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DNA-Binding and Photocleavage Studies of N,N'-Dibenzylidene-9H-Carbazole-3,6-Diamine

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Abstract: *The DNA-binding mode of **N,N'-Dibenzylidene-9H-Carbazole-3,6-Diamine** with CT-DNA was investigated by absorption spectroscopy, EB-DNA displacement, circular dichroism, thermal denaturation and viscosity measurements. Results indicated that these compounds intercalate into the base pairs of CT-DNA. The effect of ionic strength on the fluorescence property of the system indicated the presence of electrostatic interaction via phosphate backbone of DNA helix. The intrinsic binding constant values suggested that compound has DNA binding propensity. This compound promote the cleavage of plasmid pBR322. These results may be useful for the design of **N,N'-Dibenzylidene-9H-Carbazole-3,6-Diamine** with desired binding characteristics and useful to better understand the DNA binding mode of heterocyclic compound.*

Keywords: DNA binding, DNA cleavage, Absorption Spectroscopy, Fluorescence spectroscopy

I. INTRODUCTION

DNA is the molecular target of many anticancer drugs in clinical use and development. Small molecule inhibitors can interact with DNA via covalent or non-covalent interactions [1]. Their associative interactions with the DNA molecule can cause dramatic changes in the physiological functions of DNA [3-5]. Therefore, understanding the interactions of small molecules with DNA is of significance in the rational design of more powerful and selective anticancer agents [6-8].

Compounds containing carbazole core systems and their fused heterocyclic variants have attracted considerable attention from medicinal chemists because of their wide range of biological activities, such as anxiolytic, antibacterial/antifungal, antineoplastic, anticancer, DNA intercalator etc [9-11]. In this study we characterized the DNA binding mode of carbazole derivatives so as to gain better understanding of their interactions with the DNA molecule.

II. METERIALS AND METHODS

- **Material:** CT-DNA and other chemicals were purchased from sigma. The DNA concentration per nucleotide was determined by absorption spectroscopy using the molar absorption coefficient (6600 M^{-1} cm^{-1}) at 260 nm [12]. The DNA solution was stored for a short period of time at 4°C if not used immediately [13].
- **Methods:** The following spectrometric measurements were performed at 25°C in a quartz cuvette of 1 cm path length, and the sample solution was incubated for 10 min beforehand.
- **Electronic absorption titration:** Absorption titration was performed at a fixed compound concentration (10 µM) with various concentrations (0-100 µM) of CT-DNA. The absorption spectra were recorded on a Shimadzu UV-1800 spectrophotometer. The absorbance due to DNA at the measured wavelength was nullified.

2. Fluorescence Spectroscopic Studies

- **EB displacement measurement:** CT-DNA (10 µM) was pretreated with EB (10 µM) for 30 min at 25°C. Small aliquots of concentrated compound solution were added into the pretreated solution at various final concentrations (0-100 µM). The samples were excited at 480 nm and the emission spectra were observed between 550-750 nm on a Fluoromax-4 Spectrofluorometer. The slit width Ex/Em was 5 nm/5 nm.

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- **Effect of ionic strength:** CT-DNA (20 µM) was pretreated with compound (10 µM) for 30 min at 25°C. Small aliquots of a concentrated NaCl solution were added into the pretreated solution at various final concentrations (0-0.4 M). The corresponding fluorescence spectra were recorded by exciting the samples at 325 nm and the emission was observed between 400-625 nm. The slit width Ex/Em was 5 nm/5 nm.
- **Circular dichroism titration:** Circular dichroism (CD) spectra were recorded on a Jasco J-810 spectropolarimeter. CD titration was first performed at a fixed CT-DNA concentration (50 µM) with various concentrations (0-50 µM) of the compounds using the instrument parameters of 230-320 nm wavelength, scan speed 50 nm/min, 1 nm bandwidth, 100 millidegree sensitivity, and 1 s response time, with an average of three scans. From sample, the buffer and compound background were subtracted.
- **Viscosity experiment:** Viscosity measurements were carried out using an Ubbelodhe viscometer, immersed in a thermostatic water-bath that maintained at a constant temperature at 25.0 ±0.1°C. DNA samples approximately 200 base pairs in average length were prepared by sonication of CT-DNA in order to minimize complexities arising from DNA flexibility [14]. The compounds (1–80 µM) were titrated with the DNA solution (100 µM). The flow time of each sample was measured by a digital stopwatch for three times, and the average result was considered.
- **Thermal denaturation studies:** Thermal denaturation studies were carried out with a Perkin-Elmer Lambda 35 spectrophotometer equipped with a Peltier temperature-controlling programmer (±0.1°C). The absorbance at 260 nm was continuously monitored for solutions of CT-DNA (30 µM) in the absence and presence of the compound (10 µM). The temperature of the solution was increased by 1°C/min. Data were presented as A/A_0 versus temperature, where A_0, A are the initial and observed absorbance at 260 nm, respectively.
- **DNA cleavage study:** For the gel electrophoresis experiments, supercoiled pBR322 DNA (0.2 µg, 33 µM) was treated with compound in Tris–HCl buffer and the solution were then irradiated at room temperature with a UV lamp (365 nm, 10 W). A loading buffer containing 25% bromophenol blue, 30% glycerol was added and electrophoresis was carried out at 70 V for 90 min in Tris–acetic acid–EDTA buffer using 0.8% agarose gel containing 1.0 µg/mL EB and photographed under UV light.

III. RESULTS AND DISCUSSION

The **N,N'-Dibenzylidene-9H-Carbazole-3,6-Diamine** was (Fig. 1) was chosen for the DNA binding mode studies, which was purchased from sigma.

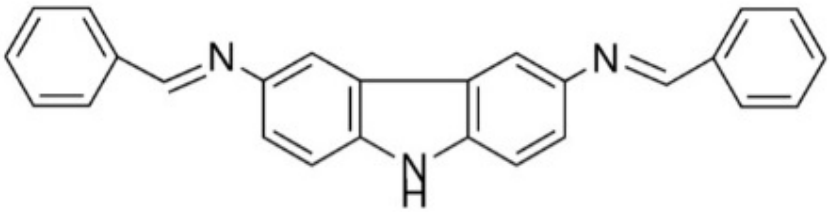

Fig. 1. Schematic structure of compounds

Electronic Absorption Titration. The electronic absorption spectroscopy is one of the most useful techniques in DNA-binding studies [15]. Fig. 2 shows the absorption spectra of compound in absence and presence of increasing amounts of CT-DNA.

Addition of increasing amounts of CT-DNA results in hypochromic and bathochromic shifts, these spectral characteristics suggest that the compounds mostly bind to DNA by intercalation [16]. From the absorption titration data, the intrinsic binding constant K_b was determined using Wolfe–Shimer equation [17]:

$$[DNA]/ (\varepsilon_a - \varepsilon_f) = [DNA]/ (\varepsilon_b - \varepsilon_f) + 1/K_b (\varepsilon_b - \varepsilon_f)$$

Where ε_a, ε_f and ε_b correspond to A_{obsd}/[compound], the extinction coefficient of the free compound and the extinction coefficient of the compound in the fully bound form, respectively. A plot of $[DNA]/ (\varepsilon_a - \varepsilon_f)$ versus [DNA] gives the K_b as the ratio of the slope to the intercept. Intrinsic binding constants K_b of 1.68×10^4 M^{-1} were measured for compounds

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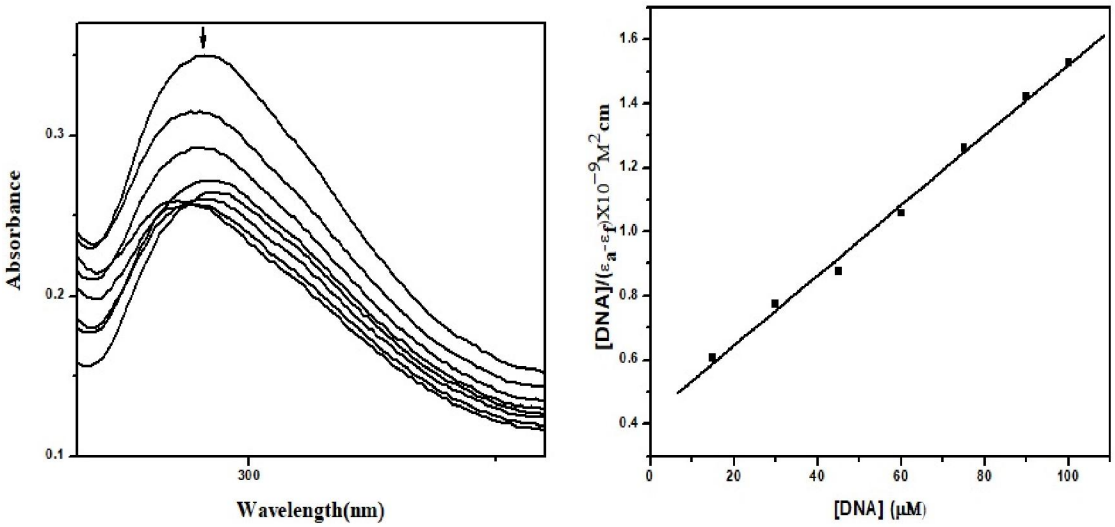

Fig. 2. Absorption spectra of compound (10 μM) in the absence and presence of increasing amounts of CT-DNA (0-100 μM) at 25 °C in 50 mM NaCl and 5 mM Tris-HCl buffer (pH 7.2). The arrow shows the absorbance changing upon increasing the DNA concentration. Inset shows the plot of [DNA]/ (ε_a-ε_f) vs [DNA] for the titration of DNA to the compounds.

Fluorescence Spectroscopic Studies

EB displacement measurement. The molecular fluorophore, EB, is known to show intense fluorescence light when bound to DNA, due to its strong intercalation between the adjacent DNA base pairs. The emission spectra of EB bound to DNA in the absence and presence of compound is shown in Fig. 3**.**

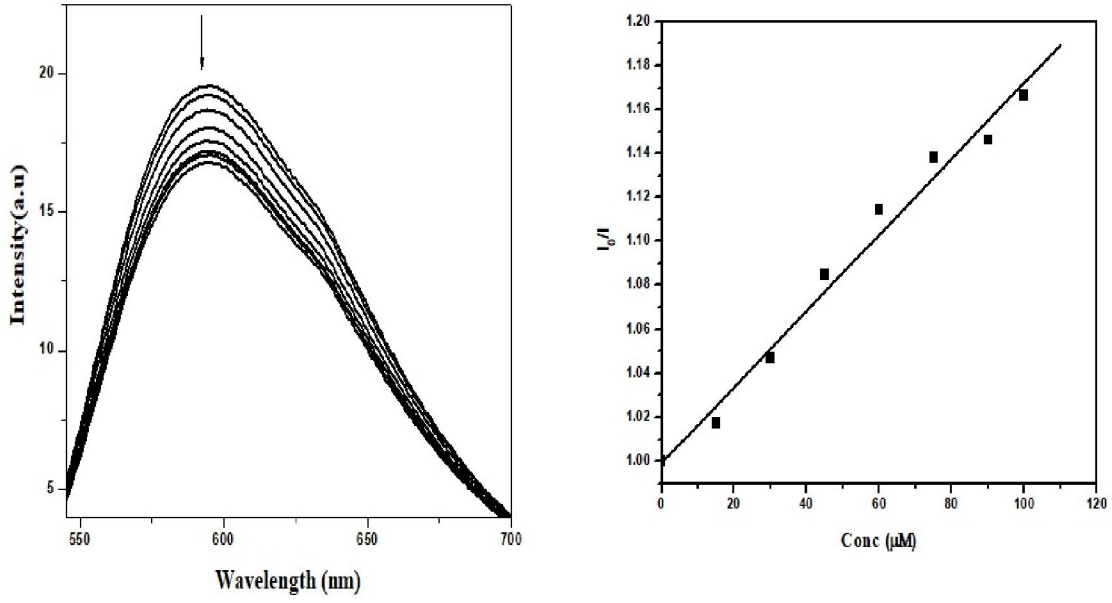

Fig. 3. Emission spectrum of EB bound to DNA in the presence of compound at 25 °C **(**[EB] = 10 μM, [DNA] = 10 μM, [compound] = 0-100 μM, ex=480 nm). The arrow shows the intensity change upon increasing compound concentrations. **d.** Plot of I_0/I vs. [Compound] for the titration of the compounds to DNA-EB system

The quenching of EB bound to DNA by the compounds is in agreement with the classical Stern–Volmer equation:

$$I_o/I = 1 + K_{sv}[Q],$$

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Linear Stern–Volmer quenching constant (K_{sv}) of 1.62×10^3 M^{-1} were determined for compounds.

Effect of ionic strength. Monitoring the spectral changes with different ionic strength is an efficient method for distinguishing the binding modes between small molecules and CT-DNA [18, 19]. The effect of ionic strength on compound–DNA binding was studied (Fig. 4).

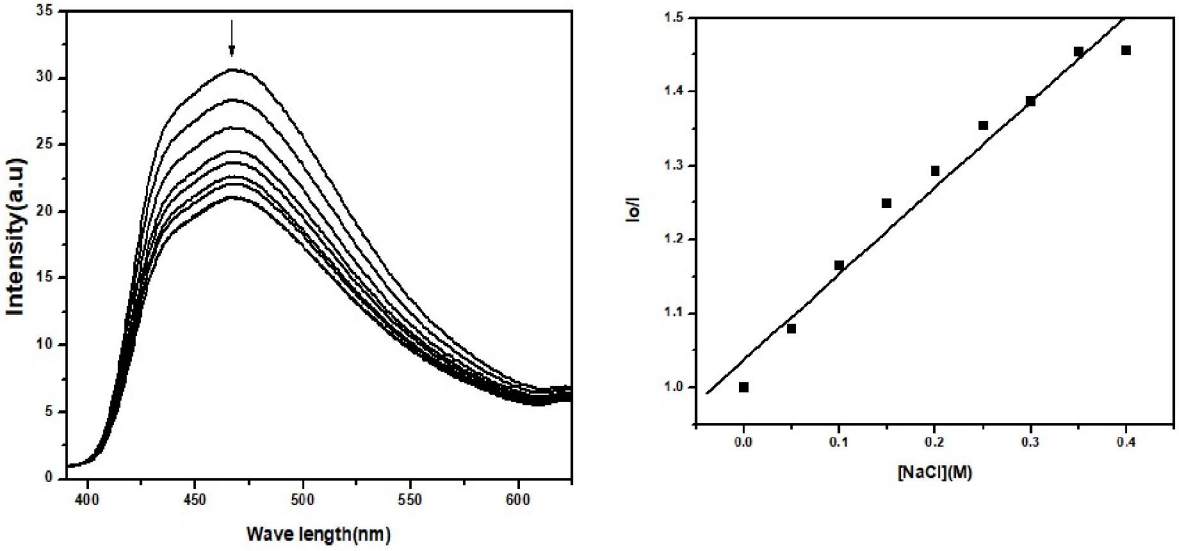

Fig. 4. Emission spectrum of compound bound to DNA in the presence of NaCl **(**[compound] = 10 µM, [DNA] = 20 µM, [NaCl] = 0-0.4 M, ex=325 nm) at 25 °C. The arrow shows the intensity change upon increasing NaCl concentrations. **d.** Plots of emission intensity I_0 / I vs [NaCl].

Linear Stern–Volmer quenching constant (K_{sv}) of 0.817×10^3 M^{-1} were determined for compounds. This result displayed a strong dependence of fluorescence intensity on ionic strength. This result indicated that the mode of binding of compounds is partially electrostatic via DNA phosphate backbone. This electrostatic interaction may be stabilizing the DNA-compound complex.

Circular Dichroism Titration. On the addition of compound to a solution of the CT-DNA, significant changes in its CD spectrum were observed (Fig. 5). The negative band shifted to higher wavelength, where as the positive signal disappeared gradually, such changes are likely to result from structural alterations induced by the compound upon binding to CT-DNA.

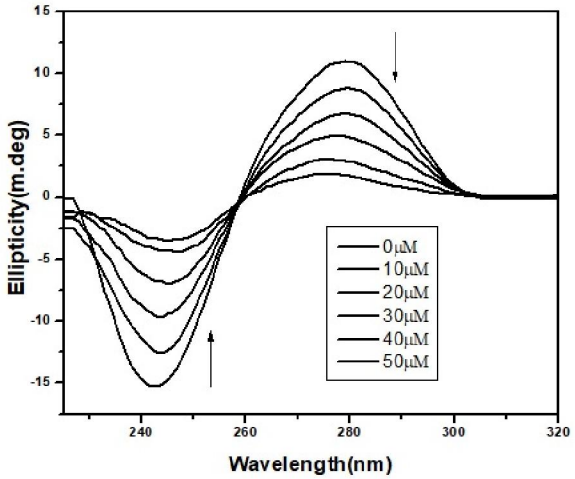

Fig. 5. CD spectra of CT-DNA (50 µM) titrated with compound [0-50 µM] at 25 °C over the wavelength range 230-320 nm.

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Viscosity experiment. To throw further light on the DNA binding mode, viscosity measurements were carried out for compounds The effect of compounds on the CT-DNA Data are presented as $(\eta/\eta_0)^{1/3}$ vs. binding ratio where η and η_0 are the viscosity of DNA in the presence and absence of complex, respectively (Fig. 6).

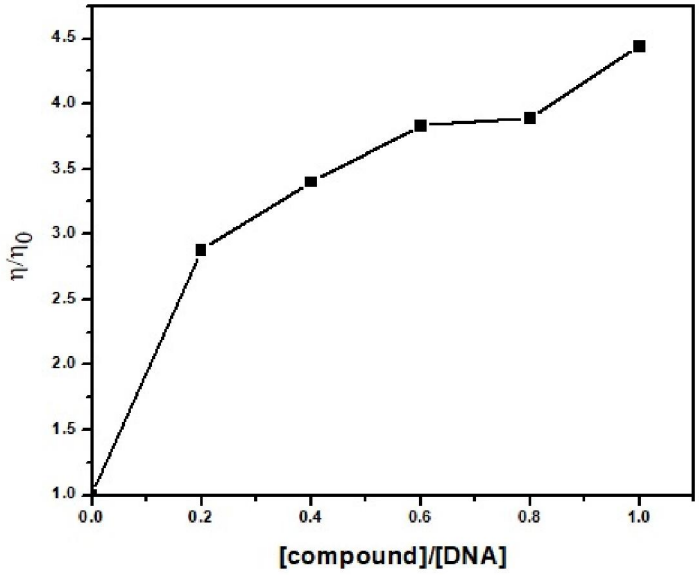

Fig. 6. Effect of increasing amounts of compound on the relative viscosity of CT-DNA at 25.0 ± 0.1 °C. [DNA] = 100 μM, r = [compound]/ [DNA] = 0-0.8.

The increased degree of viscosity may depend on the affinity of the compounds to DNA. These results suggest that the compounds intercalate between the base pairs of DNA, the difference in binding strength of the compounds is caused by the structural modification in molecule [20].

Thermal Denaturation Studies. The thermal denaturation experiment carried out for DNA in the absence of the compound revealed a T_m of 76°C under our experimental conditions. However, with the addition of compound, the T_m of the DNA increased to 76.8°C for compound **2** and (Fig. 7). These results further confirm the order of binding strength of these compounds [21, 22]

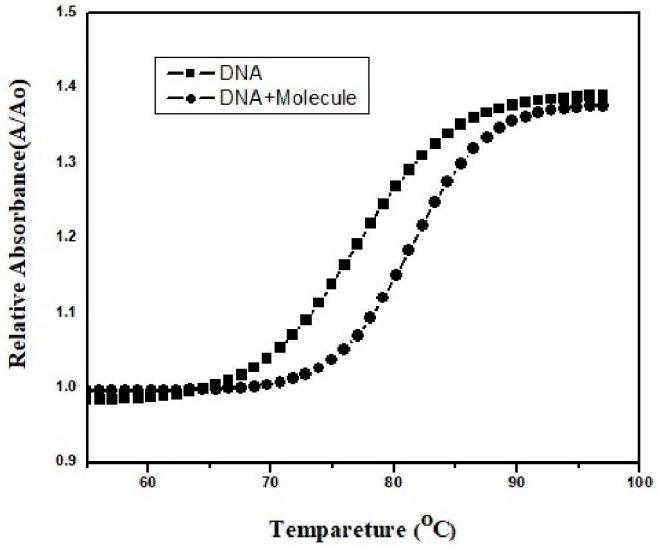

Fig. 7. Melting temperature curves of DNA in the absence and presence of compound [compound] = 10 μM and [DNA] = 30 μM.

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Cleavage of plasmid pBR322 The photo-cleavage of pBR322 DNA in the absence and presence of three compounds were carried out as shown in Fig. 8.

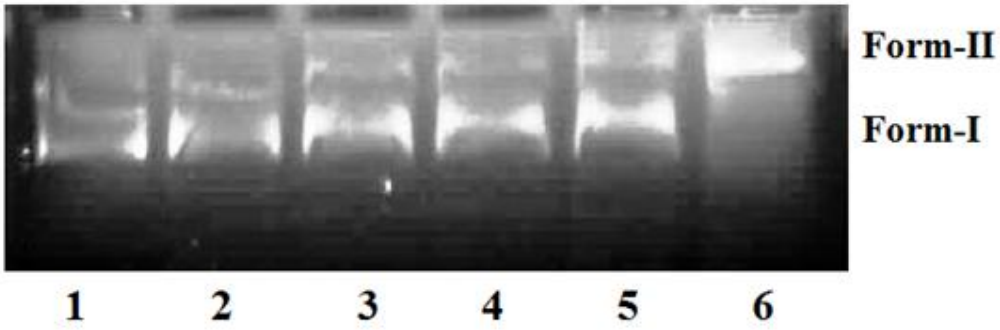

Fig. 8. Photo activated cleavage of supercoiled plasmid DNA, pBR322 [0.2 µg, 33µM] by increasing the concentration of compound. Lane 1: DNA control; Lane 2: DNA + 10 µM; Lane 3: DNA + 20 µM; Lane 4: DNA + 30 µM; Lane 5: DNA + 40 µM; Lane 6: DNA + 50 µM.

Almost no DNA cleavage was observed for the control, in which compound was absent (lane 0). With increasing concentrations of the three compounds (lanes 1–5), the amount of form I of pBR322 DNA diminishes gradually, whereas form II increases. In summary, three compounds are able to perform an efficient cleavage of pBR322 DNA [23]. All results were obtained from experiments that were performed at least in triplicate.

IV. CONCLUSION

In conclusion, we tried to understand the biological activity of the compound N,N'-Dibenzylidene-9H-Carbazole-3,6-Diamine by using Different spectral analysis and its DNA cleavage study. The examined compound showed DNA binding and cleavage properties. These results may be useful for the design of N,N'-Dibenzylidene-9H-Carbazole-3,6-Diamine with desired binding characteristics and useful to better understand the DNA binding mode of heterocyclic compound.

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