J Fluoresc (2009) 19:1045–1051
DOI 10.1007/s10895-009-0504-9

ORIGINAL PAPER

# Fluorescence Interaction and Determination of Calf Thymus DNA with Two Ethidium Derivatives

**Nuriye Akbay · Zeynel Seferoğlu · Elmas Gök**

Received: 15 January 2009 / Accepted: 5 June 2009 / Published online: 26 June 2009


**Abstract** In this paper, we reported the syntheses and investigation of the modes of binding to DNA of the two new ethidium derivatives containing benzoyl and phenylacetyl groups of both amines at 3-and 8-positions. The interactions between calf thymus DNA (*ct*-DNA) and the two derivatives, 3,8-dibenzoylamino-5-ethyl-6-phenylphenantridinium cloride *(E2)* and 3,8-diphenylacetylamino-5-ethyl-6-phenylphenantridinium chloride *(E3),* were investigated by fluorescence quenching spectra and UV-vis absorption spectra. The Stern-Volmer quenching constants, binding constants, binding sites and the corresponding thermodynamic parameters $\Delta H$, $\Delta S$ and $\Delta G$ were calculated at different temperatures. The results indicated the formation of *E2* and *E3*-DNA complexes and van der Waals interactions as the predominant intermolecular forces in stabilizing for each complex. In addition, increasing nucleophilicity of the functional groups at 3- and 8- positions exhibited the respectable increment the DNA binding affinities of derivatives. The results of absorption, ionic strength and iodide ion quenching suggested that the interaction mode of *E2* and *E3* with *ct*-DNA was intercalative binding. The limit of detection (LOD) of *ct*-DNA were $7.49\times10^{-8}$ ($n$=4) and $4.18\times10^{-8}$ mol/l ($n$=7) in presence of *E2* and *E3*, respectively.

N. Akbay · E. Gök (✉)
Department of Chemistry, Hacettepe University,
06800 Beytepe,
Ankara, Turkey
e-mail: gokoglu@hacettepe.edu.tr

Z. Seferoğlu
Department of Chemistry, Gazi University,
06500 Ankara, Turkey

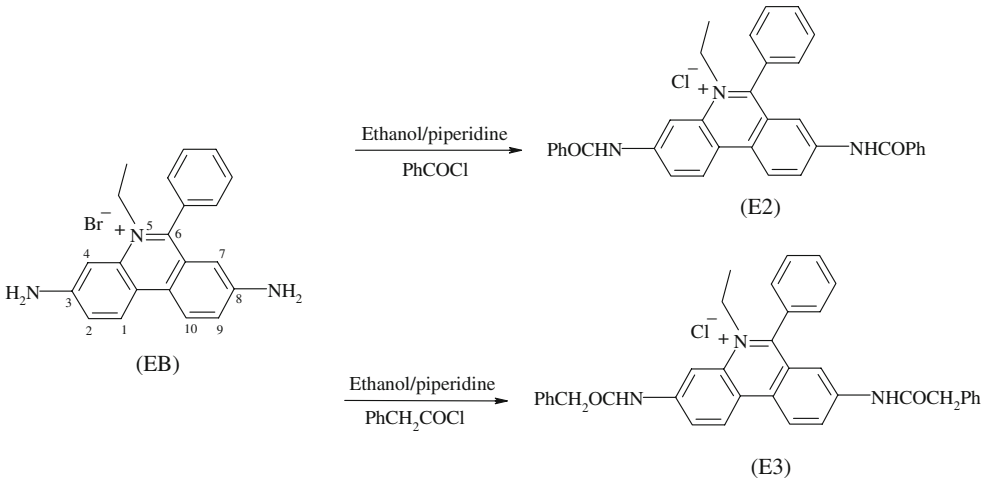
**Keywords** Calf thymus DNA · Fluorescence quenching · Stern-Volmer · Intercalation

## Introduction

The interaction studies between fluorescent dyes and DNA are significant for the desing new probes and the quantitative detection of nucleic acids. Many fluorescence assays have been reported for DNA measurement by using of fluorescent dyes [1–3], metal ions and metal complexes [4, 5] that exhibit fluorescence quenching [6, 7] or enhancement in the presence of DNA. The possible interaction models between these small molecules and DNA generally include the electrostatic or surface, the groove and the intercalation binding. Ethidium bromide (EB) is used as major intercelation agent for DNA in many years [8–10]. It has been widely utilized in biochemical and biophysical applications as a fluorescent stain. In literature, novel ethidium derivatives have been proposed as DNA probes and compared their DNA affinity and toxicity with EB. They have been synthesized via amines at 3- and 8-positions or 6-position of phenyl ring [11, 12]. 5-substitued ethidium derivatives have also been synthesized recently [13].

In the present work, the two new ethidium derivatives, *E2* and *E3*, were synthesized by binding benzoyl and phenylacetyl groups to the both amines at the 3- and 8-positions of EB (Scheme 1). They were characterized by IR, UV, MS, $^1$H-NMR and elemental analysis. The interactions of *ct*-DNA with *E2* and *E3* were investigated by fluorescence quenching method. The probable fluorescence quenching mechanisms of *E2* and *E3* by *ct*-DNA were studied by means of Stern-Volmer modeling. This

**Scheme 1** Syntheses of *E2* and *E3* from EB




study also focused on effect of differences of functional groups at 3- and 8- positions on ct-DNA binding affinities of derivatives. The experimental results showed that both derivatives occured complex formation with *ct*-DNA. In addition, the binding properties of *ct*-DNA-derivative complexes were investigated based UV-vis absorption spectra, ionic strength, iodide ion quenching and thermodynamic parameters.

## Experimental

Syntheses and characterizations of *E2* and *E3*

*3,8-dibenzoylamino-5-ethyl-6-phenylphenantridinium cloride, (E2)*

EB (197 mg, 0.5 mmol) was dissolved in mixture of absolute ethanol (30 ml) and piperidine (5 ml). The solution was cooled in salt/ice bath and followed by dropwise addition of benzoyl chloride (PhCOCl, 5 mmol, 0.4 ml) in 5 ml ethanol under constant stirring. The reaction mixture was heated at reflux on a steam bath for 1 h. and then stirred at room temperature for 4 h. The residue was filtered, washed with water and then ether. The pure product was obtained on crystallization from ethanol gave as a yellow solid. Yield: 237 mg (91 %), m.p: 306–308°C. *Rf*: 0.61 (40% Methanol/Ethyl acetate). *$^1$H NMR* (400 MHz, DMSO, δ): 11.11 (b, -$N_3$H), 10.85 (b, -$N_8$H), 9.22 (m, 2H), 9.18 (d, 1H), 8.70 (dd, 1H), 8.55 (dd, 1H), 8.20 (s, 1H), 8.09 (d, 2H), 7.91 (d, 2H), 7.83 (m, 5H), 7.68 (d, 1H), 7.60 (m, 3H), 7.50 (m, 2H), 4.70 (2H, q, -N-$\underline{CH_2}$-$CH_3$), 1.58 (3H, t, -N-$CH_2$-$\underline{CH_3}$). *IR* (KBr, $cm^{-1}$) 3408 (-NH), 3052 (aromatic C-H), 2951 (aliphatic C-H), 1657 (C=O, amide), 1599 (C=N), 1543 (C=C). MS (m/z, 100 ev): 522.2 (100.0 %) $(M-35.5)^+$; Anal. Calcd. for $C_{35}H_{28}N_3O_2Cl$: C, 75.25; H, 5.01; N, 7.52. Found: C, 75.16; H, 4.98; N, 7.61.

*3,8-diphenylacetylamino-5-ethyl-6-phenylphenantridinium chloride, (E3)*

EB (197 mg, 0.5 mmol) was dissolved in mixture of absolute ethanol (30 ml) and piperidine (5 ml). The solution was cooled in salt/ice bath and followed by dropwise addition of phenylacetyl chloride (5 mmol, 0.5 ml) in 5 ml ethanol under constant stirring. The reaction mixture was heated at reflux on a steam bath for 1 h. and then stirred at room temperature 3 h. The residue was filtered, washed with water and then ether. The pure product was obtained on crystallization from ethanol gave as a yellow solid. Yield: 266 mg (87 %), m.p: 276–278°C. *Rf*: 0.57 (40% Methanol/Ethyl acetate). *$^1$H NMR* (400 MHz, DMSO, δ): 11.20 (b, -$N_3$H), 10.80 (b, -$N_8$H), 9.13 (d, 1H), 9.10 (d, 1H), 9.06 (d, 1H), 8.42 (dd, 1H), 8.20 (dd, 1H), 8.0 (m,

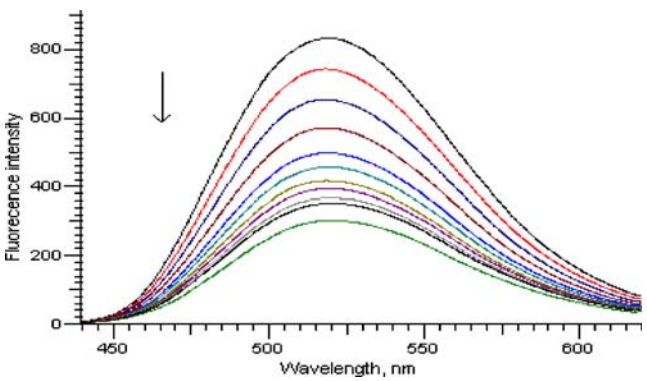



**Fig. 1** Fluorescence emission spectra of $5.0\times10^{-6}$M *E2* in the presence of *ct*-DNA. Conditions: $\lambda_{ex}$=313 nm, the concentration of *ct*-DNA from highest curve to lowest is 0, 1.5, 3.0, 4.5, 6.0, 7.5, 9.0, 10.5, 12, 13, 16.5, 19.5 ($\times10^{-7}$) mol/l

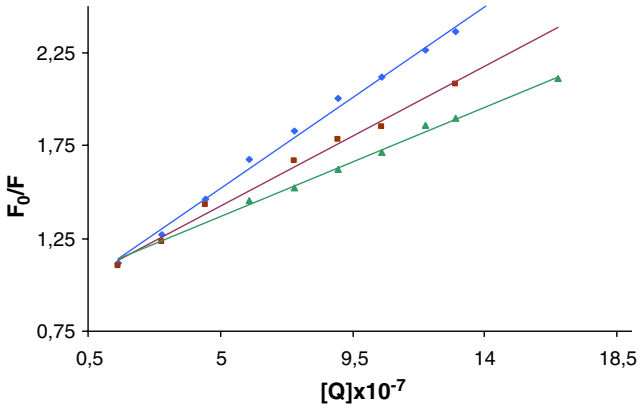



**Fig. 2** The Stern-Volmer curves for the interaction of *E2* and ct-DNA at 20°C, 30°C, 37°C (from high to low). [*E2*]=5.0×10$^{-6}$mol/l. $\lambda_{ex}$ / $\lambda_{em}$=313/519 nm

1H), 7.78 (m, 5H), 7.40–7.30 (m, 10H) 4.60 (2H, q, -N-$\underline{CH_2}$-$CH_3$), 3.83 (s, 2H), 3.64 (s, 2H), 1.50 (3H, t, -N-$CH_2$-$\underline{CH_3}$). *IR* (KBr, cm$^{-1}$) 3454 (-NH), 3032 (aromatic C-H), 2949 (aliphatic C-H), 1676 (C=O, amide), 1627 (C=N), 1599 (C=C). MS (m/z, 100 ev) 550.2 (100.0 %) (M-35.5)$^{+}$; Anal. Calcd. for $C_{37}H_{32}N_3O_2Cl$: C, 75.74; H, 5.46; N, 7.17. Found: C, 75.62; H, 5.52; N, 7.07.

## Reagents

Ethidium bromide (*3,8-diamino-5-ethyl-6-phenylphenantridinium bromide)* was purchased from Sigma. The stock solutions of *E2* and *E3* were prepared in DMSO as 2.0×10$^{-3}$mol/l. The stock solution of calf thymus deoxyribonucleic acid (*ct*-DNA, double stranded, type I, sodium salt, Sigma) were prepared in Tris-HCl buffer solution at pH 8.3 and stored in refrigerator at 4°C until used. DNA concentrations were determined by absorption spectroscopy using the molar coefficient (M$^{-1}$cm$^{-1}$) of 6,600 at the

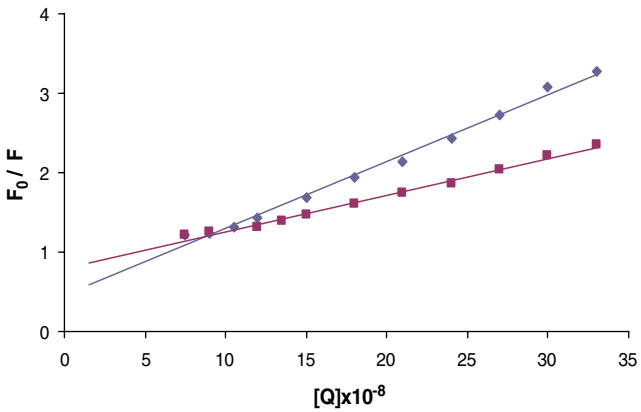



**Fig. 3** The Stern-Volmer curves for the interaction of *E3* and ct-DNA at 20 and 37°C (from high to low). [*E3*]=5.0×10$^{-6}$mol/l. $\lambda_{ex}$ /$\lambda_{em}$ = 304/517 nm

**Table 1** *Stern-Volmer* data for *E2*-DNA system

| T (°C) | Linear regression equation | $K_{sv}$ (l/mol) | $R^2$ |
|---|---|---|---|
| 20 | $F_o/F = 0.9802 + 1.082 \times 10^6[Q]$ | $1.082\times10^6$ | 0.9905 |
| 30 | $F_o/F = 1.005 + 8.380 \times 10^5[Q]$ | $8.380\times10^5$ | 0.9934 |
| 37 | $F_o/F = 1.045 + 6.520 \times 10^5[Q]$ | $6.520\times10^5$ | 0.9934 |

wavelength of 260 nm. Purity of DNA was checked by monitoring the ratio of the absorbance at 260 to that at 280 nm. The solution gave a ratio of $A_{260}/A_{280} > 1.8$, indicating that DNA was sufficiently free from protein. The all aqueous solutions used in the experiments were prepared daily from the stock solutions by appropriate dilution in doubly distilled water.

## Apparatus

Fluorescence measurements were carried out on a Hitachi F-4500 spectrofluorimeter equipped with a 150 W xenon lamp source and quartz cells of 1 cm path length. The slit widhs were 2.5 nm/2.5 nm, PMT voltage was kept at 700 V and scan speed was 20 nms$^{-1}$. All absorption spectra were performed on a *Shimadzu UV1700 (PharmaSpec) UV-Vis* spectrophotometer equipped with quartz cells. IR spectra were recorded with Mattson 1000 FTIR spectrometer with KBr. Proton NMR spectra were obtained with *Bruker 400 MHz Ultra Shielded NMR* spectrometer. Mass spectra were recorded with Agilent 1100 MSD instrument. Elements analyses were recorded on Elementar Analysensysteme GmbH (varioMICRO CHNS). pH measurements were carried out a *NeoMet* (pH-220 L) pHmeter.

## Procedure

Fluorescence spectra and intensities of *E2* and *E3* were measured at $\lambda_{ex}/\lambda_{em}$=313/519 nm and $\lambda_{ex}/\lambda_{em}$=304/517 nm in Tris HCl buffer at pH 8.3, respectively. Fluorescence titrations were performed in a 1 cm quartz cuvette by successive addition *ct*-DNA (1.5–20×10$^{-7}$mol/l) to solutions of 5.0×10$^{-6}$M *E2* and *E3* in buffer. Their fluorescence intensities decreased regularly with increase of concentration of *ct*-DNA. The titration data analyzed according to Stern-Volmer equation and investigated of interaction types of *ct*-DNA with the two derivatives.

**Table 2** *Stern-Volmer* data for *E3*-DNA system

| T (°C) | Linear regression equation | $K_{sv}$ (l/mol) | $R^2$ |
|---|---|---|---|
| 20 | $F_o/F = 0.4568 + 8.440 \times 10^6[Q]$ | $8.440\times10^6$ | 0.9935 |
| 37 | $F_o/F = 0.8025 + 4.590 \times 10^6[Q]$ | $4.590\times10^6$ | 0.9902 |

**Table 3** Binding constant K and the number of binding sites n for *E2*-DNA

| T (°C) | Double logarithm equation | K (l/mol) | n | $R^2$ |
|---|---|---|---|---|
| 20 | $\log(F_0 - F)/F = \log 6.6936 + 1.1112 \log [Q]$ | $4.939 \times 10^6$ | 1.1112 | 0.9938 |
| 30 | $\log(F_0 - F)/F = \log 6.0329 + 1.0193 \log [Q]$ | $1.079 \times 10^6$ | 1.0193 | 0.9660 |
| 37 | $\log(F_0 - F)/F = \log 5.3225 + 0.9134 \log [Q]$ | $2.101 \times 10^5$ | 0.9134 | 0.9919 |

## Results and discussion

To characterize the interaction types of the two derivatives with DNA, the fluorescence spectra of *E2* and *E3* with the presence and absence of *ct*-DNA had been studied at given conditions (see Fig. 1 for *E2*). In the interaction of *E3* with *ct*-DNA (its figure was not given), almost same effect was observed with *E2*. The results showed that the fluorescence of derivatives could be quenched by *ct*-DNA, and increasing the concentration of *ct*-DNA resulted in a gradual decrease in fluorescence intensities of *E2* and *E3*.

### Stern-Volmer quenching and binding parameters investigation

According to the Stern-Volmer equation, the quenching nature between DNA and the two derivatives can be analyzed [14]

$$F_0/F = 1 + K_{sv}[Q]$$

where, $F_0$ and F are the steady-state fluorescence intensities of *E2* and *E3* before and after the addition of *ct*-DNA, respectively, [Q] concentration of *ct*-DNA as quencher. $K_{sv}$ is the Stern-Volmer dynamic quenching constant. Linear Stern-Volmer plots were obtained from the fluorescence titrations under three different temperatures for *E2* (Fig. 2) and two different temperatures for *E3* (Fig. 3). Ksv for the interaction with *E2* and *E3* and *ct*-DNA were found from slopes of these graphs. The results showed that Ksv decreased with increased temperature, suggesting that the probable quenching mechanism of fluorescence of two derivatives by DNA was static quenching procedure. The corresponding Stern-Volmer quenching constants are shown in Tables 1 and 2.

The binding constants have been calculated by using the double logarithm regression curve of $\log(F_0–F) / F$ versus $\log[Q]$ based on the following equation [15],

$$\log(F_0 - F)/F = \log K + n \log[Q]$$

where *K* is the binding constant and site number n can be determined by the slope of double logarithm regression curve based on the equation. Tables 3 and 4 give the calculated K and n for *E2* and *E3*-DNA. It was found that the binding constants decreased with the increasing of temperature, resulting in a reduction of the stabilities of *E2* and *E3*-DNA complexes. But comparing the binding constants and binding site numbers of two groups, *E3* has more DNA binding affinity than *E2*. It can be seen that this property increases with increasing of the nucleophilic characters of functional groups.

### Interaction mechanism of *E2* and *E3* with *ct*-DNA

We proposed a series of following studies that characterize and distinguish interactions of *E2* and *E3* with *ct*-DNA in terms of surface binding, intercalation and groove binding. Steady-state queching experiments using iodide ion as quencher may provide further information about the binding of probe molecule with DNA. The groove binders are influenced from the solvent surrounding of helix much more than intercalators. In the presence of anionic quencher, base pairs below and above the intercalator would hinder the accessibility of fluorescent probe to quencher. In addition electrostatic repelling between DNA phosphate backbone and anionic quenchers will support the protection of intercalated species [16]. When KI solution was added in the presence of *ct*-DNA particular quenching was observed in *E2*-DNA system (Fig. 4). But increasing KI concentration didn't change the quenching amount. The quenching by the addition KI at the beginning was thought as quenching effect of KI on free probe. *E3*-DNA system showed the same behavior. These are consistent with intercalation rather than groove binding into the DNA helix.

The effect of the ionic strength on the binding modes between the two derivatives and *ct*-DNA was also studied with different concentrations of NaCl ranged from 0.063 to 10 mM. The addition of NaCl, in the presence of DNA, had no effect on the fluorescence of the both derivatives. These results can be explained by the protection of fluorescent probes from solvent surrounding, and indicate that the

**Table 4** Binding constant K and the number of binding sites n for *E3*-DNA

| T (°C) | Double logarithm equation | K (l/mol) | n | $R^2$ |
|---|---|---|---|---|
| 20 | $\log(F_0 - F)/F = \log 11.228 + 1.6732 \log [Q]$ | $1.690 \times 10^{11}$ | 1.6732 | 0.9943 |
| 37 | $\log(F_0 - F)/F = \log 8.7197 + 1.3267 \log [Q]$ | $5.240 \times 10^8$ | 1.3267 | 0.9940 |

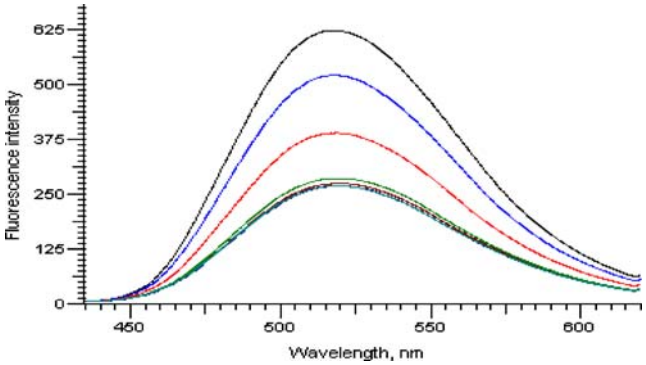



**Fig. 4** Fluorescence emission spectra of *E2*-DNA system in the presence of KI. **a** *E2* (*black line*), **b** *E2* in the presence of 1 mM KI (*blue line*), **c** *E2*-DNA system (*red line*), d *E2*-DNA system in the presence of 0.0125-1 mM KI

interaction between derivatives and ct-DNA was not surface-binding mode [17].

Figure 5 shows the absorption spectra of *E2* and *E3* in the presence of increasing amounts of *ct*-DNA to decrease in the peak intensities. Intercalation binding commonly results in hypochromic and bathochromic effect of the transition of the intercalated chromophore; generally external groove binding causes a spectral shift an a hyperchromic effect [18]. It can be seen from Fig. 5 that on addition of *ct*-DNA to *E2* and *E3* a hypochromicity was observed without any band shift. Therefore, the results indicated that it could be the indication of intercalative binding of the two derivatives between the base pairs of DNA.

## Thermodynamic parameters and nature of the binding forces

The interaction between a small molecule and biomolecule may involve hydrogen bond, van der Waals force, electro static force, hydrofobic interaction force, and so on. The thermodynamic parameters (ΔG, ΔH and ΔS) are very important for confirming binding modes of them. The log K (binding constant) values obtained at three different temperatures (20, 30 and 37°C) for *E2*-DNA system are plotted against the reciprocal of temperature (Fig. 6) according to the van't Hoff Eq. 1.

$$\log K = -\Delta H/2.303\,RT + \Delta S/2.303R \quad (1)$$

The values of ΔH and ΔS were obtained from the slope and interscept of linear van't Hoff plot. The following formulas (2, 3) were used for *E3*-DNA system at two different temperatures (20°C and 37°C).

$$\ln K_2/K_1 = \Delta H/R[1/T_1 - 1/T_2] \quad (2)$$

$$\Delta G = \Delta H - T\Delta S = -RT\ln K \quad (3)$$

$K_1$ and $K_2$ are binding constants at 20°C and 37°C, respectively. Thermodynamic parameters for the interactions of *E2* and *E3* with DNA are calculated and listed in Table 5. The neative values of ΔG reveal that the interaction processes are spontaneous.

The distinct variations in binding enthalpies are evident for the functional groups at 3- and 8- positions

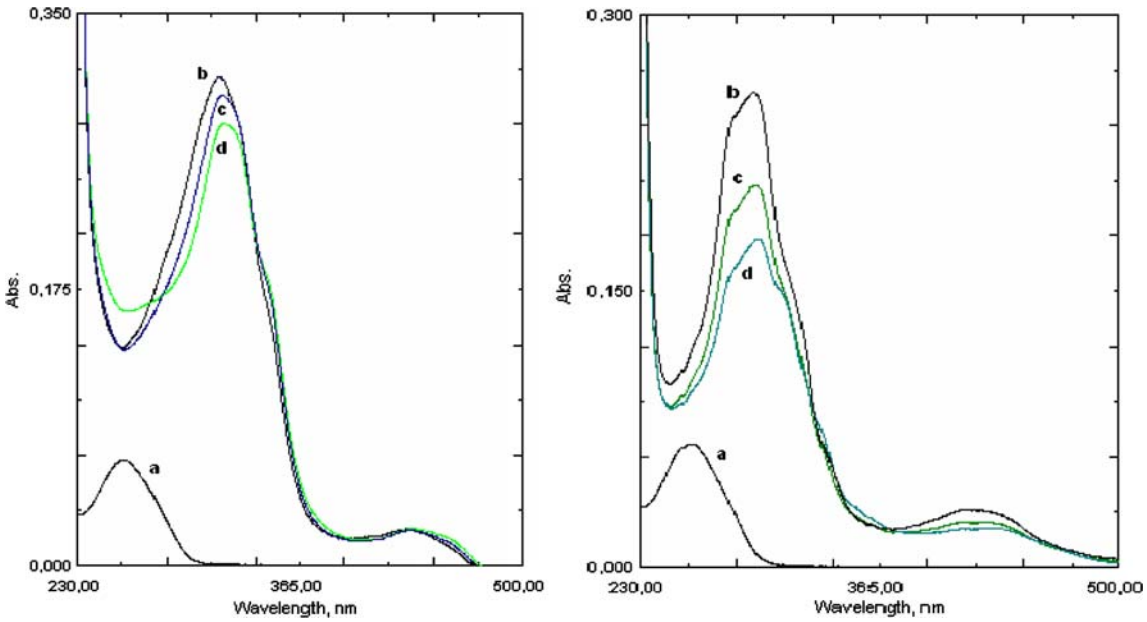



**Fig. 5** Absorption spectra of **a** $1.2\times10^{-5}$ mol/l DNA, **b** $5.0\times10^{-6}$ mol/l *E2* (*left*) and *E3* (*right*) **c** addition $6.0\times10^{-7}$ mol/l DNA **d** addition $30\times10^{-7}$ mol/l DNA

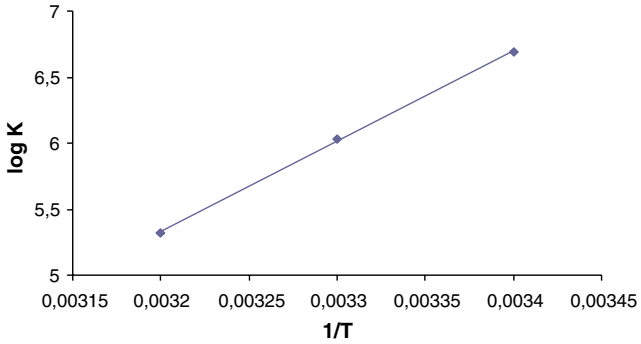



**Fig. 6** log K versus 1/T for *E2*-DNA system

of ethidium compounds are playing major role in the DNA binding interactions of ethidium derivatives. Modification of ethidium bromide from 3- and 8- amino positions changes the characteristic behaviour of EB against to DNA from enhancement to quenching. In our study we also examined the effect of similar functional groups on binding affinity of ethidium compounds. We observed that the using phenylacetyl instead of benzoyl as functional group largely increased the affinity of compound to DNA. One can be concluded that being far away of aromatic ring from carbonyl group may increase the contribution of delocalized electrons of aromatic ring system to affinity of DNA. Negative values of $\Delta H$ and $\Delta S$ mark the van der Waals interactions and hydrogen bonds in the binding mechanism of molecules to DNA [19]. Because of the incapability of forming hydrogen bonds of aromatic ring systems, we eliminate the contribution of hydrogen bonds to negative values of $\Delta H$ and $\Delta S$ in these ethidium derivatives. We proposed the van der Waals interactions as only interaction type of the our derivatives with DNA.

Calibration graphs and determination of DNA

The fluorescence quenching of *E2* and *E3* had a good relationship with the concentration of DNA. The calibration graphs were used for the determination of DNA in the presence of *E2* and *E3* under the experimental conditions above described. Calibration graphs were plotted by the changes in emission intensities against *ct*-DNA concentrations. The linear equations were $F_0/F = 0.9802 + 1.082 \times 10^6[Q]$ with $R^2=0.9905$ ($n=4$) for *E2* and $F_0/F = 0.4568 + 8.440 \times 10^6[Q]$ with $R^2=0.9935$ ($n=7$) for *E3* at 20°C. The detection and quantification limits of DNA were calculated as $3Sb/m$ and $10Sb/m$. *Sb* is the standard deviation of the intercept and *m* is the slope of the calibration graph [20]. The analytical results are given in Table 6. From the dynamic range and detection limit for *ct*-DNA, it can be seen that *E3* has more sensitive for this method.

**Table 5** The thermodynamic parameters of the binding *E2* and *E3* to DNA

| Derivatives | ΔH (kJ/mol) | ΔG (kJ/mol) | TΔS (kJ/mol) |
|---|---|---|---|
| E2 | −131.3 | −36.54 | −93.22 |
| E3 | −256.8 | −62.98 | −193.95 |

Values were calculated from data at 20°C

**Table 6** The analytical results of *ct*-DNA determination in presence *E2* and *E3*

| | E2 | E3 |
|---|---|---|
| Dynamic range of DNA (mol/l) | $1.50 - 16.5 \times 10^{-7}$ | $7.50 - 33.0 \times 10^{-8}$ |
| *Sb* standard deviation of the intercept | 0.027 | 0.118 |
| Limit of detection (LOD) (mol/l) | $7.49\times10^{-8}$ | $4.18\times10^{-8}$ |
| Limit of quantification (LOQ) (mol/l) | $2.50\times10^{-7}$ | $1.39\times10^{-7}$ |

## Conclusion

In this paper, the interactions of the two new ethidium derivatives, *E2* and *E3*, with *ct*-DNA were studied by fluorescence and UV-vis absorption spectroscopies. Their fluorescence intensities quenched by *ct*-DNA with static quenching mechanism through formation of *E2* and *E3*- *ct*-DNA complexes. Also influences of binding similar functional groups to the both amines of ethidium at 3- and 8- positions on DNA binding affinities of derivatives were investigated and considerable increment was observed it by increasing of the nucleophilic character of functional groups. The binding constants, the number of binding sites and basic thermodynamic parameters were calculated at different temperatures. The results indicated that van der Waals interactions were the predominant intermolecular forces in stabilizing for each complex. The results of absorption, ionic strength and iodide ion quenching suggested that the interaction mode of *E2* and *E3* with *ct*-DNA was intercalative binding. The limit of detection (LOD) of *ct*-DNA were $7.49\times10^{-8}$ ($n=4$) and $4.18\times10^{-8}$mol/l ($n=7$) in presence of *E2* and *E3*, respectively. Hence, the study had a great significance to explain biological activities and nucleic acid binding properties of *E2* and *E3*.

**Acknowledgements** This work was supported by the Scientific and Technical Research Council of Turkey (TUBITAK ) (project No TBAG-107 T391).

## References


1. Cao Y, He X, Gao Z, Peng L (1999) Fluorescence energy transfer between acridine orange and safranine T and its application in the determination of DNA. Talanta 49:377–383. doi:10.1016/S0039-9140(98)00386-5
2. Zhu Q-Z, Yang H-H, Li D-H, Xu J-G (1999) Determination of nucleic acids using phosphin 3R as a fluorescence probe. Anal Chim Acta 394:177–184. doi:10.1016/S0003-2670(99)00309-8
3. Li J, Wei Y-X, Wei Y-L, Dong C (2007) Study of the spectral behavior of four flıorescent dyes and their interactions with nucleic acid by the luminescence method. J Lumin 124:143–150. doi:10.1016/j.jlumin.2006.02.012
4. Wang Z-M, Lin H-K, Zhu S-R, Liu T-F, Chen Y-T (2002) Spectroscopy, cytotoxicity and DNA-binding of the lanthanum (III) complex of an L-valine derivative of 1, 10-phenanthroline. J Inorg Biochem 89:97–106. doi:10.1016/S0162-0134(01)00395-6
5. Wang Z-M, Lin H-K, Zhou Z-F, Xu M, Liu T-F, Zhu S-R, Chen Y-T (2001) Copper(II) complexes with N, Nı-Dialkyl-1, 10-phenanthroline-2, 9-dimethanamine: Synthesis, characterization, DNA-binding thermodynamical and kinetic studies. Bioorg Med Chem 9:2849–2855. doi:10.1016/S0968-0896(01)00193-6
6. Lianos P, Georghiou S (1979) Complex formation between pyrene and the nucleotides GMP, CMP, TMP and AMP. Photochem Photobiol 29:13–21. doi:10.1111/j.1751-1097.1979.tb09253.x
7. Guan Y, Zhou W, Yao X, Zhao M, Li Y (2006) Determination of nucleic acids based on the fluorescence quenching of Hoechst 33258 at pH4.5. Anal Chim Acta 570:21–28. doi:10.1016/j.aca.2006.03.106
8. Alonso A, Almendral MJ, Curto Y, Criado JJ, Rodriguez E, Manzano JL (2006) Determination of the DNA-binding characteristics of ethidium bromide, proflavine, and cisplatin by flow injection analysis: Usefulness in studies on antitumor drugs. Anal Biochem 355:157–164. doi:10.1016/j.ab.2006.06.004
9. Labieniec M, Gabryelak T (2006) Interactions of tannic acid and its derivatives (ellagic and gallic acid) with calf thymus DNA and bovine serum albumin using spectroscopic method. J Photochem Photobiol B Biol 82:72–78. doi:10.1016/j.jphotobiol.2005.09.005
10. Quiao C, Bi S, Sun Y, Song D, Zhang H, Zhou W (2008) Study of interactions of anthraquinones with DNA using ethidium bromide as a fluorescence probe. Spectrochim Acta A Mol Biomol Spectrosc 70(1):136–143. doi:10.1016/j.saa.2007.07.038
11. Garbett NC, Hammond NB, Graves DE (2004) Influence of the amino substituents in the interaction of ethidium bromide with DNA. Biophys J 87:3954–3981. doi:10.1529/biophysj.104.047415
12. Luedtke NW, Liu Q, Tor Y (2003) Synthesis, photophysical properties, and nucleic acid binding of phenanthridinium derivatives based on ethidium. Bioorg Med Chem 11:5235–5247. doi:10.1016/j.bmc.2003.08.006
13. Rangarajan S, Friedman SH (2007) Design, synthesis, and evaluation of phenanthridine derivatives targeting the telomerase RNA/DNA heteroduplex. Bioorg Med Chem Lett 17:2267–2273. doi:10.1016/j.bmcl.2007.01.070
14. Lakowicz JR (2006) Principles of fluorescence spectroscopy, 3rd edn. Springer, New York
15. Sun Y, Bi S, Song D, Qiao C, Mu D, Zhang H (2008) Study on the interaction mechanism between DNA and the main active components in *Scutellaria baicalensis* Georgi. Sens Actuators B Chem 129:799–810. doi:10.1016/j.snb.2007.09.082
16. Qui B, Guo L, Wang W, Chen G (2007) Synthesis of a novel fluorescent probe useful for DNA detection. Biosens Bioelectron 22:2629–2635. doi:10.1016/j.bios.2006.10.036
17. Hu Z, Tong C (2007) Synchronous fluorescence determination of DNA based on the interaction between methylene blue and DNA. Anal Chim Acta 587(2):187–193. doi:10.1016/j.aca.2007.01.050
18. Li JF, Dong C (2009) Study on the interaction of morphine chloride with deoxyribonucleic acid by fluorescence method. Spectrochim Acta A Mol Biomol Spectrosc 71:1938–1943. doi:10.1016/j.saa.2008.07.033
19. Ross PD, Subramanian S (1981) Thermodynamics of protein association reactions: Forces contributing to stability. Biochemistry 20:3096–3102. doi:10.1021/bi00514a017
20. Deepa S, Mishra AK (2005) Fluorescence spectroscopic study of serum albumin-bromadiolone interaction: Fluorimetric determination of bromadiolone. J Pharm Biomed Anal 38:556–563. doi:10.1016/j.jpba.2005.01.023