzothiazolamine with imidazole-2-carboxaldehyde, and its Cu(II), Co(II), Ni(II)and Zn(II) metal complexes were synthesised. These compounds were tested against HeLa and MCF-7 cancer cell lines, DNA cleavage experiment and bacterial strains. The metal complexes have shown greater activity than free ligand.

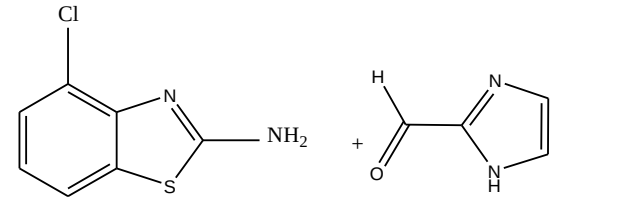
II. Experimental

2.1 Materials

All the chemicals were purchased from sigma aldrich. The solvents and reagents used were of analytical grade.

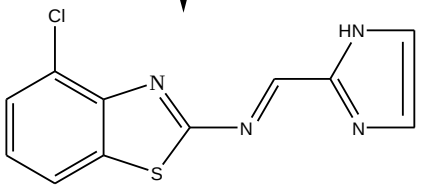
2.2 Synthesis of Ligand [L (IMCBTA)]

A solution of Imidazole-2-carboxaldehyde (5 mmol) was added dropwise to the methanolic solution of4-chloro- 2-benzothiazolamine with continuous stirring and refluxed for 6-8 h. An yellowish white amorphous precipitate is formed, which was then recrystalized from EtOH and dried in vacuo over anhydrous $Cacl_2$. The purity of the compound was tested with TLC. Yield 75%.



4-chloro-2-benzothiazolamine Imidazole-2-carboxaldehyde

Methanol Reflux 6-8 h.

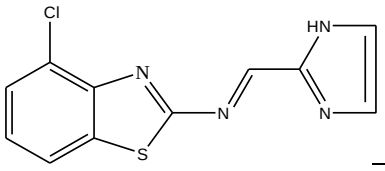


(*E*)-*N*-((1*H*-imidazol-2-yl)methylene)-4-chlorobenzo[*d*]thiazol-2-amine

(IMCBTA)

2.3 Synthesis of metal complexes

A solution of metal(II) (Cu, Co, Ni and Zn) chlorides (5mmol) dissolved in methanol was added to the methanolic solution of Schiff base (5mmol). The resulting mixture was stirred and refluxed for 3-5 hours. The coloured shining amorhous metal complex was precipitated in the rb flask, which was washed with ether, ethanol and dried.

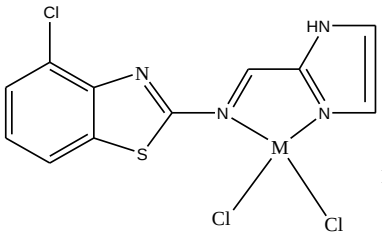


(IMCBTA)

M(II) Chloride

M=Cu,Co,Ni and Zn

Methanol, reflux stirred



M= Cu, Co, Ni, Zn.

2.3 Physical measurements

The percentage of the elements such as C, H and N present in the ligand and its metal complexes were determined by using Perkin Elmer elemental analyzer. The stretching frequencies of ligand and its metal complexes were recorded by using KBr pellets in the range 4000-400 cm^{-1} on prestige-21 instrument.The non-ionic nature of the metal complexes were carried out in DMSO (10^{-3}M) by using Elico Electronic Digital conductivity meter and 0.01 M KCl solution is used for calibration. The electronic spectra of ligand and its complexes were carried out in DMSO using a SHIMADZU UV-2600 spectrophotometer. The proton nmr of the ligand was recorded at 200 MHz and 300 MHz on Varian Gemini Spectrometer and TMS is used as an internal standard. To analyse molecular weight of the compounds VG AUTOSPEC mass spectrometer was used and which is performed through ESI technique. Thermogravimetric analysis of the metal complexes was carried on a Mettler Toledo Star system in the temperature range 50-1000^oC and heating rates were controlled by 15^oC min^{-1}. A Gouy balance model 7550 using $Hg[Co(NCS)_4]$ as standard is operated to examine the magnetic moment values of the metal complexes. By using Polmon instrument (model No. MP-96) the melting point of the ligand and decomposition temperature of the complexes were determined. The SEM/EDX images were obtained from a Hitachi SEM analyser.

2.4 Antibacterial activity

By using disc diffusion method[17]the invitro antibacterial activities of the Schiff base ligand and its metal complexes were analysed. All the metal complexes were screened against gram positive(eg. (i) Bacillus subtilis (ii) Staphylococcus aureus) and gram negative(eg.(iii) Pseudomonas putida (iv) Escherichia coli) bacterium. One day prior to the experiment, the bacterial cultures were inoculated in broth(inoculation medium) and incubated overnight at 37^0C. Inoculation medium containing 24h grown culture was added aseptically to the nutrient medium and mixed thoroughly to get the uniform distribution. This solution was poured (25mL in each dish) into petri dishes and then allowed to attain room temperature. Wells (6mm in diameter) were cut in the agar plates using proper sterile tubes. Then, wells were filled upto the surface of agar with 0.1mL of the test compounds dissolved in DMSO (200µM/mL). The plates were allowed to stand for an hour inorder to facilitate the diffusion of the drug solution. Then the plates were incubated at 37^0C for 24h and the diameter of the inhibition zones were read.

2.5 DNA cleavage studies

The compounds were dissolved in DMSO and added separately to the CT-DNA (Calf Thymus DNA) sample and add Hydrogen Peroxide. The sample mixtures were incubated at 37ºC for 1 hour. The electrophoresis of the samples was done according to the following procedure. Weigh 0.25grams of agarose and dissolve it in 25 ml of 1x TAE buffer (121.1g Tris base, pH 8.0, 0.5 M EDTA, 57.1ml of Glacial acetic acid for 1 ltr) by boiling. When the gel attains approximately 55ºC, pour it into the gel cassette fitted with comb. Let the gel to solidify. Carefully remove the comb, place the gel in the electrophoresis chamber flooded with TAE buffer. Load DNA sample with bromophenol blue carefully into the wells, along with standard DNA marker and pass the constant 100 V of electricity till the dye front reaches the end of gel. Remove the gel and carefully stain with ETBR solution (10 µg/ml) for 10-15 min and destain the gel and observe the bands under UV transilluminator.

2.6 In vitro anticancer activity

The HeLa cervical cancer cell lines and MCF- 7 breast adino carcinoma cancer cell lines were purchased from NCCS, Pune. The cells were maintained in DMEM supplemented with 10 % FBS and the antibiotics penicillin/streptomycin (0.5 mL^{-1}), in atmosphere of 5% CO_2 /95% air at 37 ^{0}C. For the MTT assay, HeLa cells were plated in 96 well plate at 5.0 X 10^3 cells were per well in culture medium and incubated overnight at37 ^{0}C.

Cell viability Cell viability of the HeLa and MCF-7 carcinoma was evaluated by the MTT Assay with three independent triplicate experiments of six concentrations of compounds (5, 10, 25, 50 75 and 100 µM). After 24 hrs of incubation, each treatment was withdrawn and MTT solution (0.5 mg / mL^{-1}) was added to each well and plates were incubated at 37 ^{0}C for 3 hrs. At the end of incubation time, precipitates are formed as a result of the reduction of the MTT salt to chromophore formazan crystals by the cells with metabolically active mitochondria. The optical density of solubilized crystals in DMSO was measured at 560 nm on a microplate reader[18].

2.7 Docking studies

The synthesized novel ligand was selected for performing molecular docking studies. In this present study GLIDE 5.6 [19] was used for molecular docking. Crystal structures of *Staphylococcus aureus* Pencillin binding protein 4 (pdb id:3HUN [20], E.coli Penicillin Binding Protein 4 -dacB (pdb id:2EXB [21]), and Homo sapiens cyclin dependent kinase (pdb id: 2XNB[22]) were downloaded from protein data bank (http://www.rcsb.org). Ligand was built using Maestro build panel and prepared by Lig Prep 2.0 application using MMFF 94s [23] force field and the corresponding low energy 3D conformers of the ligand were obtained. The protein was prepared using protein preparation wizard of Schrödinger's software, in this target preparation, all water molecules were removed and hydrogen atoms were added to the target. Grid was generated around the active site of the protein by selecting the cocrystalized ligand. Receptor van der Waals scaling for the nonpolar atoms was kept 0.9 [24]. Low energy conformation of the compound was selected and docked into the grid using standard precision docking protocol (SP). Dock pose of ligand was analyzed for interactions with the receptor.

III. Results And Discussion

3.1 Physical characteristics of the complexes

All the metal complexes were melt at higher temperatures and they were coloured, non-hygroscopic in nature and insoluble in water.

3.2 Elemental analysis

The percentage of the elements (C, H, N) present in the ligand and complexes were given in **Table.1** The experimental values were matches with the theoretical values and these results confirmed that metal to ligand ratio is 1:1. The values in brackets are calculated.

Table.1

Compound	Molecular weight	Colour	Anal. (%) found (cal)			
			C	H	N	M
Ligand $C_{11}H_7N_4SCl$	262.5	Light yellow	49.19 (50.29)	2.16 (2.67)	20.12 (21.33)	-
Cu(II)complex ($C_{11}H_7N_4SCl_3Cu$)	397	Green	31.65 (33.24)	1.71 (1.76)	13.56 (14.10)	15.56 (15.96)
Co(II)complex ($C_{11}H_7N_4SCl_3Co$)	393	Brown	31.56 (33.59)	1.75 (1.78)	14.01 (14.24)	14.98 (15.01)
Ni(II)complex ($C_{11}H_7N_4SCl_3Ni$)	393	Brick red	32.09 (33.59)	1.69 (1.78)	12.96 (14.24)	14.29 (15.01)
Zn(II)complex ($C_{11}H_7N_4SCl_3Zn$)	399	Yellow	31.08 (33.08)	1.45 (1.75)	12.95 (14.03)	15.81 (16.36)

3.3 Mass spectra and Molar conductivity

The mass spectrum of the ligand exhibits the molecular ion peak at (m/z=263), which is in agreement with its formula weight (262.5). The mass spectral values of the metal complexes were given in Table. The molar conductivity was measured for all the metal complexes in DMSO solution (10^{-3}M) to establish the charge of the metal complexes. The low molar conductance values suggests that all the metal complexes were non electrolytic in nature[25]

Table. 2 Mass And Molar Conductance Values Of The Compounds

Compound	Calculated mass	Obtained mass	Conductance ($Ohm^{-1}cm^2\ mol^{-1}$)
Ligand	262.5	263 [M^+]	------
Cu(II) complex	397	397 [M^+]	10.21
Co(II) complex	393	391 [M-2]	8.9
Ni(II) complex	393	416 [M+23]	11.2
Zn(II) complex	399	399 [M^+]	9.2

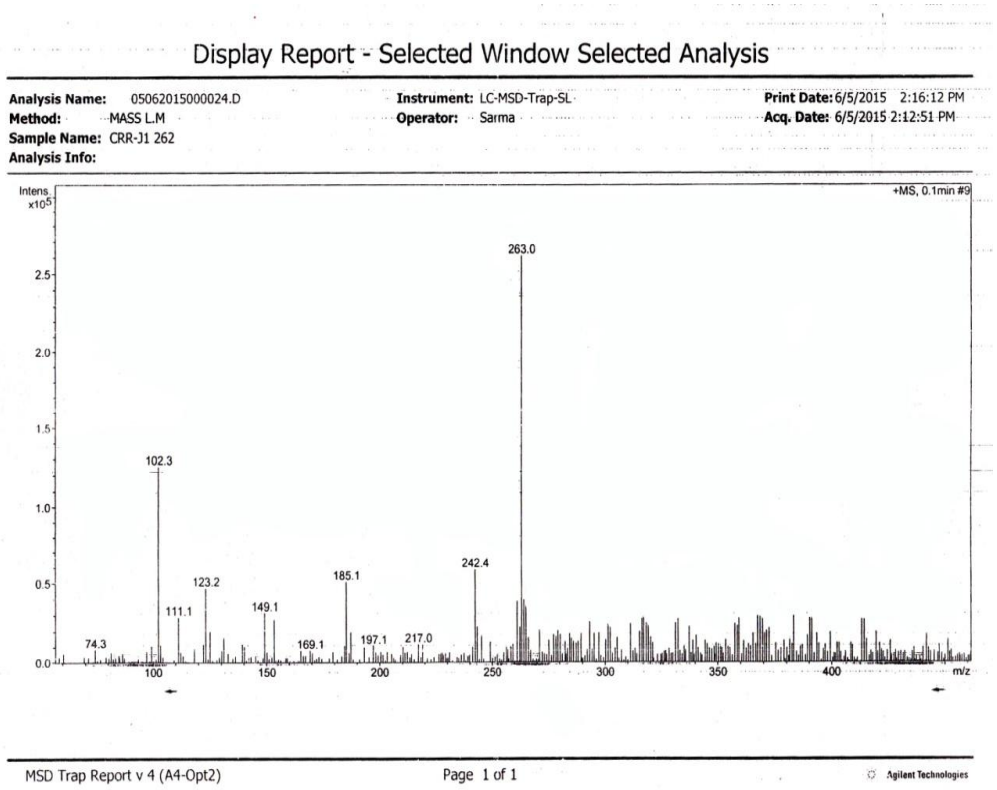


Fig. 1 Mass Spectrum Of The Schiff Base Ligand(Imcbta) (M/Z=263)

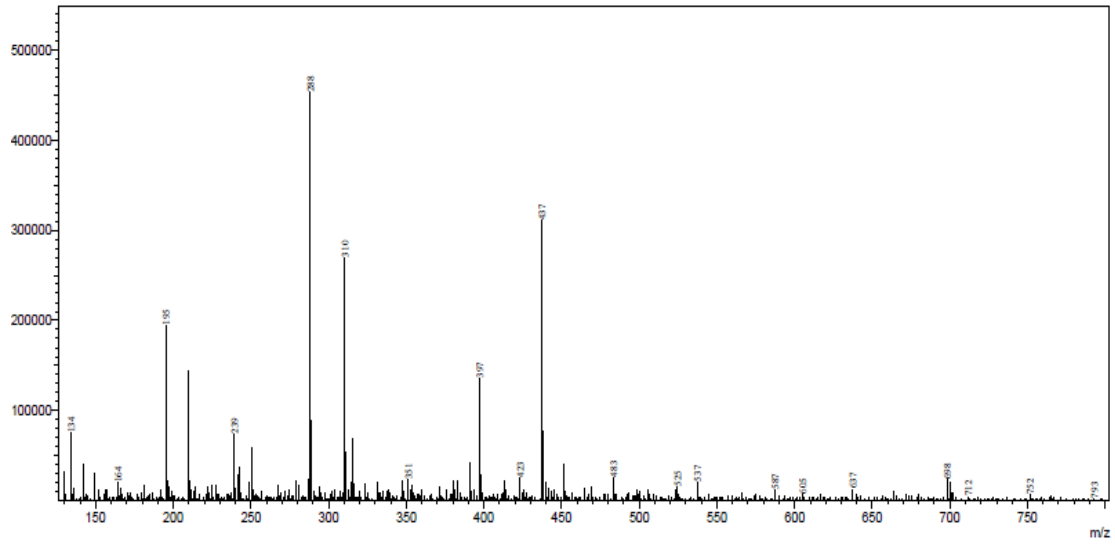


Fig. 2 Mass spectrum of the Cu(II) complex (m/z=397)

3.4 ^{1}H NMR

The ^{1}H NMR spectrum of the ligand shows a singlet at 9.80 ppm, which corresponds to the azomethine proton. The signals appeared at 7.16-7.79ppm due to aromatic protons.

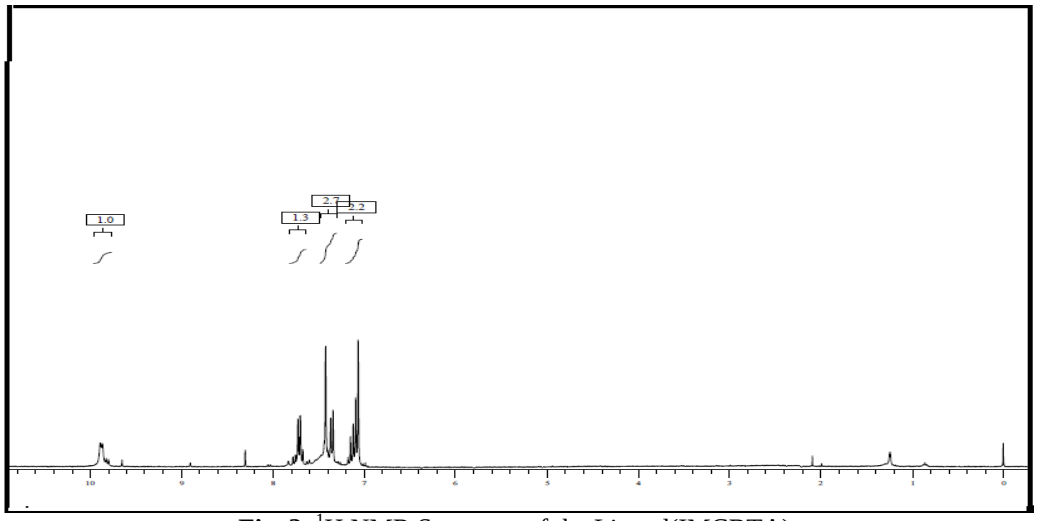


Fig. 3 ^{1}H NMR Spectrum of the Ligand(IMCBTA)

3.5 UV- Vis Spectra and Magnetic moments

The UV- Vis spectra of Schiff base ligand and its metal complexes were recorded in DMSO at room temperature. The absorption bands of a ligand observed at 279 nm and 362 nm, which corresponds to π- π^{*}(-C=C) and n- π^{*}(-C=N) transistions respectively. These bands were shifted in the complexes due to intraligand and LMCT transistions of coordinated ligand. In addition, to this the electronic spectra of the Cu complex displayed a low intensity broad band at 599 nm in the visible region is assignable to the d-d transitions [$^{2}B_{1g} \longrightarrow {}^{2}A_{1g}$][26], which is characteristic of square planar environment. Besides this the magnetic moment value 1.79 BM for Cu(II) complex confirms the square planar geometry of this complex. The electronic spectrum of Ni(II) and Co(II) complexes shown absorption bands at 542 nm and 562 nm respectively in the visible region due to d-d transistions of metal complexes. The characteristic band at 562 nm for Co(II) complex corresponds to transistion [$^{4}A_{2(F)}$- $^{4}T_{1(P)}$] convey tetrahedral environment. The observed magnetic moment value for Co(II) complex is to be 4.43BM, which is also suggesting the tetrahedral geometry for this complex[27]. The Ni(II) complex is found to be diamagnetic, with medium intensity bands in the visible region[28]. The transistion in this region clearly indicates the square planar geometry for this complex. However, the diamagnetic Zn(II) complex shown absorption bands at 272 nm and 376 nm due to intraligand and charge transfer transistions respectively, which would have expected the tetrahedral geometry around the Zn metal center [29].

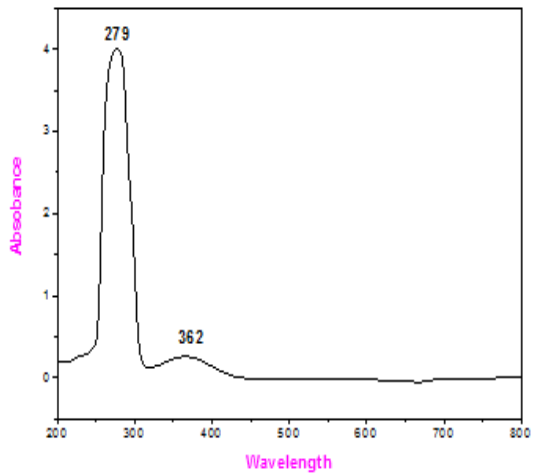


Fig.4 UV-Vis. Spectrum of ligand(IMCBTA)

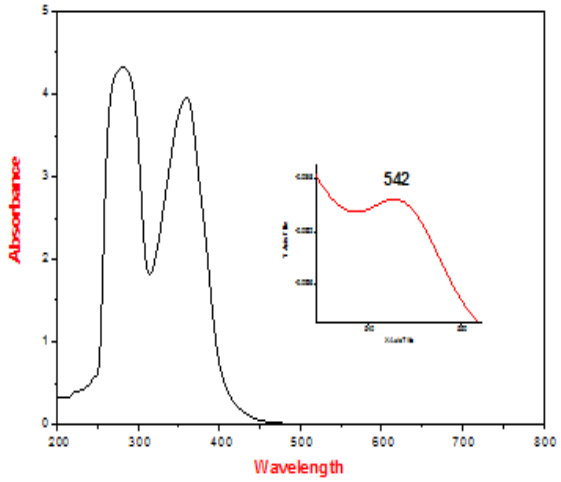


Fig.5 UV-Vis. Spectrum of Ni(II) complex

3.6 Infrared spectroscopy

A strong band at 1685 cm^{-1} in the IR spectra assigned to the Azomethine nitrogen of the ligand, which was shifted to lower frequency in the corresponding metal complexes of that ligand. The frequencies of Cu(II), Ni(II), Co(II) and Zn(II) complexes were 1621 cm^{-1}, 1630 cm^{-1}, 1621 cm^{-1} and 1620 cm^{-1} respectively. This indicates the coordination of azomethine nitrogen to the metal ion. It is due to the Imidazole nitrogen present in the ligand a stretching band occurred at 1618 cm^{-1}, This is also shifted towards lower frequency region in the metal complexes. However, this confirms the coordination of imidazole nitrogen to the metal ion. The bands observed in the region 3416 cm^{-1} and 1539 cm^{-1} corresponds to the –NH stretching frequencies of imidazole moiety[30]. The M-N bands of all the metal complexes were observed in the region 426-457 cm^{-1} [31]. The appearance of M-Cl bonds in the reion below 400 cm^{-1}[32].

Table. 3 IR spectral data of the synthesised compounds(cm^{-1})

Compound	$\upsilon_{(CH=N)azomethine}$	$\upsilon_{(C=N)imidazole\ ring}$	$\upsilon_{(M-N)}$
Ligand	1685	1618	------
Cu(II) complex	1621	1580	457
Co(II) complex	1621	1581	426
Ni(II) complex	1630	1570	457
Zn(II) complex	1620	1585	443

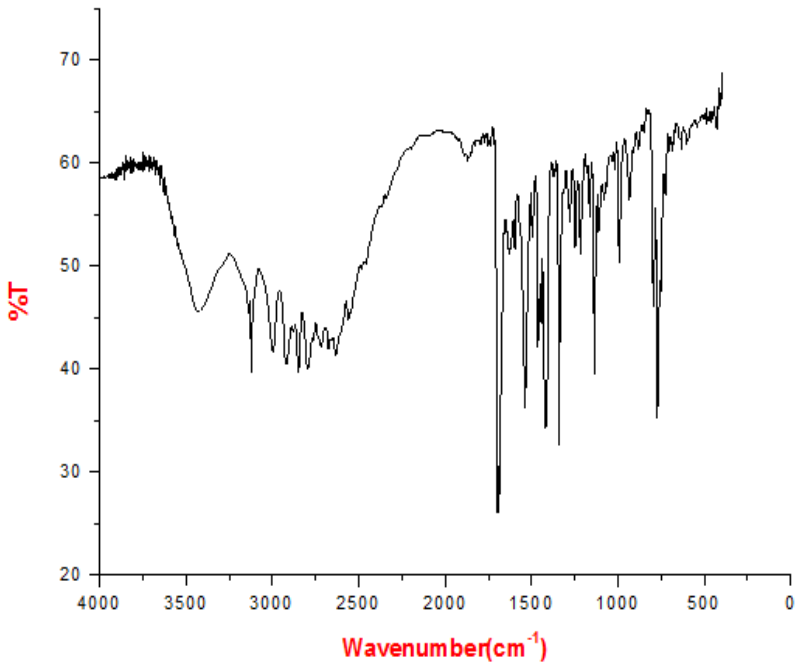


Fig.6 IR spectrum of ligand(IMCBTA)

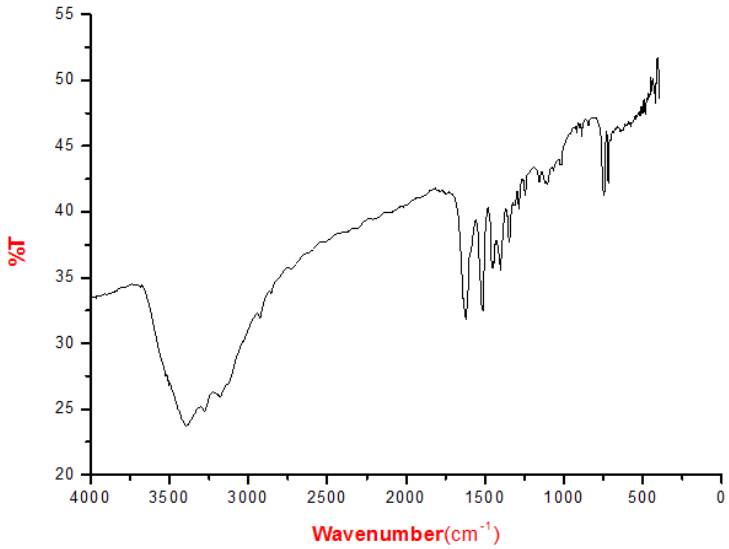


Fig.7 IR spectrum of Co(II) complex

3.7 Thermal analysis

It is observed that all the metal complexes do not show any weight loss upto 290°C, which suggests that there are no coordinated water molecules present in the complexes. It is between 320-510°C in TG curves, there is a significant weight loss occurred may be due to the removal of chlorine atoms as HCl gas, C, S and N in organic moiety as its oxide. It is after 600°C there is a straight line is obtained which indicates all the metals in the complexes were remained as their oxides. The Zn(II) and Cu(II) complexes do not show any weight loss upto 300°C, between 300-450°C gradual decline in the TG curve may be due to the removal of organic moiety. In the range 450-700°C the loss in weight corresponds to the remaining molecules present in the complexes. In all the cases remaining residues are metal oxides.

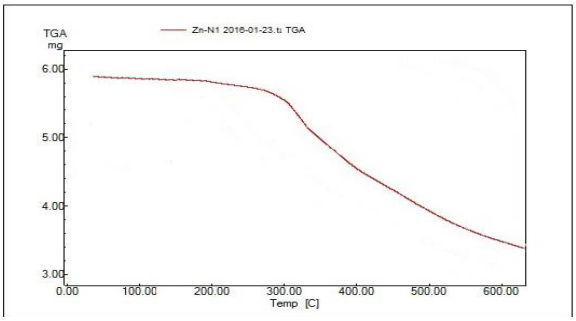


Fig.8 TG curve of the Zn(II) complex

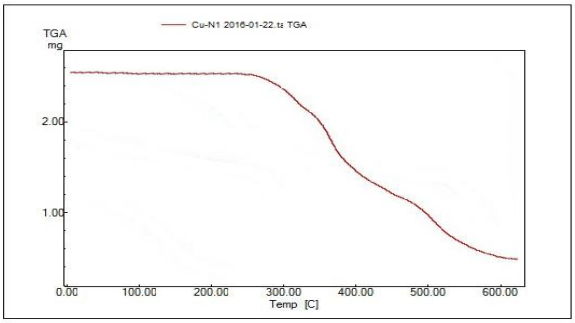
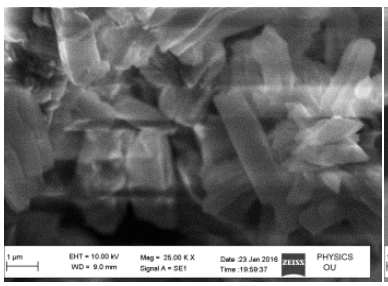


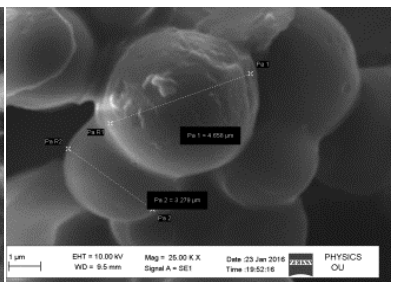
Fig.9 TG curve of the Cu(II) complex

3.8 SEM and EDX Analysis

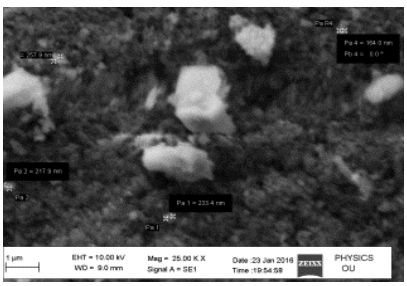
The scanning electron microscope has been used to illustrate the surface morphology and particle size of the Schiff base metal complexes. In the present work all the metal complexes showed different morphology to that of their free ligand. The composition of the elements present in the complexes were obtained from the EDX analysis.



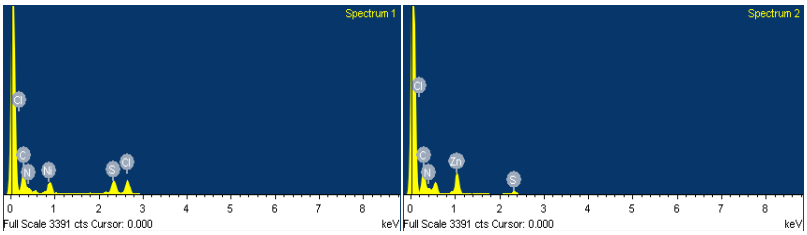
SEM image of ligand (IMCBTA)



Ni(II) complex



Zn(II) complex



EDX of Ni(II) complex Zn(II) complex

Fig. 10 SEM and EDX images of the liand and its Ni(II) and Zn(II) complexes

3.9 In vitro antibacterial activity

The zone of inhibition of the Schiff base ligand and its metal complexes against gram positive and gram negative bacterial strains was determined by using disc diffusion method. The inhibition values are tabulated in the below Table.4. It is observed that all the metal complexes have shown greater activity than free ligand. This is explained on the basis of chelation theory and overtones concept[33]. The Cu(II) and Ni(II) complexes have shown better activity against the S.aureus, E.coli and moderate activity of Co(II) complex with P.putida and E.coli. The Zn(II) and Cu(II) complexes have shown moderate activity against B.subtilis and ligands have shown less activity against B.subtilis and P.putida bacterial strains. Ampicillin and Ceftriaxone are used as standard drugs for gram positive and gram negative strains respectively. Antibacterial activity of the Schiff base ligands and their complexes E.coli and P.putida have shown in Fig.11 and Fig.12.

Table. 4 Zone of inhibition (in mm)

Compound	S.aureus	E.coli	B.subtilis	P.putida
Ligand	06	07	02	03
Cu(II) complex	11	12	07	12
Co(II) complex	10	07	8.5	08
Ni(II) complex	09	10	09	11
Zn(II) complex	09	11	06	08
Standard	15	15	13	18

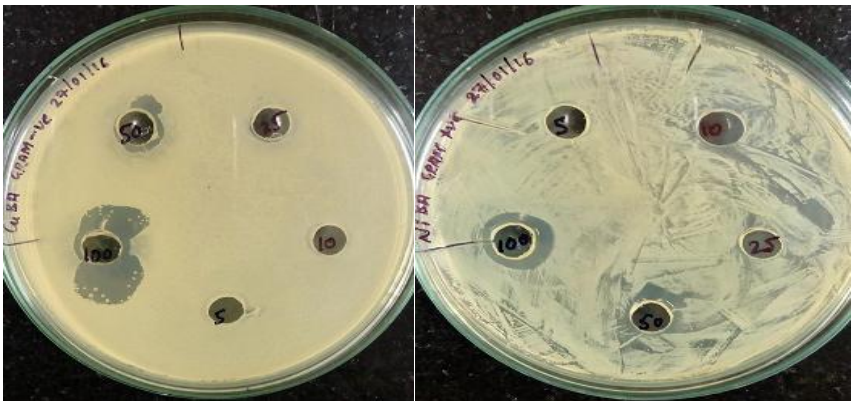


Fig.11 IMCBTA (E.coli) IMCBTA (P.putida)

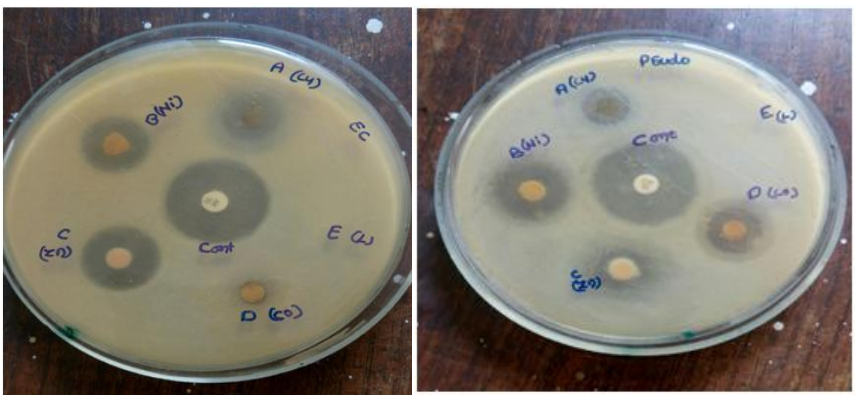


Fig.12 Antibacterial activity of IMCBTA complexes against E.coli and P.putidawhere [A] Cu complex [B] Ni complex [C] Zn complex [D] Co complex [E] No compound.

3.10 DNA cleavage experiment

In the present work CT-DNA is used for cleavage experiment. All the metal complexes can interact with CT-DNA in the presence of H_2O_2. The complete cleavage of DNA occurred with the Ni(II) and Cu(II) complexes, the Co(II) complex has shown partial cleavage. The Zn(II) complex and Schiff base ligand exhibited no cleavage activity. This clearly suggests complexes can interact with CT-DNA and effectively cleave it. The metal complexes can catalyze the production of hydroxyl radicals from H_2O_2. These hydroxyl radicals participate in the oxidation of the deoxyribose moiety, followed by the hydrolytic cleavage of sugar-phosphate backbone.

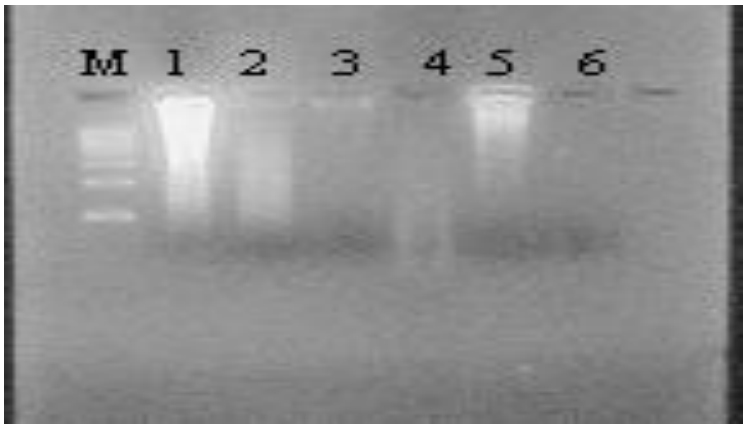


Fig.13 DNA cleavage activity of free ligand and its metal complexes
[M] Marker [1] Control(CT-DNA)+H_2O_2 [2] Ligand+DNA+ H_2O_2 [3] Ni(II) complex+DNA+H_2O_2 [4] Co(II) complex+DNA+ H_2O_2 [5] Zn(II)complex+DNA+ H_2O_2 [6] Cu(II) complex+DNA+ H_2O_2

3.11 Anticancer activity

The antiproliferative activity of the Schiff base ligand and its metal complexes were tested against the HeLa and MCF-7 cancer cell lines. MTT assay is used to check the anticancer activity of these compounds. The Inhibitory concentration 50(IC_{50}), defined as the concentration required to reduce the size of the cell population by 50%. The IC_{50} values of all the compounds are given in Table.5. All the compounds including Schiff base ligand also showed very good activity against HeLa and MCF-7 cell lines. For HeLa, the Ni(II), Cu(II) and Zn(II) complexes have shown low IC_{50} values than free ligand which indicates higher antiproliferative activity than free ligand. The Co(II) complexes have shown less activity than free ligand. For MCF-7 carcinoma ligand showed more activity than Zn(II) and Co(II) complexes which were less active than free ligand, that means these complexes showed higher IC_{50} values than free ligand.

Table. 5 IC_{50} values of the synthesized compounds

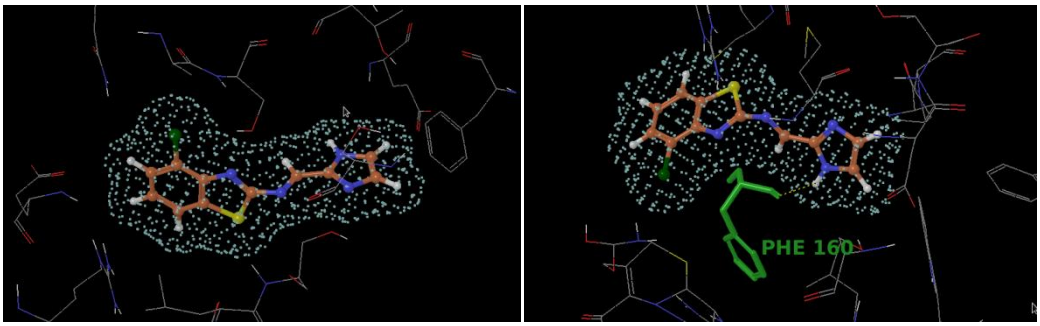
S.No.	COMPOUND	IC_{50} values (µM)	
		HeLa	MCF-7
1	Schiff base ligand	24.05	18.4
2	Zn(II) complex	22.93	115
3	Ni(II) complex	9.3	15.66
4	Co(II) complex	54.61	70.25
5	Cu(II) complex	20.23	16.7

Docking results

To gain insight of the binding mode, Ligand was docked into the ligand active site, that showed hydrogen bond interactions with PHE 160 for E .Coli Penicillin Binding Protein 4 -dacB and with GLU 12 for Homo sapiens cyclin dependent kinase.

Table.6 Dock score of Ligand

	Ligand(IMCBTA)
	Dock score(K cal/mol)
Staphylococcus aureus **(pdb id: 3HUN)**	-6.904
E.coli **(pdb.id-2EXB)**	-7.550
Homo sapiens **(pdb.id-2XNB).**	-8.497



(a) **(b)**

Fig.14 (a) Dock pose of ligand in the protein active site in in Staphylococcus aureus (pdb.id-3HUN) (b)Docked pose of ligand in the protein active site showing hydrogen bond interactions (yellow lines) with PHE160 in *E.Coli* (pdb.id-2EXB).

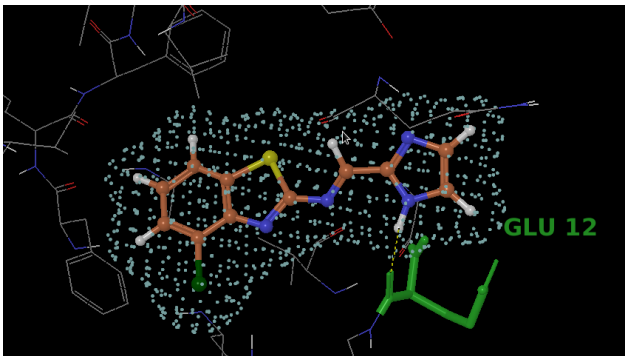


Fig.15 Docked pose of ligand in the protein active site showing hydrogen bond interaction (yellow lines) with GLU 12 in Homo sapiens cyclin dependent kinase (pdb.id-2XNB).

IV. Conclusion

In the present work, a new Schiff base ligand and its metal complexes were synthesized. All the compounds were analyzed through various spectral techniques. The core intension of the present work is to check the biological activities of the compounds. All the compounds were screened against bacterial strains and HeLa, MCF-7 cancer cell lines. Metal complexes have shown good antibacterial activity than ligand, but in case of anticancer activity both Schiff base ligand and its metal complexes have shown greater activity. The binding mode of the ligand with protein active sites were predicted using docking technique. The dock score values of ligand showed a correlation with the inhibitory activity against Staphylococcus aureus Pencillin binding protein 4, E.Coli Penicillin Binding Protein 4 (dacB) and Homo sapiens cyclin dependent kinase.

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