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# Interaction Studies of *Bis*-(*p*-aminobenzoato)diaquanickel(II) and *Bis*-(*p*-aminobenzoato)triaquacadmium(II) Complexes with Cat Genomic DNA

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*Bis*-(*p*-aminobenzoato)diaquanickel(II) [Ni(*p*-$H_2N$-$C_6H_4COO)_2 \cdot 2H_2O$] and *bis*-(*p*-aminobenzoato)triaquacadmium(II) [Cd(*p*-$H_2N$-$C_6H_4COO)_2 \cdot 3H_2O$] complexes derived from *p*-aminobenzoate are reported. The genomic DNA interactions of these complexes with cat genomic DNA have been investigated with UV spectroscopy, gel electrophoresis and viscosity measurements. Ni($H_2N$-$C_6H_4COO)_2 \cdot 2H_2O$ and Cd($H_2N$-$C_6H_4COO)_2 \cdot 3H_2O$ complexes exhibit interaction activities with DNA. The experimental results indicate that the complexes can bind to DNA *via* an intercalative mode. The intrinsic binding constants of the complexes with genomic DNA are $5.2 \times 10^5$-$2.49 \times 10^5$ $M^{-1}$, respectively.

**Key Words: Nickel (II), Cadmium(II), *p*-Aminobenzoic acid, DNA Binding, Gel electrophoresis.**

## INTRODUCTION

Recently DNA binding studies with transition metal complexes with small molecules over molecular levels get more attention[1,2]. Especially, new small complexes have get considerable attention because of their importance in the development of new therapeutic materials[3,4]. The DNA binding properties of metal complexes are being broadly studied as DNA structural probes[5-8]. There are several kinds of sites in the DNA molecule in which metal complexes binding can occur as intercalation, groove, major groove or on the outside of the helix[9]. The plenty of biological studies have already proven that DNA is the primary intracellular target of anticancer drugs because of the interaction between DNA and small molecules that may lead DNA damage in cancer cells, which prevent the division of cancer cells and leading to cell death[10-12].

Nickel(II) and cadmium complexes get more attention in order to the environmental toxicity and carcinogenic nature of some nickel and cadmium complexes and the chemotherapeutic activities of these metal complexes[13]. Some scientists have indicated that proteins or ligands can rise the toxic effects of nickel and cadmium

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ions. Though, the complete mechanism to involve carcinogenesis has not been explained[14,15]. The discover of new nickel(II) and cadmium(II) complexes that may have useful applications in medicine for DNA probes[15-17]. Hence, further researches by studying different ligands with different structures to assess and understand the factors that can determine the DNA binding modes[18].

The objective of the present study was to investigate the interactions of $Ni(H_2N\text{-}C_6H_4COO)_2{\cdot}2H_2O$ and $Cd(H_2N\text{-}C_6H_4COO)_2{\cdot}3H_2O$ complexes with cat genomic DNA using gel electrophoresis, viscosity measurements, electronic absorption spectra. The data indicate that the complexes interact with DNA *via* intercalation modes.

## EXPERIMENTAL

Genomic DNA was isolated at Kafkas University and ethidium bromide was purchased from Aldrich chemicals. All solvents and materials used in this work were analytical grade. The experiments that involve interaction of the complex with genomic DNA were conducted in doubly distilled water buffer containing 5 mM *tris*-[*tris*(hydroxymethyl)aminomethane] and 50 mM NaCl and adjusted to pH 7.1 with HCl. A solution of DNA in the buffer gave a ratio of UV absorbance of about 260 and 280 nm, which show that the DNA was free of proteins[19]. The DNA concentration per nucleotide was determined by absorption spectroscopy using the molar absorption coefficient[20] (6600 $M^{-1}$ $cm^{-1}$) at 260 nm.

**Preparation of $Ni(p\text{-}H_2N\text{-}C_6H_4COO)_2{\cdot}2H_2O$ and $Cd(p\text{-}H_2N\text{-}C_6H_4COO)_2{\cdot}3H_2O$ complexes:** Green crystals of *bis*-(*p*-aminobenzoato) diaquonickel(II), $Ni(p\text{-}H_2N\text{-}C_6H_4COO)_2.2H_2O$, were obtained by slow addition of a solution of sodium *p*-aminobenzoate to a solution of $NiSO_4$. After stirring at room temperature, a green precipitate of the $Ni(p\text{-}H_2N\text{-}C_6H_4COO)_2{\cdot}2H_2O$ (Fig. 1a) is obtained. The crystals of the compound are monoclinic[21].

The complex compound formed by cadmium halides with *p*-aminobenzoic acid have been synthesized and its properties was studied[22]. Crystals of *bis*-(*p*-aminobenzoato)triaquocadmium, $Cd(p\text{-}H_2N\text{-}C_6H_4COO)_2{\cdot}3H_2O$, were obtained by the reaction of aqueous solutions of sodium *p*-aminobenzoate and $CdCl_2$ (Fig. 1b).

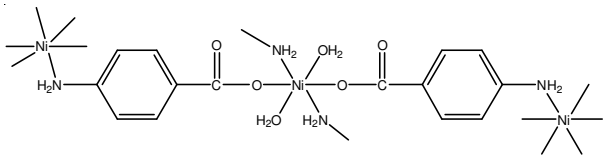



Fig. 1a. Speculate structures of $Ni(p\text{-}H_2N\text{-}C_6H_4COO)_2.2H_2O$ complex

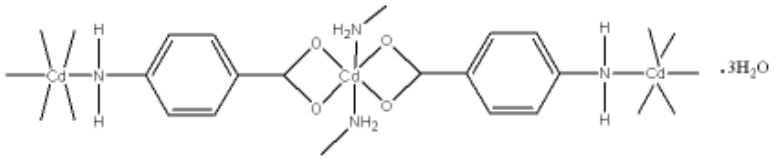



Fig. 1b. Speculate structures of $Cd(p\text{-}H_2N\text{-}C_6H_4COO)_2.3H_2O$ complex

**Gel electrophoresis study of the nickel(II) and cadmium(II) complexes with cat genomic DNA:** The *p*-aminobenzoate of the nickel(II) and cadmium(II) complexes was used as the source of sample. $Ni(H_2N\text{-}C_6H_4COO)_2{\cdot}2H_2O$ and $Cd(H_2N\text{-}C_6H_4COO)_2{\cdot}3H_2O$ complexes solutions were prepared in MilliQ water and were sterilized by passing through millipore filter. Solution of genomic DNA in the buffer containing of 1 mM *tris*-HCl at pH 7.1, 1 mM NaCl and 1 mM EDTA was used. Adequate amount of solution of the nickel(II) and cadmium(II) complexes were added to 1 mL of genomic DNA and all the volume was made up to 25 mL by adding MilliQ water in the order that the concentrations of nickel(II) and cadmium(II) complexes changed from 0.0-12 mM while that of DNA remained unchanged at 0.50 mg/mL. After the mixtures were incubated at 37 ºC for 6 h, the reaction was rapidly cooled to 0 ºC.

**Gel electrophoresis:** Agarose gel (1.5 %, w/v) in TBE buffer, pH 7.1 consisting of 0.50 mg/mL ethidium bromide was prepared. Afterward, 15 mL of each of the incubated nickel(II), cadmium(II) complexes and DNA mixtures containing loading dye was added to on the gel and the electrophoresis was performed under TBE buffer system at 100 V for 3 h. At the end of electrophoresis experiment, the gel was visualized under UV the bio-rad *trans* illuminator. In the end the gel was photographed with a polaroid camera[23,24].

**Physical measurements:** Viscosity measurement of the complexes were carried out on a Ubbelodhe viscometer, put in a thermostatic water bath at 30 ºC. About 200 base pair genomic DNA samples with average length were prepared in order to minimize complexities arising from DNA flexibility. The time of flow was determined using a digital stopwatch. Each sample was measured three times. The results were presented as $(\eta/\eta_0)^{1/3}$ *versus* the concentration of the Ni(II) and Cd(II) complexes, where $\eta$ represents the viscosity of DNA in the presence of the complexes, $\eta_0$ represents the viscosity of DNA alone[23].

Absorption spectra were performed on a Shimadzu UV-vis spectrophotometer using corvettes of 1 cm path length. Absorption spectral measurements were conducted by using DNA stock solutions interacted with the Ni(II) and Cd(ll) complexes. For the gel electrophoresis experiments, the cat genomic DNA was interacted with the Ni(II) and Cd(II) complexes in 45 mM *tris*-HCl, 18 mM NaCl buffer, pH 7.1 and the solutions were incubated for 6 h in the dark at room temperature.

## RESULTS AND DISCUSSION

In this study genomic DNA was used to carry out the gel electrophoresis for the $Ni(H_2N\text{-}C_6H_4COO)_2{\cdot}2H_2O$ and $Cd(H_2N\text{-}C_6H_4COO)_2{\cdot}3H_2O$ complexes. For $Ni(H_2N\text{-}C_6H_4COO)_2{\cdot}2H_2O$, form I and form II bands of the DNA were observed. When the concentration of $Ni(H_2N\text{-}C_6H_4COO)_2{\cdot}2H_2O$ complex was 12 mM, the interaction between the DNA and the nickel(II) complex was observed. Mobility of the band-I was decreased and visibility of band was a little faint compared to that of the untreated DNA (C) (Fig. 2). When the concentration of $Ni(H_2N\text{-}C_6H_4COO)_2{\cdot}2H_2O$

complex was 2 mM, the band (2) was observed to be faint compared to untreated DNA band (C). When 0.2 mM small amount of $Ni(H_2N\text{-}C_6H_4COO)_2{\cdot}2H_2O$ complex was added into the DNA sample, it was observed that the complex leaded to change in the mobility and intensity of the form I band (3) as compared to that observed in the untreated DNA (C), but it was found that the band (3) of the mixture was a little bright. Therefore, it can be said that the observed changes in the DNA bands in the presence of $Ni(H_2N\text{-}C_6H_4COO)_2{\cdot}2H_2O$ complex is a evidence of interaction between DNA and the complex. As a result the band (1) showed that the relative intensities and mobility of the band (1) was almost appeared to change when the concentration of the complex was increased (Fig. 2). It can be concluded that the complex interacts with genomic DNA.

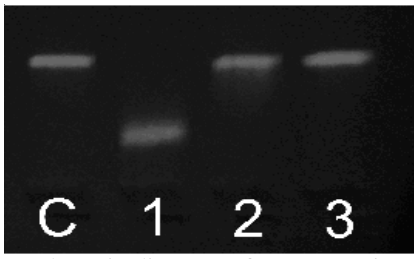



Fig. 2. Agarose gel electrophoresis diagram of cat genomic DNA-$Ni(H_2N\text{-}C_6H_4COO)_2{\cdot}2H_2O$ complex. Lane C: untreated cat genomic DNA, lanes 1-3: DNA + nickel(II) complex in the concentrations of 12, 2, 0.2 mM, respectively

As for the interaction of $Cd(H_2N\text{-}C_6H_4COO)_2{\cdot}3H_2O$ complex with genomic DNA, the DNA was incubated with cadmium(II) complex under the same reaction condition. The reaction was carried out to observe the effect of the complex on the DNA by using gel electrophoresis in the same way for the cadmium(II) complex. When 12 mM of $Cd(H_2N\text{-}C_6H_4COO)_2{\cdot}3H_2O$ complex was treated with genomic DNA, the interaction between the DNA and the complex was observed. The band (C) is untreated genomic DNA. It was observed that no change occurred in mobility and intensity of the band (C). When the concentration of cadmium (II) complex was increased to 12 mM, it was found that the band (1) was visible, and slightly faint as compared to the band (C) of the untreated DNA. Mobility and intensity of the band (1) stayed almost unchanged (Fig. 3). When the concentration of $Cd(H_2N\text{-}C_6H_4COO)_2{\cdot}3H_2O$ complex was 2 mM, the band (2) was observed to be very bright as compared to the band (C) of untreated DNA and the band (2). Also the size of band (2) was greater than those of other bands. When 0.2 mM small amount of $Cd(H_2N\text{-}C_6H_4COO)_2{\cdot}3H_2O$ complex was added, it was found that no significant change in the mobility and intensity of the form I band (3) as compared to that observed in the band (3). However, it was found that its mobility and intensity of the band decreased and the band (3) appeared brighter than those of untreated DNA and the band (1). Hence, it can be said that the observed changes in the DNA bands in the presence of $Cd(H_2N\text{-}C_6H_4COO)_2{\cdot}3H_2O$ complex is a proof of interaction

between DNA and the complex. It can be concluded that the complex interacts with genomic DNA.

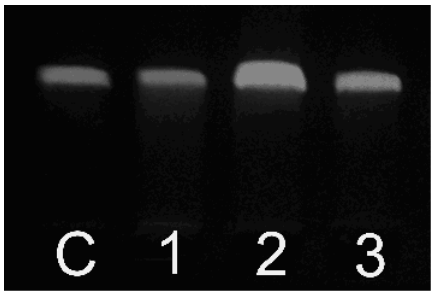
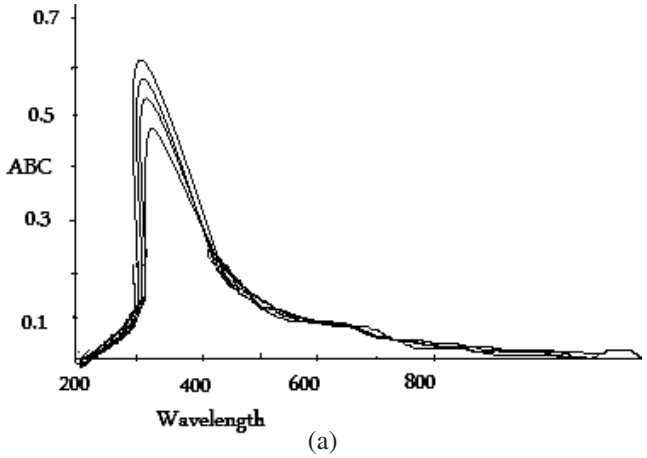
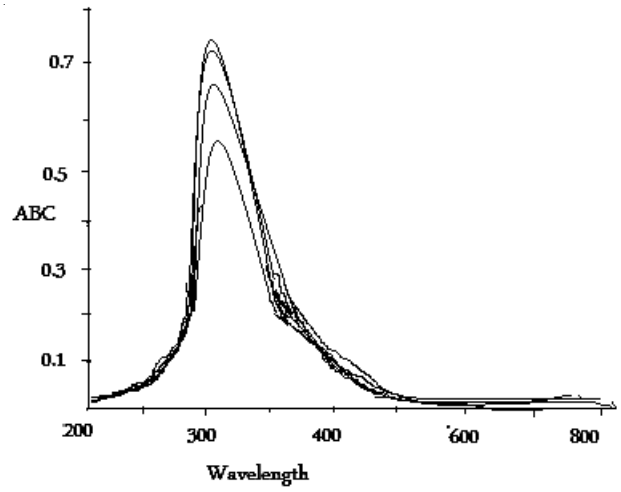



Fig. 3. Agarose gel electrophoresis diagram of cat genomic DNA-Cd($H_2N$-$C_6H_4COO)_2$·$3H_2O$ complex. Lane C: untreated cat genomic DNA, lanes 1-3: DNA + cadmium(II) complex in the concentrations of 12, 2 and 0.2 mM, respectively

The interactions of Ni($H_2N$-$C_6H_4COO)_2$·$2H_2O$ and Cd($H_2N$-$C_6H_4COO)_2$·$3H_2O$ with DNA were studied through the electronic spectra and viscosity measurements to assess their binding properties[25]. Electronic absorption spectroscopy was an efficient method in investigating the interaction with DNA[26]. The absorption spectra studies of Ni($H_2N$-$C_6H_4COO)_2$·$2H_2O$ and Cd($H_2N$-$C_6H_4COO)_2$·$3H_2O$ complexes are shown in Fig. 4a. It is seen that the addition of genomic DNA to the solutions of two complexes at the molar ratio of DNA/complexes varied from 0-10 and it caused hypochromism and bathochromism shifts[26,27]. The absorption band of Ni($H_2N$-$C_6H_4COO)_2$·$2H_2O$ was observed about 385 nm and the band of Cd($H_2N$-$C_6H_4COO)_2$·$3H_2O$ was appeared around 300 nm. The results show that Ni($H_2N$-$C_6H_4COO)_2$·$2H_2O$ and Cd($H_2N$-$C_6H_4COO)_2$·$3H_2O$ complexes interact with DNA, and the complexes bind to DNA by intercalation mode which can stabilize the DNA[27,28].



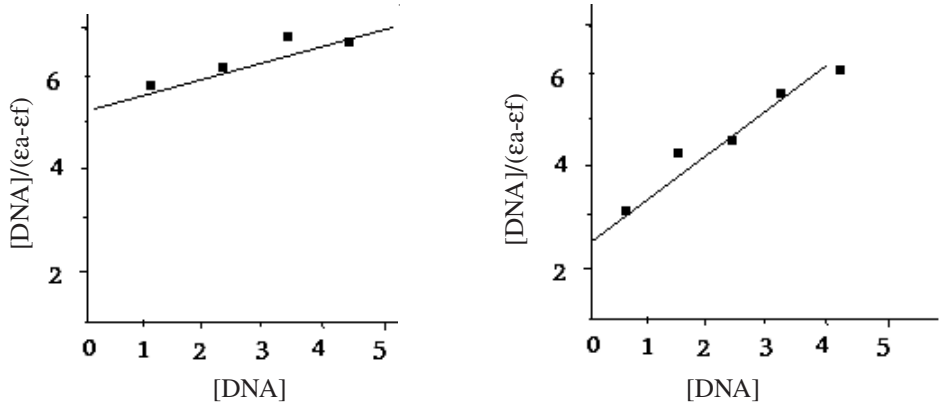

(a)




(b)

Fig. 4a. Absorption spectrums of (a) $Ni(H_2N\text{-}C_6H_4COO)_2{\cdot}2H_2O$ and (b) $Cd(H_2N\text{-}C_6H_4COO)_2{\cdot}3H_2O$ in the presence of increasing concentration of genomic DNA, [DNA] = 0-50 mM. The arrows show the absorbance changes when increasing DNA concentration

The obtained data indicate that these two complexes interact with genomic DNA. The data suggest that the complexes go into an inner site of the DNA. It can be reported that the relative binding constants of $Ni(H_2N\text{-}C_6H_4COO)_2{\cdot}2H_2O$ and $Cd(H_2N\text{-}C_6H_4COO)_2{\cdot}3H_2O$ are $5.2 \times 10^5$-$2.49 \times 10^5$ $M^{-1}$, respectively (Fig. 4b). These binding constants results show that binding strength of $Ni(H_2N\text{-}C_6H_4COO)_2{\cdot}2H_2O$ is stronger than that of $Cd(H_2N\text{-}C_6H_4COO)_2{\cdot}3H_2O$.




(a) (b)

Fig. 4b. Plots of [DNA]/(εa-εf) *vs.* [DNA] for the titrations of DNA with of (a) $Ni(H_2N\text{-}C_6H_4COO)_2{\cdot}2H_2O$ and (b) $Cd(H_2N\text{-}C_6H_4COO)_2{\cdot}3H_2O$ complexes

In addition to above the studies, viscosity measurements were also conducted to ensure the interactions between the $Ni(H_2N\text{-}C_6H_4COO)_2\cdot 2H_2O$ and $Cd(H_2N\text{-}C_6H_4COO)_2\cdot 3H_2O$ complexes and the DNA. Physical measurement studies give necessary data, but it is not effective enough to explain binding between the complexes and the DNA. The impacts of the $Ni(H_2N\text{-}C_6H_4COO)_2\cdot 2H_2O$ and $Cd(H_2N\text{-}C_6H_4COO)_2\cdot 3H_2O$ complexes on the viscosity of DNA at 30 ºC are shown in Fig. 5. The viscosities of the DNA increase with increasing amounts of the $Ni(H_2N\text{-}C_6H_4COO)_2\cdot 2H_2O$ and $Cd(H_2N\text{-}C_6H_4COO)_2\cdot 3H_2O$ complexes. This type of behaviour which increases the relative specific viscosity for the lengthening of the DNA double helix resulting from intercalation. Viscosity experimental data show that the complexes can intercalate between DNA base pairs and thus this increases the viscosity of DNA[29,30]. The $Ni(H_2N\text{-}C_6H_4COO)_2\cdot 2H_2O$ can intercalate more than that of $Cd(H_2N\text{-}C_6H_4COO)_2\cdot 3H_2O$.

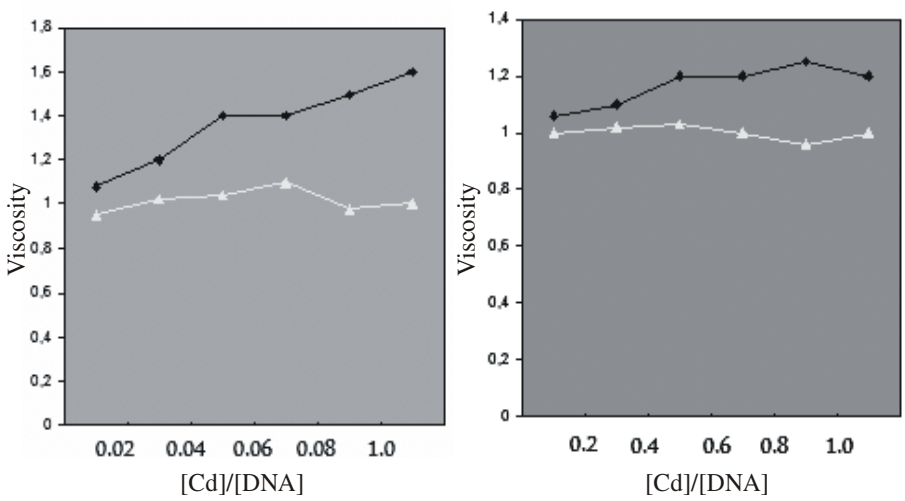



Fig. 5. Effect of increasing concentration of $Ni(H_2N\text{-}C_6H_4COO)_2\cdot 2H_2O$ (◆) (a) on the relative (▲) viscosity of cat genomic DNA at 30 ºC and $Cd(H_2N\text{-}C_6H_4COO)_2\cdot 3H_2O$ (◆) (b) on the relative (▲) viscosity of genomic DNA at 30 ºC

## Conclusion

The interaction of genomic DNA with *p*-aminobenzoate ligand of the nickel(II) and cadmium(II) complexes was investigated using gel electrophoresis, absorption spectra and viscosity measurements. The measurements show that the $Ni(H_2N\text{-}C_6H_4COO)_2\cdot 2H_2O$ and $Cd(H_2N\text{-}C_6H_4COO)_2\cdot 3H_2O$ complex interact with the DNA, but $Ni(H_2N\text{-}C_6H_4COO)_2\cdot 2H_2O$ complex can more strongly interact with the DNA than that of $Cd(H_2N\text{-}C_6H_4COO)_2\cdot 3H_2O$ complex. The interaction takes place by an intercalation mechanism. DNA-binding affinities of $Ni(H_2N\text{-}C_6H_4COO)_2\cdot 2H_2O$ are higher than $Cd(H_2N\text{-}C_6H_4COO)_2\cdot 3H_2O$.

## REFERENCES


1. D.D. Qin, Z.Y. Yang and B.D. Wang, *Spectrochim. Acta*, **68A**, 912 (2007).
2. G.M. Zhang, S.M. Shuang, C. Dong, D.S. Liu and M.M.F. Choi, *J. Photochem. Photobiol. B*, **74**, 127 (2004).
3. B.D. Wang, Z.Y. Yang and Y. Wang, *Synth. React. Inorg. Met.-Org. Nano.-Met. Chem*., **35**, 533 (2005).
4. B.D. Wang, Z.Y. Yang, Q. Wang, T.K. Cai and P. Crewdson, *Bioorg. Med. Chem*., **14**, 1880 (2006).
5. K.E. Erkkila, D.T. Odom and J.K Barton, *Chem. Rev*., **99**, 2777 (1999).
6. C. Metcalfe, and J.A. Thomas, *Chem. Soc. Rev.*, **32**, 215 (2003).
7. I. Haq, P. Lincoln, D. Suh, B. Norden, B.Z. Choedhry and J.B. Chaires, *J. Am. Chem. Soc*., **117**, 4788 (1995).
8. M. Pyle, J.P. Rehmann, R. Meshoyrer, C.V. Kumar, N.J. Turro and J.K. Barton, *J. Am. Chem. Soc*., **111**, 3051 (1989).
9. H.P. Marguerite, R.G. Michael, S.S. Heinz and M. Bernard, *J. Biol. Inorg. Chem*., **6**, 14 (2001).
10. V.S. Li, D. Choi, Z. Wang, L.S. Jimenez, M.S. Tang and H. Kohn, *J. Am. Chem. Soc*., **118**, 2326 (1996).
11. G. Zuber, J.C. Quada Jr. and S.M. Hecht, *J. Am. Chem. Soc*., **120**, 9368 (1998).
12. Z.H. Xu, F.J. Chen, P.X. Xi, X.H. Liu and Z.Z. Zeng, *J. Photochem. Photobio. A: Chem*., **196**, 77 (2008).
13. J.G. Muller, X. Chen, A.C. Dadiz, S.E. Rokita and C.J. Burrows, *J. Am. Chem. Soc*., **114**, 6407 (1992).
14. S.S. Matkar, L.A. Wrischnik, P.R. Jones and U. Hellmann-Blumberg, *Biochem. Biophys. Res. Commun*., **343**, 754 (2006).
15. H. Lu, X. Shi, M. Costa and C. Huang, *Mol. Cell. Biochem*., **279**, 45 (2005).
16. M.C. Rodriguez-Argu Elles, M.B. Ferrari, F. Biscegli, C. Pellizi, G. Pelosi and S. Pinelli, *J. Inorg. Biochem*., **98**, 313 (2004).
17. Z. Afrasiabi, E. Sinn, W. Lin, Y. Ma, C. Campana and S. Padhye, *J. Inorg. Biochem*., **98**, 1526 (2005).
18. D.M. Kong, J. Wang, L.N. Zhu, Y.W. Jin, X.Z. Li, H.X. Shen and H.F. Mi, *J. Inorg. Biochem*., **102**, 824 (2008).
19. J. Marmur and P. Doty, *J. Mol. Biol*., **3**, 585 (1961).
20. C.V. Kumar and E. H. Asuncion, *J. Am. Chem. Soc*., **115**, 8547 (1993).
21. I.R. Amiraslanov, Kh.S. Mamedov, E.M. Movsumov, F.N. Musaev, A.N. Shnulin, and G.N. Nadzhafov, *Zh. Struktur. Khim*., **19**, 1129 (1978).
22. I.R. Amiraslanov, S. Mamedov, E.M. Movsumov, F.N. Musaev, A.I. Magerramov and G.N. Nadzhafov, *Zh. Struktur. Khim*., **20**, 498 (1979).
23. T.C. Michael, R.J. Marisol and B. Allen, *J. Am. Chem. Soc*., **111**, 8901 (1989).
24. Z. Hossain and F. Huq, *J. Inorg. Biochem*., **90**, 85 (2002).
25. K.R. Fox, Drug DNA Interaction Protocols, in: Methods in Molecular Biology, Humana Press, Totowa, NJ, USA, Vol. 90 (1997).
26. J.K. Barton, A.T. Danishefsky and J.M. Golderg, *J. Am. Chem. Soc*., **106**, 2172 (1984).
27. P.Yang, M.L. Guo and B.S. Yang, *Chin. Sci. Bull*. **39**, 997 (1994).
28. E.C. Long and J.K. Barton, *Acc. Chem. Res*., **23**, 271 (1990).
29. S. Satyanarayana, J.C. Dabroniak and J.B. Chaires, *Biochemistry*, **31**, 9319 (1992).
30. S. Satyanarayana, J.C. Daborusak and J.B. Chaires, *Biochemistry*, **32**, 2573 (1993).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