Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2018, 10(2):116-127

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Evaluation of Binding Affinity with Cancer Causing Receptors, CT-DNA and Biological Activities of Mixed Ligand Complexes of Some Transition Metal Ions

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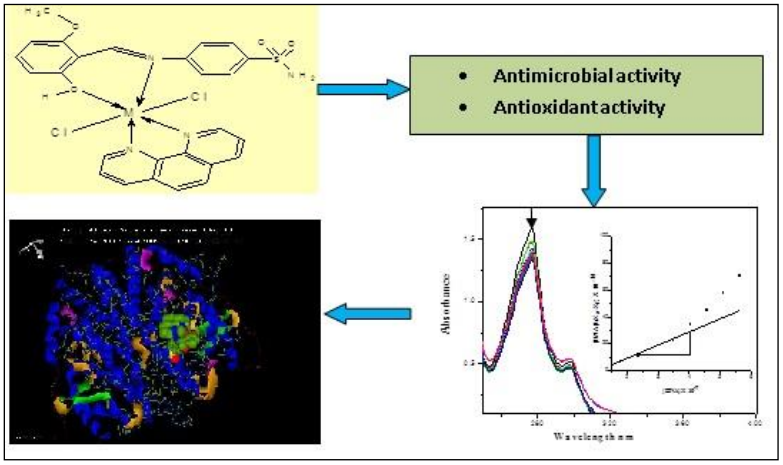
ABSTRACT

The novel complexes $[ML^1L^2Cl_2]$ and $[ML^1L^2Cl]Cl$ where M = Co(II), Cu(II), Ni(II) and Fe(III), containing bioactive ligands of the type L^1 = 4-[(2-hydroxy-6-methoxybenzylidene)amino]cyclohexa-1,5-diene-1-sulfonamide and L^2 = 1,10-phenanthroline were synthesized and structurally characterized by elemental analysis, conductivity measurement, IR, UV and ^1H NMR spectral studies. The DNA binding affinities of synthesized complexes were studied. It was found that all metal complexes were very good DNA intercalators with very high intrinsic binding constant (Kb) than the classical DNA intercalators. The minimum inhibitory concentration (MIC) values of in vitro antimicrobial and antioxidant activity (DPPH Scavenging assay) indicate that the metal complexes were more active compare to the uncoordinated ligand. The Interaction of complexes with the human estrogen receptor and tyrosine kinase (RTK) showed lowest binding energy than the free ligand and the standard indicate that the complexes have high inhibiting property for cancer causing receptors.

GRAPHICAL ABSTRACT



Keywords: Mixed ligand complexes; DNA binding; Biological activity; Docking studies

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INTRODUCTION

The therapeutic and diagnostic properties of transition metal complexes have attracted considerable attention leading to their application in many areas of modern medicine [1]. Schiff bases are the class of ligands known to coordinate with metal ions through the azomethine nitrogen atom [2] and are significant in medicinal and pharmaceutical chemistry with several biological applications [3-5]. Metal complexes of schiff bases have been widely studied because they showed antimicrobial, anticancer, herbicidal, antianalgesic, antifertility and anti-inflammatory activities [6,7]. Investigations concerning 1, 10-phenanthroline mixed ligand complexes is relevant in the field of analytical chemistry, where the use of such complexes allows the development of methods with increased selectivity, sensitivity and also has great importance in the field of biological and environmental chemistry [8-10].

It is reported that anticancer activity is due to the intercalation between the drug and the base pairs of DNA (Deoxyribonucleic acid) and interference with normal functioning of the enzyme topoisomerase II that was involved in the breaking and releasing of DNA strands [11]. Investigation of mixed ligand transition metal complexes, which bind at specific sites along a DNA strands as reactive models for protein-nucleic acid interaction, provide routes toward rational drug design as well as means to develop sensitive chemical probes for DNA. Thus, a number of ligands are of current interest for important applications in nucleic acid chemistry as probes of DNA structure in solution, reagents for mediation of strand scission of duplex DNA under physiological conditions, chemotherapeutic agents and in genomic research [12-17].

Breast cancer is the most common cancer and one of the leading causes of death among women worldwide, with nearly 1,000,000 new cases per year [18]. Breast cancer is the most diagnosed and the second leading cause of cancer deaths for women in the United States striking about 300,000 and killing about 40,000 women a year [19]. Estrogen promotes breast cancer proliferation through a number of established pathways [20]. Tyrosine kinase receptors are expressed on the surface of tumor and/or endothelial cells and represent attractive targets for new anti-cancer treatment strategies [21,22]. Developing potential inhibitors of cancer causing receptor is an area of great interest [23-26].

Bearing above facts we report here the synthesis, characterization, DNA binding studies, antimicrobial activity, antioxidant activity as well as molecular docking studies of L^1 and their mixed ligand complexes.

EXPERIMENTAL SECTION

The reagents and solvents used were of analytical reagent grade. Solvents were purified according to literature methods [27] and used. Zinc chloride, ferric chloride, cobaltous chloride, copper chloride, 1, 10-phenanthroline, sulphanilamide, 2-hydroxy-6-methoxy benzaldehyde and ethanol was purchased from Sigma Aldrich chemicals (Bangalore). Calf thymus deoxyribonucleic acid (CT-DNA) was purchased from Bangalore Gene (Bangalore, India). *Tris*-HCl buffer was prepared using deionized double distilled water. For molecular docking study we used biological databases PubMed, Drug Bank, PDB (Protein Data Bank) and software's Hex, ACD ChemSketch.

Synthesis of Schiff Base Ligand

4-[(2-hydroxy-6-methoxybenzylidene) amino] cyclohexa-1, 5-diene-1-sulfonamide L^1:

The new schiff base ligand was synthesized by following the literature procedure [28]. Sulfanilamide (0.05 mol) and 2-hydroxy-6-methoxy benzaldehyde (0.05 mol) were added into 20 mL of absolute ethanol containing a few drops of glacial acetic acid in a 100 mL round bottomed flask. The reaction mixture was refluxed for 2h. It was then cooled and ice-cold water was added .The product so formed was filtered, washed, dried and recrystallized from alcohol, Yield 85%. Analysis: ($C_{14}H_{16}N_2O_4S$). calcd. (%): C 54.48; H 5.18; N 9.08. Found (%): C 54.00; H 4.96; N 8.95 . ^{1}H NMR : 2.60 ppm (s, 3H,O-CH_3), 3.59 ppm (s, 2H, SN-H), 7.3-9.4.1 ppm (m, 7H, Ar-H), 8.56 ppm (s, H, CH=N). 14.1 ppm (s, H, Ar-OH), IR, KBr pellets (ν, cm^{-1}): 3400ν (-OH str broad band); 3300, 3236ν(NH); 1625 ν (C=N str); 1332 (S=O asymmetric); 1091(S=O symmetric); 2846 (CH-Ar str); 1462(C=C str); 771(C-S str); Electronic spectra: Band absorved around 305-395 nm.

Synthesis of Metal Complexes [$ML^1L^2Cl_2$]: General Procedure

Metal salt (1 mM) (copper chloride, zinc chloride, cobaltous chloride or ferric chloride) one at each time was dissolved in hot ethanol (10 mL) and mixed with ethanolic solution of ligand L_1 (1 mM, 10 mL). The reaction mixture was refluxed on water bath for about 30 min, then the ethanolic solution of ligand L_2(1 mM, 10 mL) (1,10-Phenanthroline) was added and continued refluxing for 3-4 h. The dark colored crystalline solid thus formed was collected by filtration, washed twice with hot ethanol (10 mL) followed by ether (10 mL) and dried under vacuum

and then recrystallized from aqueous ethanol. (Yield 50%-65%) (Figure 1).

Analytical Data of the Complexes

[Fe($C_{14}H_{16}$ N_2O_4S)($C_{12}H_8N_2$)Cl]Cl: **Complex (I),** calcd. (%) C, 50.76; H, 3.90; N, 9.11; Fe, 9.11. Found: C, 50.49; H, 3.69; N, 8.89; Fe, 8.89. IR, KBr pellets (ν, cm^{-1}): 3448 (OH broad band); 3068 (NH str); 1596.95 (C=N str); 1326 (S=O)asym; 1124 (S=O)sym; 2648 (CH-Ar str); 1510 (C=C str); 721 (C-S str); Electronic spectra : 510 nm

[Co($C_{14}H_{16}$ N_2O_4S)($C_{12}H_8N_2$)Cl_2]: **Complex (II),** calcd. (%) C, 50.52; H, 3.88; N, 9.06; Co, 9.55. Found: C, 50.38; H, 3.23; N, 8.75; Co, 9.46. IR, KBr pellets (ν, cm^{-1}): 3405 ν (OH str broad band); 3055 (N-H str); 1600 (C=N str); 1340 (S=O)asym; 1128 (S=O)sym; 2325 (CH-Ar str); 421 (C=C str); 723 (C-S str); Electronic spectra: 495 nm, 525 nm, 605 nm, 675 nm

[Cu($C_{14}H_{16}$ N_2O_4S)($C_{12}H_8N_2$)Cl_2]: **Complex (III),** calcd. (%) C, 50.15; H, 3.85; N, 9.00; Cu,10.23; Found: C, 49.37; H, 3.57; N, 8.74; Cu, 9.97. IR, KBr pellets (ν, cm^{-1}): 3448 (OH str broad band); 3057 (N-H str); 1595.02 (C=N str); 1340.43 (S=O)asym; 1149 (S=O)sym; 2354 (CH-Ar str); 1508 (C=C str); 723.26 (C-S str); Electronic spectra: 395 nm, 750 nm.

[Ni($C_{14}H_{16}$ N_2O_4S)($C_{12}H_8N_2$)Cl_2]: **Complex (IV),** calcd. (%) C, 50.03; H, 3.84; N, 8.98; Zn, 10.42. Found: C, 49.29; H, 3.19; N, 8.36; Zn, 9.98. IR, KBr pellets (ν, cm^{-1}): 3448 (OH str. broad band); 3060 (N-H str); 1602 (C=N str); 1219 (S=O)asym; 1147 (S=O)sym; 2358 (CH-Ar str); 1461 (C=C str); 727 (C-S str);

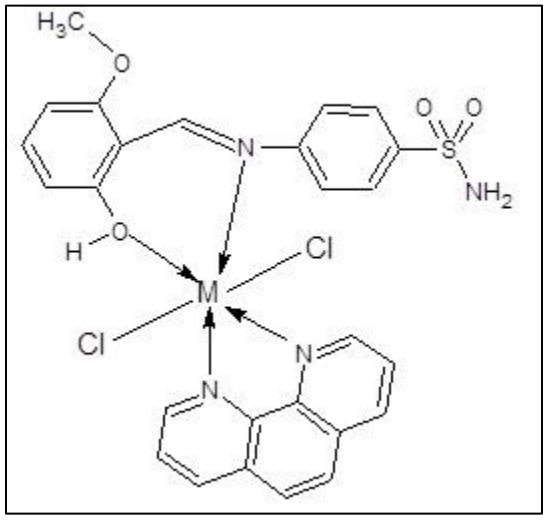

Figure 1: The structure of mixed ligand complexes

Where M = Co(II), Fe(III), Cu(II) and Ni(II).

DNA Interaction Studies

The transition metal complexes are known to bind CT-DNA via both covalent and noncovalent interactions [29,30]. In covalent binding the labile ligand of the complexes is bind DNA bases at the N7 of guanine and in noncovalent DNA interactions include intercalative, electrostatic and groove (surface) binding of metal complexes along outside of DNA helix, along major or minor groove.

Electronic absorption spectroscopy is an effective method to examine the binding mode of DNA with metal complexes [31]. If the binding mode is an intercalation, the π* orbital of the intercalated ligand can couple with the π orbital of the DNA base pairs, thus, decreasing the π $\longrightarrow$ π* transition energy and resulting in the bathochromism. On the other hand, the coupling π orbital is partially filled by electrons, thus, decreasing the transition probabilities and concomitantly resulting in hypochromism [32]. It is a general observation that the binding of an intercalative molecule to DNA is accompanied by hypochromism and significant red shift (bathochromism) in the absorption spectra due to strong stacking interaction between the aromatic chromophore of the ligand and DNA base pairs with the extent of hypochromism and red-shift commonly consistent with the strength of intercalative interaction [33]. Thus, in order to provide evidence for the possible binding of each complex

to CT DNA, spectroscopic titration of a solution of the complexes with CT DNA has been performed [34]. In general, hyperchromism and hypochromism are the spectral features of DNA concerning of its double helix structure; hyperchromism means the breakage of the secondary structure of DNA, and hypochromism means that the DNA-binding mode of complex is electrostatic effect or intercalation which can stabilize the DNA duplex, while the existence of a red-shift is indicative of the stabilization of DNA duplex [35].

All experiments involving CT-DNA were performed in *Tris*-HCl / NaCl (pH 7.2) buffer using the complexes in DMF. A solution of CT−DNA in the buffer gave a ratio of UV absorbance at 260 and 280 nm of 1.8–1.9:1, indicating that the DNA was sufficiently free of protein [36]. Stock solution of CT-DNA was stored at 4 ^{o}C and used in not more than four days. The DNA concentration per nucleotide was determined by absorption spectroscopy using the molar absorption coefficient (6600 $M^{-1}cm^{-1}$) at 260 nm. The concentrated stock solutions of complexes were prepared in DMF and the solution was diluted with *Tris*-HCl buffer. Absorption titrations were performed keeping metal complex concentration constant and varying concentration of CT-DNA (0-300µM) after equilibrium was reached (ca.5 min) the spectra were recorded against an analogous blank solution containing the same concentration of DNA. The intrinsic binding constant Kb was determined from a plot of [DNA] / (ε_a-ε_f) *versus* [DNA] equation (1).

$$[DNA] / (\varepsilon_a-\varepsilon_f) = [DNA] / (\varepsilon_a-\varepsilon_f) + 1/ Kb (\varepsilon_b-\varepsilon_f) \text{ ---------- (1)}$$

Where, ε_a, ε_f and ε_b are the extinction coefficients of apparent, free and bound form of the complex to DNA respectively [37].

Antimicrobial Activity

The *in vitro* antimicrobial activity (MIC) of synthesized schiff base ligand L_1 and their metal complexes were tested against representative bacteria *Escherichia coli, Salmonella typhi , Staphylococcus aureus, Bacillus subtilis and two fungi Aspergillus niger and Candida albicans* following the procedure described in literature [38,39]. All the tested compounds showed moderate to fairly good biological activity against microorganisms when compared to the ligand and standards.

Antioxidant Activity DPPH Free Radical Scavenging Assay

Standard: Butylated hydroxytoluene (BHT)

The antioxidant activity of the synthesized ligand and their complexes were evaluated using the DPPH free radical scavenging assay [40]. The stock solutions were prepared by dissolving the compounds 1mg in 1mL of DMSO. The test solutions (10, 20, 30, 40, 50, 60, 70, 80, 90, and 100µg/mL in DMSO) were prepared by diluting the stock solution. 1 mL of test sample solution was added to 4 mL of methanolic DPPH (40mg/100mL of methanol). The mixture was incubated for 20 minutes at room temperature and the absorbance was measured at 517 nm using BHT as standard. A blank was prepared without adding standard or test compound. Lower the absorbance of the reaction mixture indicates higher the free radical scavenging activity. The capacity to scavenge the DPPH radical was calculated using the following equation.

$$DPPH\ Scavenged\ (\%) = \frac{Abs\ Control - Abs\ Test}{Abs\ Control}$$

Where, Abs control is the absorbance of the control reaction and Abs test is the absorbance in the presence of the test compounds.

Molecular Docking using HEX 4.2

In biological system over activation of receptor tyrosine kinase (RTK) signaling pathways is strongly associated with carcinogenesis. It has become increasingly clear that impaired deactivation of RTKs may be a mechanism in cancer [41]. Further, normal cancer cells have receptors that attach to circulating estrogen and progesterone. Estrogen and progesterone bind to the receptors that may work with growth factors (e.g., oncogenes and mutated tumor suppressor genes) to cause cancer cell growth [42]. Based on the literature it has been shown clearly that the drug toremifene has been used to target the human estrogen receptor [43].

On this basis, we selected RTK and human estrogen receptor as a biological targets and the crystal structure of EGFR kinase domain (PDB ID: 2a91) and human estrogen receptor (PDB ID: 2IOK) were retrieved from protein data bank for docking study of synthesized compounds using HEX 4.2 software.

Docking of Ligand and Metal Complexes

For macromolecular docking studies, the chemical structures of synthesized ligand, metal complexes and standard toremifene were drawn using ChemDraw ultra. The 3D optimization was done in ChemDraw 3D ultra software and stored as .pdb file. Hex docking was carried out by setting suitable parameters (Table 1) this docking score can be interpreted as interaction energy.

Table 1: Parameters used for docking study

Correlation type	Shape only
Grid Dimension	0.6
Receptor range	180
Ligand Range	180
Twist range	360
Distance Range	40

Spectral Measurements

Melting points were determined in open capillaries and are uncorrected. Microanalysis (C, H, and N) were performed in Carlo-Erba 1106 model 240 Perkin-Elmer analyzer and IR spectra were recorded with Shimadzu model FT-IR spectrophotometer by using KBr pellets in the range 4000-350 cm^{-1}. The molar conductivity in Dimethylformamide (DMF) ($l0^{-3}$M) at room temperature was measured using Equiptronics digital conductivity meter. Bruker FT-NMR Spectrophotometer (400 MHz) was used for recording ^{1}H-NMR spectra at 25 °C in DMSO-d^{6} with TMS (tetra methyl silane) as the internal reference. UV-visible absorption spectra were recorded using Ocean optics HR - 4000 spectrophotometer at room temperature. DNA binding studies were carried out using Shimadzu model UV-1650PC spectrophotometer in *Tris*−HCl buffer solution (pH = 7.2) at room temperature.

RESULTS AND DISCUSSIONS

Characterization of Complexes

The elemental analysis, IR, ^{1}H NMR spectral data of the ligand and new complexes are summarized in experimental section. The elemental analysis data are agreed with the theoretical values within the limit of experimental error and confirmed the formula of the complexes. The synthesized complexes are soluble in DMF and DMSO. Molar conductance of the complexes was measured in DMSO at a concentration of 0.001 M. The observed conductance values fall in the range of 20-35 Ohm^{-1} cm^{2} mol^{-1} for complexes II, III and IV indicating that the complexes are non-electrolytes except for complex I which showed uni-uni valent behavior with conductance value of 780 Ohm^{-1} cm^{2} mol^{-1}. Infrared spectra of ligand and metal complexes are almost same with slight shift in peak position and varied intensity confirming the coordination of ligand to metal ions.

The band for C=N stretching of ligand was observed at lower frequency by 20 - 30 cm^{-1} in the metal complexes, indicating involvement of the azomethine nitrogen in the complex formation. The shift of the –OH band has appeared in the range 3405 - 3448 cm^{-1} in the metal complexes compared to the spectrum of free ligand at 3400 cm^{-1} which also indicate the involvement of the oxygen of –OH group for coordination with metal ions. Weak bands at 500 - 550 cm^{-1} indicate M-N bond and a band in the region 430 - 490cm^{-1} is due to M-O bond [44,45].

The electronic spectra of ligand and metal complexes were recorded in DMF solution. The ligand L^{1} showed a band in the region (32786 cm^{-1}) 305 nm to (25641 cm^{-1}) 390 nm due to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions respectively. Electronic spectra of high spin complex I showed one peak at (19417 cm^{-1}) 515 nm is assigned to $^{6}A1g - {}^{4}T_{1g}$ transition characteristic of octahedral structure [46-48].

The electronic spectrum of complex II displayed four band at (20202 cm^{-1}) 495 nm, (19047cm^{-1}) 525 nm, (16528 cm^{-1}) 605 nm (14814 cm^{-1}) 675 nm due to $^{4}T_{1}g(F) \rightarrow {}^{4}T_{1}g(P)$ (ν_2), $^{4}A_{2}$-$^{4}T_{1}$ (P) (ν_3), $^{4}T_{1}g(F) \rightarrow {}^{4}A_{2}g$ (F) (ν_2), $^{4}A_{2}$-$^{4}T_{1}$ (F) (ν_2) transitions respectively [49,50], these spectral transitions suggest that an octahedral geometry for Co(II) complex.

The spectrum of complex III displayed two bands. The broad band in the region (13888 cm^{-1}) 720 nm to (12987 cm^{-1}) 775 nm may be due to john-teller distortion [51,52] and a high intensity band at (25974 cm^{-1}) 385 nm is ascribed to symmetry forbidden ligand to metal charge transfer. The above result suggest distorted octahedral geometry for Cu(II) complex [53] (Figure 2).

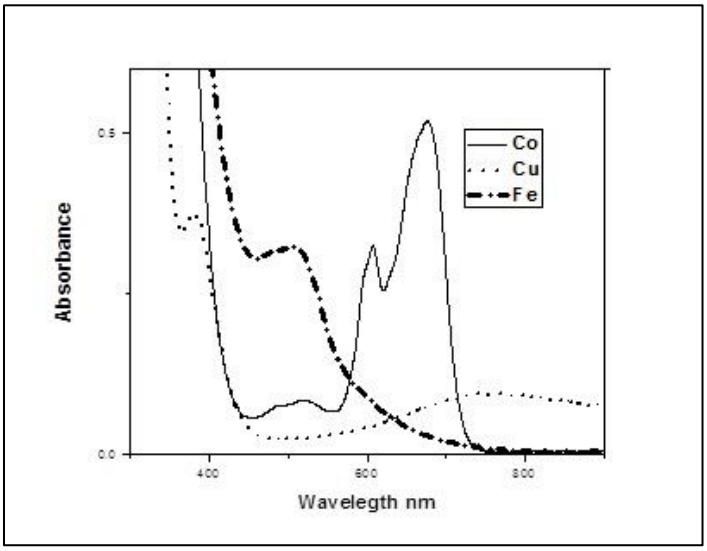

Figure 2: Electronic spectra of cobalt, copper and iron complexes

The ^{1}H NMR spectrum of ligand L^1 was recorded in DMSO-d_6 and the assignments are summarized in experimental part. It showed a singlet at 2.60 ppm for three methyl protons. The signal due to S-NH proton appeared at 3.59 ppm. The peak is due to aromatic protons obtained in the region 7.3 to 9.4 ppm and the proton of azomethine resonances at 8.56 ppm. The proton of phenolic -OH group gave a signal at 14.1 ppm (Table 2).

Table 2: Physicochemical and analytical data of the synthesized compounds

Compound	Mol. Wt.	M. P.(in °C)	Yield (%)	Color	Found (calculated) %			
					C	H	N	M
Ligand(L^1)	168	158	85	Orange	54	4.96	8.95	
					-54.48	-5.18	-9.08	---
Complex I	614	254	65	Brown	50.49 (50.76)	3.69	8.89	8.89
						-3.9	-9.11	-9.11
Complex II	617	285	57	Brown	50.38	3.23	8.75	9.46
					-50.52	-3.88	-9.06	-9.55
Complex III	621	310	60	Green	49.37	3.57	8.74	9.97
					-50.15	-3.85	-9	-10.23
Complex IV	623	295	65	Grey	49.29	3.19	8.36	9.98
					-50.03	-3.84	-8.98	-10.42

DNA Binding Experiments

The binding interaction of the complexes with CT-DNA was monitored by comparing their absorption spectra with and without CT-DNA. The Figures 3-6 represent the absorption spectra of the complexes I, II, III and IV in the presence and absence of CT-DNA respectively and all complexes exhibit well resolved bands at 270 nm to 280 nm with increasing the DNA concentration (0-300 µM). The K_b values for the complexes were determined from the decay of the absorbance in the region 270 to 280 nm with increasing concentration of DNA by using equation (1). The K_b values for complexes I, II, III and IV are 63.01 $X10^6$ M^{-1}, 19.06 $X10^6$ M^{-1}, 22.10 $X10^6$ M^{-1}, and 2.258 $X10^3$ M^{-1} respectively. The intrinsic binding constant values suggests that interactions of complexes with CT-DNA. Electronic absorption data upon addition of CT-DNA to the complexes is tabulated in Table.3.

Table 3: Electronic absorption data upon addition of *CT*-DNA to the complexes

Complex	λ_{max} (nm)		Δλ (nm)	H (%)	Binding constant K_b (M^{-1})
	Free	bound			
Complex I	272	273	1	3.95	63.01 $X10^6$
Complex II	275	276	1	5.88	19.06 $X10^6$
Complex III	277	278	1	9.14	22.10 $X10^6$
Complex IV	277	277	0	15.26	2.258 $X10^3$

The complexes can bind to the DNA in different binding modes via both covalent and/or non-covalent interactions [54]. Non-covalent interactions include intercalation between the bases, and binding to minor groove, major groove, sugar–phosphate backbone and three way junctions [55] depending on the structure, charge and type of ligands present in metal complexes. Since, the metal complexes contains Schiff base L^1 and 1,10-phenanthroline ligand, it would provide an aromatic moiety extending from the metal center through which overlapping would occur with the base pairs of DNA by intercalation. The absorption spectra of CT-DNA in the presence of complexes I and III (Figure 3 and Figure 5) accompanied by hyperchromism at λ max from 276 - 278 nm for complex I and 269 - 271 nm for complex III. The percentage hyperchromicity for the CT-DNA/I and III complex was determined from $(\varepsilon_F-\varepsilon_B)/\varepsilon_F\times100$, where ε_F is the extinction coefficient of the free complex and ε_B is the extinction coefficient of the bound complex. The hyperchromism (H% 3.95, 9.14 for complex I and II) in the band at indicate that the interaction of the complexes with CT-DNA leading to the formation of new complex with double-helical CT-DNA [56]. The intensity of absorption increases at 278 nm and 271 nm is due to the involvement purine and pyrimidine bases of DNA in binding with the complex. This causes the slight change in the conformation of DNA [57]. The hyperchromic shift observed for complex I and III is due to smaller size metal ions which enhances binding affinity with DNA and may involved in stacking interaction with DNA Via bipyridyl ring and Shiff base ligand in complexes leads to denaturation of DNA. The hypochromism and slight red shift in the absorption spectra of complexes II and IV from 274 to 300 nm is an evidence for stabilization of CT-DNA [34] and Metal ions in complexes II and IV (Cobalt and Zinc) are comparatively bigger in size and involved in groove binding or electrostatic interaction with DNA through coordinated ligands and forms a complex with DNA and enhances its stabilization.

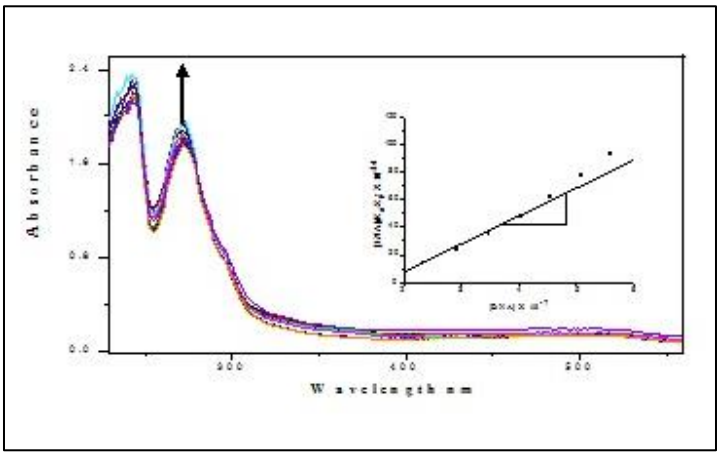

Figure 3: Absorption spectra of complex I in *Tris*-HCl buffer upon addition of DNA

[Fe] = 0.5 μM, [DNA] = 0-300μM. Arrow shows the absorbance changing upon the increase of DNA concentration. (The inset: [DNA]/ $(\varepsilon_a-\varepsilon_f)$ vs [DNA] for the titration of DNA with Fe(III) complex)

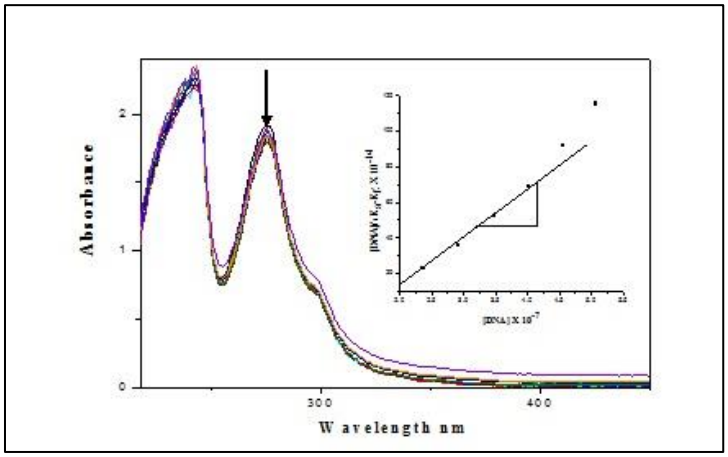

Figure 4: Absorption spectra of complex II in *Tris*-HCl buffer upon addition of DNA

[Co] = 0.5 μM, [DNA] = 0-300μM. Arrow shows the absorbance changing upon the increase of DNA concentration. (The inset: [DNA]/ $(\varepsilon_a-\varepsilon_f)$ vs [DNA] for the titration of DNA with Co(II) complex

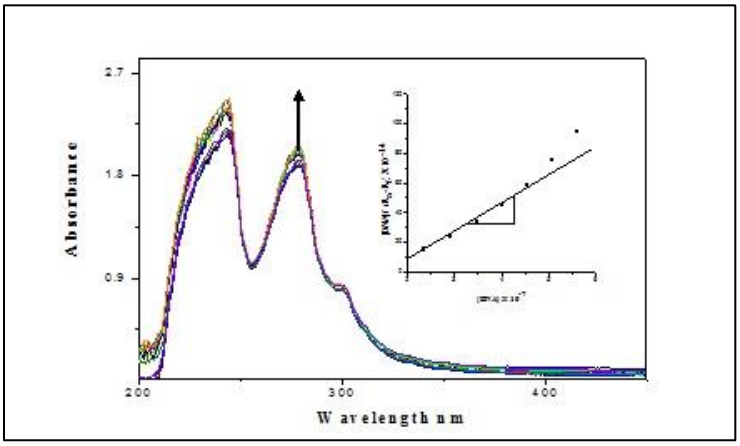

Figure 5: Absorption spectra of **complex III** in *Tris*-HCl buffer upon addition of DNA
[Cu] = 0.5 μM, [DNA] = 0-300μM. Arrow shows the absorbance changing upon the increase of DNA concentration. (The inset: [DNA]/ (ε_a-ε_f) vs [DNA] for the titration of DNA with Cu(II) complex.

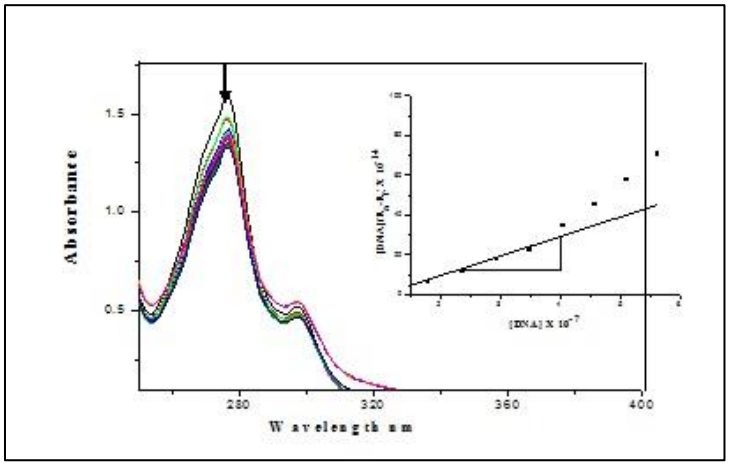

Figure 6: Absorption spectra of **complex IV** in *Tris*-HCl buffer upon addition of DNA
[Ni] = 0.5 μM, [DNA] = 0-300μM. Arrow shows the absorbance changing upon the increase of DNA concentration. (The inset: [DNA]/ (ε_a-ε_f) vs [DNA] for the titration of DNA with Ni(II) complex)

Antimicrobial Activity

The different microorganisms such as, two Gram-positive (*Staphylococcus areus*, *Bacillus subtilis*) as well as two Gram-negative (*Escherichia coli*, *Salmonella typhi*) bacteria and two yeasts (*Aspergillus niger*, *Candida albicans*) were used to study the biological activity of ligand and all the four complexes. The results are placed in Table 3.

The metal complexes exhibited fairly good activity [58,59] than the free ligand. The increased in activity of the complexes could be explained on the basis of overtones concept and chelation theory [60]. The cell permeability, the lipid membrane that surrounds the cell, favors the passage of only lipid soluble materials on the basis that liposolubility is an important factor that controls antimicrobial activity. On complexation, the polarity of the metal ion is reduced to a greater extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion. Furthermore, it increases the delocalization of p- and d-electrons over the whole complexes and enhances the lipophilicity of the complex. The increased lipophilicity enhances the penetration of the complexes into lipid membranes and blocking of metal binding sites on the enzymes and may interrupt the DNA replication of the microorganisms. The antimicrobial activity of the complexes may also affected by the geometry of the complexes which are supposed to have octahedral geometry [59]. The studied complexes showed higher activity for Gram (+) than for Gram (-) bacteria (Table 4). The highest active compound for Gram (-) bacteria was complex I while complex III was highest active for Gram (+) bacteria. The results reveal that all the four complexes are excellent antifungal agents. The higher activity of the metal complexes is may be due to the smaller ionic radius of metal ion. Since Fe(III) and Cu(II) ions in complex I and III are relatively smaller in size and enhances polar nature of the

complex to larger extent leads to the increase in lipophilicity. These results are in good correlation with the DNA interaction and docking studies of the metal complexes.

Table 4: *Invitro* antimicrobial activity of compounds and their inhibition zone (MIC) in mm

	Antibacterial activity								Antifungal activity			
	E.coli		***S.typhi***		***S.aureus***		***B.subtilis***		***A.niger***		***C. albicans***	
Comps. \Conc. (µg/mL)	100	200	100	200	100	200	100	200	100	200	100	200
Ligand (L^1)	13	15	18	23	10	12	17	20	20	25	18	24
Complex I	18	27	17	26	8	11	10	13	15	22	14	19
Complex II	14	21	15	20	23	26	21	27	18	26	15	21
Complex III	21	24	19	21	19	29	24	33	17	30	21	19
Complex IV	11	14	16	19	21	28	18	24	16	23	21	26
Std 1	25	28	24	27	25	30	28	34	-	-	-	-
Std 2	-	-	-	-	-	-	-	-	26	30	22	26
Control	-	-	-	-	-	-	-	-	-	-	-	-

Where Std1. is Chloramphenicol, Std2. is Fluconazole, Control is DMSO.

Antioxidant Activity

The synthesized ligand and the metal complexes were screened for antioxidant activity against DPPH radical at 10-100 µg/mL concentration. The antioxidant activities of the synthesized compounds are expressed by comparing with standard BHT. The uncoordinated ligand has less free radical scavenging activity compared to the metal complexes Table 5. All the complexes exhibited good antioxidant activity compared to the standard.

Table 5: Antioxidant activity of the synthesized compounds

Compounds	% of Scavenging activity at different concentrations in µg/mL									
	10	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**	**100**
Ligand (L^1)	20.27	29.91	35.43	39.82	43.7	48.21	57.97	65.47	68.21	70.37
Complex I	20.1	30.28	47.51	60.18	62.14	71.27	77.8	78.59	80.41	84.33
Complex II	17.32	28.67	35.95	44.39	46.14	65.06	73.79	79.62	83.07	90.11
Complex III	10.94	23.26	42.93	42.93	59.83	70.91	76.17	78.67	81.6	89.76
Complex IV	18.63	28.25	37.86	43.04	48.22	50.45	55.89	61.69	66.28	72.43
BHT	32.43	38.94	50.21	56.78	65.47	78.24	85.83	92.53	94.87	97.56

Docking Studies

The results of antimicrobial and DNA interaction of metal complexes revealed that the synthesized compounds are highly potent. Therefore, we have considered worth-while to do docking studies to support the *in vitro* activity. The docking was used to determine the orientation of inhibitors bound in the active site of receptors. As the metal complexes exhibited good DNA binding property in the present study we selected tyrosine kinase (RTK) and human estrogen receptor which are involved in cancer causing mechanism in biological system. Toremifene drug was used as standard for docking studies which was known to be potential inhibitor of human estrogen receptor.

The docking study was carried out using Hex 4.2 software; both the receptor and ligand/complex were uploaded in .pdb file format. The E-Total values obtained from the HEX software after completion of docking process indicate that the strong interaction of ligand/complex with the receptors. E-Total values are tabulated in Table 6. Complexes I and III showed least docking score with human estrogen receptor and tyrosine kinase respectively compare to the standard and other complexes so we considered that complex I and III are representative and potential inhibitors of cancer causing receptors. Binding of complex I with tyrosine kinase receptor and binding of complex III to the human estrogen receptor are shown in Figure 7 and Figure 8 respectively.

Table 6: Docking scores of the synthesized compounds

Compounds	**E- Total value in Kjmol^{-1}**	
	Human estrogen receptor	Tyrosinekinase (RTK)
Ligand (L^1)	-73.97	-24.97
Complex I	-105.19	-24.86
Complex II	-107.23	-18.02
Complex III	-123.4	-26.94
Complex IV	-115.53	-23.11
Toremifene	-75.28	-22.42

The docking score of ligand E-Total -73.97 which is comparable with the E-Total -75.28 of the standard toremifene for human estrogen receptor. But the E-Total values obtained for complex I, -105.19, complex II, -107.23, complex III, -123.40, complex IV, -115.53 kjmol^{-1}. In the similar manner the docking studies carried out on tyrosine kinase (RTK) receptor showed E-Total -22.42 kjmol^{-1} which is less than the uncoordinated schiff base ligand whose E-Total was -24.97 kjmol^{-1}. The docking scores obtained for complex I, complex II, complex III and complex IV was -24.86, -18.02, -26.94 and -23.11 respectively. The results of docking studies carried out using uncoordinated ligand and the complexes were compared with both the receptors. It is clear that complex III was showed highest inhibiting property against both the receptors. While other complexes exhibited fairly good inhibiting affinity than the L^1 and the standard used.

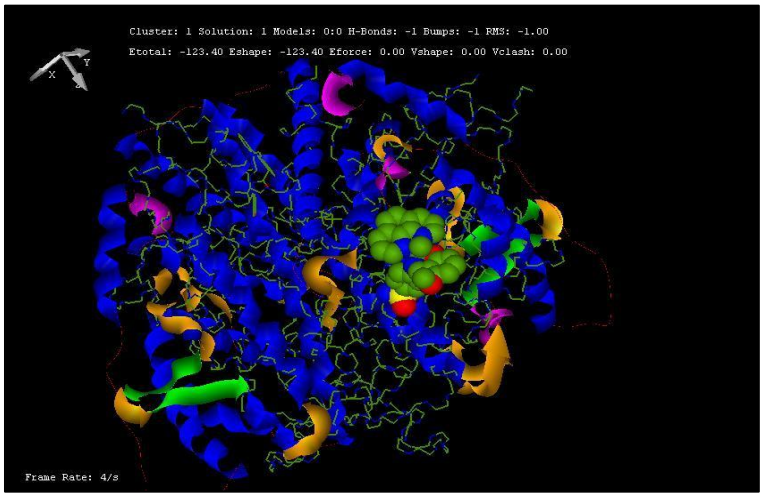

Figure 7: Binding interaction of complex III with human estrogen receptor

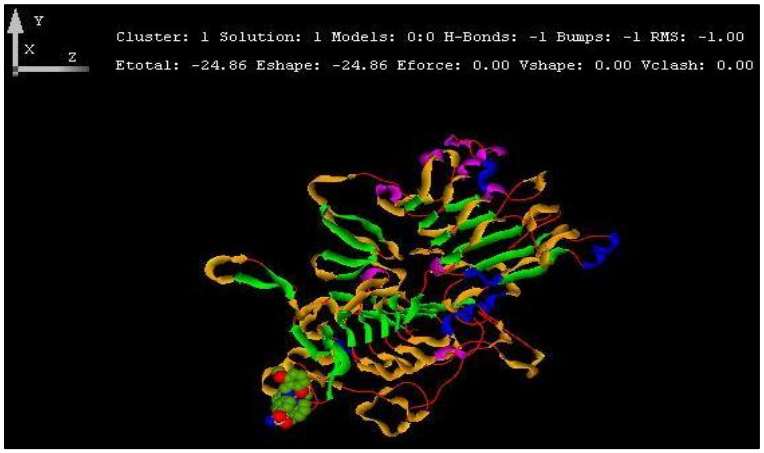

Figure 8: Binding interaction of complex I with Tyrosine kinase (RTK) receptor

CONCLUSION

The synthesized schiff base acts as bidentate ligand through the coordination of azomethine nitrogen and hydroxyl oxygen atom to the metal ion. The binding of ligand to metal ion was confirmed by the various spectral techniques like elemental analysis, conductivity, IR, ^{1}H NMR and UV-Vis spectroscopy. The interaction of complexes with CT-DNA was investigated by absorption spectroscopy. From the results, it was found that all the metal complexes bind DNA more efficiently with high intrinsic binding constant.

All the newly synthesized compounds were tested for their antimicrobial activity (MIC) against selected bacteria *Escherichia coli, Salmonella typhi, Staphylococcus aureus, Bacillus subtilis* and two fungi strains *Aspergillus niger and Candida albicans.* Iron and zinc complexes were found to be the most potent antibacterial agents and iron, cobalt and copper complexes were showed excellent antifungal activity against *Aspergillus niger and Candida*

albicans when compare to the standard drug Chloramphenicol and Fluconazole. Metal complexes showed good antioxidant property than the L^1.

Molecular docking studies revealed that metal complexes are good inhibitors of cancer causing receptors and supported the *in vitro* DNA interaction studies and biological activity of the synthesized compounds.

ACKNOWLEDGEMENT

Authors are thankful to Sri Venkateshwara Industries, Kallur-Mandli Industrial Estate Shimoga and Dr. T K Vishnuvardhan, Assistant Professor, Department of Chemistry, Acharya Institute of Technology, Bangalore, India for their support.

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