

Research Article



## Investigation on the Binding Mode of 3, 4-Dihydropyrano[c]Chromene Derivative with Double Strand DNA

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### Abstract

**Purpose:** The study on the interaction between small molecules and DNA has been very useful for investigating the structure and physical properties of DNA, elucidating the damage mechanism of DNA and significant in the design of new drugs targeted to DNA. This article describes an interaction of native calf thymus DNA (ctDNA) with a new 3, 4-dihydropyrano[c]chromene derivative, 2-amino-4-(4-chlorophenyl)-5-oxo-4H, 5H-pyrano-[3, 2-c] chromene-3-carbonitrile (4-CC) by using spectroscopic and viscometric techniques.

**Methods:** The interaction between 4-CC and ctDNA is realized from the UV absorption spectrophotometry, viscosimetry, circular dichroism and fluorescence spectroscopic techniques which shows that the successive interaction of 4-CC with ctDNA occurs.

**Results:** The experimental results revealed that 4-CC can interact with DNA through non-intercalative mode and the intrinsic binding constant ( $K_b$ ) for 4-CC with DNA was estimated to  $2.37 (\pm 0.001) \times 10^3 \text{ M}^{-1}$ . Methylene blue (MB) displacement studies revealed that 4-CC did not have any effect on MB bound DNA which is indicative of groove binding mode. Furthermore, 4-CC induces detectable changes in the CD spectrum of ctDNA as well as changes in its viscosity study corroborate the above experimental results.

**Conclusion:** These results further advance our knowledge on the molecular aspects on the interaction of 4-CC to nucleic acids.

### 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.<sup>1</sup> The study on the interaction between small molecules and DNA has been the focus of some recent researches in the scope of life science, chemistry, clinical medicine and genetics. These studies are very useful for investigating the structure and physical properties of DNA, elucidating the damage mechanism of DNA and significant in the design of new drugs targeted to DNA.<sup>2</sup> A variety of small molecules interacts reversibly with DNA, primarily through 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 drugs intercalate into the stacked base pairs of DNA.<sup>3</sup>

Dihydropyrano[c]chromenes and their derivatives have received considerable attention of synthetic and medicinal chemists because of their broad spectrum of biological and pharmaceutical activities.<sup>4</sup> These derivatives possess a wide range of biological properties, such as spasmolytic, diuretic, anticoagulant, anti-cancer, antibacterial, antiviral, antifungal and anti-anaphylactic activity.<sup>5,6</sup> 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.<sup>7</sup>

In this work, we have determined the interaction between a new pyranochromene, 2-amino-4-(4-chlorophenyl)-5-oxo-4H, 5H-pyrano-[3, 2-c] chromene-3-carbonitrile (4-CC), (Figure 1) and calf thymus DNA in simulated physiological buffer (pH 7.4) with the use of methylene blue (MB) dye as a fluorescence probe, electronic absorption, circular dichroism (CD) spectroscopy and viscosity measurement. These results should be helpful for understanding the interaction of 4-CC with nucleic acid at the molecular level and should be useful in the development of potential probes of

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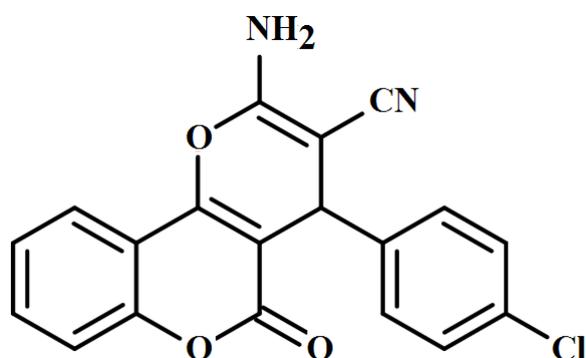
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DNA structure and conformation and the designing specific DNA-targeted drugs for some diseases.

## Materials and Methods

### Materials

Calf thymus DNA (ctDNA), Tris-HCl, dimethylsulfoxide (DMSO), methylene blue (MB) were purchased from Sigma-Aldrich. 4-CC prepared according to the previously described methods and its stock solution ( $3 \times 10^{-3} \text{ M}$ ) was prepared in absolute DMSO. ctDNA was dissolved in 10 mM Tris-HCl buffer at ambient temperature 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  $\epsilon = 6600 \text{ M}^{-1} \text{ cm}^{-1}$  at 260 nm.<sup>8</sup>



**Figure 1.** 2-amino-4-(4-chlorophenyl)-5-oxo-4H, 5H-pyrano-[3, 2-c] chromene-3-carbonitrile (4-CC).

### Methods

Absorbance spectra were recorded on a UV-visible spectrophotometer (T60, PG Instruments LTD., Leicestershire, UK) by using a cuvette of one cm path length. The absorption spectra of a DNA solution in the absence and presence of 4-CC was recorded for a constant DNA concentration and different concentrations of 4-CC. Moreover, the absorption spectra of 4-CC in the absence and presence of DNA was also recorded.

All fluorescence measurements were carried out with a JASCO spectrofluorimeter (FP6200) (Tokyo, Japan) by using a quartz cell of 1 cm path length. The competitive interaction between the methylene blue (MB), as fluorescence probes and 4-CC with ctDNA was carried out as follow: First, fixed amount of ctDNA and MB solution ( $4.9 \times 10^{-6}$  and  $5.6 \times 10^{-8} \text{ M}$ , respectively) was progressively titrated with 4-CC in the wavelength range of 660 to 720 nm with an excitation wavelength at 630 nm.

Viscosity measurements were made using a Ubbelohde-type viscometer (Julobo, MD-18 V, Germany) suspended vertically in a thermostat at 25 °C (accuracy  $\pm 1^\circ \text{C}$ ) by keeping DNA concentration constant and varying the concentrations of 4-CC. Flow time was measured with a digital stopwatch; the mean value of three replicated measurements was applied to evaluate the viscosity ( $\eta$ )

of the samples. The data are reported as  $(\eta/\eta_0)^{1/3}$  versus [compound]/[DNA] ratio, where  $\eta_0$  is the viscosity of the DNA solution alone.<sup>9</sup>

CD measurements were recorded on a JASCO (J-810) spectropolarimeter by keeping the concentration of DNA constant ( $5.0 \times 10^{-5} \text{ M}$ ) while varying 4-CC concentration from 0 to  $1.5 \times 10^{-5} \text{ M}$  ( $ri = [\text{compound}]/[\text{DNA}] = 0.0-0.3$ ).

## Results and Discussion

### Electronic absorption spectra

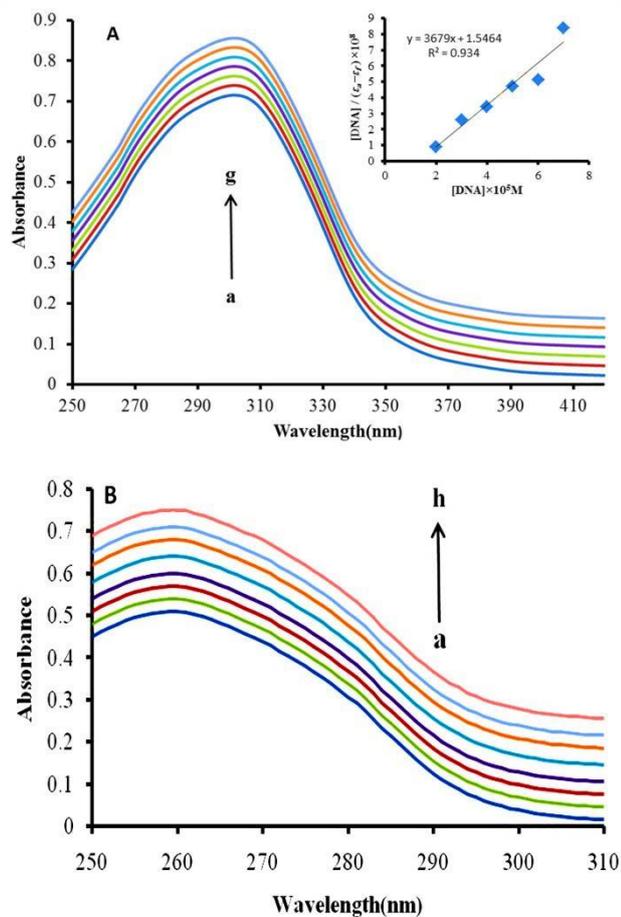
UV-vis absorption spectroscopy is often used to study the binding interactions of small molecules with DNA. In Figure 2a, the absorption spectrum of 4-CC (at a constant concentration) is shown in the absence and presence of ctDNA in Tris-HCl buffer solution. From the Figure 2a it is seen that with each addition of ctDNA to 4-CC solution, the entire absorption spectrum undergoes a hyperchromic effect without any noticeable spectral shift, which represents that the binding mode is not the intercalative binding and these changes show that the binding mode of 4-CC to DNA might be groove binding.<sup>10</sup> To confirm the mode of interaction, absorption spectra of ctDNA at constant concentration in the absence and presence of 4-CC are taken in Figure 2b. Absorption spectra of DNA increased upon increasing of 4-CC concentration, this is a typical hyperchromic effect indicate damage to the DNA double-helical structure after 4-CC binding and binding mode is non-intercalative.<sup>9</sup>

The intrinsic binding constant was calculated according to the equation<sup>11</sup>(1):

$$\frac{[\text{DNA}]}{\epsilon_a - \epsilon_f} = \frac{[\text{DNA}]}{\epsilon_b - \epsilon_f} + \frac{1}{Kb(\epsilon_b - \epsilon_f)}$$

Where [DNA] is the concentration of DNA,  $\epsilon_a$ ,  $\epsilon_f$  and  $\epsilon_b$  corresponded to the apparent extinction coefficient, the extinction coefficient for the free compound and its fully DNA-bound combination, respectively. From the plots of  $[\text{DNA}]/(\epsilon_a - \epsilon_f)$  vs. [DNA], the binding constant,  $Kb$ , was derived as the ratio of the slope to the intercept. The binding constant,  $Kb$ , for 4-CC was  $2.37 (\pm 0.001) \times 10^3 \text{ M}^{-1}$ . In comparing, the intrinsic binding constant,  $Kb$ , of 4-CC with some DNA groove binder agents, it was found that the binding mode between 4-CC and DNA was groove binding.<sup>12-14</sup>

We have recently reported the binding mode and intrinsic binding constant of another dihydropyranochromene derivative, 2-amino-4-(3-hydroxyphenyl)-5-oxo-4H, 5H-pyrano-[3, 2-c] chromene-3-carbonitrile (3-HC) with ctDNA.<sup>15</sup> It has been seen that 3-HC can bind to ctDNA through non-intercalative binding mode and the intrinsic binding constant ( $Kb$ ) of 3-HC was found to be  $(3.6 \times 10^3 \text{ M}^{-1})$  higher than 4-CC. 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.

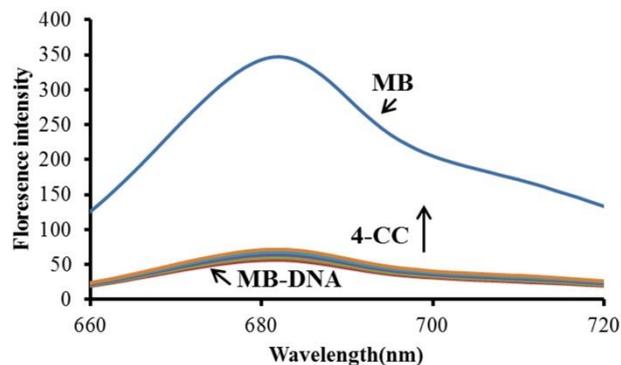


**Figure 2. Absorption spectra:** **a)** 4-CC ( $5.0 \times 10^{-5}$  M) in the absence and presence of increasing amounts of ctDNA. ( $ri = [\text{ctDNA}] / [\text{4-CC}] = 0.0, 0.4, 0.6, 0.8, 1.0, 1.2$  and  $1.4$ ); for curves *a-g*, respectively. Inset: plots of  $[\text{ctDNA}] / (\epsilon a - \epsilon_0)$  versus  $[\text{ctDNA}]$  for the titration of 4-CC with ctDNA. **b)** ctDNA ( $5.0 \times 10^{-5}$  M) in the absence and presence of increasing amounts of 4-CC. ( $ri = [\text{4-CC}] / [\text{ctDNA}] = 0.0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2$  and  $1.4$ ), for curves *a-h*, respectively.

#### Fluorescence studies (competitive studies with MB)

Fluorescence of 4-CC 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.<sup>16</sup> Acridine orange, ethidium bromide, methylene blue (MB), and similar fluorescent compounds are normally used to probe DNA structure in drug–DNA and protein–DNA interactions.<sup>17</sup> 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.<sup>18</sup> The emission spectra of the MB–DNA solution in the presence of the increasing 4-CC concentrations are shown in Figure 3. Fluorescence emission spectra of MB are significantly quenching by

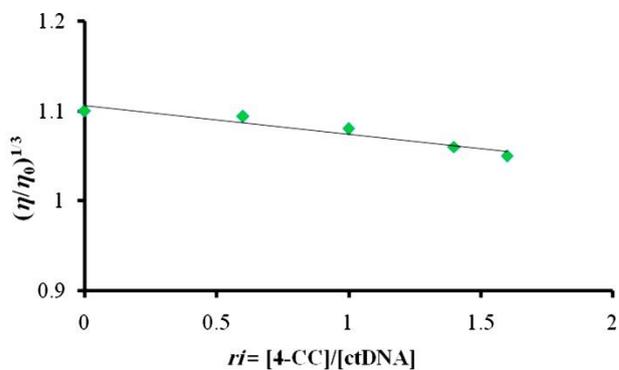
the addition of DNA. There is not any clear increase in the fluorescence intensity of the probe molecule upon adding 4-CC. These results show that MB molecules are not released from the DNA helix after addition of 4-CC and is an evidence for a non-intercalative mode of binding between 4-CC and DNA.



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

#### Viscosity measurements

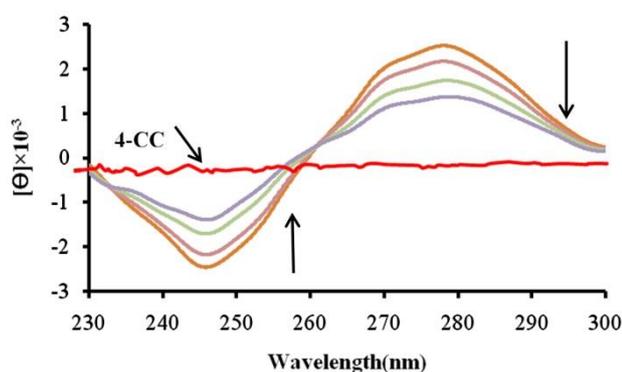
Viscosity experiment is an effective tool to decide the binding mode of small molecules and DNA. A classical intercalation binding demands the space of adjacent base pairs to be large enough to accommodate the bound ligand and to elongate the double helix, resulting in an increase of DNA viscosity.<sup>19</sup> 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.<sup>20</sup> The ctDNA viscosity experiments performed at  $25$  °C. Increasing amounts of 4-CC ( $ri = 0.0$ – $1.6$ ) were used to evaluate viscosity of ctDNA. The values of relative specific viscosity  $(\eta/\eta_0)^{1/3}$  versus  $ri$  ( $ri = [\text{compound}] / [\text{ctDNA}]$ ) were plotted (Figure 4). Little change on the viscosity of ctDNA showed that 4-CC bound to ctDNA by groove binding such as mesalamine and ketoprofen.<sup>10,15</sup>



**Figure 4.** Effect of increasing amounts of 4-CC on the viscosity of ctDNA ( $5 \times 10^{-5}$  M) in Tris–HCl buffer. ( $ri = [\text{4-CC}] / [\text{ctDNA}] = 0.0, 0.6, 1, 1.4$  and  $1.6$ ).

### Circular dichroism (CD) studies

Optical activity, as measured by CD absorption spectroscopy, is sensitive to local conformational changes in the secondary structure of DNA.<sup>17</sup> CD spectroscopy measures differences in the absorption of left-handed polarized light versus right-handed polarized light due to structural asymmetry. The CD spectrum of ctDNA exhibits a positive band at 275 nm because of base stacking and a negative band at 245 nm because of the helicity, which is characteristic of DNA in the right-handed B form<sup>17</sup> and 4-CC had no CD signal in the UV region of 230–300 nm. As shown in Figure 5, with increasing concentration of the added 4-CC, the intensities of both the positive and negative ellipticity bands were decreased. Therefore, it can be deduced that interaction of 4-CC with DNA induces certain conformational changes, such as the conversion from a more B-like to a more C-like structure with in the DNA molecule. These changes are suggestive of a non-intercalative mode of binding of 4-CC. This result is similar to the results of our previously reported data on interaction of 3-HC with ctDNA.<sup>14</sup>



**Figure 5.** CD spectra of ctDNA ( $5.0 \times 10^{-5}$  M) in Tris-HCl buffer, in the presence of increasing amounts of 4-CC. ( $r_i = [4\text{-CC}]/[\text{DNA}] = 0.0, 0.1, 0.2, \text{ and } 0.3$ ). The arrows show the CD spectra changes upon increasing 4-CC.  $[4\text{-CC}] = 1.5 \times 10^{-5}$  M (dashed line)

### Conclusion

In summary, the interaction of calf thymus DNA with a dihydropyrano[c]chromene derivative, 4-CC in physiological buffer (pH 7.4), using MB dye as a fluorescence probe, was investigated by UV-Vis absorption, CD spectroscopy coupled with viscosity measurement. With addition of DNA, the hyperchromicity of the UV-Vis absorption spectra of 4-CC was observed, while the absorption intensity of DNA increased by successive addition of 4-CC solution and the intrinsic binding constant ( $K_b = 2.37 (\pm 0.001) \times 10^3 \text{ M}^{-1}$ ) is similar to groove binders. In addition, competitive binding study with MB has revealed that 4-CC 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 a non-intercalative binding between ctDNA and 4-CC. The binding properties of drugs with DNA are greatly important in

understanding chemico-biological interactions for drug design, pharmacology and biochemistry. We have recently reported the interaction of another dihydropyranochromene with ctDNA; this work is expected to provide greater insight into the interaction of chromene derivatives with DNA.

### Ethical Issues

Not applicable.

### Conflict of Interest

The authors report no conflicts of interest.

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