Available online at www.derpharmachemica.com

Scholars Research Library

Der Pharma Chemica, 2014, 6(6):192-202
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Synthesis, molecular docking, DNA binding and biological evaluation of schiff base transition metal complexes

M. R. Lokesh[1], G. Krishnamurthy *[1], H. S. Bhojyanaik[2], N. D. Shashikumar[1], P. Murali Krishna[3] and B. Sreekanth[4]

[1]Department of Chemistry, Sahyadri Science College, Shimoga, Karnataka, India
[2]Department of PG Studies and Research in Industrial Chemistry, School of Chemical Sciences, Kuvempu University, Shankaraghatta, Shimoga, Karnataka
[3]Department of Chemistry, M. S. Ramaiah Institute of Technology, Bangalore
[4]Department of Biotechnology, Acharya Institute of Technology, Bangalore

---

ABSTRACT

Novel complexes of Fe(III), Co(II) and Ni(II) with Schiff base ligand 3-[(E)-(4H-1,2, 4-triazol-4-ylimino)methyl]quinoline-2-thiol (L) have been synthesized and characterized. The interaction of the complexes with calf thymus DNA (CT-DNA) was monitored by red shift and hypochromism and hyperchromism in the UV–vis spectra. The observed intrinsic binding constant (K_b) in the range 10^6 M^{-1} together with structural analysis of the complexes indicate the intercalative mode of binding. In vitro biological activities indicate that the metal complexes were more active compare to the uncoordinated ligand. The docking study revealed that metal complexes are potential inhibitors of cancer causing receptors.

Keywords: DNA interaction, metal complexes, docking studies, human estrogen receptor.

INTRODUCTION

Schiff bases and their metal complexes played an important role in the development of coordination chemistry ranging from physicochemical [1] and biochemically relevant studies of metal complexes [2-6]. The study of transition metal quinoline complexes is an area of great current interest [7]. The interaction of transition metal complexes of Schiff base with DNA (Deoxyribo Nucleic Acid) is a major area of research due to the utility of these complexes in the design and development of synthetic restriction enzymes, chemotherapeutic agents, foot printing agents, spectroscopic probes, site specific cleavers and molecular photo switches. A number of metal complexes of a variety of ligands were also studied in view of their affinity towards DNA and specificity for the DNA base sequence recognition [8-14].

Breast cancer is the second most common cancer in women world-wide with 1.05 million new cases being estimated in the year 2011[15]. Sex-hormones have been implicated in various human cancers such as endometrial cancer [16], breast and prostatic cancer [17]. Estrogen receptors (ERs) play a key role in normal breast development and in the development and progression of breast cancer [18]. Growth factor signaling plays a major role in prostatic oncogenesis [19]. Receptor protein tyrosine kinases associated with growth factors, control various cell functions

including proliferation, apoptosis, differentiation, cell cycle progression etc. EGFR family of transmembrane proteins contributes to the development and progression of various malignancies including prostate cancer [20, 21].

In view of above facts it was planned to make a study on the complexing ability of Schiff base chelate derived from 2-mercapto-quinoline-3-carbaldehyde with some transition metal ions and their binding affinity with CT-DNA, cancer causing receptors was evaluated and biological activities were also studied.

MATERIALS AND METHODS

Experimental

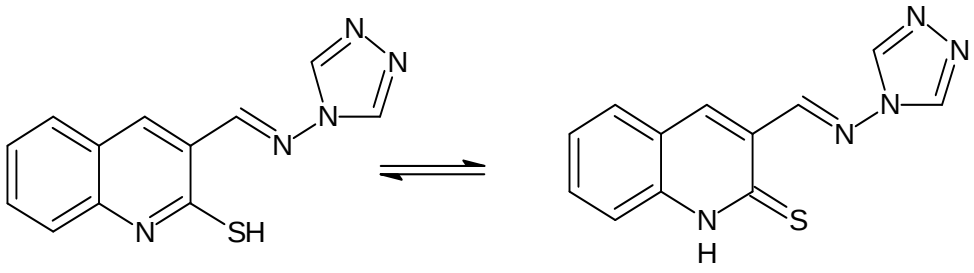
The chemicals used for preparing the ligands were of analytical grade. The solvents were distilled and dried before use by following standard literature method [22]. The acetanilide was prepared from the aniline following the standard procedure [23]. This was used as the starting material for the preparation 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. For docking study the biological databases like PubMed, Drug Bank, PDB (Protein Data Bank) and software's like Hex, ACD ChemSketch have been used.

Synthesis of Schiff base ligand:

3-[(*E*)-(4*H*-1,2,4-triazol-4-ylimino)methyl] quinoline-2-thiol

3-formyl-2- chloroquinoline was prepared according to the literature method [24]. 2-mercaptoquinoline-3-carbaldehyde was synthesized by reacting 3-formyl-2-chloroquinoline with sodium sulfide in DMF [25].

Figure. 1. Structure of 3-[(*E*)-(4*H*-1,2,4-triazol-4-ylimino)methyl]quinoline-2-thiol (L)



Thiol **Thione**

2-mercaptoquinoline-3-carbaldehyde (0.01mol) was dissolved in absolute ethanol (10mL) and added to a solution of 4-amino-1,2,4-triazole (0.01mol) dissolved in absolute ethanol (10mL). The reaction mixture was refluxed for about 2-3 h on water bath and the progress of reaction was monitored by TLC. After completion of reaction, it was cooled to RT, and then the formed precipitate was filtered, recrystallized from suitable solvent and dried in desiccator, yield 85%, M.P.202^{o}C. 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 the solution of each one metal salt at a time (1 mM) (cobaltous chloride, ferric chloride or nickel chloride) was dissolved in hot ethanol (10 mL) and mixed with ethanolic solution of ligand L (2 mM, 10mL). The reaction mixture was refluxed on water bath for about 3-4 h. During which a dark colored precipitate was separated which was filtered, washed twice with hot ethanol (10 mL) followed by ether (10 mL), recrystallized from suitable solvent and dried in a dessicator. Yield (50%-75%).

[Fe(L)$_2$ Cl$_2$]Cl: 1. C, 42.25; H, 2.67; N, 20.83; Fe, 8.30; Found: C, 42.31; H, 2.72; N, 20.92; Fe, 8.25; IR, KBr pellets (ν, cm^{-1}): 1616 ν(C=N str); 1060 ν(C=S);. Electronic spectra (nm): 305 nm, 435 nm.

$[Co(L)_2Cl]Cl$: 2. C, 45.03; H, 2.81; N, 21.89; Co, 9.21; Found: C, 45.19; H, 2.67; N, 22.01; Co, 9.00; IR, KBr pellets (ν, cm^{-1}): 1614 ν(C=N str); 1080 ν(C=S);. Electronic spectra (nm): 300 nm, 430 nm.

$[Ni(L)_2Cl_2]$: 3. C, 45.05; H, 2.81; N, 21.89; Ni, 9.18; Found: C, 44.96; H, 2.91; N, 21.63; Ni, 8.97; IR, KBr pellets (ν, cm^{-1}): 1620 ν(C=N str); 1060 ν(C=S);. Electronic spectra (nm): 300 nm, 420 nm.

DNA binding studies

DNA binding studies was carried out by following the procedure described in the literature [26]. The concentration of CT-DNA per nucleotide was measured using its known extinction coefficient at 260 nm (6600 $M^{-1}cm^{-1}$)[27] *Tris* HCl-buffer [5mM *tris*(hydroxymethyl)amino methane, pH 7.2, 50 mM NaCl] was used for the absorption experiments. For DNA binding studies an increasing known amount of complexes were added to CT-DNA until the ratio of these complexes with DNA reached ~1:1. The experiments were carried out at room temperature (25 °C) and at pH 7.2 in 5 mM NaCl, 50 mM *Tris* HCl buffer. After each addition, the mixture was shaken and kept for ~ 5 min and then the absorbance was recorded. The absorption data were analyzed for an evaluation of the intrinsic binding constant K_b using equation (1) [28].

$$\mathbf{[DNA]/(\varepsilon_a-\varepsilon_f) = [DNA]/ (\varepsilon_b-\varepsilon_f) + 1/K_b(\varepsilon_a-\varepsilon_f)} \quad \text{……..} \ (1)$$

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.

Antimicrobial activity (*in vitro)*

The *in vitro* antimicrobial activity (MIC) of synthesized schiff base ligand L and their metal complexes on selected bacteria *Escherichia coli, Salmonella typhi, Staphylococcus aureus, Bacillus subtilis* and two fungi *Aspergillus niger* and *Candida albicans* was carried out by well diffusion method [29]. The tests were carried for the concentrations of 100µg and 200µg in DMSO. The inhibition zones caused by the various compounds on the microorganisms were examined. All the tested compounds showed moderate to fairly good biological activity against microorganisms when compared to the ligand and standards chloramphenicol and flucanazole.

Anthelmintic activity (*in vitro*)

The anthelmintic assay was carried as per the method given in the literature [30]. 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^{-1} 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 [31]. Further, the 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 [32]. Based on the literature it has been shown clearly that the drug toremifene has been used to target the human estrogen receptor [33].

Bearing above facts, we have selected RTKs 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. More negative E – Total value implies that there exists a 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 was measured using Equiptronics digital conductivity meter. IR spectra were recorded with Shimadzu model FT-IR spectrophotometer by using KBr pellets 4000-350 cm^{-1}. 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. DNA binding studies were carried out using Shimadzu model UV-1650PC spectrophotometer in *Tris*−HCl buffer solution (P^H = 7.2) at room temperature.

RESULTS AND DISCUSSION

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. Synthesized complexes are soluble in DMF and DMSO.

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 20-150 Ohm^{-1} cm^2 mol^{-1}, indicating that the complexes are non-electrolytes, uni-univalent electrolyte and univalent electrolyte.

The band absorbed for uncoordinated ligand and their metal complexes are all most similar expect slight shift in the position of the peak. 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.

The electronic spectra of ligand and metal complexes recorded in DMF solution at

10^{-3} molar concentration. Schiff base ligand L showed absorption bands in the region 270 nm to 380 nm transitions due to $\pi\rightarrow\pi^*$ and $n\rightarrow\pi^*$ 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 spectra of **2** displayed absorption band in the region 420-430nm is attributed to two spin allowed d-d transition $^{4}T_1g(F)\rightarrow{}^{4}T_1g(P)$ (ν_1), $^{4}T_1g(F)\rightarrow{}^{4}A_2g$ (F), respectively [34], these transitions suggest an octahedral geometry for **2** complex. The lemon green colored **3** showed an absorption at 425nm assigned to $^{2}A_2g(F) \rightarrow {}^{2}T_1g(P)$ or $^{3}A_2g \rightarrow {}^{3}A_1g(P)$ (ν_3) of octahedral geometry [35]. The light green colored **1** exhibits the spin allowed broad absorption band in the region 400 to 430nm is assignable to the electronic absorption of $^{5}T_2g\rightarrow{}^{5}Eg$ transition obtained for octahedral configuration [36,37, 38].

The ^{1}HNMR spectrum of the ligand was recorded in DMSO-d_6. In the ^{1}HNMR spectra of ligand, a peak at 8.6 ppm is due to proton of S-H and a resonance for CH=N proton was observed at 9.1 ppm. The aromatic ring protons resonances in the range 7.5 to 8.3 ppm.

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)	27.32 (27.45)	---
1	637	234	75	Red	44.98 (45.25)	2.78 (2.82)	21.45 (21.99)	8.65 (8.77)
2	639	215	64	yellow	45.19 (45.03)	2.67 (2.81)	22.01 (21.89)	9.00 (9.21)
3	640	219	65	Green	44.96 (45.05)	2.92 (2.81)	21.63 (21.89)	8.97 (9.18)

DNA binding experiments

Electronic absorption data upon addition of CT-DNA to the complexes is listed in Table.3. The results indicate that the complexes bind to naturally occurring DNA. The addition of a soluble form of calf thymus DNA to complexes in 5mM *Tris* HCl buffer at pH 7.2 causes a slight red shift of the electronic transition of the complex from 300 to 350 nm attributed to $\pi \rightarrow \pi^*$ transitions. With increase in concentration of CT-DNA the absorption bands of the complexes were affected, resulting in decrease in intensity (hypochromic shift) for complexes **1** and **2** is 14.85% and 97.54% (H%) respectively. On the contrary for complex **3** the increase in intensity of absorption band was observed (hyperchromism). The change in absorbance with increasing amount of CT-DNA was used to evaluate the intrinsic binding constant K_b for the complexes. The Fig. 2, 3 and 4 represent the absorption spectra of the complexes **1, 2** and **3** in the presence and absence of CT-DNA respectively. The well resolved bands were noticed at 300 nm to 350 nm for all the complexes with increasing the DNA concentration (0-300µM).

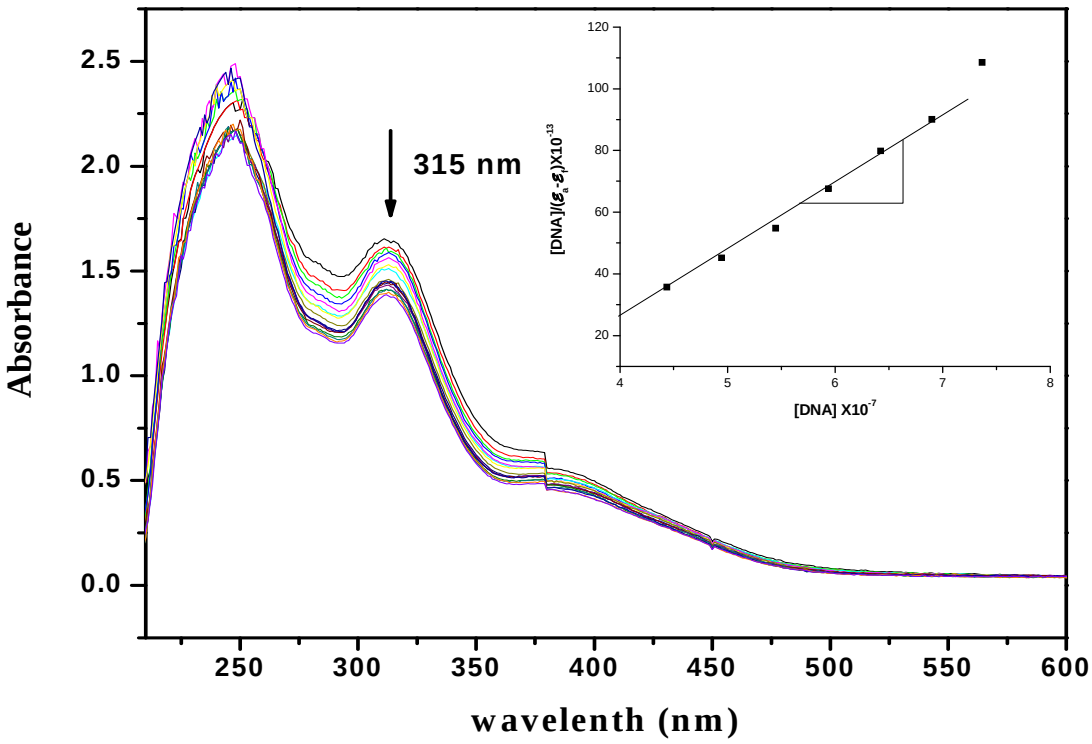


Figure.2. Absorption spectra of 1 in *Tris*-HCl buffer upon addition of DNA. [Fe(III)] = 0.5 µM, [DNA] = 0-300µM. Arrow shows the absorbance changing upon the increase of DNA concentration. (The inset: [DNA]/ (ε_a-ε_f) vs [DNA] for the titration of DNA with complex 1)

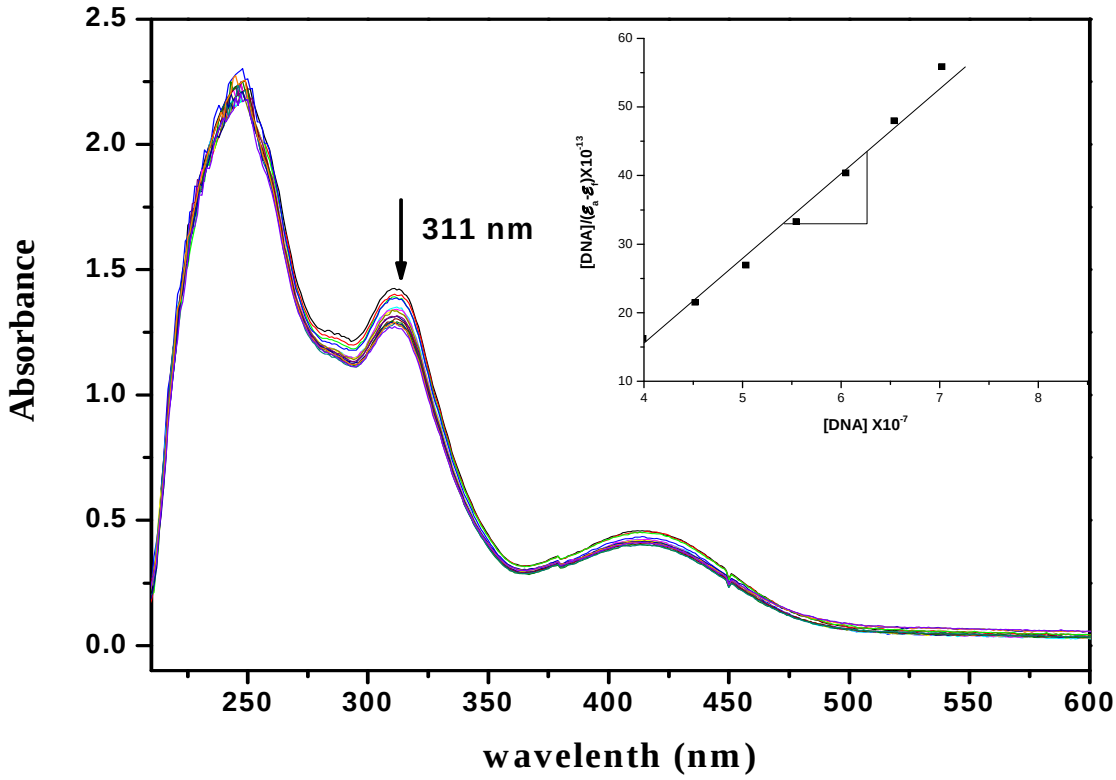


Figure.3. Absorption spectra of 2 in *Tris*-HCl buffer upon addition of DNA. [Co(II)] = 0.5 µM, [DNA] = 0-300µM. Arrow shows the absorbance changing upon the increase of DNA concentration. (The inset: [DNA]/ (ε_a-ε_f) vs [DNA] for the titration of DNA with complex 2)

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

Complex	λ_{max} (nm)		$\Delta\lambda$ (nm)	H (%)	Binding constant K_b (M^{-1})
	Free	Bound			
1	315	313	2	14.85	8.08 x 10^6
2	311	312	1	97.54	12.84 x 10^6
3	325	323	2	- 33.93	20.93 x 10^6

H (%) is percentage of hypochromism

The hypochromism, hyperchromism and slight red shift are observed for the complexes suggest that binding is intercalative mode. The K_b values for complexes **1, 2** and **3** are 8.08 X 10^6 M^{-1}, 12.84 X 10^6 M^{-1} and 20.93 x 10^6 M^{-1} respectively. So, it is obvious that the present complexes are involved in intercalative mode of interactions with CT-DNA.

Biological activity *(in vitro)*

Generally, metal complexes have been shown to be, in most cases, more effective than the free ligands. Tweedy's chelation theory predicts that chelation reduces the polarity of the metal atom mainly because of partial sharing of its positive charge with donor groups and possible electron delocalization over the whole ring. This consequently increases lipophilic character of the chelates, favoring its permeation through lipid layers of the bacterial membrane [39].

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.4.

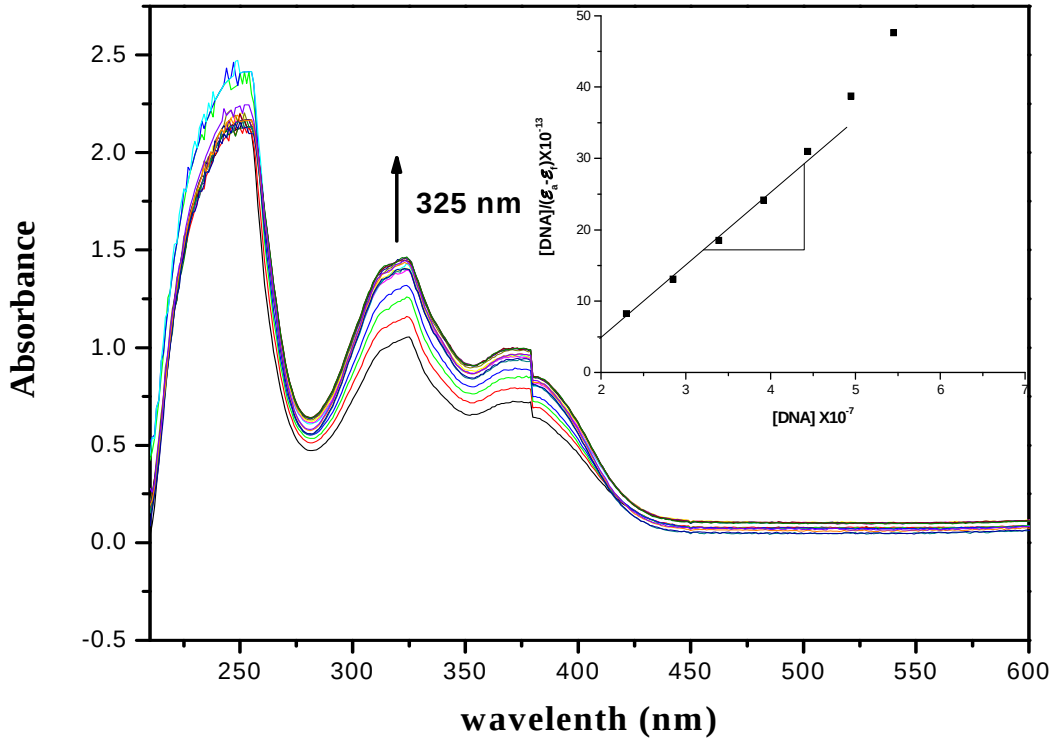


Figure.4. Absorption spectra of 3 in *Tris*-HCl buffer upon addition of DNA. [Ni(II)] = 0.5 µM, [DNA] = 0-300µM. Arrow shows the absorbance changing upon the increase of DNA concentration. (The inset: [DNA]/ (ε_a-ε_f) vs [DNA] for the titration of DNA with complex 3)

The results concerning *in vitro* antimicrobial activities of chelating agent and their complexes **1, 2** and **3** inhibition zone in (mm) was compared with chloramphenicol and flucanazole. All the compounds tested exhibit moderate to good antimicrobial activities.

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)	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
1	23	25	18	22	12	18	18	23	14	18	14	17
2	12	23	17	20	9	13	14	17	27	33	25	32
3	23	30	18	24	20	29	27	35	20	27	16	18
Std1	26	30	26	28	26	32	30	32	-	-	-	-
Std 2	-	-	-	-	-	-	-	-	26	30	24	31
Control	-	-	-	-	-	-	-	-	-	-	-	-

**Std 1 is Chloramphenicol, Std 2 is Fluconazole and Control is DMSO.*

Table.2 indicates that the **3** is more active against bacterial strains *E.coli*, *S.aureus* and *B.subtilis* and fungal strain *A.niger*. The **2** was found to be potent antifungal agent but showed moderate activity against bacterial strains. Whereas the **1** exhibited moderate antimicrobial activity.

Anthelmintic activity

The anthelmintic activity was tested on earthworms (*Pheretima Posthuma*) and results are tabulated in Table.5. The compounds are screened for activity by time taken for complete paralysis and death of worms. All the metal complexes exhibit significant activity. All the complexes are found to be more active as compared to ligand and the standard drug albendazole. 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 relate to either inhibition of energy metabolism and/or alteration in the motor activity of the parasite [40].

Table.5. Anthelmintic activity

Compounds	Time in min			
	Concentration in mg/ml	Pin pinch	Paralysis	Death
Ligand (L)	5	26.	30.56	40.23
1	5	18.58	20.03	24.52
2	5	11.16	12.54	16.04
3	5	14.12	17	23.36
Standard	5	18.01	25.00	28.20
Control	-	18.39	32.47	46.08

**Standard is Albendazole and control is DMF*

In silico docking studies

Binding models of Schiff base complex **1** with human estrogen receptor and **2** with tyrosine kinase receptor depicted in Fig 5 and Fig 6. Docking studies of the synthesized compounds was evaluated against human estrogen receptor and tyrosine kinase receptor which are responsible for breast cancer. The obtained docking scores are listed in Table.6.

The results of antimicrobial, anthelmintic activity 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 it 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 is an attempt made to evaluate their anticancer property, 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.

Table.6. Docking scores of the synthesized compounds

Compounds	Human estrogen receptor	Tyrosinekinase (RTK)
L	-73.97	-20.24
1	-122.83	-21.07
2	-120.63	-24.84
3	-129.47	-23.07
Toremifene	-75.28	-22.42

E-total value of ligand and **1, 2** and **3** complexes for human estrogen receptor are -73.97, -122.83, -120.63 and -129.47 kjmol^{-1} respectively. All the complexes showed minimum docking score than standard toremifene which showed -75.28 kjmol^{-1}. Similarly for tyrosine kinase (RTK) -20.24, -21.07, -24.84, -23.07 and -22.42 Kjmol^{-1} for ligand, **1, 2, 3** complexes and standard. All the synthesized compounds showed E-total value which is comparable with that of the standard. From above discussion it is clear that all complexes have possessed significant inhibiting property for cancer causing receptors and may be potent anticancer agents.

Figure.5. Interaction of Complex 1 with Human estrogen receptor

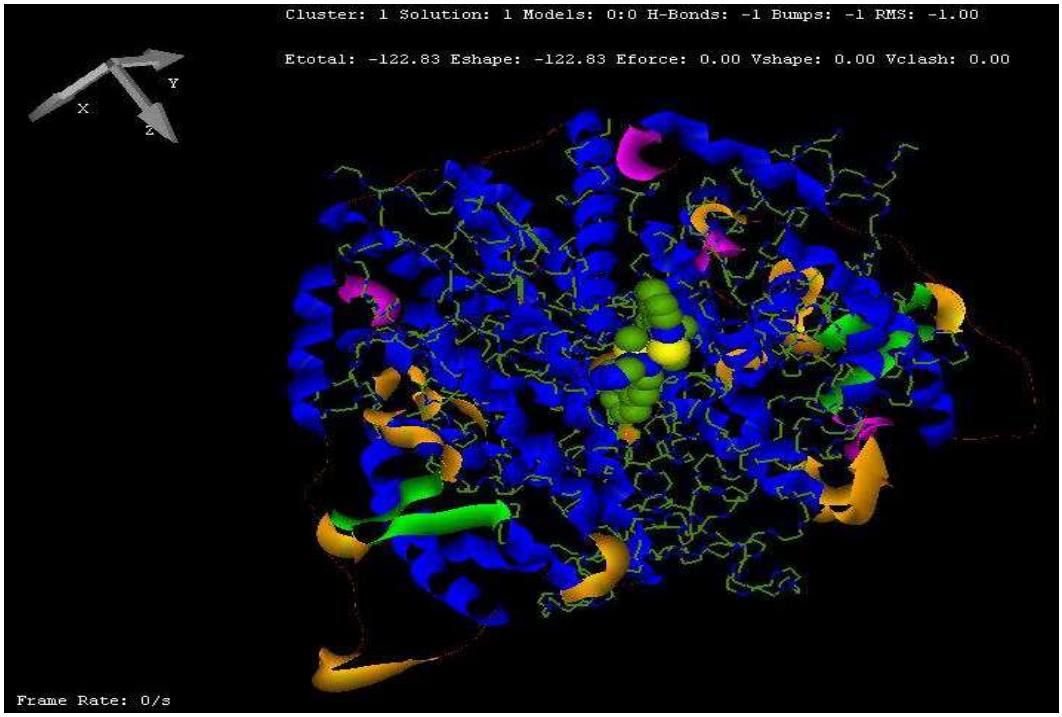
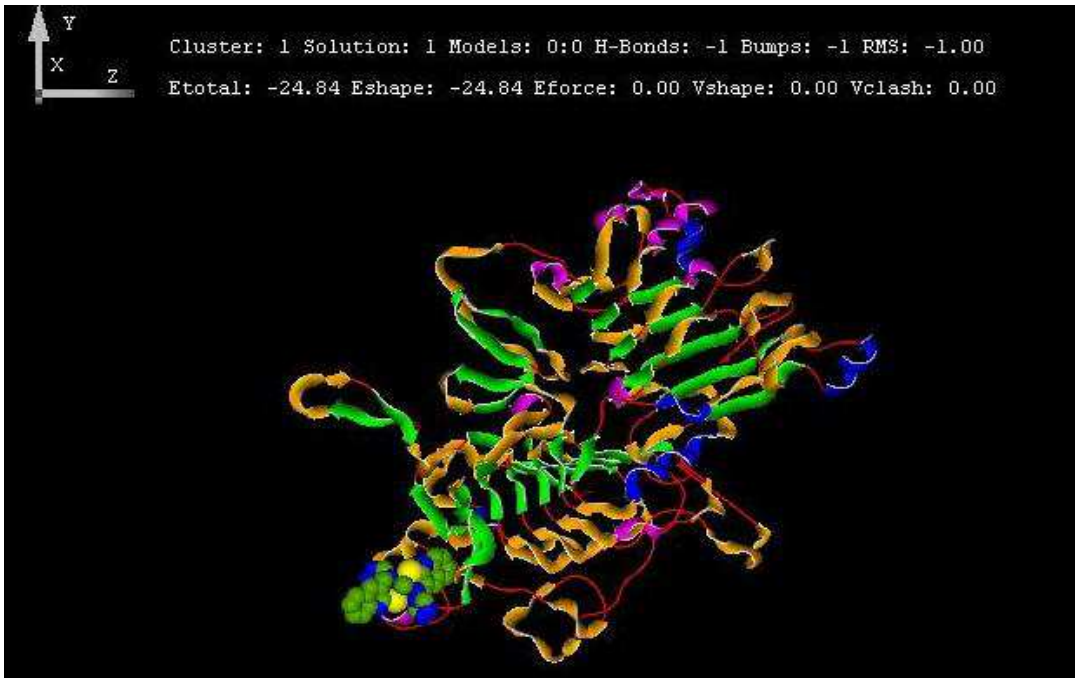


Figure. 6. Interaction of Complex 2 with Tyrosine Kinase receptor



CONCLUSION

In summary, we have synthesized and characterized quinoline schiff base ligand **L** and its complexes **1, 2** and **3**. The DNA-binding afinity of complexes were examined by absorption spectroscopy. Results indicate that all the complexes bind to CT-DNA by an intercalative mode. The antimicrobial activity results revealed that complexes

have showed more promising activities than the schiff base, especially **2** and **3** complexes showed good activity compared to standard drugs used. Anthelmintic activity of schiff base was increased on complexation with the metal ions. In silico docking studies reveals that that metal complexes were potential inhibitor of cancer causing receptors.

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-560090 for their support.

REFERENCES

[1] i) R. J. Vidmar, *Trans. Plasma. Sci*, **1992**, 21, 876-880.
ii) X. F. Luo, X. Hu, Zhao, S. H. Goh, X. D. Li, *Polymer*, **2003**, 44, 5285–5291.
[2] A. S. N. Murthy, A. R. Reddy, *J.Chem. Sci*, **1981**, 90, 519–526.
[3] V. Razakantoanina, N. K. P. Phung, G. Jaureguiberry, *Parasitology Res*, **2000**, 86, 665–668.
[4] R. E. Royer, L. M. Deck, T. Vander, J. Jagt, *J. Med. Chem*, **1995**, 38, 2427–2432.
[5] M. R. Flack, R. G. Pyle, N. M. Mullen, *J. Clin. Endocrin. & Metab*, **1993**, 76, 1019– 1024.
[6] R. Baumgrass, M. Weiwad, F. Erdmann, *J. Bio. Chem*, **2001,** 276, 47914–47921.
[7] O. Meth-Cohn, Narine, Brahmha, J.C.S. Brain Tarnoski, *Perkin*, **1981**, 1520.
[8] B. Sreekanth, G. Krishnamurthy, H. S. Bhojya Naik, M. C. Prabhakara, T. K. Vishnuvardhan, *Synth. React. in Inorg. Met-Org, and Nano-Met Chem*, **2010**, 40, 955–962.
[9] L. Singh, N. Tyagi, N.P. Dhaka, S.K. Sindhu, *Asian. J. Chem*, **1999**, 11, 503-508.
[10] B. Sreekanth, G. Krishnamurthy, H.S. Bhojya Naik, T.K. Vishnuvardhan, N.D. Shashikumar, M.R. Lokesh, *J. Chem. Pharm. Res*, **2011**, 3, 407-419.
[11] D.S. Sigman, A. Mazumder, D.M. Perrin, *Chemical Reviews*, **1993**, 93, 2295-2316.
[12] S. Routier, N. Cotelle, J.P. Catteau, *Bioorg. Med. Chem*, **1996**, 4, 1185-1188.
[13] D.J. Gravert, J.H. Grin, *Inorg. Chem*, **1996**, 35, 4837-4847.
[14] B. Sreekanth, G. Krishnamurthy, H. S. Bhojya Naik, T. K. Vishnuvardhan, *Nucleosides, Nucleotides and Nucleic Acids*, **2012,** 31, 1-13.
[15] D.M. Parkin, F. Bray, J. Ferlay, P. Pisani, *Int. J. Cancer*, **2001,** 94, 153-156.
[16] T. Van Agthoven, L.C. Timmermans Dorssers, S.C. Henzen-Logmans, *Int. J. Cancer*, **1995**, 63, 790–793.
[17] B.K. Sharma, A. Ray, *Indian. J. Clin. Biochem*, **2000,** 15, 110-117.
[18] S. Rachel, C. Kent Osborne, *Breast Cancer Research*, **2005**, 7, 205-211.
[19] P.J. Russell, S. Bennett, P. Stricker, *Cancer. Clin. Chem*, **1998,** 44, 705–23.
[20] J.M. Bartlett, D. Brawley, K. Grigor, *J. Pathol*, **2005,** 205, 522–9.
[21] H.I. Scher, A. Sarkis, V. Reuter, *Clin. Cancer. Res*. **1995**, 1, 545–50.
[22] Vogel, A. I. **1962**. A Text book of Quantitative Organic Analysis, 3rd ed ELBS Longmans Green and Co.Ltd., 1962 436-438.
[23] Vogel, A.I.A. **1962**. Text book of Quantitative Inorganic Analysis, 3rd ed ELBS Longmans Green and Co.Ltd., 1962, pp.597-601.
[24] B. Sandhya K. Suresh, *Indian. J. Chem*, **2009,** 48(B), 142-145.
[25] S. Ambika R.M. Singh, *Indian. J. Chem*, **2005,** 44(B), 1868-1875
[26] B. Sreekanth, G. Krishnamurthy, H.S. Bhojya Naik, T.K. Vishnuvardhan, B. Vinaykumar, N. Sharath, *Nucleosides Nucleotides Nucleic Acids*. **2011**, 30, 83–96.
[27] M.E. Reichmann, S.A. Rice, C.A. Thomas, P. Doty, *J. Am. Chem. Soc*. **1954**, 76, 3047–3053.
[28] A. Wolfe, G.H. Shimer, T. Meehan, *Biochemistry*. **1987**, 26, 6392–6396.
[29] Saeed-ur-Rehman, Muhammad Ikram, Sadia Rehman, Alia Faiz, Shahnawaz, *Bull. Chem. Soc. Ethiop*. **2010**, 24, 201-207.
[30] A. Sangamesh, Patila, M. Manjunatha, *Der Pharma Chemica*, **2011**, 3, 97-108.
[31] F. Cosman, *Clin. Geriatr. Med*, **2003**, 19, 371-379.
[32] J. Alex Mathew, N. Nixon Raj, "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.
[33] S. Sobha N. Raman, *Int. J. Pharm. Bio. Sci*, **2012,** 3, 116-123.
[34] R. Shakru, P. Subhashini, N.J. Shivaraj, *Heteroletters.org*, **2011,** 1, 166-175.
[35] D.K. Mishra, A.P. Mishra, *Int. J. Pharm. Res. Dev*, **2011,** 3, 24-31.

[36] G. M. Gehad, M. Mohamed, A. Omar, H. Mohamed, *Turk. J. Chem*, **2006,** 30, 361 – 382.
[37] A.B.P. Lever, Elsevier Publishing Company, Amsterdam, **1968**, 318.
[38] M. A. Aly, Hassaan, *J. Islamic. Academy. Sci*, **1991**, 4, 271-274.
[39] A. A. Ikotun, Y. Ojo, C. A. Obafemi, G. O. Egharevba, *African. J. Pure. Appl. Chem*, **2011,** 5, 97-103.
[40] E. Khatiwora, K. Mundhe, N. R. Deshpande, R.V. Kashalkar, *Der. Pharma. Chemica*, **2012**, 4, 1264-1269.