

Synthesis, Characterization of DNA Binding and Cleavage and BSA Binding Studies of a Series of Benzil Based Macrocyclic Dinuclear Zinc (II) Complexes

Vedavalli Sairaj and Kandaswamy Muthuswamy*

*Department of Inorganic Chemistry, University of Madras School of Chemical sciences, Maraimalai Campus (Guindy), Chennai 600 025, India.

E-mail: mkands@yahoo.com

Abstract- The area of homo-binuclear complexes are studied in several research areas, because of the fact that these complexes are of simple models and naturally occurring metalloenzymes and have tunable coordination environments. Binuclear Zinc(II) complexes were prepared by the template synthesis with various diamines, benzilbisdihydrazone and zinc (II) salts. The complexes were characterized by using FT-IR, electronic spectra, ESI mass and elemental analysis. The binding properties of the Zn(II) complexes with calf thymus DNA have been investigated by absorption, emission spectral studies and the binding constant (K_b) and the linear Stern-Volmer quenching constant (K_{sv}) and K_{app} were calculated. Viscosity measurements have been carried out to find out the mode of binding of the complexes with CT-DNA. DNA cleavage of the binuclear Zinc(II) complexes was studied by electrophoresis method. The binuclear Zinc(II) complexes show hydrolytic cleavage of supercoiled pBR322-DNA. The BSA binding studies of the binuclear Zinc(II) complexes were also studied by UV absorption and fluorescence spectral methods.

Keywords: Unsymmetrical binucleating macrocyclic ligands; Zn (II) complexes; DNA and BSA binding properties; DNA cleavage studies.

I. Introduction

Recently, the investigations on simple and efficient reagents, which can cleave nucleic acids under mild conditions, have been attracted considerable attentions [1,2]. Generally, in order to eliminate the possibility of significant cytotoxic side effect of reactive oxygen species, pathways that result in DNA cleavage by hydrolysis mechanisms are preferable [3,4]. Transition metal complexes as cleavage reagents have great application value in biological project, molecular biologic treatment and related fields. Complexes composed of transition metals like copper, cobalt, nickel, ruthenium *etc.*, and bidentate polypyridyl ligands like 1,10-phenanthroline have been studied extensively for numerous applications including their biological activity as artificial nucleases. [5-13]. In recent years, the studies on DNA hydrolysis cleavage by zinc complex have been attracting great interest in the filed of artificial metallonuclease [14,15] because zinc ion has been found at the catalytic sites of many natural nucleases. The results show that the interaction of the zinc complex with DNA performs mainly in intercalative mode.

In this paper, new binuclear Zn (II) complexes with various new unsymmetrical binucleating macrocyclic were prepared and characterized by using various spectroscopic methods. The interaction of the Zn (II) complexes with calf thymus DNA and BSA has been investigated using absorption and fluorescence spectroscopic experiments and viscosity methods. The cleavage reaction on plasmid DNA has been monitored by agarose gel electrophoresis.

II. Experimental

a. Materials and instruments

Zinc(II) perchlorate hexahydrate and 1,2-diaminoethane were purchased from Fluka. 1,4-diaminobutane, benzil and Zinc(II)perchloratehexahydrate were purchased from Aldrich.imethylformamide, Acetonitrile,and Diethyl ether were purchased from SRL(INDIA.) The C, H, N contents of the ligands and complexes were carried out using a Carlo Erba Elemental analyzer Model 1106. ESI mass spectra were recorded on a JEOL DX-303 EI mass spectrometer. Electrospray ionization mass spectral measurements were done using Thermo Finnigan LCQ-6000 Advantage Max-ESI mass spectrometer. The IR spectra were recorded on Perkin Elmer FTIR spectrometer with samples prepared as KBr pellets. UV-visible spectra were recorded using a Perkin Elmer Lambda 35

spectrophotometer operating in the range of 200-1100 nm with quartz cells and are given in $M^{-1} cm^{-1}$. Absorption spectral titration experiments were carried out for CT-DNA with the complexes using a Perkin Elmer Lambda 35 spectrophotometer. Stock solution of CT-DNA was prepared with a buffer (5 mM Tris-HCl/50 mM NaCl, pH 7.2) and stored at 4 °C. The intrinsic binding constant K_b was determined. The emission spectra were recorded on Perkin-Elmer LS-45 Fluorescence spectrometer. The competitive binding study was carried out by maintaining the EB (2 μ M) and CT-DNA (4 μ M) in 50 mM Tris HCl/ 1 mM NaCl buffer solution (pH 7.2, and increasing the concentrations of the complexes. The apparent binding constant (K_{app}) has been calculated. Supercoiled pBR322 DNA, CT DNA (calf thymus) and Agarose was purchased from Bangalore Genei (India). Superoxide dismutase (SOD), ethidium bromide (EB) and L-Histidine were obtained from Sigma (USA). In a typical experiment, supercoiled pBR322 DNA (5 μ l, 50 μ g/ μ L) in Tris·HCl (100 mM, pH 7.2) was treated with different concentrations of complexes (0-50 μ M), followed by dilution with the Tris·HCl buffer to a total volume of 17.5 μ L. The samples were then incubated at 37°C for different times, and loaded on a 1% agarose gel containing 1.0 μ g/mL of ethidium bromide (EB). Bands were visualized by UV light and photographed, then the intensity of the DNA bands were estimated by a Gel Documentation System.

b. Synthesis of unsymmetrical macrocyclic binuclear complexes

Synthesis of mononuclear Zn(II) complexes

To a solution of 2,6-Diformyl-4-methylphenol in warm dimethyl formamide, various diamines (1,2-diaminoethane, 1,3-diaminopropane and 1,4-diaminobutane) in warm dimethylformamide was added dropwise under constant stirring. To this solution corresponding $Zn(OAc)_2 \cdot H_2O$ was added. The resulting solution was stirred at 60 °C for 2 hrs. The resulting mononuclear complex $[ZnL]$ precipitated. The precipitate was separated by filtration and washed with isopropyl alcohol and diethyl ether.

Synthesis of unsymmetrical macrocyclic binuclear complexes

The binuclear zinc (II) complexes were prepared from a vigorously stirred suspension of mononuclear complex in acetonitrile, and acetonitrile solution of $Zn(ClO_4)_2 \cdot 6H_2O$ was added slowly and the mixture was stirred for 15 min to obtain a clear solution. Then the acetonitrile solution of corresponding benzil bis dihydrazone was added drop wise to the above solution and refluxed for 3 hrs. A resulting solution was separated on evaporating the solution at room temperature and the resulting compound was washed with ether and dried. (Scheme 1).

$[Zn_2L^1][2C1O_4]$, 1. To a boiling solution of compound PC-1 (0.25 g, 0.07 mM) in DMF (10 cm^3) was added zinc (II) acetate monohydrate (0.14 g, 0.07 mM) dissolved in acetonitrile (15 cm^3) and the mixture was refluxed for 30 min. The precipitate formed was subsequently treated with a mixture of benzil bis dihydrazone (0.16 g, 0.07 mM) and zinc (II) perchlorate hexahydrate (0.26 g, 0.07 mM) in acetonitrile (10 cm^3) and refluxed for 2 h. After cooling at room temperature, the resulting solution was filtered and the filtrate allowed to evaporate at room temperature. The dark yellow precipitate formed was filtered and washed with cold methanol and vacuum dried. Yield 0.42 g (80%).

(Found: C, 60.60; H, 4.80; N, 11.78. Calc. for $C_{36}H_{34}N_6O_{14}Cl_2Zn_2$, C, 60.59; H, 4.79; N, 11.76%)

$[Zn_2L^2][2C1O_4]$, 2. This complex was prepared by the procedure adopted for 1 except using PC-2 (0.256 g, 0.06 mM) in place of PC-1. Yield 0.43 g (82%).

(Found: C, 61.09; H, 4.99; N, 11.55. Calc. for $C_{37}H_{44}N_6O_{14}Cl_2Zn_2$, C, 61.06; H, 4.94; N, 11.51%)

$[Zn_2L^3][2C1O_4]$, 3. This complex was prepared by the method used for 1, using compound PC-3 (0.25 g, 0.07 mM) in place of PC-1. Yield 0.45 g (85%).

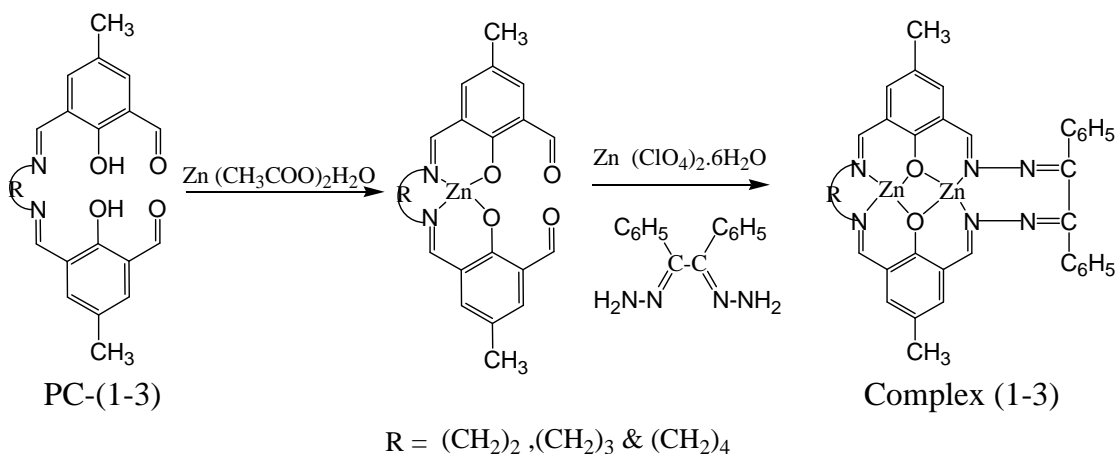
(Found: C, 61.55; H, 5.17; N, 11.33% Calc. for $C_{38}H_{46}N_6O_{14}Cl_2Zn_2$, C, 61.51; H, 5.14; N, 11.31%).

III. Results and discussion

a. Synthesis of macrocyclic binuclear zinc(II) complexes

New series of unsymmetrical binuclear zinc(II) complexes $[Zn_2L^{1-3}](ClO_4)_2$ were synthesized by Schiff base condensation of bicompartamental mononuclear complex $[ZnL]$ with various diamines like 1,2 diamino

ethane, 1,3 diamino propane and 1,4 diamino butane .Then add $Zn(ClO_4)_2 \cdot 6H_2O$ and benzilbishydrazone. The binuclear $Zn(II)$ complexes are yellow in colour. The elemental analysis data of the complexes are consistent with the calculated results from the empirical formula of each compound.



Scheme 1

IR spectra of mononuclear complex $[ZnL]$ exhibited a peak at 1630 cm^{-1} which corresponds to the azomethine group (C=N). The band observed at 1700 cm^{-1} was due to the aldehyde group (C=O). No band was seen in the region $3300\text{-}3500\text{ cm}^{-1}$ which generally corresponding to $-OH$ stretching which clearly indicates the coordination of hydroxyl group with $Zn(II)$ metal ion. The absence of carbonyl stretching frequency (C=O) and simultaneously the presence of azomethine stretching around 1615 to 1640 cm^{-1} and a sharp peak indicates the formation of binuclear complex. A strong band in the region around $1080\text{-}1110\text{ cm}^{-1}$ and a sharp peak in the region around 630 cm^{-1} was observed which was corresponds to the antisymmetric stretch and antisymmetric bending vibration of perchlorate ions, respectively. The electronic spectra of the complexes were observed as two main transitions . A moderately intense band was observed in the range $350\text{-}450\text{ nm}$ is which was due to ligand-to-metal charge transfer transition and a strong band in the range $320\text{-}220\text{ nm}$ due to intra ligand charge transfer transition. The ESI mass spectra were carried out for the binuclear zinc (II) complexes in acetonitrile. The positive ion ESI mass spectra for the zinc (II) complexes 1-3 showed a major peak at $m/z = 842, 856$ and 871 respectively corresponding to the $[Zn_2L^{1-3}.ClO_4]^+$ ion. Also mass spectra of the complexes show peaks at $372, 379$ and 386 confirm the presence of a dipositive binuclear core $[Zn_2L^{1-3}]^{2+}$ ions respectively. The analytical and ESI mass spectral data are consistent with the proposed formula of the dizinc (II) complexes.

b. Binding studies of calf thymus DNA by the zinc complex

UV Absorption studies

The electronic absorption spectra of complex ($1 \times 10^{-5}\text{ M}$) in the case of increasing amounts of DNA showed strong decreases in the peak intensities (Figure 1). Hypochromism was suggested to be due to a strong interaction between the electronic state of the intercalating chromophore and that of the DNA bases. In addition to the decrease in intensity, a small red shift was also observed in the spectra [16]. These spectral changes are consistent with the intercalation of the zinc complex into the DNA base stack. The plot of the absorption titration data gives a linear plot and results in an intrinsic binding constant (K_b) of $4 \times 10^4, 5.2 \times 10^4$ and $6.8 \times 10^4\text{ M}^{-1}$.

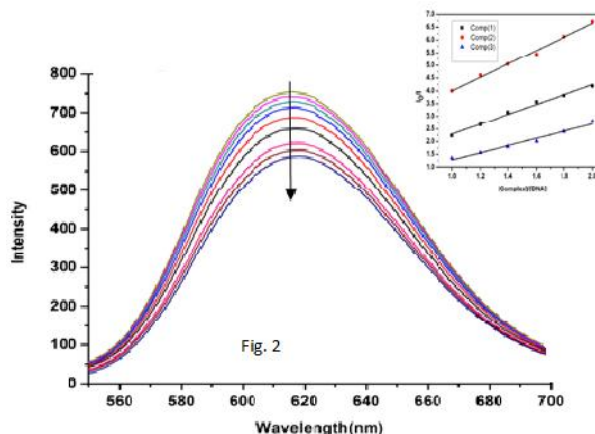
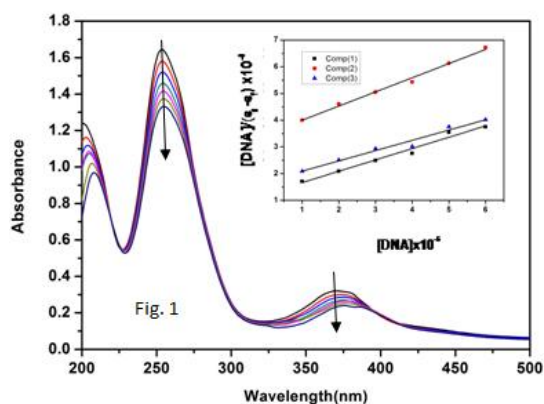


Figure 1 Absorption spectra of Zn(II) (1×10^{-5} M) in the absence and presence of increasing amounts of CT-DNA ($0-2.5 \times 10^{-3}$ M) at room temperature in 50 mM Tris-HCl / NaCl buffer (pH = 7.2). Arrow shows the absorbance changing upon increasing DNA concentrations. Insert : shows the saturation in absorption intensity hypochromism is indicated by the plot of $[DNA] / (a - f)$ vs $[DNA]$.

Figure 2 : Emission spectrum of EB bound to DNA in the presence of complexes (1-3) ($[EB] = 3.3 \mu\text{M}$, $[DNA] = 40 \mu\text{M}$, $[\text{complex}] = 0-25 \mu\text{M}$, $\lambda_{\text{ex}} = 510 \text{ nm}$). Arrow shows the absorbance changing upon increasing complex concentrations. Insert : Stern-Volmer quenching plot of EB bound to DNA by complex. I_0 is the emission intensity of EB-DNA in the absence of complex; I is emission intensity of EB-DNA in the presence of complex.

Fluorescence Spectral Studies

To further clarify the interaction of the complex with DNA, the emission spectra of DNA-EB ($1.0 \times 10^7 \text{ M}^{-1}$) adduct have been measured upon increase of the complex concentration. Ethidium bromide (EB) has long been used as a probe of DNA secondary structure as it can be intercalated into the double-stranded DNA and enhanced the sensitivity of fluorescence[17]. If the duplex of DNA decreases, the fluorescence of DNA-EB complex is quenched evidently. The fluorescence emission spectra of the zinc complex interaction with calf thymus DNA-EB with complex1 is shown in Figure 2. The fluorescence intensity of DNA-EB adducts decreases with the increasing concentrations of the complex. This may be because the intercalation of zinc complex inhibits EB from binding to DNA. The K_{app} values of the complexes (1-3) were calculate as 4.3×10^5 , 5.4×10^5 and 5.8×10^5 respectively.

Viscosity Measurement

As a means of further exploring the interaction between complex and DNA viscosity study was carried out. A classical intercalation model results in the lengthening of the DNA helix as the base pairs are separated to accommodate the binding ligand, leading to an increase in DNA viscosity[18]. Figure 3 shows that with an increasing amount of complex, the relative viscosity of DNA increases steadily, which suggests that the complex can bind to DNA by the classical intercalation.

c. Protein binding studies:

Addition of metal complexes of the solution of BSA resulted in the quenching of its fluorescence emission without any shift suggesting that the complex formed between the complexes (1-3) and BSA is responsible for the quenching of BSA. Fig 6 shows the effect of increasing the concentration of Zinc complex-1 on the fluorescence emission of BSA. The K_{sv} value obtained from the plot of I_0/I vs $\log[Q]$ was found to be 6.56×10^5 , 6.67×10^5 and $9.55 \times 10^5 \text{ M}^{-1}$ corresponding to the zinc complexes (1-3). Binding constants obtained from the plot of $\log[(F_0/F)/F]$ Vs $\log[Q]$ corresponding to the zinc complexes (1-3) were 1.58 , 1.65 and $1.28 \times 10^5 \text{ M}^{-1}$ respectively.

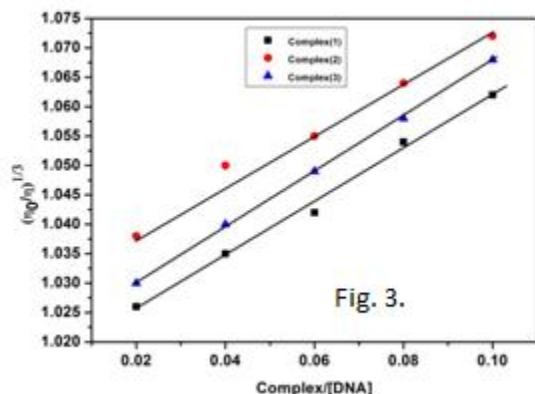


Fig. 3. The effect of the complex on the relative viscosity of DNA

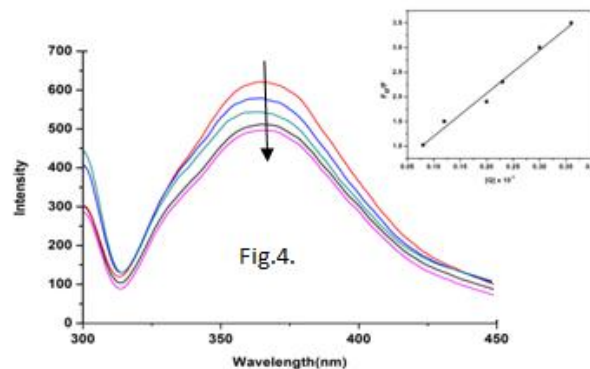


Fig.4. Changes in the Fluorence spectra of BSA[$1 \times 10^{-6} \text{M}^{-1}$] in presence of increasing complex-1 concentraion[$0-10 \times 10^{-6} \text{M}^{-1}$] at 300 K, pH=7.5 $\lambda_{\text{ex}}=280\text{nm}$ The K_{BSA} values of the complexes 1-3 are $1.55 \times 10^5 \text{M}^{-1}$, $2.32 \times 10^5 \text{M}^{-1}$ and $2.68 \times 10^5 \text{M}^{-1}$ respectively.

d. Cleavage studies of double-stranded pBR322 DNA by the zinc complex

For comparison purposes, the cleavage reactions of zinc complexes (1-3) were carried out in the presence of H_2O_2 at a concentration of $30 \mu\text{M}$. Each complex was found to possess nuclease activity. The cleavage efficiencies of complexes(1-3) (lanes 2,3,4 and 5, respectively; Figure 5) in the presence of H_2O_2 are exhibits nuclease activities.

Based upon their ability to convert the super coiled form (Form I SC) to the nicked circular (Form II NC), complex 5 was found to be an efficient chemical nuclease for double strand cleavage of DNA in comparison with complexes 3 and 4. The foregoing observations suggest that cleavage of the supercoiled form and formation of the nicked circular form.

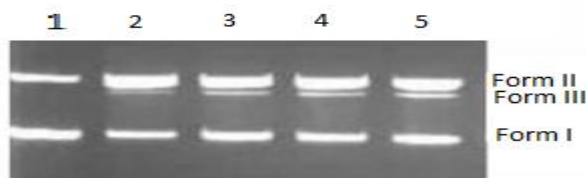


Figure 5 : Agarose gel showing cleavage of pBR322 DNA incubated by Zinc complexes (1-3) in Tris-Hcl (pH=7.2) at 37°C for 3h. Lane 1, DNA control, Lane 2, DNA+ H_2O_2 (0.08%, $1 \mu\text{l}$), Lane 3, DNA+ H_2O_2 (0.08%, $1 \mu\text{l}$) +complex 1($30 \mu\text{M}$), Lane 4, DNA+ H_2O_2 (0.08%, $1 \mu\text{l}$) +complex 2($30 \mu\text{M}$), Lane 5, DNA+ H_2O_2 (0.08%, $1 \mu\text{l}$) +complex 3($30 \mu\text{M}$).

VI. Conclusion

Binuclear Zinc(II)complexes were prepared by the template synthesis with various diamines, benzilbisdi-hydrazone. The binding properties of the Zn(II) complexes with calf thymus DNA and BSA have been investigated by absorption, emission spectral studies. Viscosity measurements have been carried out to find out the mode of binding of the complexes with CT-DNA. DNA cleavage of the binuclear Zinc(II) complexes was studied by electrophoresis method. The binuclear Zinc (II) complexes show hydrolytic cleavage of supercoiled pBR322-DNA.

Acknowledgement

The author Vedavalli Sairaj is thankful for the financial support provided by the MHRD special grants, New Delhi, INDIA under NCNSNT Program - research grants.

References

- [1] Sigman, D. S.; Bruce, T. W.; Mazumder, A.; Sutton, C. L. *Acc.* "DNA-Binding and Cleavage Studies of Zinc(II) Mixed-polypyridyl Complex" *Chem. Res.* **1993**, 26, 559.
- [2] Sigman, D.; Mazumder, S. A.; Perrin, D. M. "Chemical Nucleases" *Chem. Rev.* **1993**, 93, 2295- 2316.
- [3] Sreedhara, A.; Freed, J. D.; Cowan, J. A. "Very effective DNA cleavage by a Cu²⁺ aminoglycoside has been recently reported", *J. Am. Chem. Soc.* **2000**, 122, 8814.
- [4] Ordoukhanian, P.; Hoyce, G. F. "RNA-Cleaving DNA Enzymes with Altered Regio- or Enantioselectivity", *J. Am. Chem. Soc.* **2002**, 124, 12499.
- [5] Erkkila, K. E.; Odom, D. T.; Barton, J. K. "Recognition and Reaction of Metallointercalators with DNA", *Chem. Rev.* **1999**, 99, 2777.
- [6] Jin, L.; Yang, P. "Synthesis and dna-binding studies of a nickel(ii) coordination compound." *Microchem., J.* **1998**, 58, 144-150.
- [7] Chen, H. L.; Yang, P. Chin." Study on the Binding of Base-Mismatched Oligonucleotide d(GCGAGC)₂ by Cobalt(III) Complexes", *J. Chem.* **2002**, 20, 1529-1535.
- [8] Lu, Y. "New Transition-Metal-Dependent DNAzymes as Efficient Endonucleases and as Selective Metal Biosensors", *Chem. Eur. J.* **2002**, 8, 4588-4596.
- [9] Lu, L. P.; Zhu, M. L.; Yang, P. "Crystal structure and nuclease activity of mono(1,10-phenanthroline) copper complex.", *J. Inorg. Biochem.* 2003, 95, 31-36.
- [10] Arounaguirri, S.; Maiya, B. G. "Dipyridophenazine Complexes of Cobalt(III) and Nickel(II): DNA-Binding and Photocleavage Studies", *Inorg. Chem.* 1996, 35, 4267-4270.
- [11] Arounaguirri, S.; Easwaramoorthy, D.; Ashokkumar, A.; "Cobalt (III), nickel (II) and ruthenium (II) complexes of 1,10-phenanthroline family of ligands: DNA binding and photocleavage studies", Dattagupta, A.; Maiya, B. G. *Proc. Indian Acad. Sci. (Chem.Sci.)* 2000, 112, 1.
- [12] Sreedhara, A.; Cowan, J. A. "Catalytic hydrolysis of DNA by metal ions and complexes", *J. Biol. Inorg. Chem.* 2001, 6, 337-6341.
- [13] Burrows, C. J.; Muller, J. G. "Oxidative Nucleobase Modifications Leading to Strand Scission", *Chem. Rev.* 1998, 98, 1109-1151.
- [14] Peng, W.; Liu, P. Y.; Jiang, N.; Lin, H. H.; Zhang, G. L.; Liu, Y.; Yu, X. Q. "Dinuclear macrocyclic polyamine zinc(II) complexes linked with flexible spacers: synthesis, characterization, and DNA cleavage", *Bioorg. Chem.* 2005, 33, 374-385.
- [15] Bolognina, E.; Gatos, M.; Lucatello, L.; Mancin, F.; Moro, S.; Palumbo, M.; Sissi, C.; Tecilla, P.; Tonellato, U.; Zago, G. "Toward Efficient Zn(II)-based Artificial Nucleases" *J. Am. Chem. Soc.* 2004, 126, 4543-4549.
- [16] M. Lee, A. L. Rhodes, M. D. Wyatt, S. Forrow, J. A. Hartley, "GC base sequence recognition by oligoimidazolecarboxamide and C-terminus-modified analogs of distamycin deduced from circular dichroism, proton nuclear magnetic resonance, and methidiumpropylethylenediaminetetraacetate-iron(II) footprinting studies", *Biochemistry* 32 (1993) 4237-4245.
- [17] M. J. Al-Jeboori, H. A. Hasan, W. A. Jaafar Al-Sa'idi, "Formation of polymeric chain assemblies of transition metal complexes with a multidentate Schiff-base", *Transition Met. Chem.* 34 (2009) 593-598.
- [18] P. B. Derran, R. M. Doss, A. Marques, "Programmable DNA Binding Oligomers for Control of Transcription", *Curr. Med. Chem. Anti-Cancer Agents* vol.5, 2005 pp. 373- 387.