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DNA binding, In silico Docking and *In vitro* biological screening of some transition metal complexes of Schiff base ligand as potential blockers of cancer causing receptors

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Abstract : A series of metal(II) complexes ML where M = Cd(II), Cu(II) and Zn(II) have been synthesized from Schiff base ligand 3-[(*E*)-(4*H*-1,2,4-triazol-4-ylimino)methyl]quinoline-2-thiol (L) and characterized on the basis of elemental analysis, conductivity measurement, electronic, IR and ^{1}H NMR spectral studies. The interaction of complexes with *CT*-DNA (*Calf thymus* – Deoxyribo nucleic acid) has been studied using absorption spectroscopy. Absorption spectral studies confirmed that all the complexes exhibited potential DNA binding affinities with an intercalative mode of interaction, which involves the insertion of complexes in between the DNA base pairs was proposed. *In vitro* antimicrobial and anthelmintic activity of synthesized compounds reveals that complexes were more active than the uncoordinated ligand. Docking scores suggest that the metal complexes were potential inhibitors of human estrogen receptor and tyrosine kinase receptor (RTK), which are responsible for causing breast cancer.
Keywords: Metal complexes, antimicrobial, DNA binding, docking, human estrogen receptor.

Introduction

The quinoline ring occurs in various natural products, especially in alkaloids. The quinoline skeleton is often used for the design of many synthetic compounds with diverse pharmacological properties [1,2]. Quinoline derivatives have proven to be the potential anti-inflammatory, analgesics, anti-convulsant, antibacterial, antipyretic, antihypertensive and interferon inducing agents [3]. Many Schiff bases derived from 2-chloro-3-formyl-quinoline have been reported for their antifungal activities and as potential biodynamic agents [4]. The Schiff bases derived from 3-formyl-2-mercaptoquinoline have also proven to be important pharmaceutical agents [5].

Schiff bases play an important role in inorganic chemistry as they easily form stable complexes with most transition metal ions. The development of the field of bioinorganic chemistry has increased the interest in schiff base complexes, since it has been recognized that many of these complexes may serve as models for biologically important species [6-11]. Transition metal complexes of Schiff bases have attracted much attention due to their potent biological activities such as antifungal, antibacterial, anticancer and herbicidal applications. Investigations on the interaction between transition metal complexes and DNA have created interest due to their importance in cancer therapy and molecular biology [12].

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 [13]. 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 [14]. Estrogen promotes breast cancer proliferation through a number of established pathways [15]. Tyrosine kinase receptors are expressed on the surface of tumor and/or endothelial cells and represent attractive targets for new anti-cancer treatment strategies [16]. Literature search revealed that transition metal complexes are potential anticancer agents [17]. Developing potential inhibitors of cancer causing receptor is an area of great interest [18,19]. Hence the present study aims for the Synthesis, Characterization, biological activity, DNA interaction and molecular docking studies against cancer causing receptors, of Cd(II), Cu(II) and Zn(II) complexes with Schiff base 3-[(*E*)-(4*H*-1,2,4-triazol-4-ylimino)methyl]quinoline-2-thiol (L).

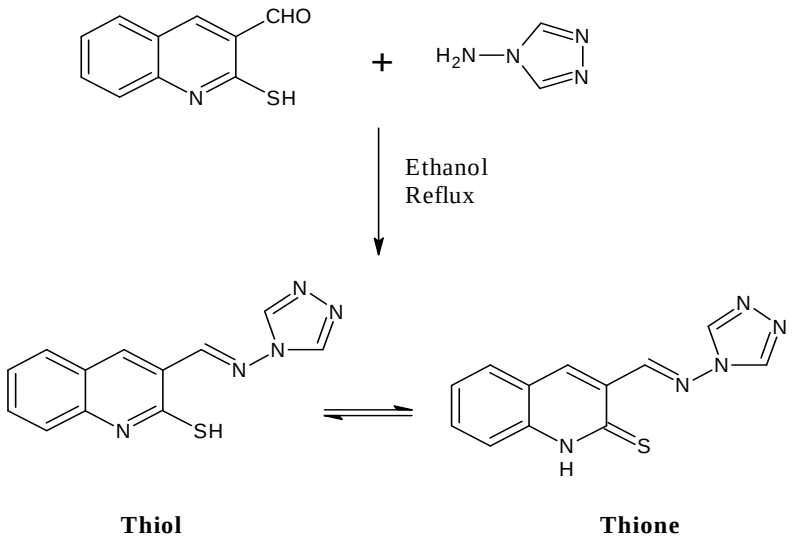
Experimental

Materials and methods

The chemicals used for preparing the ligands were of reagent grade. The solvents were distilled and dried before use by following standard procedure [20]. The acetanilide was prepared from the aniline following the standard procedure [21] and used as the starting material for the preparation of substituted 3-formyl-2-chloroquinoline. The metal chlorides and acetates used were 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.

Synthesis of Schiff base ligand: *3-[(E)-(4H-1,2,4-triazol-4-ylimino)methyl]quinoline-2-thiol*, (L)

3-formyl-2- chloroquinoline and 3-formyl-2-mercapto quinoline were prepared according to the literature method **[**22,23].



Thiol **Thione**

Fig 1. Thiol-Thione tautomerization of 3-[(*E*)-(4*H*-1, 2, 4-triazol-4-ylimino) methyl] quinoline-2-thiol (L)

A solution of 3-formyl-2-mercapto quinoline (0.01 mol) in absolute ethanol (10 mL) was added to a solution of 4-amino-1,2,4-triazole (0.01mol) in absolute ethanol (10 mL) and refluxed in water bath for 2-3 h. The Progress of the reaction was monitored by TLC. The precipitated product was filtered, recrystallized from suitable solvent and dried in desiccator, yield 85%, M.P. 202 ^{o}C, yield 85%, Anal.Calculated for $C_{12}H_9N_5S$. calcd. (%): C (28.23) H (3.52) N (27.45). Found (%): C, 28.10; H, 3.59; N, 27.32. IR, KBr pellets (ν, cm^{-1}): 1600 ν(C=N); 2360 ν(C-H, Ar-H); 1062 ν(C=S);. ^{1}H NMR (δ, ppm): 7.5-8.3 (m, 4H, Ar-H), 8.6(s 1H S-H), 9.1(s,1H, Ar-H); 9.3(s, 3H, CH=N).

General procedure for the preparation of the complexes

Metal complexes were synthesized by reacting Metal salt (1 mM) (copper acetate, zinc chloride, cobaltous chloride, ferric chloride or cadmium acetate) was dissolved in hot ethanol (10 mL) and mixed with ehtanolic solution of ligand L (2 mM, 10 mL). The reaction mixture was refluxed on water bath for about 3 - 4 h. Separated dark colored precipitates were filtered washed twice with hot ethanol (10 mL) followed by ether (10 mL), recrystallized from suitable solvent and dried in desiccator. Yield (50% - 75%).

Analytical data of the complexes

$[Cd(L)_2(OAc)_2]$: C, 45.34; H, 3.23; N, 18.89; Cd, 15.17. Found: C, 45.11; H, 3.00; N, 18.65; Cd, 14.98. IR, KBr pellets (ν, cm^{-1}): 1616 ν(C=N str); 1060 ν(C=S);. Electronic spectra (nm): 305nm, 435nm.

$[Cu(L)_2(OAc)_2]$: C, 48.54; H, 3.46; N, 20.22; Cu, 9.18. Found: C, 48.31; H, 3.29; N, 19.97; Cu, 8.96. IR, KBr pellets (ν, cm^{-1}): 1616 ν(C=N str); 1060 ν(C=S);. Electronic spectra (nm): 305nm, 435nm.

$[Zn(L)_2Cl_2]$: C, 44.58; H, 2.78; N, 21.67; Zn, 10.12. Found: C, 44.27; H, 2.43; N, 21.23; Zn, 9.87. IR, KBr pellets (ν, cm^{-1}): 1616 ν(C=N str); 1060 ν(C=S). Electronic spectra (nm): 305nm, 435nm.

DNA interaction studies

All the experiments involving the interaction of complexes with CT - DNA were conducted in *Tris* buffer (10 mm *Tris*-HCl-50 mm NaCl buffer, pH 7.2). The concentration of the DNA used for binding experiments was determined by measuring the absorption intensity at 260 nm with molar extinction coefficient value 6600 $M^{-1}cm^{-1}$ [24]. The absorbances were measured by keeping the concentration of the metal complex

constant (1×10^{-3}M) while varying the DNA concentrations (0 – 300 μL). The absorption data were analyzed for an evaluation of the intrinsic binding constant K_b using equation (1) as per reported procedure [25].

$$[DNA]/(\varepsilon_a\varepsilon_f) = [DNA]/ (\varepsilon_b-\varepsilon_f) + 1/K_b(\varepsilon_a-\varepsilon_f) \quad \ldots\ldots\ldots(1)$$

Where ε_a ε_f and ε_b are the apparent, free and bound metal complex extinction coefficients, respectively. A plot of [DNA] / (ε_b-ε_f) gave a slope of l/ (ε_b-ε_f) and intercept equal to $1/K_b$ (ε_b-ε_f), where K_b is the ratio of the slope to the y intercept.

In vitro Antimicrobial activity

Antimicrobial activity was carried out following standard method described in literature [26]. The *in vitro* biological activity of the investigated Schiff base and its metal complexes were tested against the bacteria *Escherichia coli, Salmonella typhi, Staphylococcus aureus, Bacillus subtilis* by well diffusion method using nutrient agar as the medium and chloramphenicol as the standard. The antifungal activities of the compounds were also tested against the fungi *Aspergillus niger* and *Candida albicans*, on potato dextrose agar as the medium and fluconazole as the standard. The stock solution (1mg in 1mL of Dimethylsulphoxide) was prepared by dissolving the compound in DMSO and the solution was serially diluted in order to find Minimum Inhibitory Concentration (MIC) values (100, 200 μg/mL) were prepared separately. In a typical procedure, a well was made on the agar medium inoculated with microorganism. The well was filled with the test solution using a micropipette and the plate was incubated 24 h for bacteria at 37 °C and 72 h for fungi at 30 °C. After incubation, the diameter of the clear zone of inhibition surrounding the sample is taken as a measure of the inhibitory power of the sample against the particular test organism [27].

In vitro Anthelmintic activity

The anthelmintic assay was carried as per the method given in the literature [28]. The assay was performed on adult Indian earthworms, *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings. The earthworms (*Pheretima posthuma*) collected from moist soil and washed with normal saline to remove all faecal matter were used for the anthelmintic study. The earthworms of 3 - 4 cm in length and 0.1 - 0.2 cm in width were used for all the experimental protocol.

All the synthesized compounds were subjected to anthelmintic activity studies against the earthworms at 5 mg/mL concentration and Albendazole used as a reference drug. The paralyzing and death times were noted and their mean was calculated for triplicate sets. The death time was ascertained by placing the earthworms in warm water (50 °C) which stimulated the movement, if the worm was alive.

Molecular docking using HEX 4.2.

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 [29]. 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 [30]. Based on the literature it has been shown clearly that the drug toremifene has been used to target the human estrogen receptor [31].

Bearing above facts, we selected human estrogen receptor and RTKs as a biological targets and human estrogen receptor (PDB ID : 2IOK) and the crystal structure of EGFR kinase domain (PDB ID : 2a91) 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. More negative E – Total value implies that there exists a strong strong interaction between drug and receptor and that leads to inhibition of receptor activity.

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. The molar conductivity in Dimethylformamide (DMF) ($l0^{-3}$M) at room temperature were measured using Equiptronics digital conductivity meter. IR spectra were recorded with Shimadzu model FT-IR spectrophotometer by using KBr pellets. Bruker FT-NMR Spectrophotometer (400 MHz) was used for recording ^{1}H-NMR spectra at 25°C in DMSO with TMS (Tetra methyl silane) as the internal reference. UV-visible absorption spectra were recorded using Ocean optics HR - 4000 spectrophotometer at room temperature.

Results and discussions

Characterization of complexes

The elemental analysis, UV, 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. Synthesized complexes are soluble in DMF and DMSO.

The band absorved for uncoordinated ligand and their metal complexes are all most similar expect slight shift in the position of the peak and varied intensity confirms the coordination of ligand to the metal ions. The stretching frequency observed at 1524 to 1589 cm^{-1} assigned to the C=N, the absorption band at 1500 to 1525 cm^{-1} S=C-N-H group. Stretching frequency at 1168 to 1176 cm^{-1} was assigned to C=S.

Molar conductance of the complexes is measured in DMF at a concentration of 0.001 M. The observed conductance values fall in the range of 20 to 150 Ohm^{-1} cm^2 mol^{-1}, indicating that the complexes are non-electrolytes, uni-univalent electrolyte, and univalent electrolyte.

Electronic spectral data of the ligands and their transition metal complexes were recorded in DMF solution at 10^{-3} molar concentration. The absorption bands for the complexes will help to give an idea of their structure [32]. Schiff base ligand (L) showed absorption bands in the region 270nm to 380nm transitions due to $\pi\rightarrow\pi^*$ and $n\rightarrow\pi^*$ transitions. These bands were slightly shifted to blue or red regions in all complexes, while new bands were observed in the visible region for the Cu(II) and Cd(II) complexes due to d-d transitions. The interpretations of ultraviolet spectra of metal complexes revealed that charge transfer bands occur in the same region with $\pi\rightarrow\pi^*$ and $n\rightarrow\pi^*$ transitions. Electronic spectrum of Cu(II) exhibited bands at 269nm, 321nm due charge transfer transition and one more band at 425nm assigned to $^2Eg \rightarrow {}^2T_2g$ this data substantiate for the octahedral geometry of this complex [33,34]. Cd(II) complex exhibited two bands at 267 nm and 322 nm due to charge transfer transitions and confirms coordination of ligand to metal ion.

^{1}H NMR spectral studies

^{1}H NMR spectrum was recorded in DMSO-d6. The Schiff base ligand showed peak at 9.3 (s, 3H) due to CH=N proton, pyridyl ring proton was showed peak at 9.1 (s,1 H, Ar-H), SH proton resonated at 8.6 ppm, peaks in the region 7.5 - 8.3 are due to aromatic protons(m, 4H). The peak due to SH at 8.6 was not absorved in Zn(II) complex. This confirmed the involvement of thiolate sulphur in coordination with metal via deprotanation. Disappearance of azomethine proton at 9.3 in complexes indicated the participation of azomethine nitrogen in the coordination [35].

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)	255	202	85	Brown	28.10 (28.23)	3.15 (3.52)	27.37 (27.45)	---
$[Cd(L)_2(OAc)_2]$	745	251	65	yellow	45.11 (45.34)	3.00 (3.23)	18.65 (18.89)	14.98 (15.17)
$[Cu(L)_2(OAc)_2]$	696	237	68	Green	48.31 (48.54)	3.29 (3.46)	19.97 (20.22)	8.96 (9.18)
$[Zn(L)_2(OAc)_2]$	698	225	60	Grey	44.27 (44.58)	2.43 (2.78)	21.23 (21.67)	9.87 (10.12)

DNA binding experiments

The interaction between the complexes and *CT*-DNA were monitored by UV-Vis spectroscopy. Electronic absorption data upon addition of *CT*-DNA [0 - 300 µM] to the complexes were listed in Table 3. The absorption spectra of complexes Cd(II), Cu(II) and Zn(II) in the presence and absence of *CT*-DNA are shown in Figure 3, 4 and 5. In absorbance spectra of all complexes, hyperchromism (H) and slight bathochromic shift were observed on the addition of CT-DNA. This significant hyperchromism effect suggests that there exists a strong interaction between the complexes and DNA that can be rationalized in terms of intercalative binding mode [36,37]. Intercalative mode of interactions of metal complexes was further supported by the intrinsic binding constant (K_b) values. Calculated intrinsic binding constant values for the complexes Cd(II), Cu(II) and Zn(II) was found to be $8.01 \times 10^6\ M^{-1}$, $9.49 \times 10^6\ M^{-1}$, $9.18 \times 10^6\ M^{-1}$ respectively.

Table 3. Electronic absorption data upon addition of *CT*-DNA to the complexes [*CT*-DNA = 0 – 300 µM].

Complex	λ_{max} (nm)		Δλ (nm)	H (%)	$K_b\ M^{-1}$
	Free	Bound			
Cd	324	322	2	11.21	8.01×10^6
Zn	322	322	0	21.67	9.49×10^6
Cu	322	322	0	13.88	9.18×10^6

The observable hypochromism and red shift are usually characterized by the non-covalently intercalative binding of compound to DNA helix, due to the strong stacking interaction between the aromatic chromophore of the compound and base pairs of DNA [38, 39]. Results of absorption titration for *CT*-DNA showed that no major difference was observed in the value of λ_{max}. These spectral characteristics suggest that the complexes had been bound to the base pairs DNA by intercalation and bathochromism result might be due to the decrease in the energy of $\pi \rightarrow \pi^*$ transition, when the π orbital of the base pairs of DNA coupled with the $\pi \rightarrow \pi^*$ orbital of the intercalated ligand.

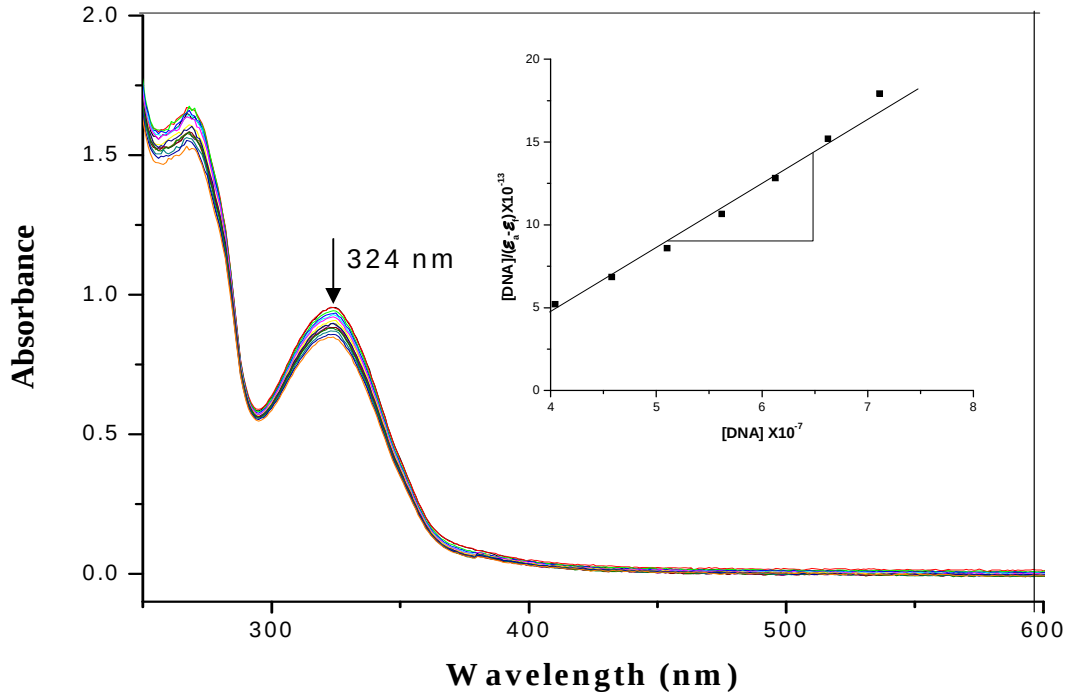


Fig 2. Absorption spectra of Cadmium complex in *Tris*-HCl buffer upon addition of DNA. [Cd] = 0.5 µM, [DNA] = 0 – 300 µM. Arrow shows the absorbance changing upon the increase of DNA concentration. (The inset: [DNA]/ (ε_a-ε_f) vs [DNA] for the titration of DNA with Cd(II) complex.)

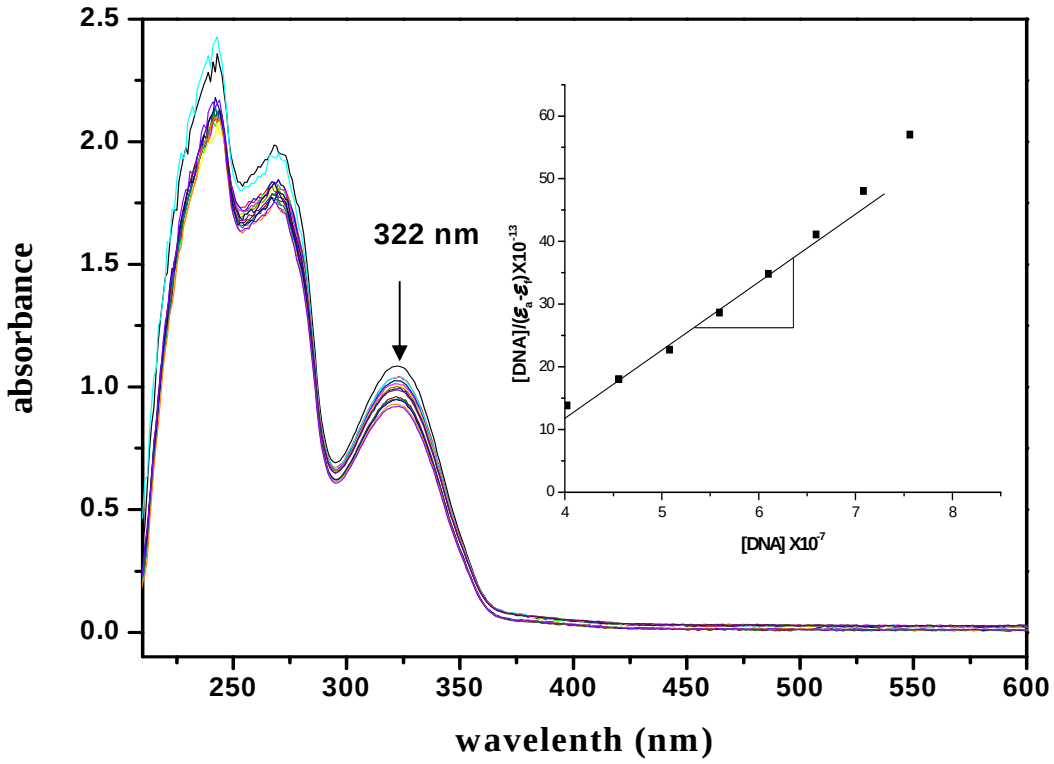


Fig 3. Absorption spectra of Copper complex in *Tris*-HCl buffer upon addition of DNA. [Cu] = 0.5 µM, [DNA] = 0 -3 00 µM. Arrow shows the absorbance changing upon the increase of DNA concentration. (The inset: [DNA]/ (ε_a-ε_f) vs [DNA] for the titration of DNA with Cu(II) complex.

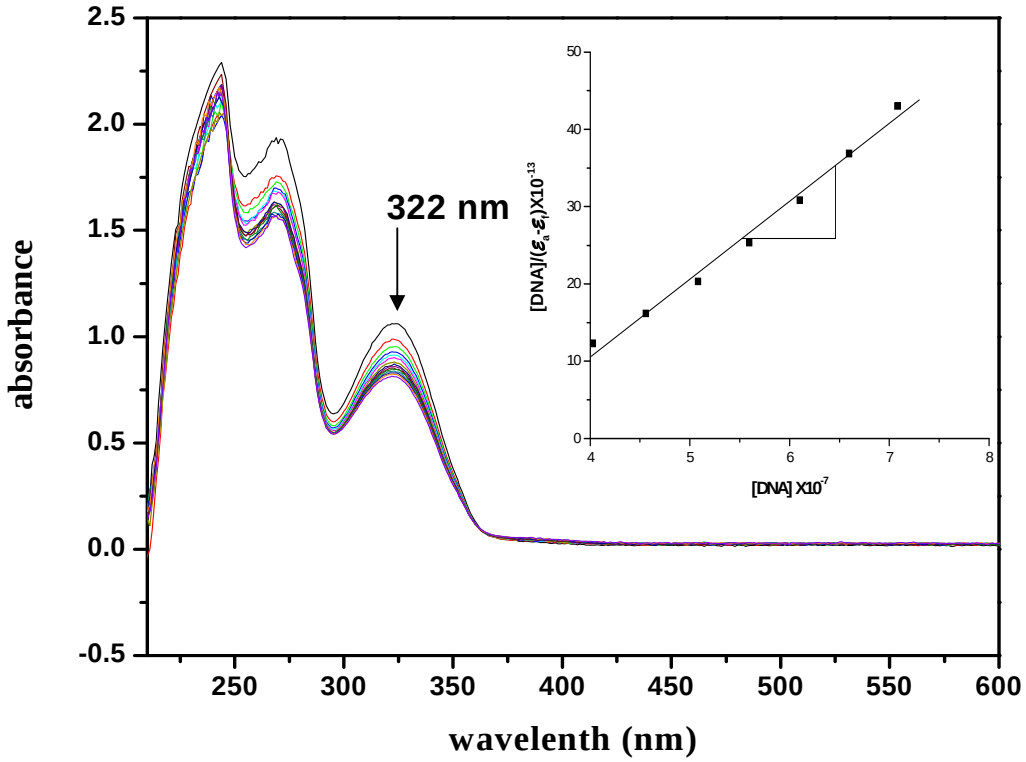


Fig 4. Absorption spectra of Zinc complex in *Tris*-HCl buffer upon addition of DNA.[Zn] = 0.5 μM, [DNA] = 0-300μM. Arrow shows the absorbance changing upon the increase of DNA concentration. (The inset: [DNA]/ (ε_a-ε_f) vs [DNA] for the titration of DNA with Zn(II) complex.

Antimicrobial activity

The synthesized compounds were evaluated for their *in vitro* antimicrobial activity against 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*) using well diffusion method. The MIC values of the investigated compounds are summarized in Table 4.

Table 4. *In vitro* antimicrobial activity of compounds and their inhibition zone (MIC) in mm

Comps. \Conc. (µg)	Antibacterial activity								Antifungal activity			
	E.coli		S.typhi		S.aureus		B.subtilis		A.niger		C. albicans	
	100	200	100	200	100	200	100	200	100	200	100	200
Ligand (L)	15	17	22	24	14	18	16	22	14	17	21	19
$[Cd(L)_2(OAc)_2]$	28	32	23	26	17	23	25	28	20	24	12	16
$[Cu(L)_2(OAc)_2]$	27	33	16	20	19	21	29	34	28	32	25	30
$[Zn(L)_2Cl_2]$	23	27	28	30	20	24	31	35	19	25	18	21
Standard 1	26	30	26	28	26	32	30	32	-	-	-	-
Standard 2	-	-	-	-	-	-	-	-	26	30	24	31
Control	-	-	-	-	-	-	-	-	-	-	-	-

Standard 1 is Chloramphenicol, Standard 2 is Fluconazole and Control is DMSO.

Data of Table 2 reveals that metal complexes are more active than the free ligand. The increased in activity of the metal chelates could be explained on the basis of overtones concept and chelation theory [40]. 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 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 the positive charge of the metal ion. Furthermore, it increases the delocalization of p- and d-electrons over the whole chelate 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 of the microorganism. The antimicrobial activity of the complexes may also affected by the geometry of the complexes which are supposed to have octahedral geometry [41].

All the synthesized complexes (Cd(II), Cu(II) and Zn(II)) showed significant activity against tested microorganisms. Cd(II) complex showed moderate antimicrobial activity but very active against *E. Coli.* Cu(II) complex showed good activity against *E. Coli* and *B.Subtilis* and found to be potent antifungal agent as it exhibited excellent activity against *A.Niger* and *C.Albicans.* Zn(II) complex is more active against *S.Typhi*, *B.Subtilis* and showed moderate activity against all other organisms. These results indicate that the increase in the size of the transition metal ions from copper to cadmium has no substantial effect on the antimicrobial activity [42].

Anthelmintic activity

The anthelmintic activity was tested on earthworms (*Pheretima Posthuma*). The compounds are screened for activity by time taken for complete paralysis and death of worms. Amongst the complexes Cd(II), Cu(II) and Zn(II) the Cu(II) and Zn(II) complexes showed more activity than the ligand and standard albendazole. Based on the above results, it may be stated that the activity of the Schiff base is enhanced on complexation with Cu(II) and Zn(II) Table 3.

Table 5. Data of anthelmintic activity

Compounds		Time in min		
	Concentration in mg/ml	Pin pinch	Paralysis	Death
Ligand (L)	5	26.	30.56	40.23
$[Cd(L)_2(OAc)_2]$	5	17.59	22.05	41.01
$[Cu(L)_2(OAc)_2]$	5	18.17	20.10	20.59
$[Zn(L)_2Cl_2]$	5	17.22	20.46	20.58
Albendazole (Std)	5	18.01	25.00	28.20
DMF (Control)	-	18.39	32.47	46.08

The biochemical mechanism of anthelmintic action of the compounds may be due to interfering with metabolic processes, interfering with neuromuscular physiology of parasites. They may inhibit the glucose uptake and depleted the glycogen content in the presence of glucose and affect the energy generating mechanism of the parasite. In general, the possible mechanism of anthelmintic action of complexes may be related to either inhibition of energy metabolism and/or alteration in the motor activity of the parasite [43].

Molecular docking studies

Binding image of copper complex with human estrogen receptor and tyrosine kinase receptor depicted in Fig 5. Docking studies of the synthesized compounds was evaluated against human estrogen receptor and tyrosine kinase receptor which are known to be responsible for causing breast cancer.

The results of antimicrobial, anthelmintic activity and DNA interaction of metal complexes revealed that the synthesized compounds are highly potent molecules. Therefore, we have considered worth-while to do docking studies to support the *in vitro* activity. The docking was used it to determine the orientation of inhibitors bound in the active site of receptors. As the metal complexes exhibited potential DNA binding property in the present study an attempt was made to evaluate their anticancer property so we selected human estrogen receptor, tyrosine kinase (RTK) which are involved in causing breast cancer. Toremifene drug was used as standard for our docking studies which was known to be potential inhibitor of human estrogen receptor. The obtained docking scores are tabulated in Table 6.

Table 6. Docking scores of the synthesized compounds

	E- total values in $kjmol^{-1}$	
Compounds	Human estrogen receptor	Tyrosinekinase (RTK)
Ligand (L)	-73.97	-20.24
$[Cd(L)_2(OAc)_2]$	-122.83	-21.07
$[Cu(L)_2(OAc)_2]$	-120.63	-30.84
$[Zn(L)_2Cl_2]$	-129.47	-23.07
Standard	-75.28	-22.42

Standard is Toremifene

For humanestrogen receptor all complexes exhibited more binding interaction energy against the receptor with least docking score compare to the ligand and standard and hence complexes may be considered as potential inhibitors of human estrogen receptor. Similarly for tyrosine kinase docking score obtained for synthesized compounds are comparable with the standard. Among all complexes docked, copper complex showed comparatively least E-total value -30.84 $kjmol^{-1}$ is significantly having more inhibiting ability towards tyrosine kinase recptor, which binds to the active site of the receptors thereby potentially inhibits the cancer causing property of the receptor. Form Table 6 it can be concluded that all complexes potentially inhibits the human estrogen receptor and tyrosine kinase (RTK) and especially copper complex was found to be potential inhibitor of both the receptors.

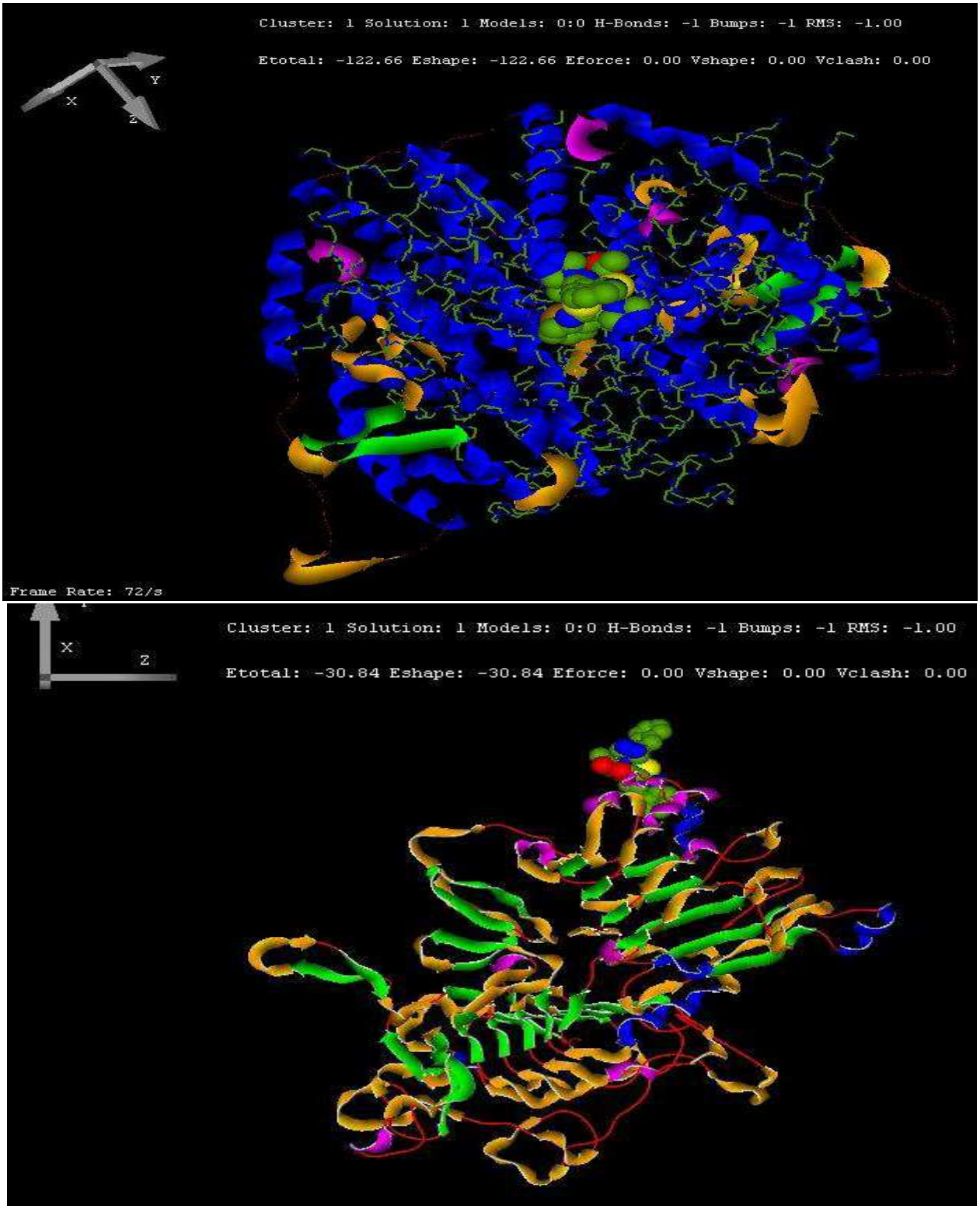


Fig 5. Binding interaction of copper complex with human estrogen receptor and tyrosine kinase receptor.

Conclusions

A Schiff base ligand (L) and its Cadium(II), copper(II), and Zinc(II) complexes were synthesized and characterized. It was determined that the bidentate behavior of the ligand was accomplished *via* the SH group and the azomethine nitrogen atom. All the synthesized compounds showed moderate to fairly good antimicrobial activity against selected bacteria and fungi. The anthelmentic activity of the Schiff base was enhanced upon complexation with metal ions. The binding behavior of the copper(II), Zinc(II) and Cadium(II) complex with calf thymus DNA was monitored by absorption spectral studies. Results indicate that the all the complexes binds more strongly to DNA *via* intercalative binding. Docking studies of the synthesized compounds reveals that metal complexes are potent anticancer agents as they exhibited least docking scores compared to the standard toremefine drug.

References

1. Hussein, S. Seleem, Chem. Central. Journal., 2011, 5, 1.
2. Jayakumarswamy, B.H.M. Fazlur Rahaman,. Revankar, V.K. Vasantakumar Pai, K. 2011, 3, 1864.
3. Morton, R. A. Rogers, E., J. Chem. Soc., 1925, 127, 2698.
4. Chen, I. S. chen, H. C. Cheng, M. J. Cheng, Y. L. Tsai. I. L., Nat. Prod. Rep., 2002, 19, 748.
5. Pande, V. Tusi, S. Tusi, Z. Joshi, M. and Bajpai. S. A., Acta. Pharm., 2004, 54, 1.
6. Chohan, Z.H. Sheazi, S.K.A., Synth. React. Inorg. Met.-Org. Chem., 1999, 29, 105.
7. Jayabalakrishnan, C. Natarajan, K. Synth. React. Inorg. Met.-Org. Chem., 2001, 31, 983.
8. Jeeworth, T. Wah, H.L. Bhowon, K. Ghoorhoo, M.G. Babooram, D., Synth. Read. Inorg. Met.-Org. Chem., 2000, 30, 1023.
9. Dharmaraj, N. Viswanalhamurthi, P. Natarajan, K., Trans. Met. Chem., 2000, 26, 105.
10. Colins, C.H.; Lyne, P.M. Microhiul Melfwds University Park Press: Baltimore, MD, USA, 1970, 422.
11. Nora, H. Al-Shaalan., Molecules., 2011, 16, 8629.
12. Rakesh, K. P. Shiva Prasad, K. Shridhara Prasad, K. 2012, 2, 221.
13. Ferlay, J., Bray, F.Pisani P., Parkin.D.M., Cancer Incidence and Mortality Worldwide. Lyon: IARC Cancer Base No 5. 2001, GLOBOCAN 2000.
14. Jamal A., Murry T., Ward E., Samuels A., Tiwari R.C., Ghafoor A., Feuer E.J., Thun MJ Cancer Statistics, *CA*, Cancer. J. Clin., 2005, 56, 10.
15. Yager, J.D. Davidson, N.E. N., Engl. J. Med., 2006, 354, 270.
16. Jochen Rossler, Birgit Geoerger, Melissa Taylor and Gilles Vassal., 2008, 8, 76.
17. Shazia Rafique, Muhammad Idrees, Anwar Nasim, Haji Akbar and Amin Athar., Biotech.Mol. Bio.Rev., 2010, 5, 38.
18. Raymond Wai-Yin Sun, Dik-Lung Ma, Ella Lai-Ming Wong and Chi-Ming Che., Dalton Trans., 2007, 4884.
19. (*a*) Zhang, C. X. and Lippard, S., J. Curr. Opin. Chem. Biol., 2003, **7**, 481.
    (*b*) Guo, Z. and Sadler, P. J. Adv. Inorg. Chem., 2000, 49, 183. (*c*) Guo, Z. and Sadler, P. J., Angew. Chem., Int. Ed., 1999, 38, 1512.
20. Vogel, A. I. A Text book of Quantitative Organic Analysis, 3rd edition ELBS Longmans Green and Co.Ltd.,1962, 436.
21. Vogel, A. I. A Text book of Quantitative Inorganic Analysis, 3rd edition ELBS Longmans Green and Co.Ltd.,1962, 597.
22. Sandhya Bawa and Suresh kumar., Indian J. Chem., 2009, 48, 140.
23. Ambika srivastava and Singh, R.M., Indian J. Chem., 2005, 44, 1868.
24. Sreekanth, B. Krishnamurthy, G. Bhojya Naik, H. S. Vishnuvardhan, T. K. Vinaykumar, B.and Sharath N., Nucleosides, Nucleotides and Nucleic. Acids., 2011, 30, 83.
25. Sreekanth, B. Krishnamurthy, G. Bhojya Naik, H.S. Vishnuvardhan, T.K. Lokesh, M.R. Shashikumar, N.D., Res. J. Pharm., Biol. Chem. Sci., 2011, 2, 201.
26. Mahmoud, M. Mashaly, Zinab, H. Abd-Elwahab and Abeer, Faheim, A., J. Chinese. Chem. Soc., 2004, 51, 901.
27. Golebiewski, W.M. Wilkowska, E., Polish J.Chem., 2000, 74, 759.

28. Sangamesh, A. Patila, Manjunatha, M., Der. Pharma. Chemica., 2011, 3, 97.
29. Cosman, F., Clin. Geriatr. Med., 2003, 19, 371.
30. Alex Mathew, J. Nixon Raj, N. "Proceedings of the International Multi Conference of Engineers and Computer Scientists" Hong Kong Docking Studies on Anticancer Drugs for Breast Cancer Using Hex, IMECS; 2009, March 18 - 20.
31. Sobha, S. Raman, N., Int. J. Pharm. Bio. Sci., 2012, 3, 116.
32. Salawu, O .W. and Abdulsalam, A.O., J. Physical. Sci. Innovation., 2011, 3, 51.
33. Mishra, A.p. and Mishra, D.K., Int. J. Pharm. Bio. Sci., 2011, 2, 430.
34. Pulin Nath and Sreedhar, D. Dhumwad., J. Chem. Pharm. Res., 2012, 4, 851.
35. Saha, N.C. Butcher, R.J. Chaudhari, S. Saha, N., Polyhedron., 2003, 22, 383.
36. Ahmed, I.T., Spectrochim. Acta. Part A., 2006. 65, 5.
37. Sreekanth,B. Krishnamurthy, G. Bhojya Naik, H. S. Vishnuvardhan, T. K. Vinaykumar, B. and Sharath N., Nucleosides. Nucleotides and Nucleic Acids., 2011, 30, 83.
38. Barton,J.K. Danishefsky, A.T. Goldberg, J.M., J. Am. Chem. Soc., 1984, 106, 2172.
39. Lu, H.L. Liang, J.J. Zeng, Z.Z. Xi, P.X. Liu, X.H. Chen, F.J. Xu, Z.H., Trans Metal Chem., 2007, 32, 564.
40. Raman, N. Kulandaisamy, A. Thangaraja, C. Manisankar, P. Viswanathan, S. Vedhi, C., Trans. Met. Chem., 2004, 29, 129.
41. Canpolat, E. Kaya, M. Gur, S., Turk. J. Chem., 2004, 28, 235.
42. Anil Kumar and Devinder Kumar., ARKIVOC., 2007, 9, 117.
43. Khatiwora, E. Mundhe, K. Deshpande, N. R. and Kashalkar, R. V., Der. Pharma. Chemica., 2012, 4, 1264.

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