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RESEARCH ARTICLE

FLUORESCENCE AND SPECTROSCOPIC STUDIES OF THE BINDING OF COBALT IMIDAZOLE PHENANTROLINE DERIVATIVE TO CT-DNA: A THERMODYNAMIC APPROACH

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ABSTRACT

In this study, a cobalt (II) complex with 1,10-phenanthroline based ligand, $[Co(FIP)_2](OAC)_2$ with FIP = 2-(Furan-2-yl)-1H-Imidazole[4,5-f] [1,10] phenanthroline as ligand, was synthesized and characterized by spectroscopic methods and elemental analysis. The results show that the interaction is endothermic and the driving force is entropy. In addition, the denaturation curves of ct-DNA solution in the absence and presence of $[Co(FIP)_2](OAC)_2$ were studied between 24 - 86°C. The results showed that increase of the $[Co(FIP)_2](OAC)_2$ concentration, ct-DNA will be more stable. Finally, the $[Co(FIP)_2](OAC)_2$ complex bind to ct-DNA via hydrophobic mode as illustrated by hyperchromism in the UV/Vis absorption band of $[Co(FIP)_2](OAC)_2$, and also the decreasing of ethidium bromide (EtBr)-DNA solution fluorescence. Moreover, the competitive binding with a standard intercalator like ethidium bromide (EB) indicate that the binding mode of $[Co(FIP)_2](OAC)_2$ with DNA is not a classical intercalation mode.

INTRODUCTION

There is a strong tendency toward studying biologically active compounds such as metal complexes which are applied as antitumor drugs in biochemistry and medicine [1, 2].Regarding metal complexes, studying their interaction with nucleic acid is of utmost importance since they have an important role in developing new drugs which have therapeutic effects[3,4]. Generally, metal complexes can interact with DNA via covalent interaction which incorporates the coordination of the nitrogenous base or the phosphate moiety of the nucleic acid to the central metal ion or non-covalent interaction. This latter case includes intercalation, groove binding or external electrostatic binding [5]. DNA repair can be hampered by metal complexes through their interference with enzymes or proteins involved in DNA replication or DNA repair. Imidazole is incorporated into many important biological molecules. The high therapeutic properties of the imidazole related drugs have encouraged the medicinal chemists to synthesize a large number of novel chemotherapeutic agents. Imidazole has become an important part of many pharmaceuticals. Synthetic imidazoles are present in many fungicides and antifungal, antiprotozoal, and antihypertensive medications [6-13]. Imidazole and its derivatives are reported to be physiologically and pharmacologically active and find applications in the treatment of several diseases. In view of all of the above, it was thought worthwhile to study ct-DNA binding of Cobalt complex of Phenantroline-Imidazole derivative. This complex can be synthesized in high yield using inexpensive starting materials. Its interaction with calf thymus DNA (ct-DNA) was investigated by electronic absorption, thermal denaturation, and Fluorescence measurements.

Experimental

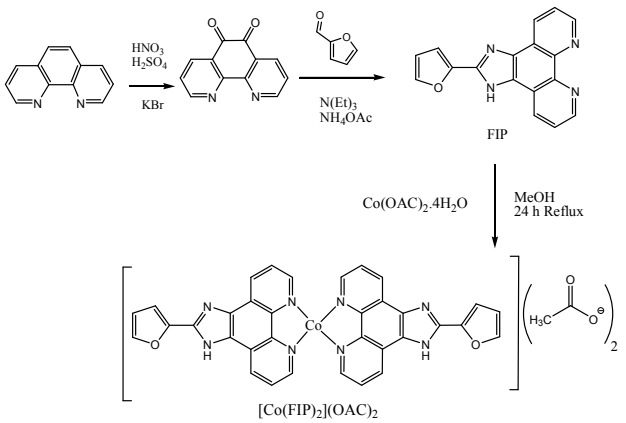
Materials and Instruments

All reagents and solvents used were supplied by Merck chemical company and were used without further purification. Double strand Calf thymus DNA (ct-DNA) was purchased from sigma. The stock solutions of $[Co(FIP)_2](OAC)_2$ complex (1 mM) was made in Tris-HCl buffer bygentle stirring and heating at 308 K and the stock solution of ct-DNA (4 mg/mL) was prepared by dissolving of ct-DNA in 20 mM NaCl- 20 mM Tris-HCl buffer at pH= 7.0overnight and was stored at 277 K for about a week. The ct-DNA concentration per nucleotide was determined by absorption spectroscopy using the molar absorption coefficient (33000 M^{-1} cm^{-1}) at 260 nm. Solutions of ct-DNA gave a ratio of UV-Vis absorbance of 1.8-1.9 : 1 at 260 and 280 nm, indicating that the ct-DNA was sufficiently free of protein [14]. Double distilled water was used to prepare all stock solutions for ct-DNA binding studies.

UV-Vis spectrum was recorded on a Perkin-Elmer lambda-25 recording spectrophotometer. Fluorescence intensity changes were studied by using a Scinco spectrofluorimeter, FS-2 model, equipped with a thermostatically controlled cuvette compartment. The following spectrometric measurements were all performed in a quartz cuvette of 1 cm path length.

Synthesis of the ligand Cobalt complex of Phenantroline-Imidazole ([Co(FIP)$_2$](OAC)$_2$

A solution of cobalt(II) acetate tetrahydrate (0.747 g, 1 mmol) in absolute ethanol (10 ml) was added to a solution of ligand in absolute ethanol (10 ml).The mixture was refluxed for 24 h, the dark brown precipitates obtained were washed well with cooled diethyl ether and dried. The yield of [Co(FIP)$_2$](OAC)$_2$ was 0.42 g(64%). Anal. Found for $C_{38}H_{26}CoN_8O_6$:C,60.73; H, 3.47; N,14.24, Calc: C,60.89; H, 3.50; N, 14.95 %. The chemical structure of Cobalt complex of Phenantroline-Imidazole was shown in Scheme 1.



Scheme1 Structure of Cobalt complex of Phenantroline-Imidazole

General procedure

The absorbance measurements were carried out using UV-Vis, Perkin Elmer Lambda25 double beam Spectrophotometer, operating from 200 to700 nm in 1.0 cm quartz cells. The absorbance titrations were performed at a fixed concentration of the [Co(FIP)$_2$](OAC)$_2$complexes varying the concentration of ct-DNA. In order to prevent interferences due to ct-DNA absorption, the data were obtained by keeping the same concentration of ct-DNA in the reference cuvette.

Melting curves were performed using an UV-vis Perkin Elmer Lambda 25 double beam spectrophotometer in conjunction with a thermo stated cell compartment. The measurements were carried out in 20 mMTris/HCl buffer, pH 7. The temperature inside the cuvette was determined with a platinum probe and has increasedover the range 25-86 °C at a heating rate of 0.5 °C/min(Thermal software). The melting temperature, T_m was obtained from the mid-point of the hyperchromic transition. In allof the experiments, for the pH measurement, we used a potentiometer (Metrohm model, 744).

Ethidium bromide (EB), one of the most sensitive fluorescence probed, has a planar structure binds with DNA through intercalative mode [15]. At first, DNA (13.5 μM) was added to 1μM aqueous ethidium bromide solution and maximum quantum yield for ethidium bromide was achieved and emission was observed in the range of 550–700 nm. To this solution (containing EB and DNA) different concentrations of the [Co(FIP)$_2$](OAC)$_2$complex (0.1 μM) were added. Addition of any of these metal complexes to DNA-EB system causes obvious reduction in fluorescence intensity. These metal complexes do not exhibit emission in the presence of DNA and there is no influence on the emission intensity of free EB in the absence of DNA. Thus the competitive DNA-binding of metal complexes with EB could provide evidence of the interaction of the metal complexes between DNA base pairs.

RESULTS AND DISCUSSION

Electronic

Absorption Study

Electronic absorption spectroscopy is usually utilized to determine the binding of complexes with the DNA helix. The absorption spectrum of the [Co(FIP)$_2$](OAC)$_2$ complex in the absence and at various concentration of ct-DNA is shown in Figure 1.In the UV-vis region, the [Co(FIP)$_2$](OAC)$_2$ complex exhibit two intense absorption bands: one at~286 nm which is attributed to the metal-to-ligand charge transfer absorption (MLCT) and the other at ca. 228 nm, which is assigned to the $\pi\rightarrow\pi^*$ transition of the aromatic chromophore. A spectral change of the [Co(FIP)$_2$](OAC)$_2$ complex dueto addition of ct-DNA was shown in Figure 1. For obtaining these spectra, the fixed amount of [Co(FIP)$_2$](OAC)$_2$ complex in Tris/HClbuffer solution, pH = 7 was titrated with a stock solution ofct-DNA. It exhibited the low hyperchromism in all spectral regions and negligible red shift due to the incremental addition of ct-DNA. Hypochromism and hyperchromism are both spectral feature of ct-DNA concerning changes in its double helix structure. Hypochromism happens when the DNA- binding mode of a complex has an electrostatic effect or an intercalation which stabilizes the DNA duplex. While hyperchromism may probably be due to dissociation of aggregated ligand or external contact with DNA [16, 17]. Asimilar hyperchromism has been observed for the soretbands of certain porphyrins when they interact with ct-DNA [18]. The apparent binding constant, K_{app}, for the interaction between the [Co(FIP)$_2$](OAC)$_2$ complex and ct-DNA can be determined by analysis of absorption spectro photometric titration data atroom temperature using Eq. (1):

$$[DNA]_{total}/(|\varepsilon_{app} \quad \varepsilon_f|) = [DNA]_{total}/(|\varepsilon_b \quad \varepsilon_f|) + 1/K_{app}(|\varepsilon_b \quad \varepsilon_f|) \quad (1)$$

Where ε_{app}, ε_f and ε_b correspond to $A_{observed}/[Co(FIP)_2](OAC)_2]$, the extinction coefficient for the free [Co(FIP)$_2$](OAC)$_2$ complex and the extinction coefficient for the [Co(FIP)$_2$](OAC)$_2$ complex in the fully bound form, respectively. In the plot of $[DNA]_{total}/(|\varepsilon_{app} \quad \varepsilon_f|)$versus $[DNA]_{total}$ that was shown in Figure 2, K_{app} is givenby the ratio of the slope to the intercept [18-21].

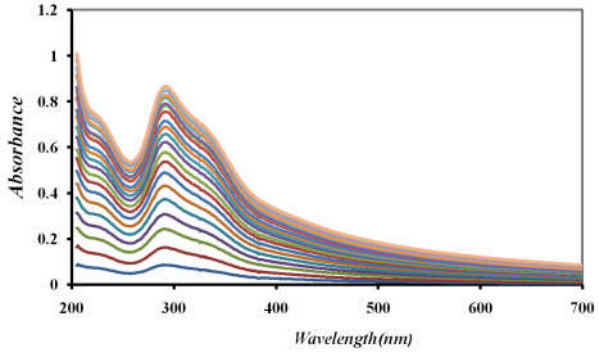


Figure 1 Electronic Absorption spectra of [Co(FIP)$_2$](OAC)$_2$complex

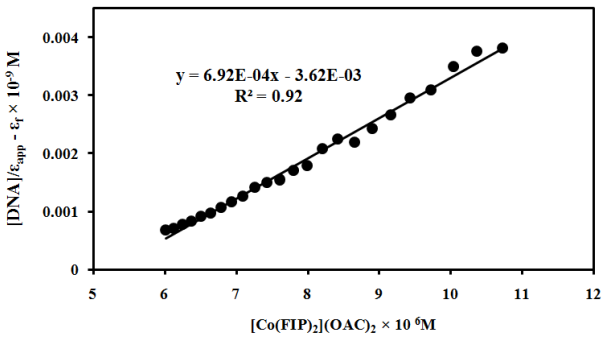


Figure 2 The plot of [ct-DNA]/(|ε_{app} ε_f|) versus [ct-DNA].

The apparent binding constant of $[Co(FIP)_2](OAC)_2$complex was estimated and used for calculation of Gibbs free energy change of reaction atvarious temperaures.

Thermodynamics of ct-DNA-[Co(FIP)$_2$](OAC)$_2$ Interaction

The energetics of DNA-$[Co(FIP)_2](OAC)_2$equilibrium can be conveniently characterized by three thermodynamic parameters, standard Gibbs free energy, ΔG°, standard enthalpy, ΔH° and standard entropy changes, ΔS°. ΔG° can be calculated from the equilibrium constant, K, of the reaction using the familiar relationship, ΔG° = RT LnK, in which R and T refers to the gas constant, and the absolute temperature, respectively. Furthermore, K is the apparent equilibrium constant and cons equently ΔG° is the apparent Gibbs free energy change. If heat capacity changes for the reaction are essentially zero, the van't Hoff equation (Eq. 2) gives a linear plot of LnKversus 1/T (Fig. 3).

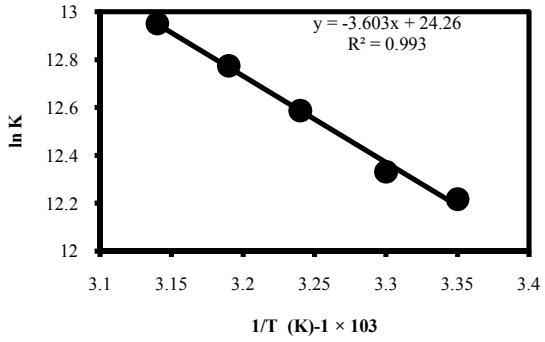


Figure 3 The Van't Hoff plot $[Co(FIP)_2](OAC)_2$complex binding to ct-DNA.

$$d\ln K/d(1/T) = \Delta H°/R \quad (2)$$

The apparent standard enthalpy change ΔH°, can be calculated from the slope of the straight line, ΔH°/R and the apparent standard entropy change from its intercept, ΔS°/R. The van't Hoff plots for interaction of $[Co(FIP)_2](OAC)_2$ complex with ct-DNA is shown in Figure 3 and the calculated thermodynamic parameters with their uncertainties are reported in Table 1. It has been revealed that the standard Gibbs free energy changes for ct-DNA-$[Co(FIP)_2](OAC)_2$interaction is negative, representing the relative affinity of the $[Co(FIP)_2](OAC)_2$ complex to ct-DNA. It has also been indicated that the binding process is endothermic disfavored (ΔH° > 0) and entropy favored (ΔS°> 0). As proposed by Ross when ΔH° < 0 or ΔH° ≈ 0, ΔS°> 0, the mainly acting force is electrostatic; when ΔH° < 0,ΔS° < 0, the mainly acting force is van der Waals or hydrogen bonding and when ΔH° > 0, ΔS° > 0, the mainly force is hydrophobic. Therefore, in the cases of the present system, we presumed that hydrophobic interaction might be the mainacting force in the binding of the $[Co(FIP)_2](OAC)_2$ complex and ct-DNA. From the thermodynamic data, it was quite clear that the interaction processes were endothermic disfavored butentropy favored (ΔH° > 0, ΔS° > 0).

Table 1 Thermodynamic parameters and binding constants for binding of $[Co(FIP)_2](OAC)_2$complex to ct-DNA in 20 mMTris/HCl buffer, pH 7 at various temperatures.

T(K)	Ln K_b	ΔH^0 (*cal/mol*)	ΔG^0 (*cal/mol*)	ΔS^0 (*cal/mol K*)
298	12.2±0.2	30015.7±6.7	-30256± 5.7	202.25±1.9
303	12.3±0.2	30015.7 ±6.7	-31050± 5.8	201.53±1.9
308	12.5±0.2	30015.7 ±6.7	-32215± 5.9	202.05±1.9
313	12.7 ± 0.2	30015.7 ± 6.7	-33225 ± 6.3	202.04 ± 1.9
318	12.9 ± 0.2	30015.7 ± 6.7	-34222 ± 6.1	202.00 ± 1.9

Thermal Denaturation Measurements

Other strong evidence for the binding mode between the metal complexes and ct-DNA was obtained from ct-DNA melting studies. The intercalation of small molecules into the double helix DNA is known to significantly increase the melting temperature of ct-DNA, at which the double helix denatures into single-stranded ct-DNA However, the T_m will slightly increase (< 0.6 ^{0}C) on the interaction of small molecules with ct-DNA through nonspecific electrostatic interactions with the phosphate backbone of ct-DNA . The extinction coefficient of ct-DNA bases at 260 nm in the double helical form is much less than that in the single stranded form, hence the melting of the helix leads to an increase in the absorption at this wavelength (Fig. 4). Thus, the helix to coil transition temperature can be determined by monitoring the absorbance of ct-DNA at 260 nm as a function of temperature(T_m). Obtained data show that interaction of the $[Co(FIP)_2](OAC)_2$]complex with ct-DNA leads to relatively moderate stabilization of duplex structure (Table 2). The results of thermal denaturation experiments presented are consistent with the absorption spectral profiles which demonstrate a non-interactive mode. This indicates that $[Co(FIP)_2](OAC)_2$ complex binds strongly to ct-DNA mostly in the outside-binding and hydrophobic interaction modes.

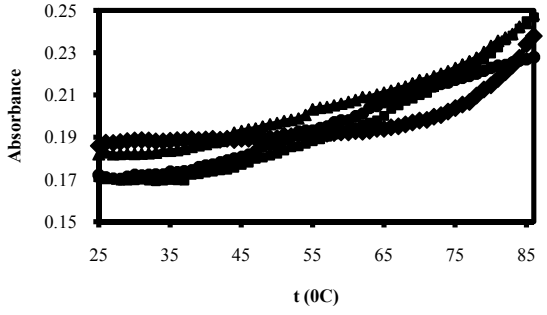


Figure 4 Melting profiles (λ = 260 nm) at various molar ratios (R= ([Co(FIP)$_2$](OAC)$_2$]/[ct-DNA]). R_1 = 0.0(◆), R_2 = 0.013(▲), R_3 = 0.026 (■) and R_4 = 0.052(●) in 20 mMTris/HClbuffer, pH 7 and in range of temperature 25 °C-86 °C.

Table 2 Melting temperature of free ct-DNA in the absence and in the presence of various stoichiometric ratios (R=[[Co(FIP)$_2$](OAC)$_2$]/[ct-DNA])

$\frac{[Co(FIP)_2](OAC)_2}{[ct-DNA]}$	**0**	**0.013**	**0.026**	**0.039**
T_m (K) (exp)	318	326	330	334

Fluorescence Titration Studies

The fluorescence of ethidium bromide (EB) increased after intercalation in DNA. If the complex intercalates into DNA, it leads to a decrease in the binding sites of DNA available for EB-DNA system [22, 23].

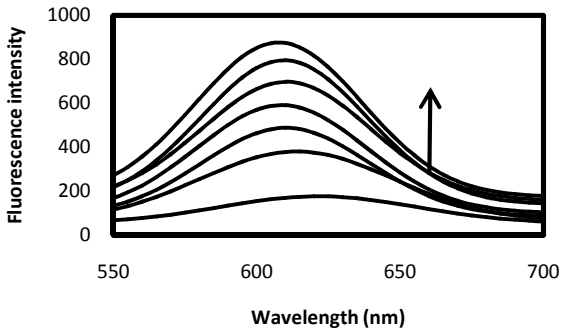


Figure 5 Fluorescence emission spectra of interacted EB-DNA

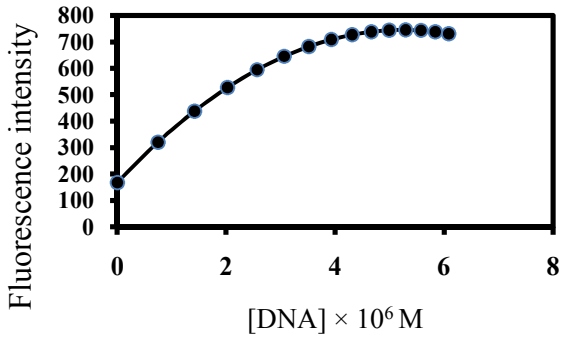


Figure 6. Fluorescence emission spectra of interacted EB-DNA

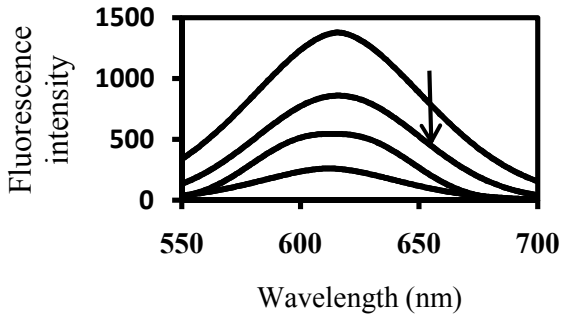


Figure 7. Fluorescence emission spectra of interacted EB-DNA in the absence and presence of different concentrations of $[Co(FIP)_2](OAC)_2$ complex.

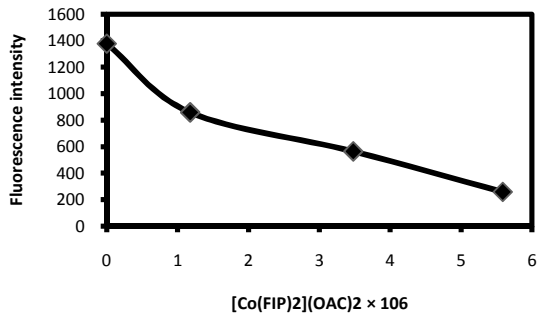


Figure 8 Fluorescence emission spectra of interacted EB-DNA in the absence and presence of different concentrations of $[Co(FIP)_2](OAC)_2$ complex.

Figs. 5 show fluorescence emission spectra of intercalated EB in DNA, with increasing concentrations of $[Co(FIP)_2](OAC)_2$ complexes at 25 °C. Figs. 5, 6, 7 and 8 also show a significantly reduction of the ethidium intensity by adding the different concentrations of $[Co(FIP)_2](OAC)_2$ complex. These results suggest that the above metal complexes presumably intercalate in DNA. As indicated in Figs. 5, the fluorescence intensity of DNA intercalated ethidium bromide is quenched far better in the presence of complex (Fig. 5).

CONCLUSION

In summary, we investigated the binding of ct-DNA with a Cobalt(II) complex,$[Co(FIP)_2](OAC)_2$ which contained imidazole derivatives of 1,10-phenanthroline ligand .According to the results of UV/Vis and fluorescence spectroscopies, there is a complex (1) bind to ct-DNA via hydrophobic mode. The thermodynamic parameters ($\Delta H^o > 0$ and $\Delta S^o > 0$) showed that the hydrophobic interaction leads to the increasing entropy which is brought about by interaction with the complex. The negative ΔG^o values for interaction of ct-DNA with the complex indicate the spontaneity of the complexation.

References

1. J.B. Chaires, Energetics of drug–DNA interactions, Bioploymers 44(1998) 201-215
2. A.M. Pyle, J.P. Rehman, R. Meshoyrer, C.V. Kumar, N.J. Turro,J.K. Barton, Mixed-ligand complexes of ruthenium (II): factors governing binding to DNA, *J. Am. Chem. Soc.* 111(1989) 3051-3058.
3. K. Naing, M. Takahashi,M. Taniguchi,A. Yamagishi, Interactions of Chiral Ru(II) Complexes with Calf Thymus DNA as Studied by Circular Dichroism and Electric Dichroism Measurements, Bull. Chem. Soc. Jap. 67(1994) 2424-2429.
4. K.E. Erkkila, D.T. Odom, J.K. Barton, Recognition and reaction of metallointercalators with DNA, Chem. Rev. 99(1999) 2777-2796
5. N.Shahabadi, L. Heidari, Synthesis, characterization and multi-spectroscopic DNA interaction studies of a new platinum complex containing the drug metformin. Spectrochim. Acta Part A128(2014) 377-385
6. G. Roman, J.G. Riley, J. Z. Vlahakis, R.T. Kinobe, J.F. Brien, K. Nakatsu, W.A. Szarek, Heme oxygenase inhibition by 2-oxy-substituted 1-(1*H*-imidazol-1-yl)-4-phenylbutanes: Effect of halogen substitution in the phenyl ring, Bioorg. Med. Chem. Lett. 15(2007) 3225–3234.
7. M. S. Han, D. H. Kim,Effect of zinc ion on the inhibition of carboxypeptidase A by imidazole-bearing substrate analogues, Bioorg. Med. Chem. Lett. 11(2001) 1425- 1427.
8. T. Nakamura, H. Kakinuma, H. Umemiya, H. Amada, N. Miyata, K.Taniguchi, K. Bando and M. Sato, Imidazole derivatives as new potent and selective 20-HETE synthase inhibitors, Bioorg. Med. Chem. Lett.14(2004)333–336.
9. A.M. Venkatesan,A. Agarwal, T. Abe, H.O. Ushirogoch i, D. Santos, Z. Li, G. Francisco, Y.I. Lin,P.J. Peterson, Y. Yang, W.J. Weiss, D.M. Shales, T.S. Mansour,5,5,6-Fused tricycles bearing imidazole and pyrazole 6-methylidene penems as broad-spectrum inhibitors of β-lactamases, Bioorg. Med. Chem. Lett. 16(2008) 1890–1902.
10. D.L. Banville, L.G. Marzilli, J.A. Strickland, W.D.J. Wilson, Comparison of the effects of cationic

porphyrins on DNA properties: Influence of GC content of native and synthetic polymers, Biopolymers 25(1986)1837-1858.
11. R.Vijayalakshmi, M.Kanthimathi, V.Subramanian, B. Unni Nair,Interaction of DNA with [Cr(Schiff base)(H(2)O)(2)]ClO(4), Biochim. Biophys. Acta 1475(2000)157-162.
12. F. Arjmand, M. Aziz, M.J. Chauhan, Synthesis, spectroscopic studies of new water-soluble Co(II) and Cu(II) macrocyclic complexes of 4,15-bis(2-hydroxyethyl)-2,4,6,13,15,17-hexaazatricyclodocosane: their interaction studies with calf thymus DNA and guanosine 5′ monophosphate, *J. Incl. Phenom. Macrocycl.Chem*.61(2008) 265-275.
13. R.F. Pasternack, E.J. Gibbs, J.J. Villafranca,Interactions of porphyrins with nucleic acids, Biochem. 22(1983)5409–5417.
14. J.Onuki, A.V. Ribas, M.H.G. Medeiros, K. Araki, H.E. Toma, L.H. Catalani,P.D. Mascio, Supramolecular Cationic Tetraruthenated Porphyrin Induces Single-Strand Breaks and 8-Oxo-7,8-dihydro-2′-deoxyguanosine Formation in DNA in the Presence of Light, *J. Photochem. Photobiol*. 63(1996)272-277.
15. S.Mettah, B.R. Munson, R.K. Pandey,DNA Interaction and Photocleavage Properties of Porphyrins Containing Cationic Substituents at the Peripheral Position, Bioconj. Chem.10 (1999) 94-102.
16. L.W.Zhang, K. Wang, X.X. Zhang, Study of the interactions between fluoroquinolones and human serum albumin by affinity capillary electrophoresis and fluorescence method, Anal.Chim.Acta 603(2007) 101-110.
17. M.P.Foloppel, S. Rault, D.E. Thurston, T.C. Jenkins, M. Robbal, DNA-binding properties of pyrrolo[2,1-c][1,4]benzodiazepine N10-C11 amidines, *Eur. J. Med. Chem*.31(1996) 407-410.
18. L. Peres-Flores, A. J. Ruiz-Chica, J. G. Delcros, F. M. Sanches,F. J. Ramirez, Effect of spermine conjugation on the interaction of acridine with alternating purine–pyrimidine oligodeoxyribonucleotides studied by CD, fluorescence and absorption spectroscopies, Spectrochim. Acta Part A 69(2008)1089-1096.
19. S. Das, G.S. Kumar, Molecular aspects on the interaction of phenosafranine to deoxyribonucleic acid: Model for intercalative drug–DNA binding, *J. Mol. Struct*.872(2008)872, 56-63
20. S.C. Bi, D. Qiao, D. Song, Y. Tian, D. Gao, Y. Sun, H. Zhan, Study of interactions of flavonoids with DNA using acridine orange as a fluorescence probe, Sens Actuators B Chem.119(2006) 199-208,
21. T.R. Li, Z. Yang, B. Wang, .D.D. Qin, Synthesis, characterization, antioxidant activity and DNA-binding studies of two rare earth (III) complexes with naringenin-2-hydroxy benzoyl hydrazone ligand, *Eur. J. Med. Chem*. 43(2008)1688-1695
22. J.Olmsted, D.R. Kearns, Mechanism of ethidium bromide fluorescence enhancement on binding to nucleic acids, Biochem. 16(1977) 3647-3654.
23. Y.Ni, D. Lin, S. Kokot, Synchronous fluorescence, UV–visible spectrophotometric, and voltammetric studies of the competitive interaction of bis (1,10-phenanthroline) copper (II), complex and neutral red with DNA, Anal. Biochem.325(2006) 231-242