Journal of Reports in Pharmaceutical Sciences 2016, 5(2), 80-82

DNA Binding Study of Dihydropyrano [3, 4-*C*] Chromene Derivative by some Spectroscopic Techniques

Mahvash Farajzadeh Dehkordi, Gholamreza Dehghan*, Majid Mahdavi, Mohammad Ali Hosseinpour Feizi

Department of Biology, Faculty of Natural Sciences, University of Tabriz, Tabriz, Iran.

ARTICLE INFO

Article Type:
Research Article

Article History:
Received: 2016-06-17
Revised: 2016-07-24
Accepted: 2016-08-15
ePublished: 2016-08-23

***Keywords*:**
Binding constant
Chromone derivative
Circular dichroism
Groove binding

A B S T R A C T

This study was designed to examine the interaction of dihydropyranochromene derivative, 2-amino-4-phenyl-5-oxo-4H, 5H-pyrano-[3, 2-c] chromene-3-carbonitrile (4-PC) with calf thymus DNA (ctDNA) by absorption spectroscopy, competitive fluorescence studies, circular dichroism (CD) and viscosity measurements. It was found that 4-PC could interact with ctDNA through non- intercalative mode and the intrinsic binding constant, K_b, for 4-PC with ctDNA was estimated to be $2.74 \times 10^3 M^{-1}$. Methylene blue (MB) displacement studies revealed that 4-PC could not replace ctDNA bounded MB, which is indicative of groove binding mode of 4-PC with DNA. Furthermore, 4-PC induced detectable changes in the CD spectrum of ctDNA as well as changes in its viscosity, corroborate the above experimental results.

***Corresponding Author:** GholamrezaDehghan**, E-mail:** dehghan2001d@yahoo.com & gdehghan@tabrizu.ac.ir

Introduction

Deoxyribonucleic acid (DNA) is an important genetic substance in the organism, which plays an extremely significant role in the process of human life, such as gene expression, gene transcription, mutagenesis, and carcinogenesis[1]. Furthermore, the primary target molecule for most anticancer and antiviral therapies according to cell biologists is DNA and among the different therapeutic strategies to eradicate cancer cells through DNA damage, the view of using small molecules, capable of binding to DNAas anticancer drugs, is a challenging issue in chemotherapy[2].. Furthermore,many natural or synthetic drugs serve as analogs in the research of protein–nucleic acid recognition, sensitive probes of local nucleic acid structure and provide site-specific affinity for molecular biology. Therefore, the investigation of the interaction between drug molecules and DNA is very helpful in understanding the molecular mechanisms of the drug action and designing new and specific DNA-targeted drugs[3]. The possible interaction models between small molecules and DNA generally include three modes: (i) electrostatic or surface binding between the cationic species and the negatively charged DNA phosphate backbone, which is along the external DNA double helix and do not possess selectivity; (ii) groove binding in which the small molecules bound on nucleic acids are located in two grooves of DNA double helix involving hydrogen bonding or van der Waals interaction with the nucleic acid bases in the major or minor groove of the DNA helix; (iii) intercalative binding that drug molecules intercalate into the stacked base pairs of DNA[4]. These weak acting forces are deterministic factors in life phenomenon on the molecular level and determination of the main mechanism of gene transcription and adjusting[5]. The intercalative mode is the most important mode in which drug molecules can intercalate between the pair-bases of double helix DNA, forming π-π overlapping interaction, simultaneously lengthening and unwinding the helix. In minor groove- binding, the crescent-shaped ligand fits into the minor groove with little steric hindrance and with little perturbation of the DNA structure[6,7].

Pyranochromenes are an important class of oxygen containing heterocycles that have attracted significant importance in the field of organic and natural product chemistry [8].Chromene, the basic skeleton of pyranochromenes is a group of naturally occurring compounds that is ubiquitous in nature especially in plants [9]. Further, pyranochromenes and their derivatives are of considerable interest as they possess a wide range of biological properties, such as spasmolytic, diuretic, anticoagulant, anti-cancer, antibacterial, antiviral, antifungal and anti-anaphylactic activities [8,10]. In addition, they can be used as cognitive enhancers for the treatment of neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, AIDS associated dementia and Down's syndrome as well as for the treatment of schizophrenia and myoclonus [11].

In this report, the binding properties of dihydropyranochromene derivative, 2-amino-4-phenyl-5-oxo-4H,5H-pyrano-[3,2-c]chromene-3-carbonitrile (4-PC) with calf thymus DNA (ctDNA) was investigated *in vitro,* , as compound effecting DNA by various spectroscopic techniques including UV–visible spectroscopy, fluorescence spectroscopy, circular dichroic spectral together with viscosity measurements. Determination of the binding mode and affinity between 4-PC and ctDNA should be helpful for understanding the mechanism of action of dihydropyranochromenes at the molecular level and to designing therapeutic interventions.

Materials and methods

Materials

Calf thymus DNA (ctDNA), Tris–HCl, dimethylsulfoxide (DMSO), methylene blue (MB) were purchased from Sigma-Aldrich. 4-PC prepared according to the previously described methods and its stock solution(3×10^{-3} M) was prepared in absolute DMSO. The stock solution of ctDNA was prepared by dissolving of ctDNA in 10 mM of the Tris–HCl buffer (pH = 7.4) and the purity of it was verified by monitoring the ratio of absorbance at 260 nm to that at 280

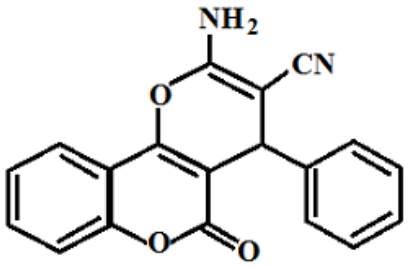
nm, which was in the range 1.8–1.9. The concentration of the DNA was determined spectrophotometrically using ε= 6600 $M^{-1}cm^{-1}$ at 260 nm [12].

Methods

The UV-visspectra for DNA–4-PC interactions were obtained using a UV-visible spectrophotometer (T60, PG Instruments Ltd., Leicestershire, UK). Solutions of ctDNA and 4-PC were scanned using a 1-cm quartz cuvette. The absorption spectra of a 4-PC solution in the absence and presence of ctDNA was recorded for a constant 4-PC concentration and different concentrations of ctDNA. Moreover, the absorption spectra of ctDNA in the absence and presence of 4-PC was also recorded.

All fluorescence measurements were carried out with a JASCO spectrofluorometer (FP6200) (Tokyo,Japan). The competitive interaction between the methylene blue (MB), as a fluorescence probe and 4-PC with ctDNA was carried out as follow: fixed amount of ctDNA and MB solution (4.9×10^{-6} and 5.6×10^{-8}M, respectively) was progressively titrated with 4-PC solution in the wavelength range of 660 to 750 nm with an excitation wavelength at 630 nm. The CD spectra of ctDNA incubated with concentration of ctDNA constant (5.0×10^{-5}M), while varying 4-PC concentration (*ri* = [4-PC]/[DNA] = 0–0.8) were measured on a JASCO (J-810) spectropolarimeter at wavelengths between 230 and 300 nm.

Viscosity measurements were performed using Ubbelohde viscometer which was thermostated at 298 K in a constant temperature bath. The concentration of ctDNA in Tris–HCl buffer solution (pH = 7.4) in the absence and presence of 4-PC was fixed at 5×10^{-5}M and the flow time was measured using a digital stop watch. The mean values of three replicated measurements were used to evaluate the relative specific viscosity $(\eta/\eta_0)^{1/3}$, where η_0 and η are the specific viscosity contributions of DNA in the absence and presence of 4-PC, respectively [13].



4-PC

Fig.1. 2-amino-4-phenyl-5-oxo-4H, 5H-pyrano-[3, 2-c]chromene-3-carbonitrile (4-PC)

Results and discussion

Electronic absorption spectra

Electronic absorption spectroscopy is an efficient method for studying the binding mode of DNA with small molecules. The absorption spectra of 4-PC in the absence and presence of ctDNA are indicated in Figure 2a. As shown in Fig. 2a, addition of increasing amounts of DNA to 4-PC solution results in the obvious hyperchromic tendency of the absorption bands without any noticeable spectral shift , suggesting a strong interaction between 4-PC and DNA which is different from the classical intercalation binding and these changes show that the binding mode of 4-PC to DNA might be groove binding [14].

To confirm the mode of interaction, absorption spectra of ctDNA at constant concentration in the absence and presence of increasing amounts of 4-PCare taken in Fig. 2b. Absorption spectra of ctDNA increased upon increasing of 4-PC concentration, this is a typical hyperchromic effect indicate damage to the DNA double-helical structure after 4-PC binding, indicating a non-intercalative mode of interaction [13].

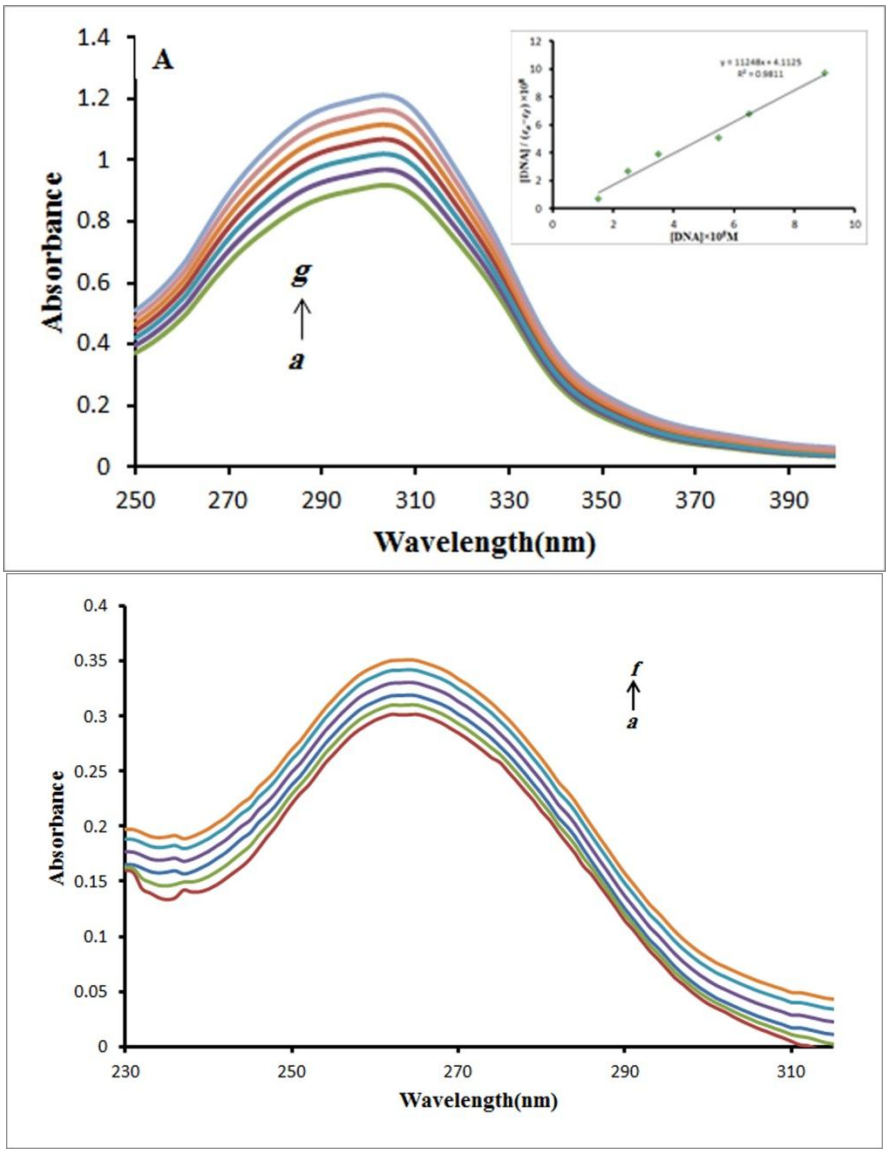


Fig. 2. Absorption spectra: **a)** 4-PC (5.0×10^{-5}M) in the absence and presence of increasing amounts of ctDNA. (*ri*= [ctDNA]/ [4-PC] =0.0, 0.3, 0.5, 0.7, 1.1, 1.3 and 1.6); for curves *a-g*, respectively. Inset: plots of [ctDNA]/ ($\varepsilon a - \varepsilon_f$) versus [ctDNA] for the titration of 4-PC with ctDNA. **b)** ctDNA (5.0×10^{-5}M) in the absence and presence of increasing amounts of 4-PC. (*ri* = [4-PC]/[ctDNA] = 0.0, 0.3, 0.5, 0.7, 1.1and 1.4), for curves *a-f*, respectively.

In order to further illustrate the binding strength of 4-PC with ctDNA, the intrinsic binding constant, K_b, was determined from the spectral titration data using the following equation:

$$\frac{[\mathbf{DNA}]}{\varepsilon \boldsymbol{a} - \varepsilon \boldsymbol{f}} = \frac{[\mathbf{DNA}]}{\varepsilon \boldsymbol{b} - \varepsilon \boldsymbol{f}} + \frac{\mathbf{1}}{\boldsymbol{Kb}(\varepsilon \boldsymbol{b} - \varepsilon \boldsymbol{f})} \qquad \textbf{(1)}$$

Where [DNA] is the concentration of DNA, $\mathcal{E}_a$, $\mathcal{E}_f$ and $\mathcal{E}_b$ corresponded to the apparent extinction coefficient, the extinction coefficient for the free compound and its fully DNA-bound combination, respectively. From the plots of [DNA]/ (ε_a–ε_f) vs. [DNA], the binding constant, K_b, was derived as the ratio of the slope to the intercept [15].

The binding constant, K_b, for 4-PC was 2.74 $\times 10^3$ M^{-1}($y = 11.26 \times 10^{-5} x + 4.11 \times 10^{-8}$, R^2= 0.981). In comparing, the intrinsic binding constant, K_b, of 4-PC with some DNA groove binder agents such as mesalamine,2- imidazolidinethione and levetiracetam (K_b=1.27×$10^3$$M^{-1}$, 1.4×$10^3$ $M^{-1}$and (4.9 ± 0.2) ×$10^3$$M^{-1}$) respectively, it was found that the binding mode between 4-PC and DNA was groove binding[16-18]. We have recently reported the binding mode and intrinsic binding constant of another dihydropyranochromene derivatives ,2-amino-4-(3-hydroxyphenyl)-5-oxo-4H, 5H-pyrano-[3, 2-c] chromene-3-carbonitrile (3-HC) with ctDNA [19]. It has been seen that 3-HC can bind to ctDNA through non- intercalative binding mode and the intrinsic binding constant, K_b, of 3-HC was found to be 3.6×10^3 M^{-1}. This variation likely reflects the different ability of these compounds to hydrogen bonding or van der Waals interaction with the nucleic acid bases of DNA.

Fluorescence studies (competitive studies with MB)

Fluorescence of 4-PC was not detectable either in aqueous solution or in the presence of ctDNA at room temperature. Although DNA has a natural fluorescence, the intensity is so weak that the direct use of the fluorescence emission of DNA is limited to study its properties [20].Acridine orange, ethidium bromide, methylene blue, and similar fluorescent compounds are normally used to probe DNA structure in drug–DNA and protein–DNA interactions [21].There are two modes of interaction between methylene blue and DNA: strong binding (semi-intercalation) and weakbinding [22].

Interestingly, the emission intensity of MB is quenched on adding ctDNA. This emission quenching phenomenon reflects the change in the excited state structure as a consequence of the electronic interaction in the MB-ctDNA complex. The emission-quenching phenomenon and the hypochromic and red shift effects in the absorption spectra attribute to the intercalative mode of MB to DNA[23]. The emission spectra of the MB-DNA solution in the presence of the increasing 4-PC concentrations are shown in Fig. 3. Fluorescence emission spectra of MB is significantly quenched by the addition of DNA. There is not any clear increase in the fluorescence intensity of the probe molecule upon adding 4-PC, also the K_b of MB is K_{MB} = 2.13 ×10^4 M^{-1} higher than that of 4-PC, so in this binding competition MB is winner, Additionally, MB binding to DNA with alternating AT base sequence: minor groove binding is favored over intercalation[24-25]. These results supported the view that 4-PC could interact as a non-intercalative binder.

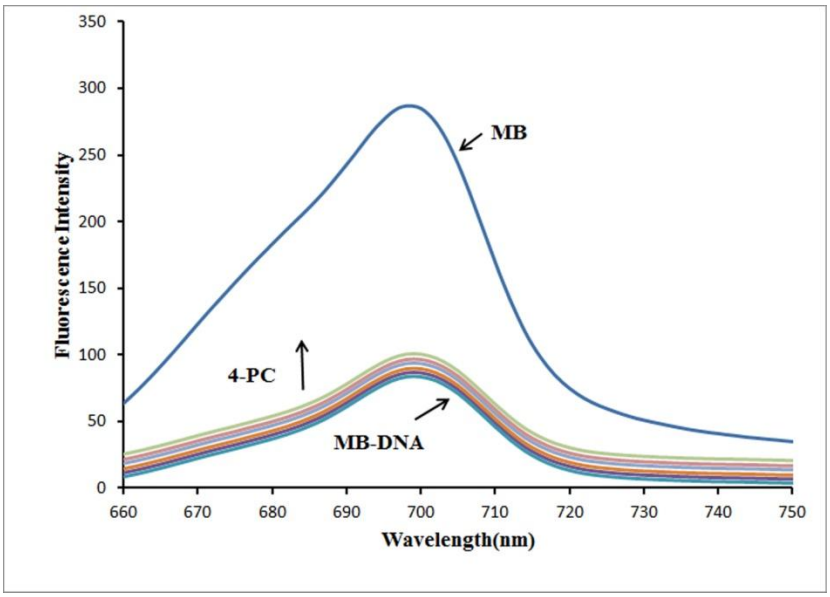


Fig. 3. Emission spectra of MB–ctDNA complex in the presence of increasing amounts of 4-PC. (*ri* = [4-PC]/ [MB + ctDNA] = 0.0, 0.2, 0.4, 0.6, 0.8 and 1.2) at 298 *K* and λ_{ex}= 630 nm.

Circular dichroism (CD) studies

A solution of ctDNA exhibits a positive band (275 nm) from base stacking interactions and a negative band (245 nm) from the right-handed helicity of DNA. 4-PC had no CD signal in the UV region of 230–300nm. The interaction of 4-PC with DNA induces a change in the CD spectrum of the B-DNA. As shown in Fig. 4, with increasing concentration of 4-PC, the intensities of both the positive and negative ellipticity bands were decreased. Therefore, it can be deduced that the interaction of 4-PC with DNA induces certain conformational changes, such as the conversion from a more B-like to a more C-like structure within the DNA molecule. These changes are suggestive of a non-intercalative mode of binding of 4-PC. This result is similar to the results of our previously reported data on interaction of 3-HC with ctDNA [19-23].

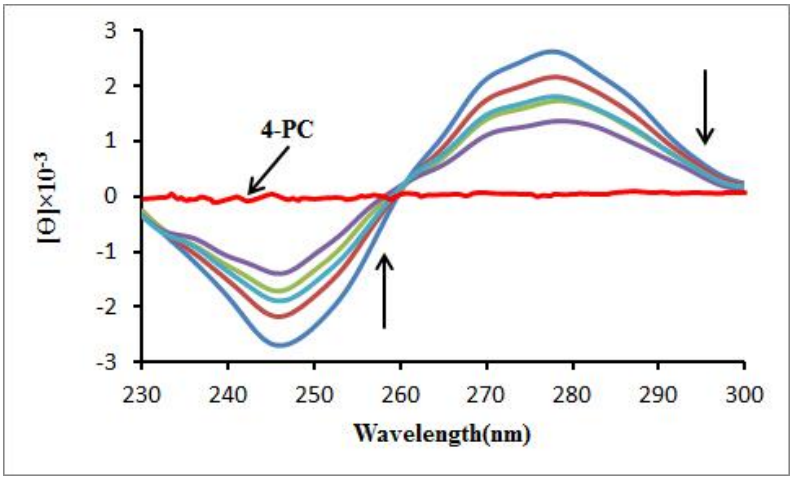


Fig. 4. CD spectra of ctDNA (5.0 × 10 $^{-5}$ M) in Tris–HCl buffer, in the presence of increasing amounts of 4-PC. (*ri* = [4-PC]/[DNA] = 0.0, 0.1, 0.3, 0.6 and 0.8). The arrows show the CD spectra changes uon increasing 4-PC. [4-PC] =1.5×10^{-5}M (dashed line).

Viscosity measurements

Viscosity measurement, which is sensitive to the changes in the length of the DNA molecule, is regarded as the least ambiguous and the most critical means of studying the binding mode of small molecules with DNA in solution. A classical intercalation binding demands the space of adjacent base pairs to be large enough to accommodate the bound ligand and to elongate the double helix, resulting in an increase of DNA viscosity [26]. In contrast, drugs those bind exclusively in the DNA grooves by partial and/or non- classical intercalation, under the same conditions, typically cause less executed (positive or negative) or no change in DNA solution viscosity [27]. The viscosities of ctDNA in the Tris–HCl buffer solution in the absence and presence of 4-PC was measured and the results were shown in Fig. 5. As shown in Fig. 5, little change on the viscosity of ctDNA with the increasing concentration of 4-PC, indicating that 4-PC bound to ctDNA by groove binding such as 3-HC[19].

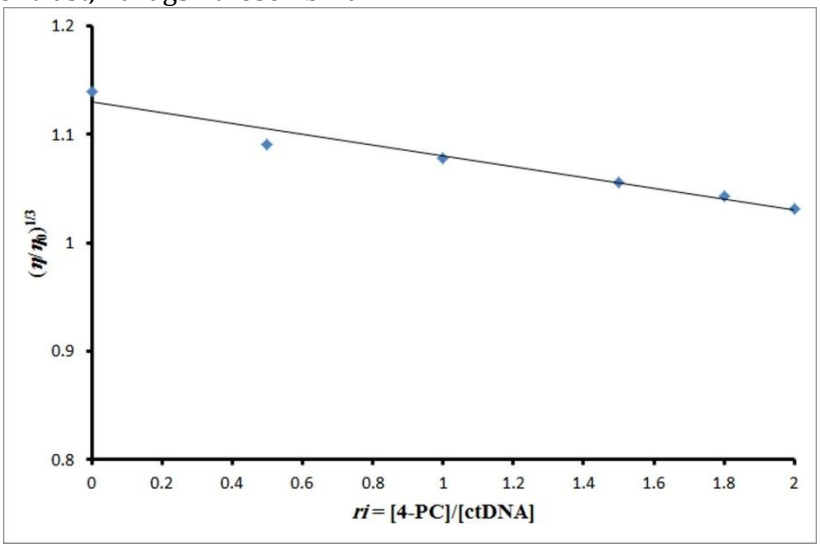


Fig. 5. Effect of increasing amounts of 4-PC on the viscosity of ctDNA (5×10^{-5}M) in Tris–HCl buffer. (*ri* = [4-PC]/[ctDNA] = 0.0, 0.5, 1, 1.5,1.8 and 2.3).

Conclusion

In this work, we explored the binding interaction of calf thymus DNA with a dihydropyrano[c] chromene derivative, 4-PC in physiological buffer by UV–vis, CD spectroscopy, viscosity measurement and MB dye as a fluorescence probe. Through the addition of DNA, the hyperchromicity of the UV–vis absorption spectra of 4-PC was observed, while the absorption intensity of DNA increased by successive addition of 4-PC solution and the intrinsic binding constant (K_b= 2.74 $\times 10^3$ M^{-1}) is similar to groove binders. In addition, competitive binding study with MB has revealed that 4-PC couldn't release MB dye, indicating that non-intercalation is a possible mode of its interaction with ctDNA. CD measurements in combination with DNA viscosity measurement revealed that an non-intercalative binding between ctDNA and 4-PC. We have recently reported the interaction of another dihydropyranochromene with ctDNA; this work is expected to provide greater insight into the interaction ofchromene derivatives with DNA.

Conflict of interest

There is no conflict of interest related to study from the authors.

References

[1] Kathiravan A, Renganathan R. Photoinduced interactions between colloidal TiO 2 nanoparticles and calf thymus-DNA. Polyhedron. 2009;28:1374-8.

[2] Rescifina A, Zagni C, Varrica MG, Pistarà V, Corsaro A. Recent advances in small organic molecules as DNA intercalating agents: Synthesis, activity, and modeling. European journal of medicinal chemistry. 2014;74:95-115.

[3] Cui F, Huo R, Hui G, Lv X, Jin J, Zhang G, et al. Study on the interaction between aglycon of daunorubicin and calf thymus DNA by spectroscopy. Journal of Molecular Structure. 2011;1001:104-10.

[4] Bera R, Sahoo BK, Ghosh KS, Dasgupta S. Studies on the interaction of isoxazolcurcumin with calf thymus DNA. International journal of biological macromolecules. 2008;42:14-21.

[5] Waring M. Variation of the supercoils in closed circular DNA by binding of antibiotics and drugs: evidence for molecular models involving intercalation. Journal of molecular biology. 1970;54:247-79.

[6] Chaires JB. A thermodynamic signature for drug–DNA binding mode. Archives of biochemistry and biophysics. 2006;453:26-31.

[7] Lu Y, Lv J, Zhang G, Wang G, Liu Q. Interaction of an anthracycline disaccharide with ctDNA: investigation by spectroscopic technique and modeling studies. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2010;75:1511-5.

[8] Emmadi NR, Atmakur K, Chityal GK, Pombala S, Nanubolu JB. Synthesis and cytotoxicity evaluation of highly functionalized pyranochromenes and pyranopyrans. Bioorganic & medicinal chemistry letters. 2012;22:7261-4.

[9] Parveen M, Malla AM, Yaseen Z, Ali A, Alam M. Synthesis, characterization, DNA-binding studies and acetylcholinesterase inhibition activity of new 3-formyl chromone derivatives. Journal of Photochemistry and Photobiology B: Biology. 2014;130:179-87.

[10] Abdolmohammadi S, Balalaie S. Novel and efficient catalysts for the one-pot synthesis of 3, 4-dihydropyrano [c] chromene derivatives in aqueous media. Tetrahedron Letters. 2007;48:3299-303.

[11] Khurana JM, Nand B, Saluja P. DBU: a highly efficient catalyst for one-pot synthesis of substituted 3, 4-dihydropyrano [3, 2-c] chromenes, dihydropyrano [4, 3-b] pyranes, 2-amino-4H-benzo [h] chromenes and 2-amino-4H benzo [g] chromenes in aqueous medium. Tetrahedron. 2010;66:5637-41.

[12] Dehghan G, Dolatabadi JEN, Jouyban A, Zeynali KA, Ahmadi SM, Kashanian S. Spectroscopic studies on the interaction of quercetin–terbium (III) complex with calf thymus DNA. DNA and cell biology. 2011;30:195-201.

[13] Shaghaghi M, Dehghan G, Jouyban A, Sistani P, Arvin M. Studies of interaction between terbium (III)-deferasirox and double helix DNA by spectral and electrochemical methods. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2014;120:467-72.

[14] Guo H, Cai C, Gong H, Chen X. Multi-spectroscopic method study the interaction of anti-inflammatory drug ketoprofen and calf thymus DNA and its analytical application. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2011;79:92-6.

[15] Arvin M, Dehghan G, Hosseinpourfeizi MA, Moosavi-Movahedi AA. Spectroscopic and electrochemical studies on the interaction of carmoisine food additive with native calf thymus DNA. Spectroscopy Letters. 2013;46:250-6.

[16] Shahabadi N, Fili SM, Kheirdoosh F. Study on the interaction of the drug mesalamine with calf thymus DNA using molecular docking and spectroscopic techniques. Journal of Photochemistry and Photobiology B: Biology. 2013;128:20-6.

[17] Ahmadi F, Alizadeh A, Bakhshandeh-Saraskanrood F, Jafari B, Khodadadian M. Experimental and computational approach to the rational monitoring of hydrogen-bonding interaction of 2-Imidazolidinethione with DNA and guanine. Food and chemical toxicology. 2010;48:29-36.

[18] Shahabadi N, Hadidi S. Spectroscopic studies on the interaction of calf thymus DNA with the drug levetiracetam. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2012;96:278-83.

[19] Dehkordi MF, Dehghan G, Mahdavi M, Feizi MAH. Multispectral studies of DNA binding, antioxidant and cytotoxic activities of a new pyranochromene derivative. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2015;145:353-9.

[20] Udenfriend S, Zaltzman P. Fluorescence characteristics of purines, pyrimidines, and their derivatives: measurement of guanine in nucleic acid hydrolyzates. Analytical biochemistry. 1962;3:49-59.

[21] Piétrement O, Pastré D, Landousy F, David M-O, Fusil S, Hamon L, et al. Studying the effect of a charged surface on the interaction of bleomycin with DNA using an atomic force microscope. European Biophysics Journal. 2005;34:200-7.
[22] Vardevanyan P, Antonyan A, Parsadanyan M, Shahinyan M, Hambardzumyan L. Mechanisms for binding between methylene blue and DNA. Journal of Applied Spectroscopy. 2013;80:595-9.
[23] Ahmadi SM, Dehghan G, Hosseinpourfeizi MA, Dolatabadi JEN, Kashanian S. Preparation, characterization, and DNA binding studies of water-soluble quercetin–molybdenum (VI) complex. DNA and cell biology. 2011;30:517-23.
[24] Nafisi S, Saboury AA, Keramat N, Neault J-F, Tajmir-Riahi H-A. Stability and structural features of DNA intercalation with ethidium bromide, acridine orange and methylene blue. Journal of Molecular Structure. 2007;827:35-43.
[25] Rohs R, Sklenar H. Methylene blue binding to DNA with alternating AT base sequence: minor groove binding is favored over intercalation. Journal of Biomolecular Structure and Dynamics. 2004;21:699-711.
[26] Lerman L. Structural considerations in the interaction of DNA and acridines. Journal of molecular biology. 1961;3:18IN3-30IN14.
[27] Shahabadi N, Falsafi M. Experimental and molecular docking studies on DNA binding interaction of adefovir dipivoxil: Advances toward treatment of hepatitis B virus infections. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2014;125:154-9.

