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Synthesis and Biological Evaluation of DNA Targeting Flexible Side-Chain Substituted β-Carboline Derivatives

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Abstract—A series of 3-substituted-β-carboline derivatives was synthesized from L-tryptophan. The intercalating binding mode of these compounds with DNA, the effects of the flexible alkylamine side chain on the intercalating ability and their antitumor activity were studied, which agreed well with the molecular modeling results.

The studies of the interaction between the small molecules and DNA, especially the effects of the structural characteristics of the small molecules on the DNA binding mode and binding strength, are important in the design of DNA targeting new antitumour drugs. In the early 1960s Lerman[1] first suggested that planar aromatic compounds could bind to DNA by an intercalation mode to explain the binding mode of the acridine derivative proflavine to DNA. Since then, considerable attention has been devoted to the study of the binding of small molecules and anticancer drugs with DNA by a combination of ^{1}H NMR spectroscopy, X-ray diffraction, scanning force microscopy (SFM) and other conventional techniques in this field.[2–10] Those studies provided further understanding of the intercalative mechanism that a polycyclic planar aromatic nucleus is required and revealed that specific atomic sites on DNA, such as the N atom of base and the C_1', C_4', C_5' atoms of deoxyribose,[10] were often the targets for drug covalent actions, but it was still challenging to conceive how the substituents of small molecules and anticancer drugs bound to those atoms by non-covalent actions, thus governing the binding mode and binding affinity. Some simple natural products, such as β-carboline derivatives, were found to bind with DNA in an intercalative binding fashion[11] and their DNA binding studies indicated that they showed high selectivity to the G–C base pair.[12] The biological activity of some β-carboline derivatives, such as harman, harmanol, harmine, etc., and their DNA binding studies were also reported[13] in our previous work.

As an extension of those studies to gain insight into the relative importance of the key structural feathers on the intercalative binding mode, six new β-carboline derivatives **6–11** with different substituents at position 3 had been synthesized, the structures of those compounds (Scheme 1) were confirmed by ^{1}H NMR and electron ionization mass spectra. The binding mode and binding strength of those compounds to DNA were studied by means of thermal denaturation measurements, viscosity titrations, and spectroscopic studies. Our work was especially focused on how the structure factors of the flexible alkylamine side chain contributed to the intercalating ability and the antitumor activity of the newly synthesized compounds **6–11**, which agreed well with the results of molecular modeling.

Synthesis

The route used for the preparation of the compounds **6–11** was carried out as outlined in Scheme 1. β-Carboline-3-carboxylic acid **5** was synthesized according to a method as described in the literature.[14–16] In the first route, using the usual procedure, treatment of the β-carboline-3-carboxylic acid **5** with thionyl chloride provided its acid chloride, the excess thionyl chloride was distilled and the last traces of it were removed by codistillation with toluene under vacuum, which reacted with corresponding diamine to obtain the compounds **6–10**; in the second route, 3-carbomethoxy-β-carboline **4** reacted directly[17] with 1,4-diaminobutane in DMF under reflux to obtain compound **11**. All the compounds **6–11** were purified either by recrystallization in methanol or by silica gel column chromatography.

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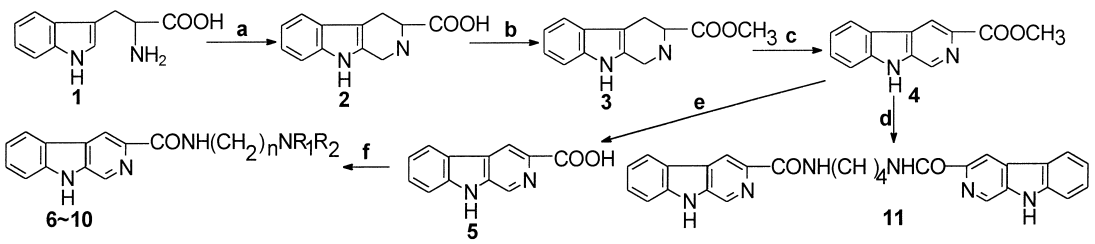

Scheme 1. (a) HCHO, OH^-; (b) $SOCl_2$, CH_3OH; (c) S, xylene, dioxane; (d) $NH_2(CH_2)_4NH_2$; (e) 2 N NaOH; (f) $NH_2(CH_2)_nNR_1R_2$.

Compd	*n*	R_1	R_2
6	2	H	H
7	2	C_2H_5	C_2H_5
8	4	H	H
9	4	CH_3	CH_3
10	6	H	H
11	4	H	

Antitumor Activity

Biological tests in vitro showed that all those compounds (except compound **11**) had relative inhibitory interaction to several human cancer cell lines (Table 1). Compound **8**, especially, showed high activities for HL-60 (human leukemia cancer) cells and BGC (human gastric cancer) cells.

DNA Binding Studies

In order to reveal the molecular mechanism of antitumor action and the structure factors of the flexible alkylamine side chain of compounds **6–11** contributing to the antitumor activity, the DNA binding studies were carried out by means of biochemical techniques as follows.

Thermal denaturation

The alteration of the denaturation temperatures of DNA depended on its binding strength with drugs. Hence, thermal denaturation profiles provided the simplest means for detecting binding and also asserting relative binding strength. Melting curves were measured at 260 nm by the traditional method as previous described.[18] The melting temperature (T_m) of calf thymus DNA (CT-DNA) in the presence and absence of compounds **6–11** were obtained from melting curves and the results are shown in Figure 1. Under these conditions, CT-DNA shows a T_m of 63.0 °C. Binding of any of the compounds studied resulted in T_m being raised, indicating stabilization of DNA double strand. Furthermore, a duplex stabilizing effect of compound **8** was relatively stronger ($\Delta T_m = 5.7$ °C) than the other compounds. This stabilization of DNA double helix probably contributed to the inhibition of DNA replication, thus having antitumor activity.

Table 1. The IC_{50} values (M) of compounds **6–11** to three cancer cell lines[a]

Compound	HL-60	KB	Hela	BGC
6	8.1×10^{-5}	1.4×10^{-4}	—	—
7	1.3×10^{-4}	2.5×10^{-4}	—	9.8×10^{-5}
8	1.9×10^{-6}	4.1×10^{-6}	—	8.6×10^{-6}
9	—	—	1.4×10^{-4}	—
10	5.1×10^{-4}	—	—	—
11	—	—	—	—
5-Fu (positive control)	1.0×10^{-6}	3.5×10^{-6}	1.6×10^{-6}	6.8×10^{-5}

[a]An RPMI 1640 was used as the culture medium. The cell lines used in this study were HL-60, KB, Hela and BGC with the concentration of $8–10\times10^5$ cells/mL. After the compounds were added separately, the cells were incubated, respectively, in 5% CO_2 at 37 °C for 48 h. The tetrazolium salt (MTT) was added each time at a concentration of 5 mg/mL. After 4 h incubation, the measurements were carried out using a Bio-Rad Model 450 Microplate reader.[13]

Binding mode

To further investigate the DNA binding properties, the binding mode of the six compounds **6–11** with CT-DNA was determined by viscometric titration. Viscosity measurement is regarded as one of the least ambiguous and most important critical tests for the classical intercalation model in the absence of X-ray and NMR structure data.[19,20] The classical intercalation mode demands that the DNA helix lengthen as base pairs are separated to accommodate the binding compound, leading to the increase of DNA viscosity. The effect of the test compounds on the viscosity of CT-DNA solution is shown in Figure 2. All the compounds increased the viscosity of DNA as expected for the lengthening of the double

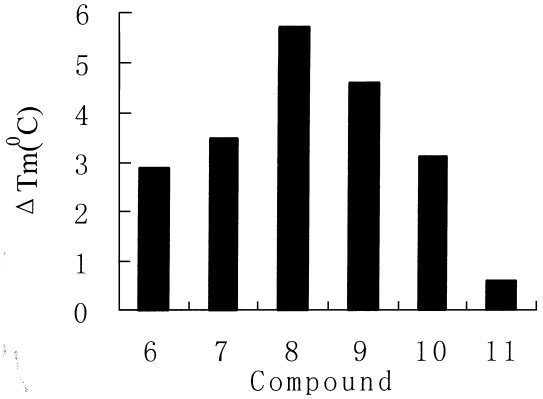

Figure 1. Effect of binding by compounds **6–11** on the thermal stability of the CT-DNA. T_m measurements were performed in PE buffer (1 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.60) in a thermostatically controlled cell hold, and the quartz cuvette (1 cm path length) was heated by circulating water at a heating rate of 0.4 °C/min from 20 to 85 °C. In all cases, the ratio of compound to CT-DNA is 0.4.

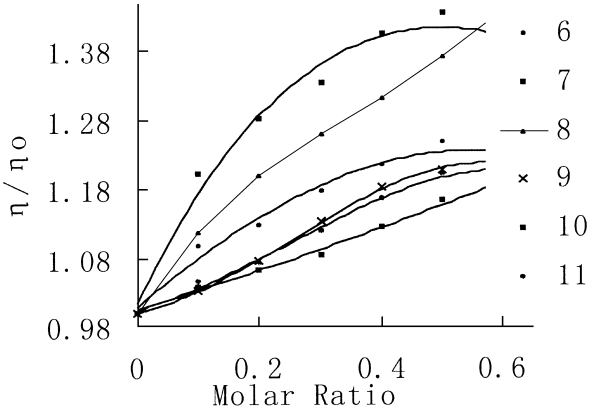

Figure 2. Effect of increasing amounts of binding ligand on the relative specific viscosity of CT-DNA. Viscometric titration of sonicated CT-DNA was conducted at 30.2(±0.1)°C in pipes00 buffer (0.01 M 1,4-piperazinediethane sulfonic acid, 0.001 M EDTA, pH 7.00) buffer in a Cannon-Fenske modification of the Ostwald viscometer.

helix by intercalation. On the basis of these experiments and combination with the previous studies of β-carboline derivatives binding with DNA,[13,21,22] we have concluded that those compounds bound to CT-DNA also by intercalation.

Binding strength

Absorption measurements in the UV–vis region were made on a pharmacia LKB Biochem 4060. Spectra for each compound in the presence and absence of CT-DNA were recorded directly by a PC microcomputer using the program of WAVESCAN in SWIFT. The curves in Figure 3 were the spectra for compound **8** and compound **11**, which was similar to those for the other compounds in these studies. A signification hypochromism was observed when CT-DNA was added to compound **8**, which suggested that compound **8** had a strong interaction with the CT-DNA double helix. However for compound **11**, many fewer spectral changes were observed in the presence of CT-DNA.

In order to determine in detail the effects of the substituents on the binding affinity, the intrinsic binding constant for the interaction of compounds **6–11** with the nucleic acid were determined by spectrophotometric titration. According to the site exclusion equation of McGhee and von Hippel,[21]

$$\gamma/c = K_b\,(1 - n\gamma)\{(1 - n\gamma)/[1 - (n - 1)\,\gamma]\}$$

where γ is the moles of compound per DNA base pair, c is the free compound concentration and n is the number of base pairs per binding site. K_b and n determined by Scatchard plots analysis using a non-linear least square procedure were collected in Table 2. The measured binding affinities of all compounds toward DNA were consistent with the binding mode proposed. In the intercalation model, the planar β-carboline ring was sandwiched between adjacent base pairs, stabilized by extensive van der Waal and hydrogen-bonding interaction along the groove of the DNA helix. It is apparent from the data in Table 2 that compound **8** had higher binding constants than the corresponding analogues.

Table 2. The intrinsic binding constant and number of site for compounds **6–11** with CT-DNA[a]

Compd	K ($M^{-1}\times10^{-3}$)	n
6	31.57	1.69
7	20.72	1.80
8	45.03	1.80
9	25.04	1.80
10	6.83	1.77
11	3.80	1.90

[a]Spectrometric titration was carried out at 300 nm in pipes00 buffer (as previously described).

Molecular modeling

Moreover, molecular modeling experiments were also performed in an attempt to better understand the DNA-binding properties. The docking calculation of the six small molecules **6–11** with target DNA sequence of d $(CCGGCGGT)_2$ containing the code 12 of c-Ha-Ras oncogene was carried out using the Biosym modeling package (Biosym Technologies) in Insight II. The suggested intercalative binding mode is shown in Figure 4 and the modeling results implied that the planar β-carboline ring system is a good match to G–C base pairs and hydrogen bonds formed between the terminal $-NH_2$ of the carboxamide side chain and N_7 of the guanine at the

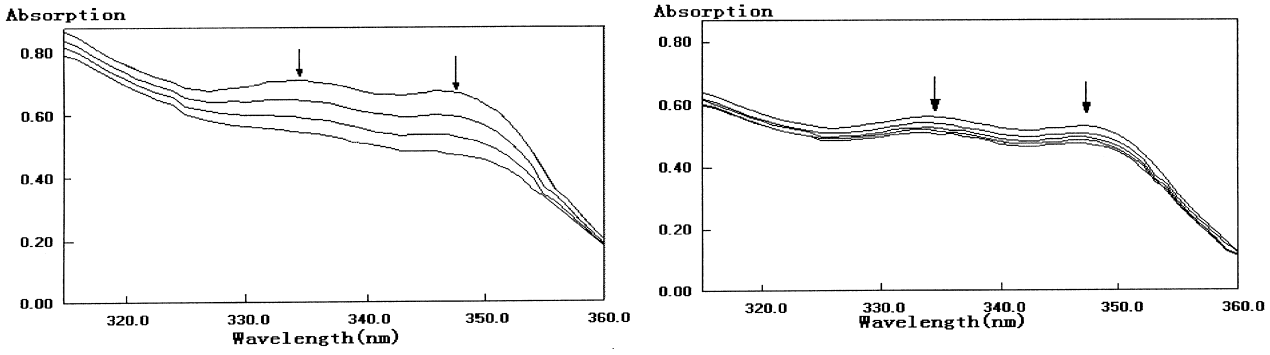

Figure 3. Absorption spectra for compound **8** (1.19×10^{-4} M) (left) and compound **11** (9.43×10^{-5} M) (right) in 1 mL pipes00 buffer (as previously described) at different molarities of calf thymus DNA base pairs: top curve (0.0) and bottom curve (6.75×10^{-4} M) were recorded in quartz cells (10 mm path length) by a UV–visible spectrophotometer at room temperature. Arrows show the absorbance changes upon increasing DNA concentration.

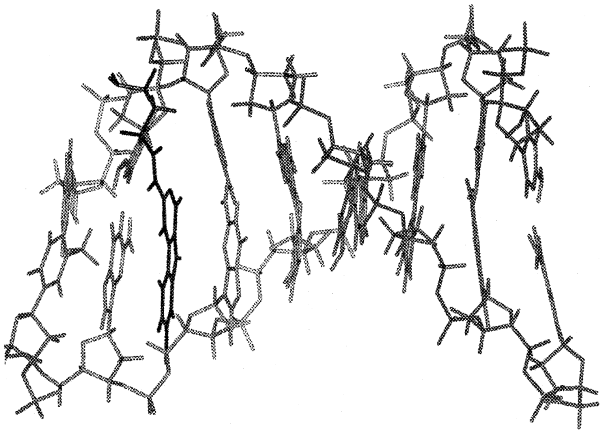

Figure 4. Models illustrating the binding of compound **8** with β-carboline ring system being intercalated into d(CCGGCGGT)$_2$. The models were generated from a molecular mechanics energy minimization as previous described.

Table 3. Optical conformation energies for compounds **6–11** and their minimization binding energies with d(CCGGCGGT)$_2$

Compd	E_1[a] (kJ/mol)	E_2[b] (kJ/mol)
6	82.30	−34.19
7	102.37	−70.52
8	78.79	−167.28
9	79.59	−96.93
10	82.26	−99.73
11	149.18	−139.32

[a]Optical conformation energy.
[b]Minimization binding energy. Compounds **6–11**, d(CCGGCGGT)$_2$ and their complexes were minimized using DISCOVER with a steepest descents algorithm in an AMBER.FRC potential forcefield on the SGI (Silicon Graphics Inc.) VX24 workstation, respectively.

intercalation site. The theoretical calculating energies are also presented in Table 3. Compound **8** exhibited the lowest optical conformation energy (78.79 kJ/mol) and the lowest docking-binding energy (−167.28 kJ/mol) among the six compounds, which indicated that compound **8** was more stable than the other analogues; and for R_1 and R_2 substituted compounds, the steric hindrance caused the distortion of the sugar phosphate backbone, thus resulting in the relative higher docking energy. Those data agreed well with our previous experimental results and the antitumor activity. Using the program, DISCOVER, water molecular and metal cation have been excluded in the calculation, our experimental data of the binding between the compounds **6–11** and DNA are consistent with the estimation by calculation (Fig. 4).

Conclusion

All the experiments reported here together with the previous studies of others[11,12,19,21] as well as ours[13] have left no room for doubt that the newly designed compounds **6–11** bound to DNA by intercalation. The *N*-containing rigid β-carboline ring system is a planar aromatic structure, which served as an intercalating chromophore to stack in base pairs of nucleic acids and stabilized electronically in the helix by $\pi-\pi$ stacking and dipole–dipole interactions, this intercalation into DNA may well contribute, at least partially, to the biological activity.

The results of these varied experiments also proved that the alkylamine side chain at position 3 played a crucial role for double helix affinity, little modification of which would lead to great changes in their binding affinities with CT-DNA. For all the compounds, where the intercalation β-carboline chromophore was kept constant and the only structural modification was a modification of the alkylamine side chain, the tendencies of each of the substituents might be compared. In the series of **6**, **8**, **10**, compound **8** exhibited the strongest stabilization of CT-DNA ($\Delta T_m = 5.7\,^{\circ}C$), the greatest binding affinity ($K = 4.503\times10^4\,M^{-1}$), the lowest docking-binding energy and showed high inhibition rate for HL-60 cells and BGC cells. The results from several different techniques indicated that this variation ($n = 4$) was favorable for the terminal amine group to form hydrogen binds with the N_7 of the guanine along the groove of the DNA helix. As for the series of **8**, **9**, **11**, an increase in the size of the N-terminal substituents (R_1, R_2) decreased the binding affinity, which suggested that steric hindrance might be responsible for a negative role of the substituents to the intercalating ability. Especially in the compound **11**, the length of the linking chain, $-CONH(CH_2)_4NHCO-$, might be shorter than the length required for full bifunctional intercalation, therefore, the second bulk hydrophobic β-carboline ring showed great steric effect and dramatically decreased the binding affinity according to our experiments.

Studies are in progress to try to test the further in vivo biological activity of compound **8**.

These studies may provide an experimental and theoretical basis for the understanding of intercalation at molecular levels for the DNA targeting antitumor drug design.

Acknowledgements

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