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Cytotoxicity and DNA binding property of triphenylethylene–coumarin hybrids with two amino side chains

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ABSTRACT

Novel triphenylethylene–coumarin hybrids containing two amino side chains were designed and synthesized. Some of these 3,4-diphenyl coumarins, **7b–c** (the double chains at 4-position on 3-,4-phenyl, respectively), and **13b–f** (the double chains at 4-position on 3-phenyl and 7-position, respectively), showed a broad-spectrum and good anti-proliferative activity against five tumor cells and low cytotoxicity in osteoblast. UV–vis, fluorescence, and circular dichroism (CD) spectroscopies and thermal denaturation exhibited that compounds **7b** (R = piperidinyl), **7e** (R = NEt_2), and **7f** (R = 4-methylpiperazinyl) had significant interactions with Ct-DNA by the intercalative mode of binding. Structure activity relationships (SARs) analysis suggested that the location of the two amino alkyl chains would play an important role both in the compounds against tumor cells proliferation and their interactions with DNA.

Coumarins are a wide group of naturally occurring compounds and have a variety of biological activities such as anti-cancer, anti-HIV, antimicrobial, and anti-inflammatory.[1] They represent a significant source of inspiration for the new drugs discovery.[2] As the anti-tumor agents, they can act on the cancer formation by interfering with the cellular proliferation.[1a,b,3] They can block cell cycle,[4] induce cell apoptosis,[5] modulate estrogen receptor (ER),[6] or inhibit the DNA-associated enzymes, such as topoisomerase or gryase.[7] Due to their potential applications in cancer therapy, extensive studies have been carried out on the design and synthesis of coumarin derivatives with improved anticancer activity.[8] Among them, coupling coumarin with different bioactive molecules is one of the effective ways. Adopting this approach, chalcone- pyrazole-, and stilbene-commarin hybrids (compounds **A**, **B**, **C** in Fig. 1)[2,8a,c] have exhibited significant anti-tumor activities.

Recently,[9] we have also found that triphenylethylene-coumarin hybrids (compound **D** in Fig. 1) containing indispensable two amino (except morpholinyl) side chains showed a broad-spectrum and excellent anti-tumor activity possibly by acting on DNA via the intercalative mode. The positions of the side chains were randomly chosen and the amount of them had important impact on both their anti-tumor activity and DNA binding property. So far only the 3,4-diphenyl coumarin SP500263, a structural resemblance to the triphenylethylene–coumarin hybrids, was studied as the new selective estrogen receptor (SERM).[6] In order to shed more light on the structure–activity relationships (SARs) and demonstrate how the positions of the two side chains affect their anti-proliferative activity, we herein report the design and synthesis of the novel triphenylethylene–coumarin hybrids with two amino side chains at different positions based on our previous helpful results.[9] The interactions of the compounds with Ct-DNA were also tested by UV–vis, fluorescence, and circular dichroism (CD) spectroscopies and thermal denaturation experiment.

Since the compounds containing three amino side chains at 4-position on 3-,4-phenyl and 7-position, respectively, had been explored to exhibit weak anti-tumor activity but strong DNA

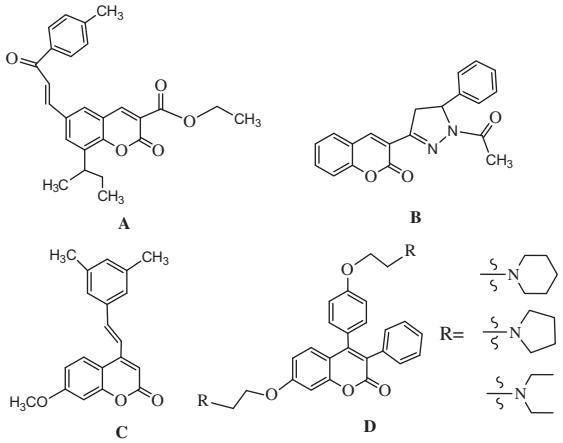

Figure 1. The hybrid derivatives of coumarin.

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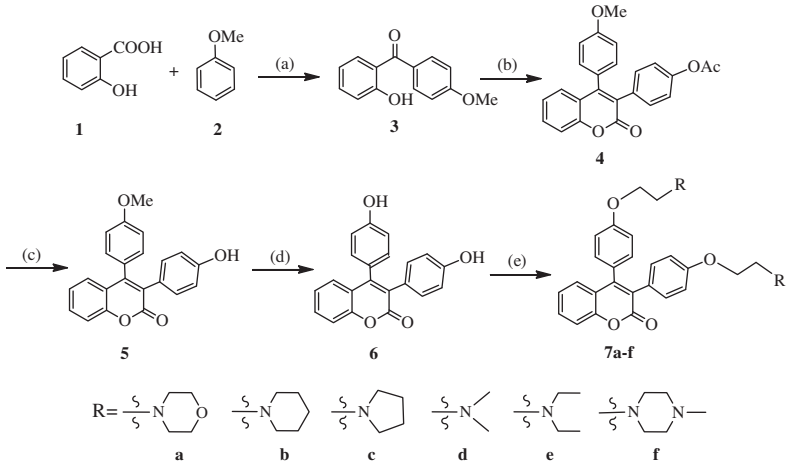
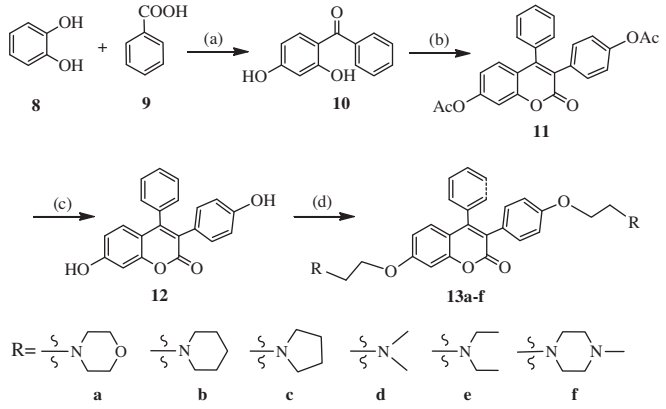


Scheme 1. Synthesis of compounds **7a–f**. Reagents and conditions: (a) $POCl_3$, $ZnCl_2$, 65 °C, 2.5 h, 45%; (b) 4-hydroxyphenylacetic acid, Ac_2O, K_2CO_3, 130 °C, 5 h, 50%; (c) 3 N HCl, ethanol, reflux, 2 h, 85%; (d) BBr_3, CH_2Cl_2, ice bath, 1 h, 80%; (e) $ClCH_2CH_2R$, dry acetone, K_2CO_3, KI, 65 °C, 8 h, 21–73%.



Scheme 2. Synthesis of compounds **13a–13f**. Reagents and conditions: (a) $POCl_3$, $ZnCl_2$, 65 °C, 3 h, 30%; (b) 4-hydroxyphenylacetic acid, Ac_2O, K_2CO_3, 135 °C, 5 h, 67%; (c) 3 N HCl, ethanol, reflux, 2 h, 96%; (d) $ClCH_2CH_2R$, dry acetone, K_2CO_3, KI, 65 °C, 8 h, 22–71%.

binding property,[9] except for the reported two positions in compound **D** (Fig. 1), the corresponding other two positions of the chains were chosen for this study (compound **7** in Scheme 1 and **13** in Scheme 2).

Synthesis of the 3,4-diphenyl coumarins (**7a–f**) containing two amino alkyl chains at 4-position on 3-,4-phenyl, respectively, was achieved according to the previous procedures[6,9] as outlined in Scheme 1. Briefly, commercially available 2-hydroxybenzoic acid (**1**) reacted with anisole (**2**) to yield the benzophenone (**3**) under Fries reaction condition. The treatment of **3** with 4-hydroxyphenyl acetic acid mainly gave the acylated 3,4-diaryl coumarin derivative (**4**) via Perkin reaction route. The deprotection of acetyl group was performed in 3 N HCl to achieve **5**, and then methyl group was removed in BBr_3 solution to obtain **6**. The S_N2 nucleophilic substitutions of **6** with a variety of 2-chloroethanamine provided the final products (**7a–f**). Following the same procedures, the target products (**13a–f**) containing two amino alkyl chains at 4-position on 3-phenyl and 7-position, respectively, were synthesized by using 1,2-benzenediol and benzoic acid as the starting materials (Scheme 2).

The structures of all the newly compounds were determined by NMR, MS, and elemental analysis. Both analytical and spectral data of compounds are in agreement with the proposed structures.

Compounds **7a–f** and **13a–f** were subjected to anti-proliferative tests against the following cancer cell lines, Hela (cervical carcinoma), A549 (human lung cancer), K562 (chronic myeloid leukemia), and MCF-7 (human breast cancer). Cisplatin was used as positive controls. As shown in Table 1, compounds **13b–f** and **7b–c** exhibited significant anti-proliferative activity against four cancer cells at IC_{50} of near 10 μM, and better than the positive control except **7c** against MCF-7. It seemed that these compounds exhibited a broad-spectrum anti-proliferative activity. Compounds

Table 1
Cytotoxicity of compounds against the cancer cell lines

Compds	IC_{50} (μM)			
	Hela	A549	K562	MCF-7
7a	>100[a]	>100	>100	>100
7b	4.36 ± 0.46	7.28 ± 0.84	5.44 ± 0.51	4.29 ± 0.30
7c	8.25 ± 0.94	8.79 ± 1.17	7.54 ± 1.99	16.65 ± 1.22
7d	>100	>100	99.56 ± 9.48	71.59 ± 12.48
7e	45.24 ± 6.62	46.31 ± 5.01	56.25 ± 12.49	50.13 ± 4.70
7f	60.52 ± 8.65	55.61 ± 7.34	53.25 ± 9.40	78.33 ± 26.40
13a	>100	>100	>100	>100
13b	5.08 ± 0.56	5.21 ± 0.62	4.90 ± 0.22	6.53 ± 0.59
13c	4.70 ± 0.44	7.35 ± 0.58	5.46 ± 0.88	6.63 ± 0.54
13d	8.49 ± 0.75	9.13 ± 0.82	7.19 ± 1.01	6.99 ± 0.42
13e	9.58 ± 1.72	8.97 ± 0.98	2.73 ± 0.55	5.95 ± 0.60
13f	3.68 ± 0.39	4.70 ± 0.73	3.23 ± 0.38	3.47 ± 0.51
Cisplatin	10.82 ± 1.01	9.21 ± 1.22	8.00 ± 0.71	7.93 ± 0.67

[a] No activity.

Table 2
Cytotoxicity of compounds **7b–c** and **13b–c** towards osteoblast[a]

Compds	% Cell cytotoxicity				
	1 μM	10 μM	20 μM	40 μM	100 μM
7b	−3.2 ± 2.2[b]	1.6 ± 2.1*	10.6 ± 0.9*	22.3 ± 0.6*	40.3 ± 0.2***
7c	0.1 ± 2.4*	−0.5 ± 2.9	8.4 ± 1.1	20.4 ± 1.5*	39.5 ± 0.4**
13b	−6.5 ± 3.1	3.7 ± 1.1	4.9 ± 1.3*	18.6 ± 0.5*	33.4 ± 0.7**
13c	−2.5 ± 2.8	−5.6 ± 2.4*	5.2 ± 0.8**	16.4 ± 0.7*	32.8 ± 0.6*
13d	−0.6 ± 3.1	−0.2 ± 3.4	4.3 ± 1.7	13.8 ± 1.5	31.5 ± 0.3*
13e	−7.2 ± 2.9	−1.5 ± 1.2*	3.7 ± 1.4	15.8 ± 0.9*	31.7 ± 0.6**
13f	−2.4 ± 2.2*	0.6 ± 1.9	3.2 ± 0.8*	14.6 ± 0.8*	31.6 ± 0.4**

[a] Primary cultured osteoblasts (separated from newborn mice skull) were treated with active coumarin compounds at varying concentrations for 48 h and induced cell death was measured with MTT assay.
[b] The data presented are means ± SEM and six replicates were performed.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$ with respect to the untreated cells.

7e–**f** showed weak anti-tumor activities and **7d** showed no activity. Compounds **7a** and **13a** (R = morpholinyl) didn't express anti-proliferative activity, which was similar with the previous observation[5] that the weaker basic amino group on the side chain, for example the morpholinyl, demonstrated a detrimental effect on the anti-proliferative activity. It should be mentioned that compounds **6** and **12** without the amino side chains also showed no anti-proliferative effects on K562 and MCF-7 (not listed), which further reflected the importance of the two amino side chains.[10] Compounds **13d**–**f** were more active than **7d**–**f** implied that the farther location of the two side chains was favor to the anti-tumor activities of such compounds. In addition, the anti-proliferative activities of compounds **7b**–**c** were more superior to those of **7d**–**f** suggested that the basic amino group had certain effects on their activity when the two side chains were close to each other.

The active compounds **7b**–**c** and **13b**–**f** were further evaluated for possible cytotoxicity in normal cell, for example, osteoblast (Table 2). It could be found that all the tested compounds did not significantly affect the growth of osteoblasts, suggesting that these molecules had selectivity for inducing growth inhibition of tumor cells. The results suggested that these coumarins might be suitable as potential drug candidate for cancer chemotherapy.

In order to evaluate the interactions with DNA, UV–vis, fluorescence, and CD spectroscopies and DNA thermal denaturation experiment were used to ascertain the DNA binding properties of compounds **7b**, **7e**, **7f**, **13b**, **13e**, and **13f** with calf thymus (Ct) DNA.

The DNA binding properties of compounds **7b**, **7e**, **7f**, **13b**, **13e**, and **13f** with Ct-DNA were investigated by UV–vis spectra in phosphate buffer (10 mM, pH 7.4) containing 50 mM NaCl at 25 °C. As shown in Figure 2 and Table 3, the absorption intensities of compounds **7b**, **7e**, and **7f** enhanced with increased Ct-DNA concentrations in high hyperchromities of 16%, 16% and 17%, respectively, while the intensities of compounds **13b**, **13e**, and **13f** increased in middle hyperchromities of 6%, 8% and 8%, respectively. The maximum absorption (λ_{max}) of the tested coumarin derivatives **7b**, **7e**, and **7f** showed obviously blue shift (2.5–5.0 nm) and **13b**, **13e**, and **13f** had little hypsochromic shift (0.5–1.0 nm) in the presence of increasing concentration of Ct-DNA. These results demonstrated that the 3,4-diphenyl coumarin derivatives **7b**, **7e**, and **7f** containing two amino alkyl chains at 4-position on 3-, 4-phenyl, respectively, had stronger binding capacity with DNA than **13b**, **13e**, and **13f** containing the two chains at 4-position on 3-phenyl and 7-position, respectively. This suggested that the closer the distance of the two side chains was, the stronger the DNA binding

Table 3
Photometric properties of the coumarin derivatives **7b**, **7e**, **7f**, **13b**, **13e**, and **13f** with Ct-DNA investigated by UV–vis spectra and binding constants (K) by fluorescence spectra

Compds	Hypsochromic shift (nm)	Hyperchromicity[a] (%)	K_b (M^{-1})
7b	2.5 (324.5–322)	16	1.2×10^4
7e	4.5 (318–313.5)	17	1.0×10^4
7f	5.0 (323–318)	17	1.4×10^4
13b	0.5 (335–334.5)	6	7.0×10^3
13e	0.5 (334.5–334)	8	6.0×10^3
13f	1.0 (339–338)	8	5.2×10^3

[a] Obtained at λ_{max}.

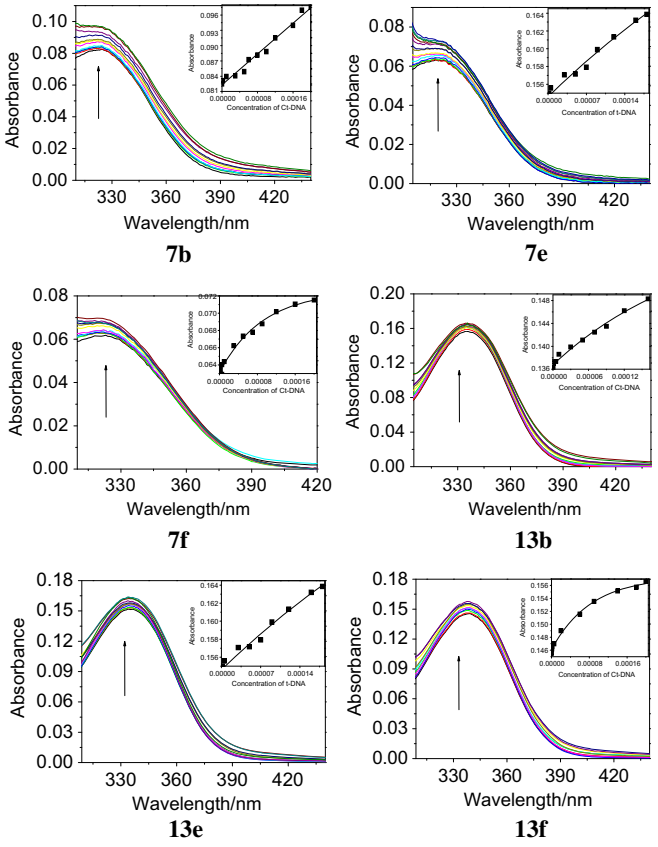


Figure 2. UV–vis spectral changes of compounds **7b**, **7e**, **7f**, **13b**, **13e**, and **13f** at the concentration of 1.0×10^{-5} M upon addition of Ct-DNA (0 –200 μM) in phosphate buffer (10 mM, pH 7.4) containing 50 mM NaCl and 2% DMSO at 25 °C. Inset: the fitting plots for **7b**, **7e**, **7f**, **13b**, **13e**, and **13f** with Ct-DNA obtained at the maximum absorption.

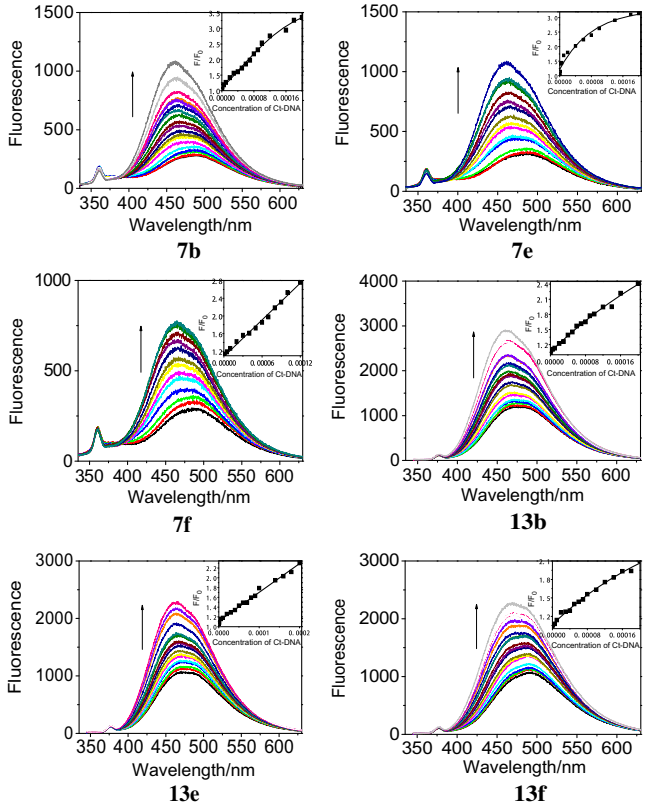


Figure 3. Fluorescence spectral changes of **7b** (λ_{ex} = 324.5 nm), **7e** (λ_{ex} = 318 nm), **7f** (λ_{ex} = 323 nm), **13b** (λ_{ex} = 335 nm), **13e** (λ_{ex} = 334.5 nm), and **13f** (λ_{ex} = 339 nm) at the concentration of 5.0×10^{-5} M upon addition of Ct-DNA (0–200 μM) in phosphate buffer (10 mM, pH 7.4) containing 50 mM NaCl and 2% DMSO at 25 °C. Both excitation and emission slit widths were 10.0 nm. Inset: Stern–Volmer plots for the observed fluorescence enhancement upon addition of Ct-DNA to the coumarin derivatives.

properties of such compounds had. Additional, the observed spectral changes (significant blue shift and hypsochromism) in compounds **7b**, **7e**, and **7f** implied that they would insert into the base pairs of DNA as DNA-intercalating agents,[11] while **13b**, **13e**, and **13f** (little blue shift and middle hypsochromism) possibly acted in a same binding mode with DNA.[12]

The fluorescence properties were performed to investigate the interactions between compounds **7b**, **7e**, **7f**, **13b**, **13e**, and **13f** and Ct-DNA in phosphate buffer (10 mM, pH 7.4) containing 50 mM NaCl at 25 °C. As shown in Figure 3, all compounds showed similar binding properties with Ct-DNA around 455 nm in the fluorescence spectra. Upon addition of DNA, fluorescence intensities increased markedly, possibly due to the coplanar modulation of the conformation of 3- and 4-phenyl groups induced by Ct-DNA.[13] The maximum emission bands were blue shifted which implied that these compounds entered Ct-DNA-stacking region with lower polarity than the buffer solution,[14] and intercalated into the bases of the Ct-DNA.[13a] The observed fluorescence intensities were quantified by plotting F/F_0 as a function of Ct-DNA concentrations, where F_0 and F are the fluorescence intensity without and with Ct-DNA, respectively. Stern–Volmer analysis gave deep insight into the binding efficiency of fluorescence enhancement of the coumarins with increasing concentrations of Ct-DNA.[13a] The calculated binding efficiency (Table 3) were $1.2 \times 10^4\ M^{-1}$, $1.0 \times 10^4\ M^{-1}$, and $1.4 \times 10^4\ M^{-1}$ for compounds **7b**, **7e**, and **7f**, and $7.0 \times 10^3\ M^{-1}$, $6.0 \times 10^3\ M^{-1}$, and $5.2 \times 10^3\ M^{-1}$ for compounds **13b**, **13e**, and **13f** (Fig. 3, inset), respectively.[15] The Stern–Volmer plots indicate that the fluorescence of compounds **7b**, **7e**, and **7f** possessing two amino side chains at 4-position on 3-, 4-phenyl, respectively, is higher sensitive to the Ct-DNA concentrations than those of **13b**, **13e**, and **13f**. These results are consistent with the observations by UV, that is, the closer distance of the two side chains is of benefit to improving the DNA binding capacity of these coumarins.

CD is a useful technique to investigate the conformational changes in DNA morphology during small molecules-DNA interactions. As shown in Figure 4, the CD spectrum of free Ct-DNA showed a negative band at 246 nm due to the polynucleotide helicity, and a positive band at 277 nm due to the base staking, which indicated that the Ct-DNA existed in the right-band B form.[16] Upon addition of all the tested compounds, the intensity of the positive band at 277 nm increased obviously, while the intensity of the negative band at 246 nm decreased (without significant wavelength change). This observation implied that these compounds could intercalate DNA and induce a B to A conformational change on Ct-DNA.[17] The order of the intensity change of **7b**, **7e**, and **7f** (strong intercalation) **>13b**, **13e**, and **13f** (poor intercalation), which was in agreement with the UV–vis analysis. Additional, very weak positive induced circular dichroisms (ICD) signals were observed in the region of the characteristic absorption of these

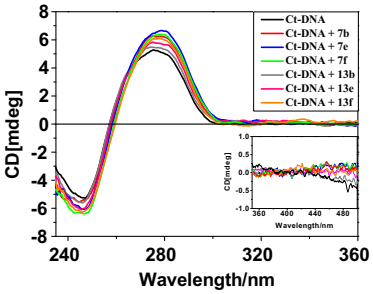


Figure 4. CD spectra of Ct-DNA (5.0×10^{-5} M) in the absence and presence of **7b**, **7e**, **7f**, **13b**, **13e**, and **13f** (2.0×10^{-5} M) in phosphate buffer (10 mM, pH 7.4) containing 50 mM NaCl and 2% DMSO at 25 °C.

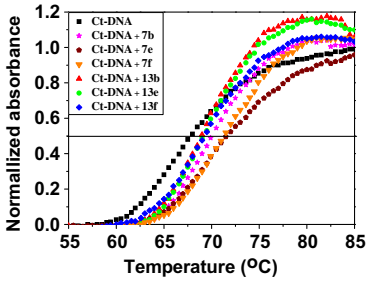


Figure 5. DNA melting curves for Ct-DNA (5.0×10^{-5} M) in the absence and presence of **7b**, **7e**, **7f**, **13b**, **13e**, and **13f** with concentration of 5.0×10^{-6} M in phosphate buffer (1 mM, pH 7.4) containing 5 mM NaCl and 2% DMSO at 25 °C.

Table 4
Average T_m and ΔT_m for Ct-DNA in the absence and presence of **7b**, **7e**, **7f**, **13b**, **13e**, and **13f**

Compds	T_m (°C)	ΔT_m (°C)
Ct-DNA	67.0	0
7b	70.0	3.0
7e	71.5	4.5
7f	71.0	4.0
13b	68.2	1.2
13e	68.8	1.7
13f	69.2	2.2

triphenylethylene–coumarin hybrid derivatives (350–500 nm), which indicated that **7b**, **7e**, **7f**, **13b**, **13e**, and **13f** intercalated DNA with a vertical orientation in the intercalation pocket.[18]

It is well known that the temperature at which a half of a DNA sample melts is known as the melting temperature (T_m). A change of T_m may be observed if a molecule binds with DNA.[19] Thus the thermal behavior of DNA in the presence of the triphenylethylene–coumarin hybrid derivatives provides useful information on the conformational changes and the strength of the DNA-compound complexes. The melting curves of Ct-DNA in the absence and presence of **7b**, **7e**, **7f**, **13b**, **13e**, and **13f** are illustrated in Figure 5 and Table 4, respectively. The T_m value for the free Ct-DNA was 67.0 °C. Upon addition of **7b**, **7e**, and **7f**, obvious changes in the DNA melting temperature were observed. The T_m values increased to 70.0, 71.5 and 71.0 °C, respectively, and the levels of the increased melting temperature (ΔT_m) induced by DNA-compound interactions were 3.0, 4.5 and 4.0 °C, respectively. Compounds **13b**, **13e**, and **13f** possessed lower DNA melting temperature than **7b**, **7e**, and **7f**, and the ΔT_m values were 1.2, 1.7, and 2.0 °C, respectively. These results further illuminated that compounds **7b**, **7e**, and **7f** with various amino alkyl chains at 4-position on 3-,4-phenyl, respectively, exhibited strong affinities with Ct-DNA, consistent with the results from the fluorescence data.

Considering all the above results, we could conclude that the novel triphenylethylene-coumarin hybrid derivatives with two amino side chains possessed the intercalative mode of binding properties with DNA, such as **7b**, **7e**, **7f**, **13b**, **13e**, and **13f**. When the two chains locate at 4-position on 3-,4-phenyl, respectively, like **7b**, **7e**, and **7f**, the intercalative binding is more efficient possibly due to the different noncovalent force between the chains and DNA grooves.[20] The similar binding constants of **7b**, **7e**, and **7f** indicated that the basic amino group (except morpholinyl) had little effects on their interactions with DNA, although they affected their anti-tumor activities. These compounds possessed potential application as novel DNA staining agent. Furthermore, it should be noticed that the anti-tumor activity of such coumarins did not tie in very closely with the observed DNA binding, for example **7e**, **7f** and **13e**, **13f**. That is, compounds **7e** and **7f** with low

anti-tumor activities intercalate into DNA more strongly than compounds **13e** and **13f** with good anti-proliferative effects. It was difficult to exactly explain this discrepancy. In the absence of any other evidence, we hypothesized that the possibility can not be excluded that these compounds may be targeting one or more other bio-macromolecular,[21] such as DNA topoisomerase or ER (estrogen receptor). These additional binding forces might also contribute to the compounds' anti-proliferative efficacy.[22] Therefore, DNA might be one of the potential targets but not the only one or not the most pivotal target for such triphenylethylene–coumarin hybrids as anti-tumor drug candidates. This and other possible targets remain to be explored in subsequent studies.

In summary, a series of novel triphenylethylene–coumarin hybrids possessing two amino side chains were designed and synthesized based on the triphenylethylene template. Compounds **13b–f** and **7b–c** showed a broad-spectrum and excellent anti-tumor activity and low cytotoxicity in osteoblast, which suggested that they might be suitable as potential drug candidate for cancer chemotherapy. The structure–activity relationships (SARs) suggested that the positions of the two amino alkyl chains had profound effects on their anti-proliferative activities and DNA binding properties.

Acknowledgments

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Supplementary data

Supplementary data (experimental procedures and characterization data for compounds **7a–f** and **13a–f**) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.12.084.

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