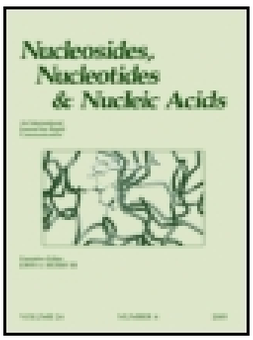


Nucleosides, Nucleotides and Nucleic Acids

ISSN: 1525-7770 (Print) 1532-2335 (Online) Journal homepage: http://www.tandfonline.com/loi/lncn20

Multispectroscopic Studies on the Interaction of a Copper(II) Complex of Ibuprofen Drug with Calf Thymus DNA

Nahid Shahabadi & Farshad Shiri

To cite this article: Nahid Shahabadi & Farshad Shiri (2016): Multispectroscopic Studies on the Interaction of a Copper(II) Complex of Ibuprofen Drug with Calf Thymus DNA, Nucleosides, Nucleotides and Nucleic Acids, DOI: 10.1080/15257770.2016.1223305

To link to this article: http://dx.doi.org/10.1080/15257770.2016.1223305

Published online: 11 Nov 2016.

NUCLEOSIDES, NUCLEOTIDES AND NUCLEIC ACIDS
2016, VOL. 0, NO. 0, 1–24
http://dx.doi.org/10.1080/15257770.2016.1223305

Taylor & Francis
Taylor & Francis Group

Multispectroscopic Studies on the Interaction of a Copper(II) Complex of Ibuprofen Drug with Calf Thymus DNA

Nahid Shahabadi[a,b] and Farshad Shiri[a]

[a]Inorganic Chemistry Department, Faculty of Chemistry, Razi University, Kermanshah, Iran; [b]Medical Biology Research Center (MBRC) Kermanshah University of Medical Sciences, Kermanshah, Iran

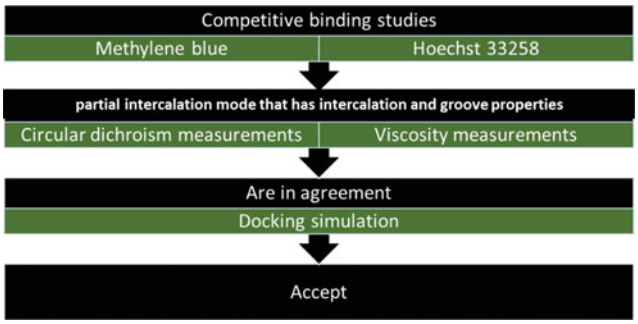
ABSTRACT

The interaction of copper(II)–ibuprofenato complex with calf thymus DNA (ct-DNA) has been explored following, UV-visible spectrophotometry, fluorescence measurement, dynamic viscosity measurements, and circular dichroism spectroscopy. In spectrophotometric studies of ct-DNA it was found that $[Cu(ibp)_2]_2$ can form a complex with double-helical DNA. The association constant of $[Cu(ibp)_2]_2$ with DNA from UV-Vis study was found to be 6.19×10^4 L mol^{-1}. The values of K_f from fluorescence measurement clearly underscore the high affinity of $[Cu(ibp)_2]_2$ to DNA. The experimental results showed that the conformational changes in DNA helix induced by $[Cu(ibp)_2]_2$ are the reason for the fluorescence quenching of the DNA-Hoechst system. In addition, the fluorescence emission spectra of intercalated methylene blue (MB) with increasing concentrations of $[Cu(ibp)_2]_2$ represented a significant increase of MB intensity as to release MB from MB-DNA system. The results of circular dichroism (CD) suggested that copper(II)–ibuprofenato complex can change the conformation of DNA. In addition, the results of viscosity measurements suggest that copper(II)–ibuprofenato complex may bind with non-classical intercalative mode. From spectroscopic and hydrodynamic studies, it has been found that $[Cu(ibp)_2]_2$ interacts with DNA by partial intercalation mode which contains intercalation and groove properties.

ARTICLE HISTORY
Received 27 March 2016
Accepted 1 August 2016

KEYWORDS
Copper(II)-ibuprofen complex; DNA interaction; partial intercalation; Spectral probes

GRAPHICAL ABSTRACT



CONTACT Nahid Shahabadi n.shahabadi@razi.ac.ir Inorganic Chemistry Department, Faculty of Chemistry, Razi University, Kermanshah, Iran, and Medical Biology Research Center (MBRC), Kermanshah University of Medical Sciences, Kermanshah, Iran

Introduction

Development of cancer drugs is slow and costly. One approach to accelerate the availability of new drugs is to reposition approved drugs for other indications as anticancer agents.[1] A class of drugs that are produced and consumed in large quantities and can be used for this purpose is nonsteroid anti-inflammatory drugs or NSAIDs. Out of several NSAIDs, ibuprofen that derived from propionic acid is widely used as analgesic and antipyretic.[2] Ibuprofen is often used for the treatment of rheumatoid arthritis and osteoarthritis, mild to moderate pain, dysmenorrhea, fever and also shows anticancer activity.[3,4] The binding mode between ibuprofen and DNA helix is intercalation that is most effective mode of the drugs targeted to DNA and is related to the antitumor activity of this compound.[5] In addition, the investigation of the complexes of metal ions such as Cu, Zn, and Ni which are vital for biological systems and can be present at the foods, is important because it can serve the clarification of clinical results.[6] Among these ions, copper is an essential trace element for all living organisms and is a universally important cofactor for many hundreds of metalloenzymes.[7] Copper(II) complexes have found possible medical uses in the treatment of many diseases including cancer[8,9] as well as have demonstrated a wide range of pharmacological activities such as antiviral[10] and anti-inflammatory.[11] Copper(II) complexes of NSAIDs are often more active and desirable drugs than the parent ligands themselves.[12] Copper ibuprofenate is a chemical complex consisting of copper(II) and the chelate form of the anti-inflammatory drug ibuprofen which showed better anti-ulcer protective effect than the parent drug.[13] Therefore, investigations on copper complexes and drugs such as ibuprofen, which can be used in the goals that discussed above, are becoming more prominent in the research area of bioinorganic chemistry. On the other side, the DNA binding properties of substitution-inert transition-metal complexes have lately captured an extensive interest due to their possible applications in cancer therapy and molecular biology. Metallointercalators and transition metal complexes which bind DNA primarily as classical or non-classical intercalation, are considered as the most effective class of molecules in these applications.[14,15] In the present study, the interaction between copper ibuprofenate complex ($[Cu(ibp)_2]_2$, [ibp = Ibuprofen]) (Figure 1) and ct-DNA was investigated with the use of Hoechst 33258 and methylene blue (MB) dyes as spectral probes by the application of UV–vis and fluorescence spectroscopic techniques, CD spectroscopy, viscosity measurements, and docking simulation.

Material and methods

Reagents

The ibuprofen drug was supplied by Sigma Co, $Cu(CH_3COO)_2.H_2O$ and NaOH were obtained from Merck. $[Cu(ibp)_2]_2$ were prepared according to the published method.[13,16] The ct-DNA, Tris–HCl and Hoechst 33258 were purchased from Sigma Co and were used without further purification. Methylene blue

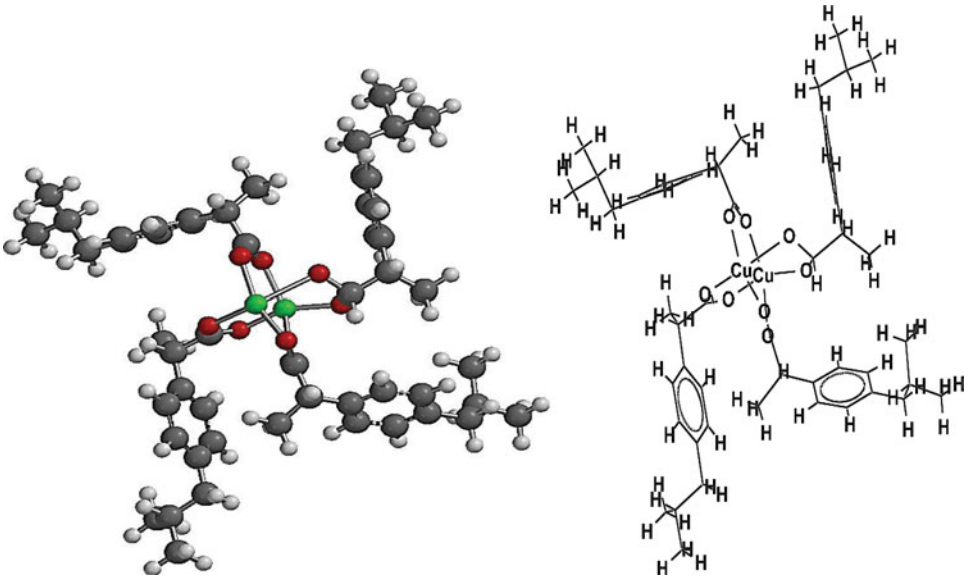


Figure 1. The chemical structure of $[Cu(ibp)_2]_2$ complex (optimized by Spartan 10 program).

(MB) was provided from Merck. Tris–HCl buffer solution was prepared from tris-(hydroxymethyl)-amino-methane–hydrogen chloride and pH was regulated to 7.4. The stock solution (1×10^{-3} mol.L^{-1}) of $[Cu(ibp)_2]_2$ was prepared by dissolving its crystals in 80% (v/v) ethanol/Tris–HCl buffer. Calf thymus DNA dissolved in Tris–HCl buffer as stock solution and was stored at 4°C, the purity of ct-DNA was checked by monitoring the ratio of $A_{260}/A_{280} > 1.80$, and the concentration of ct-DNA was determined by the absorption of ct-DNA at 260 nm ($\varepsilon_{260} = 6600$ L mol^{-1} cm^{-1}).[17]

Synthesis of $[Cu(ibp)_2]_2$complex

The $[Cu(ibp)_2]_2$ complex was prepared according to the following procedure: 2.0 mmol (0.412 g) of ibuprofen reacted with 2.0 mmol (0.08 g) of NaOH in 20 mL of distilled water, to give the sodium salt of the ibuprofen. Then 1.0 mmol (0.199 g), $Cu(CH_3COO)_2.H_2O$ was added by stirring. The turquoise precipitate formed was collected by filtration, washed several times with distilled water and dried at room temperature. The formation of this complex was confirmed by elemental analysis, UV-visible and also FT-IR spectroscopy according to the published methods[13,16] as follows: The UV-vis characteristic bands of ibuprofen alone and along with the $[Cu(ibp)_2]_2$ complex from 200 to 800 nm (Figure 2) are listed in Table 1. Free ibuprofen shows bands at 228 and 262 nm assigned to π - π * transitions. Clear hyperchromic due to metal- ligand charge transfer for the complex is attributed to complexation of ibuprofen. The IR spectra of $[Cu(ibp)_2]_2$ complex and free ibuprofen were measured as KBr discs and the assignments are given in Table 2. The ν(C=O) and ν(OH) of carboxylic acid appeared at 1721 and 3000–3350 cm^{-1} for the free ligand, but disappeared in the spectrum of the complex. IR spectrum of the complex

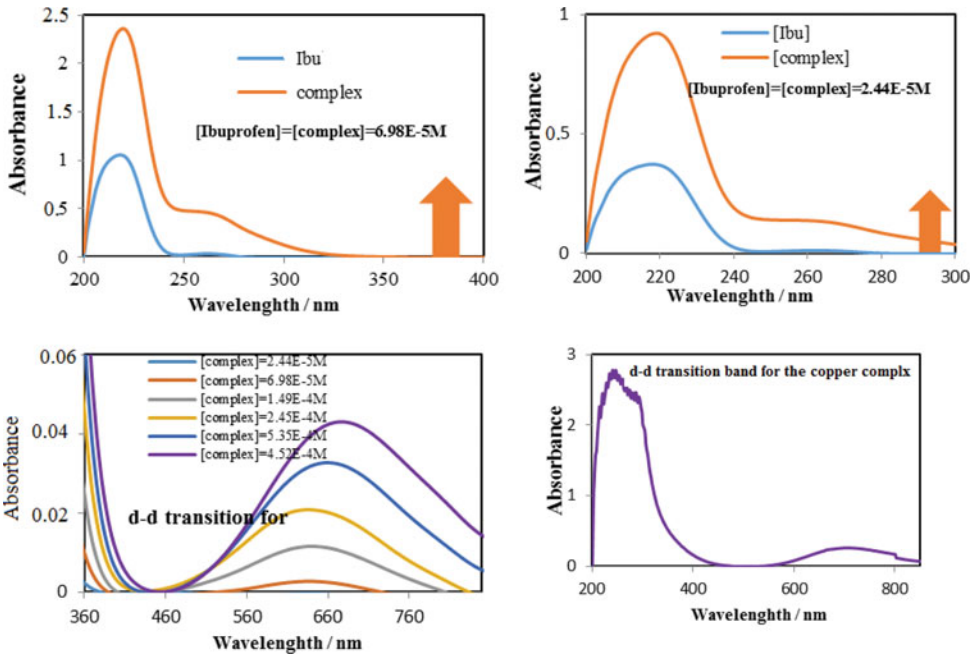


Figure 2. UV- Vis spectra of ibuprofen (ligand) and $[Cu(ibp)_2]_2$ complex.

Table 1. Characteristic bands in UV-vis absorption spectra of ibuprofen and $[Cu(ibp)_2]_2$ complex.

Ibuprofen	$[Cu(ibp)_2]_2$ complex	Assignment
228	230	π - π^* transitions
262	268	
—	~ 218–260	Metal- Ligand charge transfer
—	~ 670	d-d transition

Table 2. Principal FT-IR absorption bands for ibuprofen and $[Cu(ibp)_2]_2$ complex.

Ibuprofen	$[Cu(ibp)_2]_2$	Assignment
3350–3000 w	—	$\nu_{OH}(H_2O)$; ν_{OH}(COOH)
3046–2869m-w	2953–2869 m-w	$\nu_{asym.}$ CH_3,CH_2 $_{sym.}$ CH_3,CH_2
1721vs	—	$\nu_{C=O}$(COOH)
—	1587.5 vs	$\nu_{asym.}$ (COO)
1508 m-w	1515 w	ν_{C-C} ring str.
1462 m-w	1459 w	ν_{C-C} ring str.
1420 m	1407 m	$\nu_{sym.}$ (COO)
1380–1183.6 m-w	1368–1294w	asym. CH_3,CH_2,CH sym. CH_3,CH_2,CH
1067.6 w	1072w	i.p. C-H (ring)
1008 w	1021vw	i.p. C-H (ring)
936 m	—	o.p. C-H (ring)
866 w	853 vw	o.p. C-H (ring)
779.8 m	799 vw	o.p. C-H (ring)
668 w	—	i.p. CH (ring)
—	553.6 w	ν_{Cu-O}

s = strong, v = very, m = medium, w = weak, str = stretching, asym = asymmetric, sym = symmetric, i.p. = in planer, o.p. = out of planer

shows two very strong bands at 1587 cm^{-1} and 1407 cm^{-1} which were assigned to ν(COO) asymmetric and symmetric stretches, respectively, with an average $\delta\nu$ of ~180 cm^{-1}, indicating bidentate coordination of carboxylate. Coordination via oxygen of carboxylate is confirmed by ν(Cu–O) band at 553.6 cm^{-1}. Thus, ibuprofen is coordinated through oxygen of carboxylates. Elemental analysis also confirms the synthesis of the complex as, C52H68Cu2O8Calc.: C; 70.98 and H; 7.73. Found: C, 71.87 and H, 7.43.

Apparatus

The UV—visible absorption measurements were obtained with an Agilent 8453 spectrophotometer, using a 1.0 cm cell. Fluorescence measurements were carried out with a JASCO spectrophotometer (FP 6200). Viscosity measurements were performed in a viscometer (SCHOT AVS 450), which was kept in a constant temperature bath at 29 ± 0.2°C. CD measurements were recorded on a JASCO (J-810) spectrophotometer. The elemental analysis was performed using a Heraeus CHN elemental analyzer. The pH values were measured with a pH meter (Jenway Model: 3020) supplied with a glass-combined electrode.

UV–visible absorption spectral measurements

The UV–vis absorption spectra of ct-DNA in the absence and presence of $[Cu(ibp)_2]_2$ complex and the absorption spectra of corresponding concentrations of $[Cu(ibp)_2]_2$ solutions were measured in the range of 200–350 nm and pH 7.4 in Tris–HCl buffer solution at room temperature.

Fluorescence emission spectral measurements

The fluorescence intensity was measured with the excitation and emission wavelengths at 265 and 292 nm, respectively at 283, 298, and 315 K. The spectra band widths of excitation and emission slits were both kept at 5.0 nm. To eliminate the probability of reabsorption and inner-filter effect arising from UV—vis absorption of ct-DNA, the fluorescence data of $[Cu(ibp)_2]_2$ complex were corrected for absorption of excitation and emitted light according to Eq. 1[18,19]

$$F_c = F_m 10^{\frac{(A_1 + A_2)}{2}}, \tag{1}$$

where F_c and F_m are the corrected and measured fluorescence, respectively, and A_1 and A_2 are the absorbance of the ct-DNA solutions at 265 and 292 nm, respectively.

Competitive experiments

The Uv-vis and fluorescence spectra of the competitive reaction between $[Cu(ibp)_2]_2$ complex and the probes (MB and Hoechst) with ct-DNA were performed in Tris–HCl buffer solution (pH 7.4) at room temperature. This part of

experiment was carried out by titration of constant amounts of the probes and ct-DNA with increasing amounts of $[Cu(ibp)_2]_2$ complex solution and analysis of the changes in absorbance and fluorescence intensities.

Circular dichroism measurements

The CD spectra of DNA in the presence of $[Cu(ibp)_2]_2$ complex were conducted in the wavelength range of 200–350 nm by keeping the concentration of ct-DNA constant at 4.81×10^{-5} mol L^{-1} while varying the $[Cu(ibp)_2]_2$ concentration from 0 to 5.76×10^{-5} molL^{-1}.

Viscosity measurements

Viscometric measurements were carried out using a viscometer, which was kept at (29 ± 0.2)°C by a constant temperature bath. The solution of (DNA + $[Cu(ibp)_2]_2$) was prepared in Tris–HCl buffer and the flow times of ct-DNA alone and its mixtures with different ratios of $[Cu(ibp)_2]_2$ to ct-DNA through the capillary were then measured in seven replicates using a digital stopwatch with an accuracy of $\pm$ 0.02 s.

Docking simulation

Docking simulation was performed using MGL tools 1.5.4 with AUTOGRID 4 and AUTODOCK version 4.2. The DNA sequence obtained from the Protein Data Bank (PDB NDB: DD0018) and the complex was optimized by SPARTAN 10 program and both the complex and DNA were prepared for using in AUTODOCK tools. The DNA was enclosed in a box with number of grid points in $x \times y \times z$ directions, $40 \times 40 \times 80$.

Results and discussion

UV–Vis absorption spectroscopy

The application of electronic absorption spectroscopy is one of the most useful techniques for studying both the stability of DNA and the interactions with molecules.[20]

The binding of metal complexes to DNA helix has been characterized through absorption spectral titrations by following the changes in absorbance and shift in wavelength.[21] The spectral change process reflects the corresponding changes in the helix structure and the steric configuration of DNA after a compound binds to DNA.

The UV spectra of DNA at about 260 nm in the absence and presence of $[Cu(ibp)_2]_2$ complex is shown in Figure 3. The absorption band exhibits hyporchromism without any clear shifts in the band position, suggesting that there exists an interaction between the $[Cu(ibp)_2]_2$ complex and ct-DNA, which indicates formation of an adduct between DNA and $[Cu(ibp)_2]_2$ and change in DNA

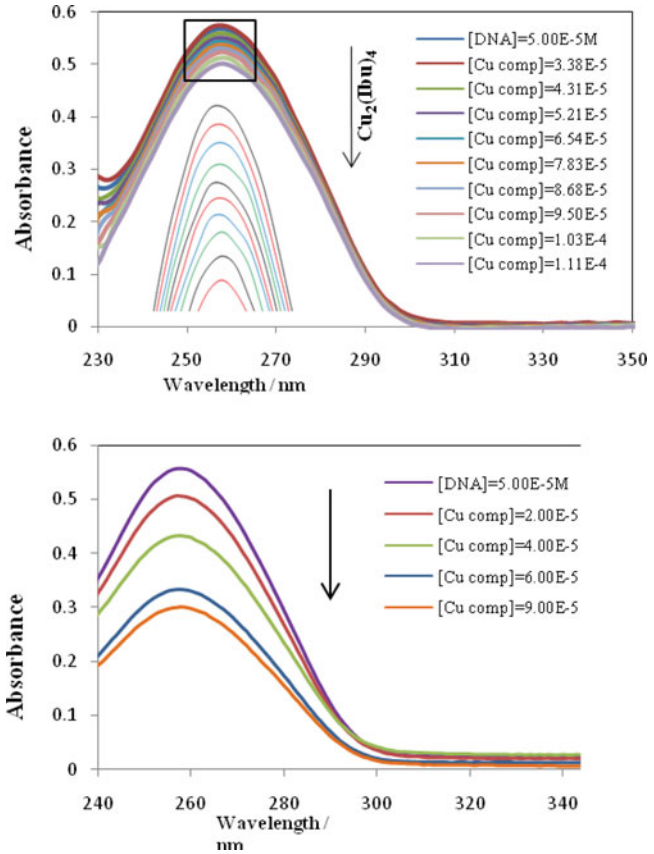


Figure 3. Changes of UV spectra of ct-DNA in the presence of different concentrations of $[Cu(ibp)_2]_2$ complex at pH 7.4 and room temperature. UV-Vis spectra of ibuprofen (ligand) and $[Cu(ibp)_2]_2$ complex at different concentrations as depicted in the insets of figures. (A) By titration; (B) Over 22 h at ambient temperature.

conformation. The observed large hypochromism during the interaction of the $[Cu(ibp)_2]_2$ complex with DNA strongly indicates that the distance between the $[Cu(ibp)_2]_2$ complex and the DNA bases is small and suggests a strong interaction between the electronic states of the chromophore and DNA bases (Figure 3B). This hypochromism usually results in complex binding with DNA, through intercalation or groove binding mode. In intercalation binding mode, the π^* orbital of the intercalated molecule can couple with the π orbital of the DNA base pairs, the coupling π - π^*orbital is partially filled by electrons, thus, decreasing the transition probabilities and concomitantly resulting in hypochromism. In the case of the groove binding mode between DNA and drug molecules, the hypochromic effect can be associated with the overlapping of the electronic states of the chromophore of the complex with the nitrogenous bases in the grooves of DNA.[22]

A large part of our existing knowledge of noncovalent binding is based on the measurement of binding constant. Binding constant afford the scientist information on the mechanism of the chemical process involved. In general, the basic process

can be distilled down to the association, or binding of one or more ligands to a host molecule. A scheme for interaction between substrate and drug may be represented by:

$$D + nd = d_nD, \tag{2}$$

where n signifies the maximum number of binding sites on substrate (D or DNA), d, denotes the drugs that are free to bind to each site and D_nd is DNA-drug complex. Hence the binding constant (K_b) is:

$$K_b = \frac{[D_nd]}{[D]\,[d]^n}. \tag{2a}$$

The binding constant and the number of $[Cu(ibp)_2]_2$ complex bound to one DNA molecule has been developed before ($n = 1$).[23] It is obvious that all the binding sites are independent and the Beer's law is followed by all species. A wavelength is considered at which the molar absorptivity of the DNA (ε_D) and the molar absorptivity of the DNA-drug ($\varepsilon_{d\text{-}D}$) are not same. At whole concentration of the DNA (D_0), and the light path length (b), the solution absorbance is introduced as in the following equation (in the absence of drug):

$$A_0 = \varepsilon_D b[D]_0. \tag{2b}$$

As though the absorbance of a solution containing the same concentration of whole DNAs, by role playing the total concentration of drug ($[d]_0$) is obtained as follows:

$$A = \varepsilon_D b\,[D] + \varepsilon_d b\,[d] + \varepsilon_{d-D} b\,[d_nD]\,, \tag{2c}$$

where $[D]$ is the concentration of the uncomplexed DNA, $[d]$ the concentration of the free drug, and $[d_nD]$ is the concentration of the formed complex. By applying equation (2c) with the mass balance on $[D]$ and $[d]$, equation (2d) can be introduced:

$$A = \varepsilon_D b[D]_0 + \varepsilon_L b[d]_0 + \Delta\varepsilon_{d-D} b\,[d_nD]\,, \tag{2d}$$

And $\delta\varepsilon_{d-D} = \varepsilon_{d-D} - \varepsilon_D\text{- n}\varepsilon_d$($\varepsilon_d$, molar absorptivity of the drug). By evaluating absorbance of the solution against a blank containing whole concentration of drug $[d]_0$, the calculated absorbance is given as:

$$A = \varepsilon_D b[D]_0 + \Delta\varepsilon_{d-D} b\,[d_nD]\,. \tag{2e}$$

By combining Eq. (2a) and Eq. (2b) with Eq. (2e), whereas $\delta A = A - A_0$ and A_0 is the absorbance of DNA at 260 nm in the absence of $[Cu(ibp)_2]_2$:

$$\Delta A = K_b \Delta\varepsilon_{d-D} b\,[D]\,[d]^n. \tag{2f}$$

From this mass balance equation, $[D]_0 = [D] + [D_nd]$,

Table 3. The K_b, n, and other parameters in Eq. (2h) by curve fitting on this equation.

model	$\frac{1}{A} = \frac{1}{K_b \Delta\varepsilon_{d-D}[D]_0[d]^n} + \frac{1}{\Delta\varepsilon_{d-D}[D]_0}$
R-square	0.994
$K_b \Delta\varepsilon_{d-D}[D]_0$	$-1.592E+04$
$\Delta\varepsilon_{d-D}[D]_0$	-0.2471
N	1.01
K_b	$6.19E+04$ (L.mol^{-1})

we get $[D] = [D]_0/(1 + K_b[d]^n)$:

$$\frac{\Delta A}{b} = \frac{K_b \Delta\varepsilon_{d-D}[D]_0[d]^n}{K_b[d]^n + 1}. \tag{2g}$$

The binding isotherm (Eq. 2g) can be introduced which shows the hyperbolic dependence on not-bonded drug concentration. The K_b and n can be estimated from the following equation that is 1/(Eq. 2g):

$$\frac{b}{\Delta A} = \frac{1}{K_b \Delta\varepsilon_{d-D}[D]_0[d]^n} + \frac{1}{\Delta\varepsilon_{d-D}[D]_0}. \tag{2h}$$

This equation only yields the binding stoichiometry when there is only one binding site or in the case of infinite cooperativity; i.e., the occupation of one binding site favors ligand association. In the otherwise, n is just a phenomenological parameter (the Hill coefficient), that is a qualitative measure of the degree of cooperativity and it is experimentally less than the actual number of binding sites present.[19,24]

The K_b and n for the $[Cu(ibp)_2]_2$–DNA complex were obtained from the optical absorption at 260 nm at the various $[Cu(ibp)_2]_2$ complex concentrations, by the plotting of $1/(A–A_0)$ versus $C_{[Cu(ibp)2]2}$ and curve fitting by use of MATLAB software (Table 3 and Figure 4).

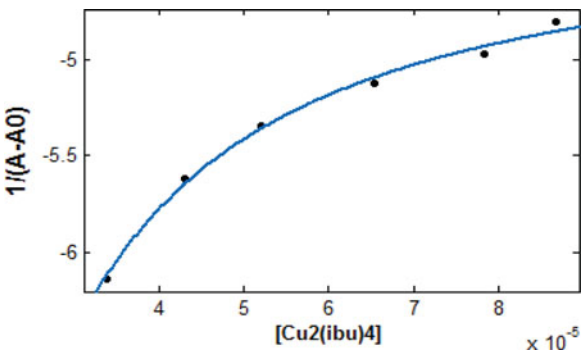


Figure 4. Representative binding curve for effect of various $[Cu(ibp)_2]_2$ concentrations on ct-DNA absorption at 260 nm.

Fluorescence studies

Determination of binding stoichiometry by the job's plot

To realize the correctness of the above discussion and that really n is near to one, the continuous variation method was used (or Job plot), that is one potential method to determine binding stoichiometry.[24] In this method, the total molar concentration [DNA+ Cu(II) complex] is held constant, but their mole fractions are varied. A measurable parameter, that is proportional to formed complex concentration, is plotted against the mole fraction of one of these two components. A special point is determined from the plot. The binding stoichiometry is calculated from the mole fraction of DNA and Cu(II) complex at that specific point.[25]

In this case, fluorescence intensity of the complex was selected as the parameter that is proportional to the complex concentration. The relation between the Cu(II) complex concentration and the fluorescence intensity can be described by:

$$\frac{[\mathrm{Cu}]_t}{[\mathrm{Cu}]_0} = \frac{F}{F_0}, \tag{3}$$

where $[\mathrm{Cu}]_0$ is the total $[\mathrm{Cu(ibp)_2}]_2$ concentration and $[\mathrm{Cu}]_t$ the concentration of uncomplexed$[\mathrm{Cu(ibp)_2}]_2$. Defining the concentration of the formed complex as $[D{-}\mathrm{Cu}] = [\mathrm{C}]_0 - [\mathrm{C}]_t$ and it can be shown using Eq. 4:

$$[D - \mathrm{Cu}] = \frac{F_0 - F*}{F_0}[\mathrm{Cu}]_0. \tag{4}$$

The plot of [D-Cu] versus mole fraction of $[\mathrm{Cu(ibp)_2}]_2$ (The Job plot) is shown in Figure 5. The obtained results, presented in Figure 5, show that the maximum of the Job's curve occurs at mole fraction of $[\mathrm{Cu(ibp)_2}]_2 = 0.5$, indicating the existence of a complex with a 1:1 stoichiometry and $n = 1$.

Fluorescence quenching studies

To elucidate the mode of binding, fluorescence quenching of DNA bound $[\mathrm{Cu(ibp)_2}]_2$ complex was studied in relation to the fluorescence quenching of the

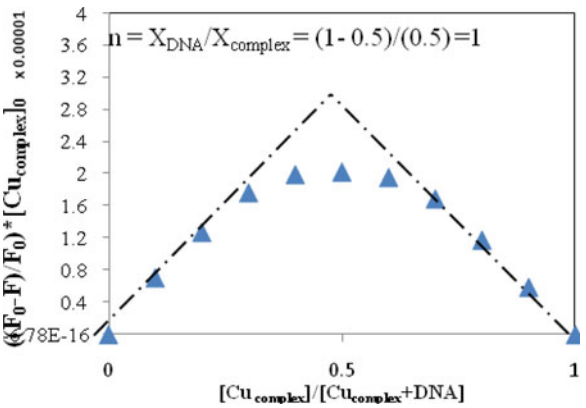


Figure 5. Job's plot for DNA–Cu(II) complex. [D–Cu] versus mole fraction of Cu(II) complex at total concentration [DNA + Cu(II) complex] = 8.0×10^{-5} mol.L^{-1}.

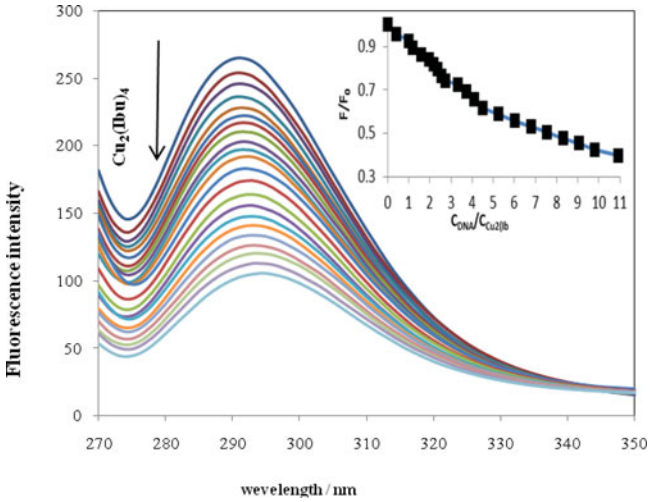


Figure 6. Emission spectra of the copper(II)–ibuprofenato complex in Tris-HCl buffer at 10°C in the absence and presence of ct-DNA at different concentrations. $C_{Cu(II)\ complex} = 9.85 \times 10^{-6}$ mol.L^{-1}, $C_{DNA} = 0.00$ to 1.083×10^{-4} mol.L^{-1}. The inset of figure shows $[Cu(ibp)_2]_2$ fluorescence intensity versus $C_{DNA}/C_{Cu(II)\ complex}$.

drug in pH 7.4 Tris–HCl buffer solution. Figure 6 shows the characteristic changes in fluorescence emission spectra during the titration of $[Cu(ibp)_2]_2$ complex with ct-DNA. Upon subsequent addition of ct-DNA there is a gradual quenching of the fluorescence intensity without any significant change in emission maxima which is direct evidence of the interaction between $[Cu(ibp)_2]_2$ complex and ct-DNA.[26]

The fluorescence quenching effect might result from a variety of processes such as excited state reactions, ground state complex formations, and collisional processes. Quenching can occur by different mechanisms which are usually classified as dynamic and static quenching. The static quenching was due to the formation of ground state complex between fluorophores and quencher and the dynamic quenching was due to the process in which the fluorophore and the quencher come into contact during the transient existence of the exited state. Because, the collisional quenching or dynamic quenching resulted from the collisions between fluorophores and quencher, it could be mathematically expressed by the Stern–Volmer equation (eq. 3)[27]:

$$\frac{F_0}{F} = 1 + K_q \tau_0 [Q] = 1 + K_{sv} [Q], \tag{5}$$

where F_0 = the initial fluorescence intensity of copper(II)–ibuprofenato complex, F= the fluorescence intensity of $[Cu(ibp)_2]_2$ complex after the addition of the quencher (DNA), K_q is the apparent bimolecular quenching rate constant, K_{sv} the Stern–Volmer quenching constant which is considered to be a measure of efficiency of fluorescence quenching by DNA, τ_0 is the average lifetime of $[Cu(ibp)_2]_2$ complex without the quencher[28] and [Q] is the concentration of the quencher, the K_{sv} can be obtained by the slope of the plot of F_0/F versus [Q] (Figure 7).

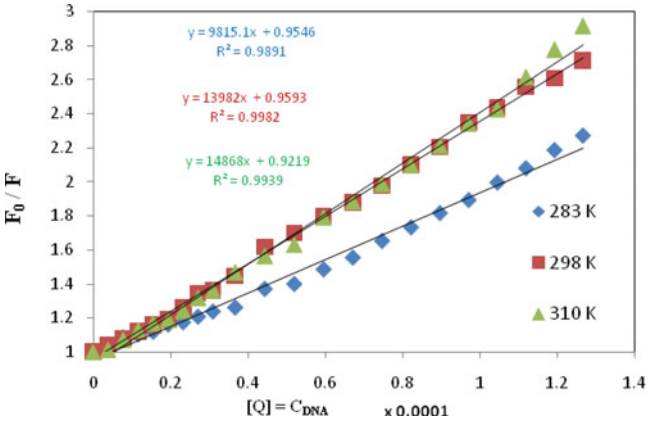


Figure 7. Plots of F_0/F versus [Q].

Dynamic and static quenching can be distinguished by their different dependence on temperature. Dynamic quenching depends on diffusion. Since higher temperatures result in larger diffusion coefficients, in the dynamic quenching process, the elevated temperature is conducive to effective collision between molecules. So the quenching rates accelerate, K_q or K_{sv} increases. In contrast, an increase in temperature is likely to result in a decrease in the stability of complexes, and thus, lower the values of the static quenching constants.[29]

Also, the limiting diffusion rate constant of biomolecule is known to be around 2.0×10^{10} $M^{-1}S^{-1}$, thus if the value of K_q is higher than the limiting diffusion rate constant, the quenching process is static rather than dynamic.[30]

In this work, the Stern–Volmer plot is linear, indicating that only one type of quenching process occurs, either static or dynamic quenching.[31] The results in Table 4 indicate that Ksv has been increased with increasing temperature (dynamic quenching) and K_q is higher than the limiting diffusion rate constant (static quenching). Thus, UV–vis absorption spectrum was used to give some more evidences for the actual quenching process.

Dynamic quenching only affects the excited state; no changes are expected in the ground state. Alternatively, a static process only involves complex formation in the ground state.[32] As shown in Figure 3 there is some changes in the UV–vis spectra of the DNA in the presence of various concentrations of the complex, so an adduct of DNA and copper complex forms in static quenching, whereas dynamic quenching has no such changes and UV spectra of DNA would have no detectable changes if the quenching was a dynamic mechanism.[33] Therefore, according to the UV spectra,

Table 4. The quenching constants of copper(II)–ibuprofenato complex by ct-DNA at different temperatures.

T (K)	K_{SV} ($\times 10^4$ L.mol^{-1})	Kq ($\times 10^{12}$ L.mol^{-1})	R^2
283	0.99	0.98	0.989
298	1.40	1.40	0.998
310	1.50	1.50	0.994

Table 5. Binding parameters of ibuprofen–Cu(II) complex with DNA at different temperatures.

T(K)	F_c(a.u.)	K_d ($\times 10^{-5}$ mol.L^{-1})	K_f($\times 10^4$ L.mol^{-1})	R^2
283	0.000	10.7	0.93	0.989
298	0.000	7.13	1.40	0.995
310	0.009	6.88	1.45	0.989

the fluorescence quenching of the complex in this case seems to be primarily caused by complex formation between the copper(II)–ibuprofenato complex and ct-DNA and confirming that the quenching process is static rather than dynamic.

Determination of the binding constant

The binding constant (K_f) for the complex formation between $[Cu(ibp)_2]_2$ complex and ct-DNA were measured according to the published method,[24] using the Eq. 6 that is most generally valid equation to analyze fluorescence changes upon formation of a 1:1 complex.

$$\frac{F_0 - F}{F_0 - F_c} = \frac{[Cu]_t + [D]_t + K_d - \sqrt{\left([Cu]_t + [D]_t + K_d\right)^2 - 4*[Cu]_t*[D]_t}}{2*[Cu]_t} \quad (6)$$

$$K_f = \frac{1}{K_d}, \quad (7)$$

where F is the measured fluorescence, F_0 the fluorescence in the absence of the DNA, F_c the fluorescence of the fully complexed $[Cu(ibp)_2]_2$, K_d the dissociation constant, $[D]_t$ the concentration of added ct-DNA and $[Cu]_t$ the concentration of $[Cu(ibp)_2]_2$, In the following, the values of K_d, K_f, and F_c were obtained for different temperatures by fitting the experimental data with Eq. 6. The obtained results are presented in Table 5.

The binding constants calculated for the $[Cu(ibp)_2]_2$-DNA are comparable with those of the K values estimated by Stern–Volmer (discussed above) and suggest high affinity of $[Cu(ibp)_2]_2$-DNA binding by increasing in temperature.[34–36]

Thermodynamic parameters of DNA binding

The interaction forces between drug and biomolecule may involve hydrophobic forces, electrostatic interactions, van der Waals interactions, hydrogen bonds, etc. According to the data obtained from enthalpy changes ($\Delta H°$) and entropy changes ($\Delta S°$), the model of interaction between a drug and biomolecule can be concluded[37]:

(a) $\Delta H° > 0$ and $\Delta S° > 0$, hydrophobic forces; (b) $\Delta H° < 0$ and $\Delta S° < 0$, van der Waals interaction and hydrogen bonds; (c) $\Delta H° < 0$ and $\Delta S° > 0$, electrostatic interactions.[38]

If the temperature does not vary significantly, the enthalpy change can be regarded as a constant. By utilizing the Vant–Hoff relation,[39] which describes the dependence of equilibrium constant on temperature, thermodynamic parameters of

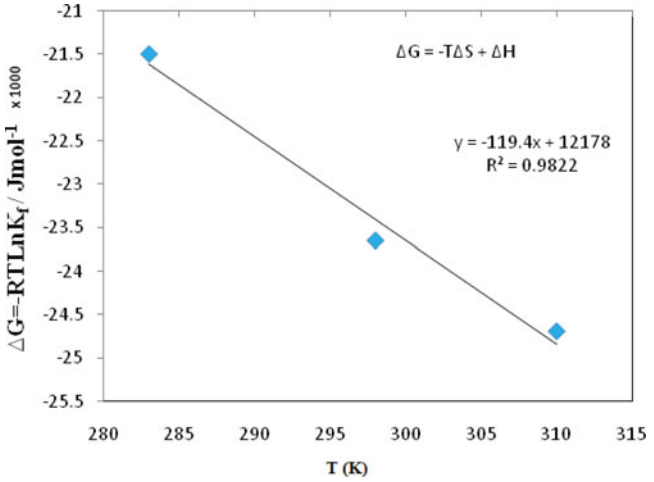


Figure 8. Plot of ΔG (ΔG = −RTLnK$_f$) versus T.

equilibrium have been determined:

$$\mathrm{LnK_f} = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} \tag{8}$$

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ = -RT\mathrm{LnK_f}, \tag{9}$$

where K_f is the binding constant at the corresponding temperature, ΔH° is the molar enthalpy change, ΔS° is the molar entropy change, $R = 8.31$ J.mol^{-1}K the universal gas constant, and T is the Kelvin temperature. A linear regression of ΔG° versus T is shown in Figure 8, which determines enthalpy and entropy change of the reaction from the slope and intercept of the linear plot. The results show that ΔH°>0 and ΔS°> 0 (Table 6), therefore, hydrophobic forces are probably the main forces in the binding of the complex to ct-DNA.

Competitive binding studies with methylene blue and hoechst 33258

Methylene blue as classical intercalator[40,41] and Hoechst 33258 as groove binder[42] probes were used, respectively to clarify the nature of the interaction between $[Cu(ibp)_2]_2$ and DNA.

Initially, with the addition of $[Cu(ibp)_2]_2$ to a solution of DNA-Hoechst complex, some Hoechst molecules were released into the solution after displacing with the $[Cu(ibp)_2]_2$, so result was fluorescence quenching (Figure 9), this assert the view that $[Cu(ibp)_2]_2$ could interact as a groove interaction.

Table 6. Thermodynamic parameters for the binding of copper(II)–ibuprofenato complex to ct-DNA.

T (K)	ΔG° (× 10^3 J.mol^{-1})	ΔH° (× 10^3 J.mol^{-1})	ΔS° (J.mol^{-1}K^{-1})
283	−21.50	12.18	119.4
298	−23.65	12.18	119.4
310	−24.70	12.18	119.4

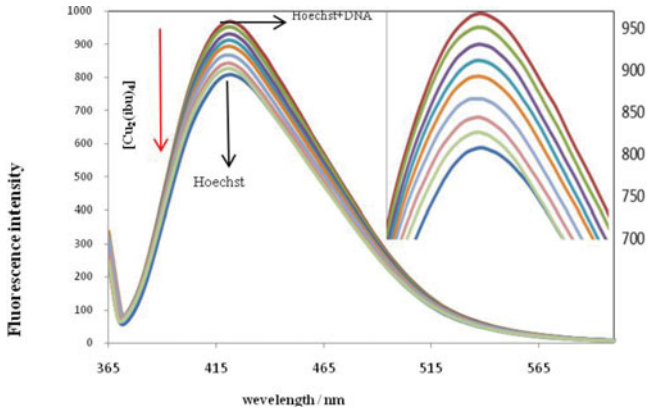


Figure 9. Fluorescence spectra of the competition between $[Cu(ibp)_2]_2$ and Hoechst 33258. $C_{DNA} = 8.55 \times 10^{-5}$ mol.L^{-1}, $C_{Hoechst} = 7.50 \times 10^{-6}$ mol.L^{-1} , $C_{Cu(II)\ complex} = 0.00, 0.49, 0.99, 1.47, 2.20, 3.14, 4.07$ and 4.54×10^{-5} mol.L^{-1}.

But it was later shown that the dominant binding mode was not only groove interaction. The significant change in the fluorescence intensity of the DNA-methylene blue complex with adding $[Cu(ibp)_2]_2$ indicated that MB molecules were released from the DNA helix after addition of the $[Cu(ibp)_2]_2$. So, binding mode of $[Cu(ibp)_2]_2$ and DNA is an intercalative mode, too (Figure 10).

To find out that the changes in fluorescence intensity is only resulted of releasing probe molecules into the solution after displacing with the $[Cu(ibp)_2]_2$ complex and are not reason of: (1) the binding between $[Cu(ibp)_2]_2$ complex and probe molecules and (2) formation aspecial complex with fluorescence emission and increase in fluorescence intensity of the system. This theory was examined by using Uv-vis and fluorescence spectroscopy. Since there are not any changes in the fluorescence intensities of MB and Hoechst upon adding $[Cu(ibp)_2]_2$ when DNA is absence, the first reason is not true. For testing the second reason UV-vis spectroscopy was used. If

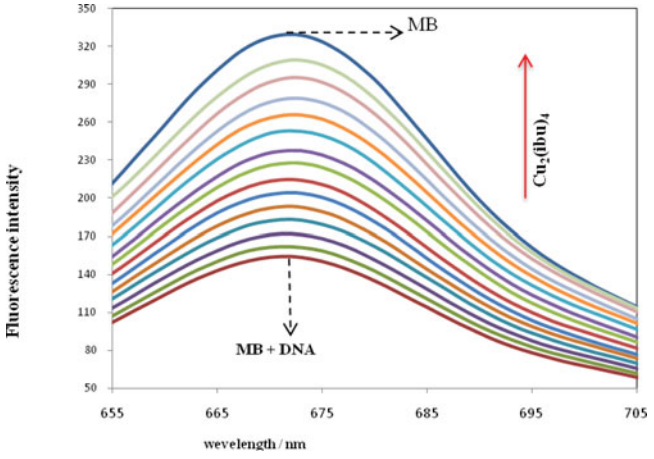


Figure 10. Fluorescence spectra of the competition between $[Cu(ibp)_2]_2$ and MB. $C_{DNA} = 8.55 \times 10^{-5}$ mol.L^{-1}, $C_{MB} = 7.50 \times 10^{-6}$ mol.L^{-1}, $C_{Cu(II)\ complex} = 0.00, 0.99, 1.96, 3.38, 4.52, 5.88, 7.19, 8.04, 8.88, 10.11, 11.30, 12.08, 16.49$, and 19.83×10^{-5} mol.L^{-1}.

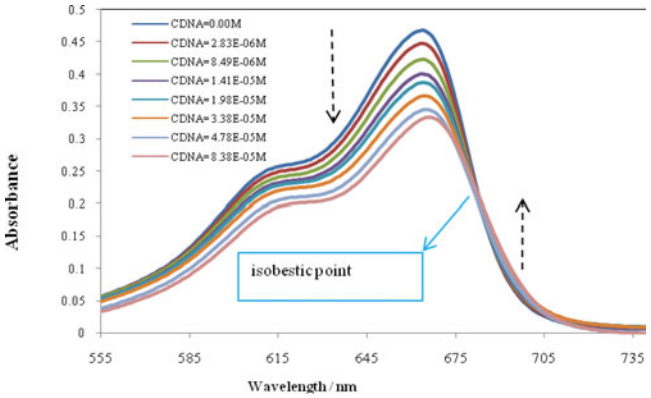


Figure 11. Absorption spectra of MB in the presence of different DNA concentrations.

the increase in fluorescence intensity of MB-DNA system is due to the formation of acomplex with fluorescence emission, absorption spectra of the MB-DNA should not be changed upon adding $[Cu(ibp)_2]_2$ complex.

The absorption spectra of the MB dye upon addition of DNA are shown in Figure 11. It is apparent from this figure that the absorption peak of the MB at around 650 nm showed gradual decrease with the increasing concentration of DNA, and a new band at around 700 nm developed. This was attributed to the formation of the new DNA-MB complex. An isobestic point at 683 nm confirmed DNA-MB complex formation.

As shown in Figure 12 upon addition of $[Cu(ibp)_2]_2$ complex the spectra exhibited the reverse process. The observed changes in the absorption spectra with increasing amounts of $[Cu(ibp)_2]_2$ to the DNA-MB complex solution suggest that the addition of complex induces conformational changes on DNA, which lead to the free form of MB.

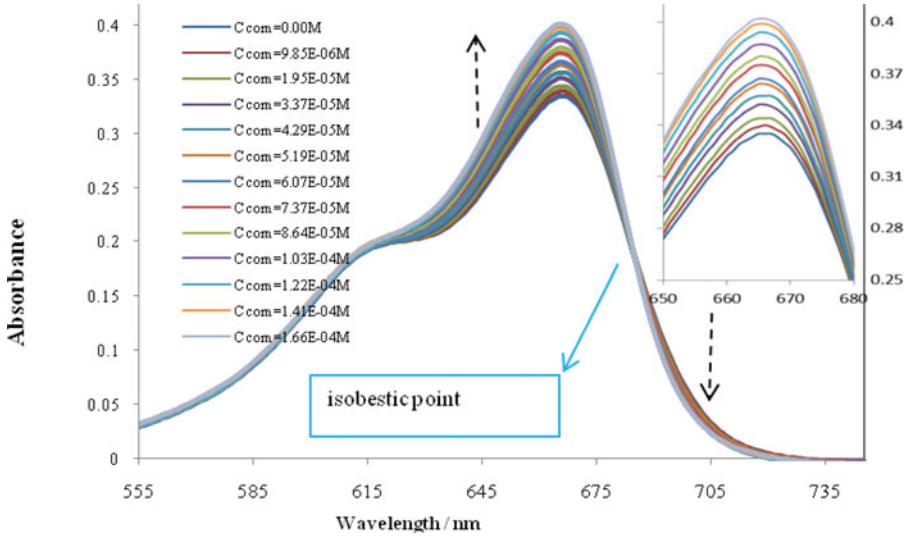


Figure 12. Effect of the $[Cu(ibp)_2]_2$ complex on the absorption spectra of MB-DNA.

So, with the addition of copper(II)–ibuprofenato complex into the solutions of DNA-MB complex and DNA-Hoechst complex, the MB and Hoechst molecules were released into the solutions after displacing with the $[Cu(ibp)_2]_2$ and results are changes in the fluorescence intensities. This asserts that $[Cu(ibp)_2]_2$ could interacts as both groove and intercalation interaction.

As to the unexpected and adverse binding mode, it may be concluded that an unusual DNA binding mode is existed and could be explained by a special type of binding mode, like partial intercalation or a special type of binding with a partially intercalation portion and the same in the groove portion[43,44]; an unusual DNA binding mode with significantly slower association and dissociation rates compared with classical intercalation. In this interaction the situation is obviously different than the penetration by ordinary intercalators in the bound state, a part of the molecule is partially intercalated between the base pairs, and the bulky substituents are located one in each groove. To reach this final state the propeller-like bulky end of this molecule must insert through the DNA base pairs, which transiently requires the opening of base pairs, i.e., local DNA melting, to an unknown extent. $[Cu(N\text{-}N)(L)(EtOH)]\ (NO_3)_2.\ 2H_2O$,[45] and bisantrene[46] fall in this category of interaction.

Viscosity measurements

In addition to spectroscopic data, viscosity experiment was carried out which is regarded as less ambiguous and the most critical tests of DNA binding model in solution.[47] Generally, a classical intercalation binding causes an increase in the viscosity of DNA solution because it demands a large enough space of adjacent base pairs to accommodate the ligand and to lengthen the double helix. However, a partial or nonclassical intercalation of the ligand may bend or kink the DNA helix, resulting in a decrease of its effective length and concomitantly, its viscosity.[48] In contrast, there is little or no increase of DNA viscosity if electrostatic or groove binding occurs in the binding process.[49]

The relative viscosity of DNA in the absence and presence of $[Cu(ibp)_2]_2$ complex were calculated from the Eq. 7:

$$\eta = \frac{t - t_0}{t}, \tag{10}$$

where t_0 and t were the observed flow time in the absence and presence of $[Cu(ibp)_2]_2$ complex. Data has been presented as (η/η_0) versus 1/R (R= [copper complex]/[DNA]), where η is the viscosity of DNA in the presence of the complex and η_0 is the viscosity of DNA alone (Figure 13).

As shown in Figure 13 the viscosity of DNA remarkably decreased upon the addition of $[Cu(ibp)_2]_2$ concentration. Such behavior is consistent with bending or kinking of the helix upon binding copper(II)–ibuprofenato complex to DNA. The results suggest that $[Cu(ibp)_2]_2$ complex may bind with a nonclassical intercalative mode like partial intercalation.

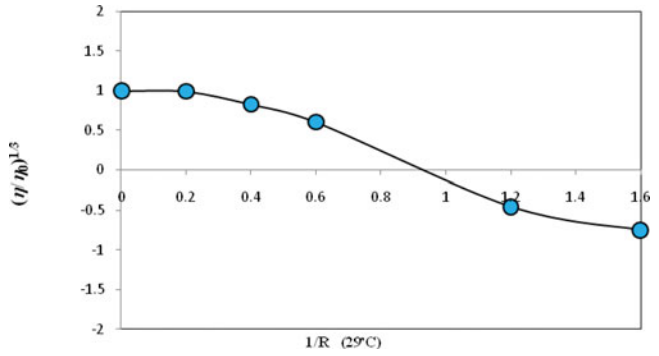


Figure 13. The effect of increasing amounts of $[Cu(ibp)_2]_2$ on the viscosity of ct-DNA; $C_{DNA} = 5 \times 10^{-5}$ mol.L^{-1}, $C_{Cu(II)\ complex} = 0.0, 1.0, 2.0, 3.0, 6.0,$ and 8.0×10^{-5} mol.L^{-1}.

Circular dichroic (CD) spectral studies

It is well known that the circular dichroism (CD) is very sensitive to the environment of the metal complexes, so to establish in more detail whether binding of the complex brings about any significant conformational change of the DNA double helix, CD spectra of ct-DNA were recorded. The changes in CD signals of DNA observed on interaction with molecules may be assigned to the corresponding changes in DNA structure, as a positive band at 275 nm from base stacking and a negative band at 246 nm from helicity of DNA are quite sensitive to the mode of DNA interactions with molecules.[50] The effect of increasing amounts of copper(II)–ibuprofenato complex on the conformation of the structure of DNA was studied (Figure 14).

As shown in Figure 14, in the presence of the $[Cu(ibp)_2]_2$ complex, both the positive and the negative bands decreased. The observed decreases in the positive and negative bands are consistent with a conformational change in DNA. The observed reduction in the positive DNA dichroic signal is likely due to a transition from the extended nucleic acid double helix to the more denatured structure.[51,52] These

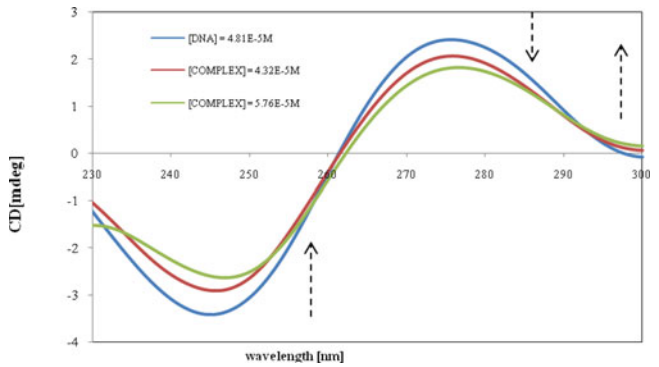


Figure 14. CD spectra of DNA in the presence of increasing amounts of $[Cu(ibp)_2]_2$ complex.

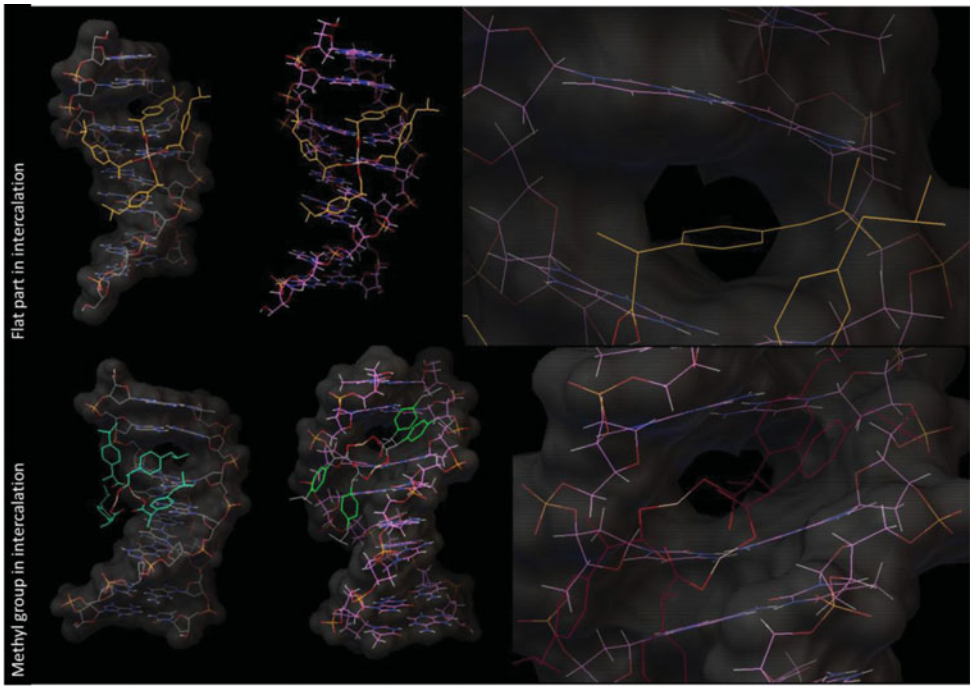


Figure 15. Molecular docked models of energy–minimized structure of $[Cu(ibp)_2]_2$ complex with DNA.

changes correlate with helix unwinding and can be associated with a kind of intercalative action by the $[Cu(ibp)_2]_2$ complex.[53] Also it should be noted that, remarkably reductions in molar ellipticity in the negative band (245 nm) are related to destabilization and helix unwinding.[54,55]

In assumed binding mode, a part of the complex is partially intercalated between the base pairs, and other substituents are located in the groove position, so both signals of base stacking and helicity at 275 nm and 246 nm should be changed. Therefore, these changes in CD signals are in agreement with the proposed mode of interaction.

Docking simulation results

Molecular docking techniques are well-documented computational tools to understand the Drug–DNA interactions for structure-based drug design and discovery, as well as mechanistic examination by replacing a small molecule into the binding site of the target region of the DNA highly in a non-covalent mode.[56,57] Here, molecular docking studies of the complex were carried out with DNA duplex to search the proper binding site. The energetically most favorable conformation of the docked pose is picked up from the 50 runs. As a result of these interactions, it was clear that the complex was located in both groove and intercalation sites of double-helix DNA (Figure 15). This again asserts that $[Cu(ibp)_2]_2$ could interacts as both groove and intercalation modes that is in agreement with the proposed mode of interaction.

Table 7. Binding constants (K_b) of some of the copper complexes which studied previously.

Complex	K_b / L.mol^{-1}	Ref.
$[CuL^3]Cl_2$ L^3 = *3,10-bis(2- hydroxyethyl)-1,3,5,8,10,12 hexaazacyclotetradecane*	7.80×10^2	[58]
$[CuL^2]Cl_2$ L^2 = *3,10-bis(2-propionitrile) - 1,3,5,8,10,12 hexaazacyclotetradecane*	4.00×10^3	[58]
$[Cu(L^2)_2(H_2O)_2](ClO_4)_2$ L^1 = *N-(furan-2-ylmethyl)-2-pyridinecarboxamide*	1.13×10^4	[59]
$[Cu(N\text{-}N)(L)(EtOH)]\ (NO_3)_2.2H_2O$	2.90×10^4	[45]
$[Cu(L^2)_2(H_2O)](NO_3)_2$ L^2 = *N-(thiophen-2-ylmethyl)-2-pyridinecarboxamide*	3.94×10^4	[59]

Outlook

Conclusion

One goal in the study of molecules that target the reproduction of tumor cells is the investigation on complexes which have a high affinity to DNA, as well as slow dissociation from DNA. Based on the results mentioned above it was found that copper(II)–ibuprofenato complex molecules could bind with DNA by both groove and intercalation modes, something likes a partial intercalation interaction with the following properties: (I)Time consumption and temperature dependence of the interaction (there are more changes in the UV–visible absorption spectra during the experiment and there is an increase in the binding constant with the temperature increasing). (II) Temperature stable interaction (free energy changes are decreased with increasing temperature). (III) A part of the complex is partially intercalated between the base pairs, and other substituents are located in groove positions (Whole of the MB (classical intercalator) and Hoechst (groove binder) molecules were released into solution after displacing with the $[Cu(ibp)_2]_2$ complex which indicates blocking of both locations by copper ibuprofenate complex). As well as the results of CD spectroscopy and Docking simulation prove exists of both groove and intercalation modes. In addition of high desire to interaction with DNA by this complex, because of high equilibrium constant ($6.19 \times 10^4\ M^{-1}$)in contrast to other copper complexes, (Some of these complexes are shown in Table 7[45,58,59]),it also has a new binding mode with DNA which has the properties of both the intercalation and groove binding modes. Therefore this complex can occupy both the active site of DNA namely groove and intercalation sites and can deeply affect DNA. These results again can reveal that this complex is more effective than others copper complexes with just groove[60–62] or intercalation[59,63] mode of bindings and can be a new direction in the research of DNA interaction, cancer therapy and molecular biology.

Acknowledgments

This work was supported by Razi University of Kermanshah-Iran.

References

1. Shim, J.S.; Liu, J.O. Recent advances in drug repositioning for the discovery of new anticancer drugs. *Int. J. Biol. Sci.* **2014**, *10*, 654–663.
2. Chowdhury, B.; Roy, D.; Chavan, U.; Mukhopadhyay, S. Preparation and characterization of ibuprofen nanoparticles by using solvent/ antisolvent precipitation. *Med. Sci. Res.* **1996**, *24*, 801–802.
3. Manrique, J.; Martinez, F. Solubility of ibuprofen in some ethanol + water cosolvent mixtures at several temperatures. *Lat. Am. J. Pharm.* **2007**, *26*, 344–354.
4. Endo, H.; Yano, M.; Okumura, Y.; Kido, H. Ibuprofen enhances the anticancer activity of cisplatin in lung cancer cells by inhibiting the heat shock protein 70. *Cell Death and Disease* **2014**, *5*, e1027.
5. Husain, M.A.; Sarwar, T.; Rehman, S.U.; Ishqi, H.M.; Tabish, M. Ibuprofen causes photocleavage through ROS generation and intercalates with DNA:A combined biophysical and molecular docking approach. *Phys. Chem. Chem. Phys.* **2015**,*17*, 13837–13850.
6. Nagesh, G.Y.; Raj, K.M.; Mruthyunjayaswamy, B.H.M. Synthesis, characterization and biological relevance of some metal (II) complexes with oxygen, nitrogen and oxygen (ONO) donor Schiff base ligand derived from thiazole and 2-hydroxy-1-naphthaldehyde. *J. Mol. Struct.* **2015**, *1079*, 423–432.
7. Angelova, M.; Asenova, S.; Nedkova, V.; Koleva-Kolarova, R. Copper in the human organism. *Trakia J. Sci.* **2011**, *9*, 88–98.
8. Sorenson, J.R.J.; Sigel,H. ed, In *Metal Ions in Biological Systems*, Marcel Dekker, NewYork, **1984**.
9. Grouch, R.K.; Kensler, T.W.; Oberley, L.W.; Sorenson, J.R.J. In *Biological and Inorganic Copper Chemistry*, ed., K.D., Karlin; J. Zubieta Adenine Press, Guilderland, New York, 1986.
10. West, D.X.; Owens, M.D. DNA binding, DNA cleavage, and cytotoxicity studies of two new Copper (II) complexes. *Transit. Methods Chem.* **1998**, *23*, 87–91.
11. Andrade, A.; Namora, S.F.; Woisky, R.G. Analysis and chemical speciation of copper and zinc in wound fluid. *J. Inorg. Biochem.* **2000**, *81*, 23–27.
12. Sorenson, J.R.J. *Biology of Copper Complexes*, Humana Press, New York, **1987**, 243–257.
13. Kowcun, K.; Frańska, M.; Frański, R. Binuclear copper complexes with non-steroidal anti-inflammatory drugs as studied by electrospray ionization mass spectrometry. *Cent. Eur. J. Chem.* **2012**, *10*, 320–326.
14. Fox, K. R.; Brassett, C.; Waring, M. Kinetics of dissociation of nogalamycin from DNA: Comparison with other anthracycline antibiotics. *J. Biochim. Biophys.Acta.* **1985**, *840*, 383–392.
15. Zeman, S. M., Depew, K. M., Danishefsky, S. J., Donald, M., Crothers, D. M. Simultaneous determination of helical unwinding angles and intrinsic association constants in ligand–DNA complexes: The interaction between DNA and calichearubicin *B. Proc Natl Acad Sci U S A.* **1998**, Apr 14; *95*(8), 4327–4332.
16. Tita, B.; Bandur, G.; Tita, D. Anti-inflammatory drugs interacting with Zn(II)metal ion synthesis, characterization and thermal behaviour of the complex with ketoprofen. *Rev. Chim.* **2013**, *62*, 569–572.
17. Kdlly, J.M.; Tossi, A.B.; McConnell, D.J.; OhUigin, C. A study of the interactions of some polypyridylruthenium (II) complexes with DNA using fluorescence spectroscopy, topoisomerisation and thermal denaturation. *Nucleic Acids Res.* **1985**, *13*, 6017–6034.
18. Puchalski, M.M.; Morra, M.J.; yon Wandruszka, R. Fluorescent labeling of human albumin using the new aromatic dialdehyde labels and the study of innerfilter effect. *Fresenius J. Anal Chem.* **1991**, *340*, 341–344.
19. Weert, M.; Stella, L. Fluorescence quenching and ligand binding: A critical discussion of a popular methodology. *J. Mol. Struct.* **2011**, *998*, 144–150.

20. Brton, J.K.; Danishefsky, A.; Goldberg, J. Tris(phenanthroline)ruthenium(II): Stereoselectivity in binding to DNA. *J. Am. Chem. Soc.* **1984**, *106*, 2172–2176.
21. Bloomfield, V.; Crothers, D.M.; Tinoco Jr., I. *Physical Chemistry of Nucleic acids*, Harper and Row, New York, 1974.
22. Marty, R.; Nsoukpoe-Kossi, N.Ch.; Charbonneau, D.; Weinert, C.M.; Kreplak, L.; Tajmir-Riahi, H.A. Structural analysis of DNA complexation with cationic lipids. *Nucleic Acids Res.* **2009**, *37*, 849–857.
23. Dubeau, S.; Bourassa, P.; Thomas, T.J.; Riahi, H.A.T. Biogenic and synthetic polyamines bind bovine serum albumin. *Biomacromolecules* **2010**, *11*, 1507–1515.
24. Hirose, K. A. Practical guide for the determination of binding constants. *J. Pheno. Chem.* **2001**, *39*, 193–209.
25. Huang, C.Y. Determination of binding stoichiometry by the continuous variation method: The job plot. *Methods Enzymol.* **1982**, *87*, 509–525.
26. Zou, H.Y.; Wu, H.L.; Zhang, Y.; Li, S.F.; Nie, J.F.; Fu, H.Y.; Yu, R.Q. Low-cost fabrication of paper-based microfluidic devices by one-step plotting. *J. Fluoresc.* **2009**, *19*, 955–966.
27. Lakowicz, J.R. Principles of fluorescence spectroscopy. Plenum Press, New York, **1983**, 257–257.
28. Ling, X.; Zhong, W.; Huang, Q.; Ni, K. Spectroscopic studies on the interaction of pazufloxacin with calf thymus DNA. *J. Photochem. Photobiol.* **2008**, *93*, 172–176.
29. Baguleyand, B.C; Bret, M.L. Quenching of DNA-ethidium fluorescence by amsacrine and other antitumor agents: A possible electron-transfer effect. *Biochemistry* **1984**, *23*, 937–943.
30. Lakowicz, J.R.; Weber, G. A model of dynamic quenching of fluorescence in globular proteins. *Biochemistry* **1973**, *12*, 4171–4179.
31. Eftink, M.R.; Ghiron, C.A. Fluorescence quenching studies with proteins. *Anal. Biochem.* **1981**, *114*, 199–227.
32. Li, T.R.; Yang, Z.Y.; Wang, B.D.; Qin, D.D. Synthesis, characterization, cytotoxic activities, and DNA-binding properties of the La(III) complex with Naringenin Schiff-base. *Eur. J. Med. Chem.* **2008**, *43*, 1688–1695.
33. Tian, F.; Jiang, F.; Han, X.; Xiang, C.; Ge, Y.; Li, J.; Zhang, Y., et al. Synthesis of a novel hydrazonederivative and biophysical studies of its interactions with Bovine Serum Albumin by spectroscopic, electrochemical, and molecular docking methods. *J. Phys. Chem. B* **2010**, *114*, 14842–14853.
34. Dufour, C.; Dangles, O. Flavonoid-serum albumin complexation: Determination of binding constants and binding sites by fluorescence spectroscopy. *Biochim. Biophys. Acta.* **2005**, *1721*, 164–173.
35. Arnold, K.; Bordoli, L.; Kopp, J.; Schwede, T. The SWISS-MODEL workspace: A web-based environment for protein structure homology modeling. *Bioinformatics* **2006**, *22*, 195–201.
36. Rost, B. Twilight zone of protein sequence alignments. *Protein Eng.* **1999**, *12*, 85–94.
37. Nike, D.B.; Moorty, P.N.; Priyadarsini, K.I. DNA interaction studies of a new Platinum(II) complex containing different aromatic Dinitrogen Ligands. *Chem. Phys. Lett.* **1990**, *168*, 533–538.
38. Rahman, M.H.; Maruyama, T.; Okada, T.; Yamasaki, K.; Otagiri, M. Spectroscopic studies on the binding of bioactive phenothiazine compounds to human serum albumin. *Biochem.Pharmacol.* **1993**, *46*, 1721–1731.
39. Antonov, L.; gergov, G.; Petrov, V.; Kubista, M. UV-Vis spectroscopic and chemometric study on the aggregation of ionic dyes in water. *J. Nygren. Talanta.* **1999**, *49*, 99–106.
40. Sinha, A.P.B. *Spectroscopy in Inorganic Chemistry*, Academic Press, New York, 1971.
41. Kumar, C.V.; Asuncion, E.H. DNA binding studies and site selective fluorescence sensitization of an anthryl probe. *J. Am. Chem. Soc.* **1993**, *115*, 8547–8553.
42. Vega, M.C.; Garcia Saez, I.; Aymami, J.; Eritja, R.; Van Der Marel, G.A.; Van Boom, J.H.; Rich, A.; Coll, M. Three-dimensional crystal structure of the A-tract DNA dodecamer

d(CGCAAATTTGCG)complexed with the minor-groove-binding drug Hoechst 33258. *Eur. J. Biochem.* **1994**, *222*, 721–726.
43. Andersson, J.; P. Lincoln, P. Kinetic Characterization of an extremely slow DNA binding equilibrium. *J. Phys. Chem. B.* **2011**, *115*, 14768–14775.
44. Lokey, R.S.; Kwok, Y.; Guelev, V.; Pursell, C.J.; Hurley, L.H.; Iverson, B.L. Threading polyintercalators with extremely slow dissociation rates and extended DNA binding sites. *J. Am. Chem. Soc.* **1997**, *119*, 7202–7210.
45. Shahabadi, N; Darabi, F; Maghsudi, M; Kashanian, S. DNA Binding and Gel Electrophoresis Studies of a Copper (II) Complex Containing Mixed Aliphatic and Aromatic Dinitrogen Ligands. *DNA Cell Biol.* **2010**, *29*, 329–336.
46. Wilson, W.D.; Ratmeyer, L.; Zhao, M.; Strekowski, L.; Boykin, D. The binding mode of drugs to the TAR RNA of HIV-1 studied by electric linear dichroism. *Biochemistry* **1993**, *32*, 4098–4104.
47. Xi, P.; Xu, Z.; Chen, F.; Zeng, Z.; Zhang, X. Synthesis and assembly of Barium-doped Iron Oxide nanoparticles and nanomagnets. *Inorg. Biochem.* **2009**, *103*, 210–218.
48. Satyanarayana, S.; Dabrowiak, J.C.; Chaires, J.B. Tris (phenanthroline)ruthenium(II) enantiomer interactions with DNA: Mode and specificity of binding. *Biochemistry* **1993**, *32*, 2573–2584.
49. Bi, S.; Qiao, C.; Song, D.; Tian, Y.; Gao, D.; Sun, Y.; Zhang, H. Publishers note : New editorial structure for sensors and actuators B: Chemical. *Sens. Actuators.* **2006**, *119*, 199–208.
50. Shahabadi, N.; Kashanian, S.; Darabi, F. DNA binding and DNA cleavage studies of a water soluble cobalt (II) complex containing dinitrogen Schiff base ligand: The effect of metal on the mode of binding. *Eur. J. Med. Chem.* **2010**, *45*, 4239–4245.
51. Kashanian, S.; Shahabadi, N.; Roshanfekr, H.; Shalmashi, K.; Omidfar, K. Proinflammatory cytokines in response to insulin-induced hypoglycemic stress in healthy subjects. *Biochemistry (Moscow)* **2008**, *73*, 929–936.
52. Malinge, J.M.; Leng, M. Reaction of nucleic acids and cis-diamminedichloroplatinum(II) in the presence of intercalating agents. *EMBO J.* **1984**, *3*, 1273–1279.
53. Grueso, E.; López-Pérez, G.; Castellano, M.; Prado-Gotor, R. Thermodynamic and structural study of phenanthroline derivative ruthenium complex/DNA interactions: Probing partial intercalation and binding properties. *J. Inorg. Biochem.* **2012**, *106*, 1–9.
54. Tajmir-Riahi, H.A.; Naoui, M.; Ahmad, R. Does DNA acid fixation produce left-handed Z structure. *Biopolymers* **1993**, *33*, 1819–1827.
55. Vaidyanathan, V.G.; Nair, B.U. Photooxidation of DNA by a cobalt (II) tridentate complex. *J. Inorg. Biochem.* **2003**, *94*, 121–126.
56. Trott, O.; Olson, A.J. AutoDockVina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J. Comput. Chem.* **2010**, *31*, 455–461.
57. Gilad, Y.; Senderowitz, H. Docking studies on DNA intercalators. *J. Chem. Inf. Model.* **2014**, *54*, 96–107.
58. Liu, J.; Zhang, T.; Lu, T.; Qu, L.; Zhou, H.; Zhang, Q.; Ji, L. D NA-binding and cleavage studies of macrocyclic copper(II) complexes. *J. Inorg. Biochem.* **2002**, *91*, 269–276.
59. Mondal, B.; Sen, B.; Maji, A.; Zangrando, E.; Chattopadhyay, P. Synthesis, crystastructure, redox behavior and DNA-binding properties of a series of copper(II) complexes with two new carboxamide derivatives. *J. Coord. Chem.* **2015**, *68*, 4038–4054.
60. Sarkar, S.; Sen, S.; Dey, S.; Zangrando, E.; Chattopadhyay, P. Coordination behavior of 3,4 bis(2-pyridylmethylthio)toluene with copper(II) ions: Synthesis, structural characterization and reactivity, and DNA binding study of the dinuclear copper(II) complex. *Polyhedron* **2010**, *29*, 3157–3163.
61. Dey, S.; Mukherjee, T.; Sarkar, S.; Evans, H.S.; Chattopadhyay, P. 5-Nitro-1,10-phenanthroline bis(*N,N*-dimethylformamide-)-bis(perchlorato) copper(II): Synthesis,

structural characterization, and DNA-binding study. *Transition Met. Chem.* **2011**, *36*, 631–636.
62. Dey, S.; Sarkar, S.; Mukherjee, T.; Mondal, B.; Zangrando, E.; Sutter, J.P.; Chattopadhyay, P. An oxamato bridged trinuclear copper(II) complex: Synthesis, crystal structure, reactivity, DNA binding study and magnetic properties. *Inorg. Chim. Acta.* **2011**, *376*, 129–135.
63. Golcu, A. Ofloxacin metal complexes: Synthesis, characterization, analytical properties, and DNA binding studies. *Inorg. Chem.* **2014**, *44*, 1509–1520.