Synthesis and Biological Evaluation of Novel β-Carbolines as Potent Cytotoxic and DNA Intercalating Agents

Zhiyong CHEN,[a] Rihui CAO,[a] Buxi SHI,[a] Wei YI,[a,c] Liang YU,[a] Huacan SONG,*[,a] and Zhenhua REN*[,b]

[a] *School of Chemistry and Chemical Engineering, Sun Yat-sen University;* [b] *State Key Laboratory of Biocontrol, School of Life Science, Sun Yat-sen University; 135 Xin Gang West Road, Guangzhou 510275, P.R. China: and* [c] *Departement de Pharmacochimie Moleculaire, UMR-CNRS 5063, Faculte de Pharmacie de Grenoble; 5 Avenue de Verdun, BP 138, Meylan 38243, France.* Received January 31, 2010; accepted March 24, 2010; published online April 14, 2010

A series of novel water-soluble β-carbolines bearing a flexible amino side chain was designed, synthesized and evaluated as potent cytotoxic and DNA intercatalating agents. The N^9-arylated alkyl substituted β-carbolines represented the most interesting cytotoxic activities. The results suggested that (1) the N^9-arylated alkyl substituents of β-carboline nucleus played a very important role in the modulation of the cytotoxic potencies; (2) the length of the alkylamino side chain significantly affected their cytotoxic potency, and *N*,*N*-dimethylaminopropylamino substituent were more favorable. In addition, these compounds were found to exhibit significant DNA intercalating potencies.

Key words β-carboline; synthesis; cytotoxic; intercalating; melting temperature

β-Carboline alkaloids represent a large number of naturally and synthetic indole alkaloids associated with a broad spectrum of biochemical effects and pharmaceutical properties.[1)] Previous investigations focused on the effects of β-carboline alkoloids on the central nervous system (CNS).[2—9)] Recent reports[10—17)] have pointed out β-carbolines as a class of potential antitumor agents, which was discovered to function their antitumor activity through multiple mechanisms, such as intercalating into DNA,[11,18)] inhibiting topoisomerase I and II,[13)] CDK,[19—21)] MAPKAP-K2,[22)] MK-2,[23)] PLK1[24)] and kinesin Eg5.[25)]

Recently, our group investigations[26—31)] on the synthesis of a variety of β-carboline derivatives and the evaluation of their antitumor activities unraveled that β-carbolines had potent antitumor activities and the activities were correlated to both the planarity of the molecule and the presence of the ring substituents. Structure–activity relationships (SARs) analysis suggested that the introduction of appropriate substituents into position-2, 3 and 9 played a vital role in determining their antitumor effects, and the *n*-butyl, benzyl or phenylpropyl substituents at position-9 was suitable pharmacophoric group giving rise to some potent antitumor agents.

In continuing search for novel and effective antitumor agents, we designed and synthesized a series of water-soluble β-carbolines bearing a flexible alkylamino side chain at position-3 and a alkyl or arylated alkyl substituent at position-9, respectively. The selective introduction of a methyl, *n*-butyl, benzyl, 4-fluorobenzyl and phenylpropyl substituents into position-9 of β-carboline nucleus was based on the previous SARs analysis results, and the design of the amino substituents was limited to diethylaminoethylamino, dimethylaminopropylamino diethylaminopropylamino and diethylaminobutylamino group. The purpose of this study was to investigate effect of the flexible amino side chain moiety on the antitumor activity, with the ultimate aim of developing novel antitumor β-carbolines with improved water solubility and bioavailability.

Chemistry

The synthetic routes of novel β-carbolines **1a**, **2a**—**d**, **3a**—**d**, **4a**—**d**, **5a**—**d** and **6a**—**d** are outlined in Chart 1. 1-Methyl-β-carboline-3-carboxaldehydes **1**—**6** were prepared from the L-tryptophan *via* six steps including the Pictet–Spengler condensation, esterification, aromatization, *N*-alkylation or *N*-arylation, reduction and oxidation as previously described.[26—28)] The reaction of compounds **1**—**6** with the corresponding diamines to form schiff bases took place readily at room temperature in good yield. The crude schiff bases without further purification were directly reduced with $NaBH_3CN$ in anhydrous methanol to give the target β-carbolines **1a**, **2a**—**d**, **3a**—**d**, **4a**—**d**, **5a**—**d** and **6a**—**d** (Table 1) in 40—78% yield. The chemical structures of all the synthesized compounds were characterized by MS, IR, ^{1}H-NMR, and ^{13}C-NMR spectra.

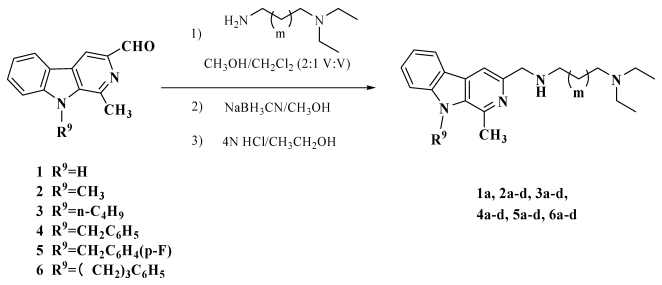


Chart 1. Synthesis of β-Carboline Derivatives

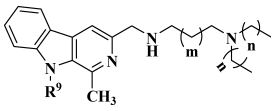
Results and Discussion

Cytotoxic Activity *in Vitro* The cytotoxic potential of all newly synthesized β-carbolines was evaluated *in vitro* against a panel of human tumor cell lines according to procedures described in our previous reports.[26)] The tumor cell line panel consisted of renal carcinoma (769-P), epidermoid carcinoma of the nasopharynx (KB), gastric carcinoma (BGC-823), renal carcinoma (786-0 and OS-RC-2), liver carcinoma (HepG2), melanoma (A375), colon carcinoma (HT-29), prostate carcinoma (22RV1) and breast carcinoma (MCF-7). The results were summarized in Table 1.

As shown in Table 1, compound **1a** without substituent at position-9 and compounds **2a**—**d** bearing a methyl group at

* To whom correspondence should be addressed. e-mail: yjhxhc@mail.sysu.edu.cn; Renzhh@mail.sysu.edu.cn

Table 1. Cytotoxicity of β-Carboline Derivatives *in Vitro*[c] (IC_{50},[a] μM)



Compound	R^9	*m*	*n*	769-P	KB	BGC-823	786-0	HepG2	A375	HT-29	OS-RC-2	22RV1	MCF-7
1a	H	1	0	>50	>50	>50	>50	25.4	>50	>50	46.4	>50	>50
2a	CH_3	0	1	24.6	11.0	>50	>50	>50	>50	>50	>50	>50	>50
2b	CH_3	1	0	38.6	38.3	25.4	>50	48.9	>50	24.4	>50	>50	>50
2c	CH_3	1	1	>50	11.3	>50	>50	>50	>50	>50	>50	>50	>50
2d	CH_3	2	1	47.1	>50	>50	>50	>50	42.6	24.9	>50	>50	>50
3a	*n*-C_4H_9	0	1	8.0	11.0	18.0	21.1	7.7	41.9	4.6	>50	25.3	>50
3b	*n*-C_4H_9	1	0	5.7	40.3	16.0	6.2	13.5	20.7	12.7	14.4	7.3	36.6
3c	*n*-C_4H_9	1	1	5.3	>50	17.4	7.2	22.1	25.9	15.1	17.6	7.6	>50
3d	*n*-C_4H_9	2	1	14.4	7.9	13.4	4.3	4.2	13.6	12.7	>50	9.1	12.6
4a	$CH_2C_6H_5$	0	1	19.8	28.2	29.5	48.1	34.1	28.9	19.6	>50	>50	40.8
4b	$CH_2C_6H_5$	1	0	1.6	7.6	5.1	5.8	6.2	17.1	1.6	14.1	4.6	25.7
4c	$CH_2C_6H_5$	1	1	18.1	>50	10.0	17.8	12.7	11.4	8.1	21.9	30.8	24.0
4d	$CH_2C_6H_5$	2	1	23.2	36.1	13.3	18.8	13.3	19.3	9.0	32.8	32.7	31.7
5a	$CH_2C_6H_4$(p-F)	0	1	26.5	27.8	17.3	14.3	12.8	26.0	18.9	38.7	5.3	19.5
5b	$CH_2C_6H_4$(p-F)	1	0	1.5	4.0	5.0	5.6	3.2	14.1	2.0	14.1	5.4	30.5
5c	$CH_2C_6H_4$(p-F)	1	1	15.8	32.2	5.3	2.9	12.0	14.3	10.9	33.3	5.2	11.1
5d	$CH_2C_6H_4$(p-F)	2	1	12.1	7.8	8.6	3.8	3.2	9.9	11.6	39.3	6.8	13.6
6a	$(CH_2)_3C_6H_5$	0	1	8.7	30.1	7.9	13.9	10.0	15.1	27.1	11.4	8.1	12.7
6b	$(CH_2)_3C_6H_5$	1	0	3.6	10.9	3.8	7.1	5.5	11.9	4.2	11.3	4.8	33.7
6c	$(CH_2)_3C_6H_5$	1	1	6.4	25.6	5.7	5.0	12.8	22.5	9.5	25.4	3.2	8.8
6d	$(CH_2)_3C_6H_5$	2	1	25.7	12.3	20.4	11.4	13.0	13.3	15.8	21.7	8.9	11.4
	Cisplatin			19.2	4.6	13.4	4.9	16.0	9.4	>50	3.4	4.6	12.4
	Paclitaxel			7.1	0.08	1.5	<0.08	<0.08	0.81	0.38	<0.08	0.08	1.3

a) Cytotoxicity as IC_{50} for each cell line is the concentration of compound, which reduced by 50% the optical density of treated cells with respect to untreated using the MTT assay. *b*) Cell lines include renal carcinoma (769-P), epidermoid carcinoma of the nasopharynx (KB), gastric carcinoma (BGC-823), renal carcinoma (786-0 and OS-RC-2), liver carcinoma (HepG2), melanoma (A375), colon carcinoma (HT-29), prostate carcinoma (22RV1) and breast carcinoma (MCF-7). *c*) The data represent the mean values of three independent determinations.

position-9 of β-carboline nucleus were almost inactive to all human tumor cell lines at the concentration of 50 μM. As predicted, introducing an *n*-butyl, benzyl, 4-fluorobenzyl or phenylpropyl substituent into position-9 of β-carboline core led to compounds **3a**—**d**, **4a**—**d**, **5a**—**d** and **6a**—**d** respectively, which all showed significant cytotoxic activity against most of human tumor cell lines with IC_{50} values lower than 20 μM. Interestingly, the N^9-arylated alkyl substituted β-carbolines **4a**—**d**, **5a**—**d** and **6a**—**d** displayed the most interesting cytotoxic activities, and compounds **4b**, **5b** and **6b** bearing a dimethylaminopropylamino group were found to be the most potent compounds with IC_{50} values lower than 20 μM against nine human tumor cell lines. These results indicated that (1) the N^9-arylated alkyl substituents of β-carboline nucleus played an important role in the modulation of the cytotoxic potencies; (2) the length of the alkylamino side chain significantly affected their cytotoxic potency, and *N*,*N*-dimethylaminopropylamino substituent were more favorable.

UV Spectral Absorbance In order to elucidate the DNA-binding ability of this series of β-carboline derivatives, the modification of the UV absorption spectra of the selected compounds **1a**, **2b**, **3b**, **4b** and **6b** in the absence and presence of increasing concentrations of calf thymus DNA (CT-DNA) was examined by UV spectroscopy based on the principle that the UV spectra of a substance changes if it interacts with the DNA.[11,32] Figure 1 illustrates the absorption spectra of compounds **1a**, **2b**, **3b**, **4b** and **6b** in the phosphate ethylenediaminetetraacetic acid (PE) buffer (1 mM Na_2HPO_4, 0.1 mM ethylenediaminetetraacetic acid (EDTA), pH 7.4) in the presence of increasing amounts of CT-DNA. In all cases, the binding of the drugs to CT-DNA results in considerable spectral changes, characterized by a slight bathochromic shift and a marked hypochromic effect. Bathochromic shifts and hypochromic effects are considered to be evidence of interaction of small molecules with DNA. Therefore, these results indicated that this class of β-carboline derivatives had a significant interaction with CT-DNA double helix.

DNA Thermal Denaturation Studies Intercalation of small molecules into DNA double helix is known to increase the DNA helix melting temperature.[11,32,33] In order to confirm the DNA intercalating ability of those compounds, we investigated the stabilization of the DNA helix by the selected β-carbolines **1a**, **2b**, **3b**, **4b** and **6b** using melting temperature (T_m) studies to evaluate relative affinity for DNA of the selected compounds. The T_m of CT-DNA in the presence and absence of compounds **1a**, **2b**, **3b**, **4b** and **6b** were obtained from melting curves (not shown) and the results of T_m analysis performed with CT-DNA are shown in Fig. 2. CT-DNA which melt at a low temperature (53.5 °C in PE buffer) affords a sensitive determination of the DNA binding capacity of the studied molecules. As indicated in Fig. 2, compound **1a** without substituent at position-9 stabilized CT-DNA against heat denaturation with ΔT_m value ($\Delta T_m = T_m^{\text{drug-DNA complex}} - T_m^{\text{DNA alone}}$) of 19.3 °C. 9-Methyl (compound **2b**) and 9-*n*-butyl (compound **3b**) substituted β-carboline congeners markedly stabilized CT-DNA against heat denaturation with ΔT_m value of 24.4 and 21.4 °C, respectively. Whereas 9-benzyl (compound **4b**) and 9-(3-phenyl)propyl (compound **6b**) substituted β-carboline congeners exhibited more weaker effect on CT-DNA thermal sta-

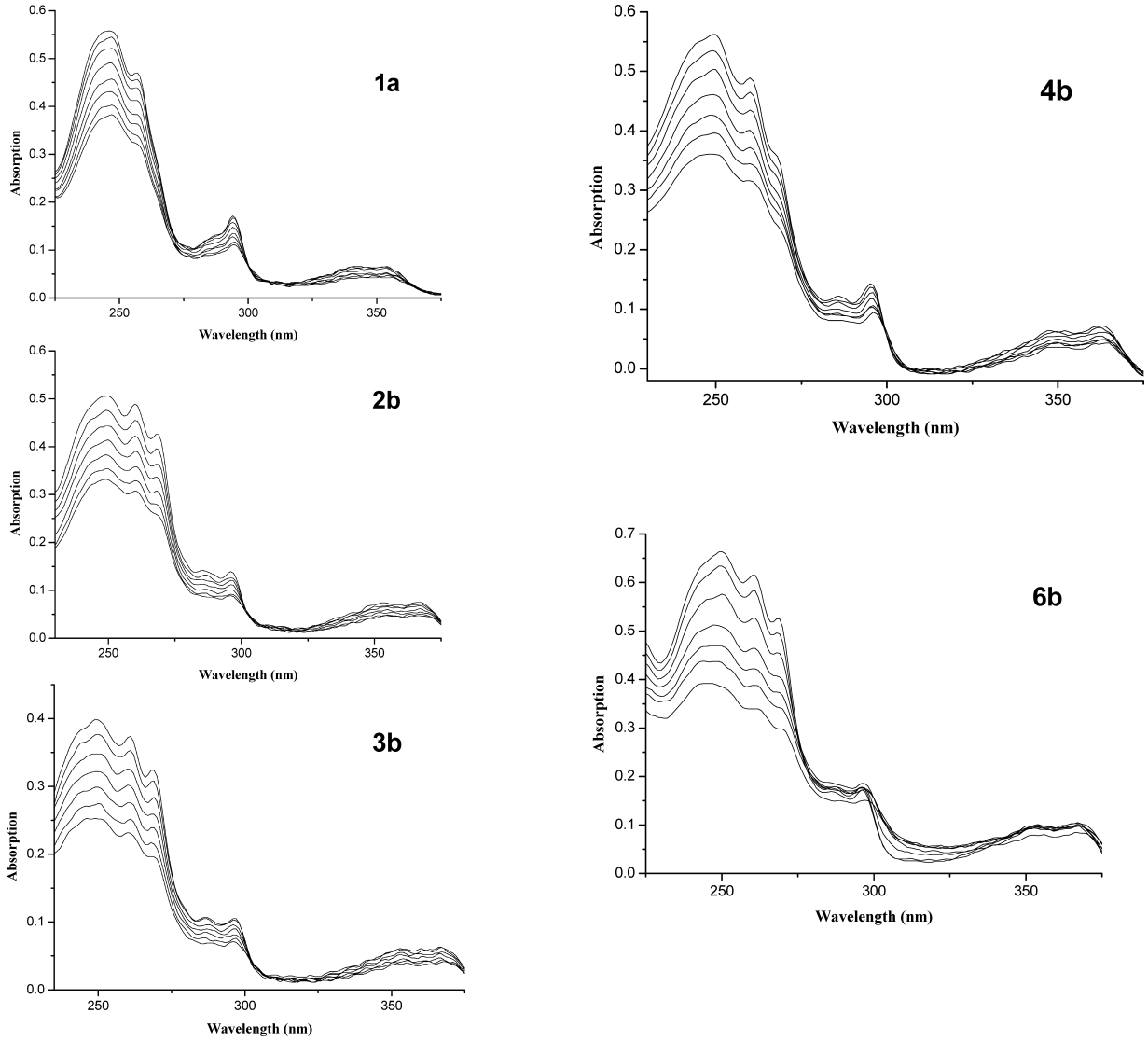


Fig. 1. Absorption Spectra for Compounds **1a**, **2b**, **3b**, **4b** and **6b** in 1 ml PE Buffer (1 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.4) at Different Molarities of CT-DNA

Top curve (0.0) and bottom curve (0.2 μM) were recorded in quartz cells (10 mm path length) by a UV–visible spectrophotometer at room temperature.

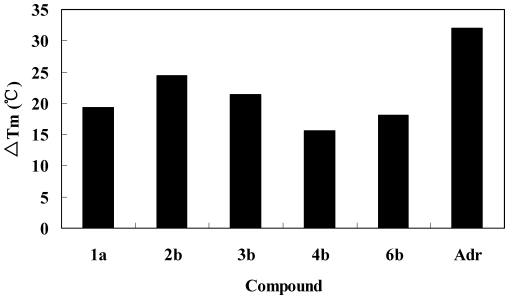


Fig. 2. Variation of the ΔT_m of the Complexs between the Tested Compounds and CT-DNA

Melting temperature measurements were performed in PE buffer (1 mM Na_2HPO_4, 0.1 mM EDTA) at pH 7.4 with a drug/DNA ration of 0.2. Adr is abbreviation of adriamycin (doxorubicin hydrochloride).

bility with ΔT_m value of 15.6 and 18.0 °C, respectively. The results suggested that (1) these compounds could significantly stabilize the double helix of CT-DNA; (2) the introduction of appropriate substituent into position-9 of β-carboline nucleus facilitated the intercalating potency, and short alkyl group was superior to arylated aklyl substituent. Unexpectedly, these data showed no obvious correlation between their cytotoxic activities and DNA binding potencies.

Fluorescence Studies Fluorescence spectra measurement is another useful technique to determine the binding mode of small molecules with DNA helix.[16,32] Compounds **1a**, **2b**, **3b**, **4b** and **6b** present intrinsic fluorescence properties which could be used to evaluate their respective DNA-binding potency. The effect of CT-DNA on the fluorescence intensity of the selected β-carbolines **1a**, **2b**, **3b**, **4b** and **6b** was determined by the use of fluorescence spectroscopy. From the titration of compounds **1a**, **2b**, **3b**, **4b** and **6b** (Fig. 3), the fluorescence intensity of all investigated compounds gradually decreased with the increasing concentrations of added CT-DNA. When the concentration of added CT-DNA was increased to 90 μM, the fluorescence intensity of all investigated compounds was lowered to their minimum. In addition, over the course of the fluorescence quenching of all investigated compounds, a slight bathochromic shift was also

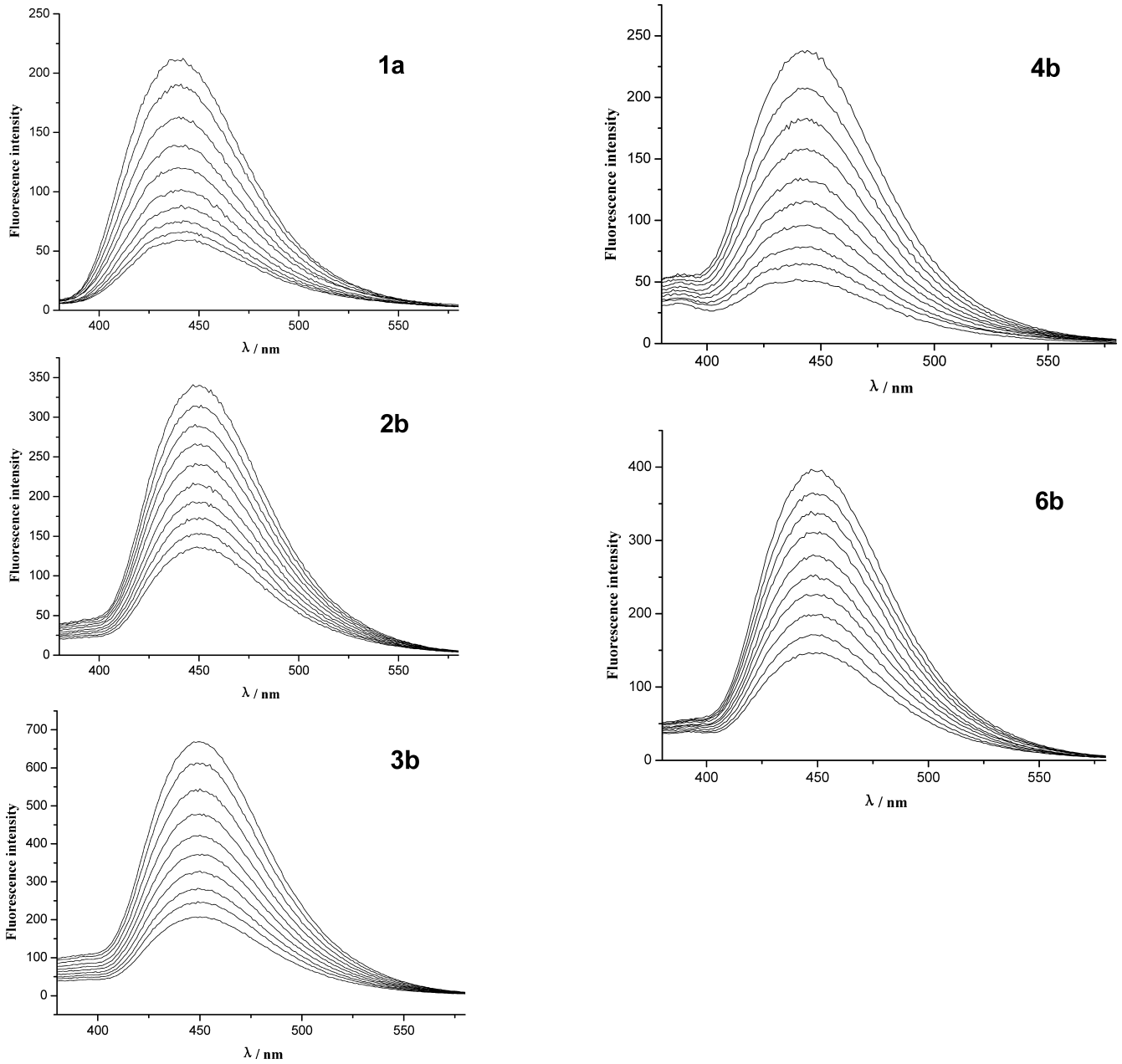


Fig. 3. Fluorescence Spectra of the Various β-Carbolines Upon Incubation with Graded Concentration of CT-DNA

A fixed concentration of the various β-carbolines (10 μM) was incubated with increasing concentration of CT-DNA from 0 to 90 μM in PE buffer (1 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.4).

observed. These results indicated that such β-carbolines could effectively interact with DNA.

Conclusion

A series of water-soluble β-carbolines bearing a flexible amino side chain described in this paper were proved to be significantly cytotoxic activities. The N^9-arylated alkyl substituented β-carbolines represented the most interesting cytotoxic activities. These results indicated that (1) the N^9-arylated alkyl substituents of β-carboline nucleus played an important role in the modulation of the cytotoxic potencies; (2) the length of the alkylamino side chain moiety also affected their cytotoxic potency, and *N*,*N*-dimethylaminopropylamino substituent were more favorable. In addition, the significant spectral changes (bathochromic shifts and hypochromic effects), elevating T_m value and fluorescence quenching effects of these compounds in the presence of CT-DNA were observed, suggesting that these compounds could significantly interact with DNA and remarkedly stabilizes the double helix of DNA. However, these data showed no obvious correlation between cytotoxic activity and DNA binding capacity of the newly synthetic β-carbolines.

In order to confirm the utility of the water-soluble β-carbolines bearing a flexible amino side chain moiety as an interesting antitumor agent, compounds **4b**, **5b** and **6b** are now selected and submitted to further acute toxicity and antitumor activity studies in animal models, and the relative possible results will be reported in due course.

Experimental

Reagents and General Procedures NMR spectra were recorded on a Varian Mercury-Plus 300 spectrometers, Bruker AVANCE 400 and Varian INOVA500NB in D_2O or DMSO-d_6 or D_2O+DMSO-d_6 at 25 °C. All chemical shifts (δ) are quoted in parts per million downfield from tetramethylsilane (TMS) and coupling constants (J) are given in Hz. Mass spectra were obtained from VG ZAB-HS and LCMS-2010A. High-resolution mass spectrometry (HR-MS) was performed on MAT95XP. Infrared (IR) spectra were measured on VECTOR 22 spectrometer using a potassium bromide (KBr) disk, scanning from 400 to 4000 cm^{-1}. All reactions were monitored by TLC (Merck Kieselgel GF_{254}) and spots were visualized with UV light or iodine. All commercially available reagents and solvents were used without further purification.

General Procedures for the Synthesis of Substituted β-Carboline Derivatives 1a, 2a—d, 3a—d, 4a—d, 5a—d and 6a—d A mixture of 1-methyl-β-carboline-3-carboxaldehyde (1 mmol), diamine (1.2 mmol), anhydrous methanol (6 ml) and anhydrous CH_2Cl_2 (3 ml) was stirred at room temperature overnight. The solvent was evaporated under vacuum to give the crude schiff base, which was used directly in the next step without further purification.

$NaBH_3CN$ (10 mmol) was added to a solution of the crude schiff base in anhydrous CH_3OH (10 ml) at 0 °C. The mixture was stirred at room temperature for 24 h and then concentrated under vacuum. The residue was dissolved in CH_2Cl_2 (50 ml) and washed with aqueous Na_2CO_3 (pH 10, 50 ml). The organic layer was separated, dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue was purified by flash chromatography on silica gel (CH_2Cl_2/CH_3OH/NH_4OH, 95 : 5 : 1) to gain yellow oil. The oil was dissolved in 4 N HCl/ethanol (20 ml) and stirred at room temperature for 30 min, then removed the solvent under reduced pressure to obtain yellow solid.

3-[*N*-(3-Dimethylamino)-propyl]-aminomethyl-1-methyl-β-carboline Hydrochloride Salt (**1a**): Yield 68%; IR (KBr, cm^{-1}) ν: 2937, 2745, 1630, 1473, 1384, 1343, 1241, 1050, 761; ^{1}H-NMR (500 MHz, D_2O) δ: 8.22 (s, 1H), 7.90 (d, J=8.0 Hz, 1H), 7.55—7.58 (m, 1H), 7.36 (d, J=8.0 Hz, 1H), 7.22 (t, J=7.5 Hz, 1H), 4.62 (s, 2H), 3.29—3.32 (m, 2H), 3.24—3.28 (m, 2H), 2.89 (s, 6H), 2.75 (s, 3H), 2.20—2.25 (m, 2H); ^{13}C-NMR (100 MHz, D_2O+dioxane) δ: 142.8, 139.6, 133.1, 131.9, 131.4, 129.8, 122.5, 121.9, 119.1, 117.3, 112.3, 54.2, 47.4, 44.7, 42.9, 21.3, 15.7; electrospray ionization (ESI)-MS m/z: 297.4 $(M+1)^+$.

3-[*N*-(2-Diethylamino)-ethyl]-aminomethyl-1,9-dimethyl-β-carboline Hydrochloride Salt (**2a**): Yield 70%; IR (KBr, cm^{-1}) ν: 2942, 2685, 1628, 1466, 1387, 1341, 1136, 1027, 770; ^{1}H-NMR (500 MHz, D_2O) δ: 8.31 (s, 1H), 7.81 (d, J=8.0 Hz, 1H), 7.46—7.49 (m, 1H), 7.21 (d, J=8.0 Hz, 1H), 7.11 (t, J=7.5 Hz, 1H), 4.76 (s, 2H), 3.71—3.74 (m, 5H), 3.61—3.64 (m, 2H), 3.30—3.34 (m, 4H), 2.97 (s, 3H), 1.31 (t, J=7.5 Hz, 6H); ^{13}C-NMR (100 MHz, D_2O+dioxane) δ: 144.0, 140.0, 133.2, 131.9, 131.7, 129.9, 122.0, 121.8, 118.2, 117.1, 110.2, 48.2, 47.6, 46.8, 41.9, 31.9, 17.9, 8.2; ESI-MS m/z: 325.4 $(M+1)^+$.

3-[*N*-(3-Dimethylamino)-propyl]-aminomethyl-1,9-dimethyl-β-carboline Hydrochloride Salt (**2b**): Yield 78%; IR (KBr, cm^{-1}) ν: 2954, 2733, 1628, 1470, 1384, 1344, 1252, 1057, 773; ^{1}H-NMR (500 MHz, D_2O) δ: 8.29 (s, 1H), 7.82 (d, J=8.0 Hz, 1H), 7.46—7.49 (m, 1H), 7.21 (d, J=8.5 Hz, 1H), 7.12 (t, J=7.5 Hz, 1H), 4.71 (s, 2H), 3.73 (s, 3H), 3.35 (t, J=8.0 Hz, 2H), 3.27—3.30 (m, 2H), 2.97 (s, 3H), 2.91 (s, 6H), 2.23—2.30 (m, 2H); ^{13}C-NMR (100 MHz, D_2O+dioxane) δ: 144.0 139.9, 133.2, 131.9, 131.7, 129.9, 122.1, 121.8, 118.2, 117.1, 110.2, 54.2, 47.2, 44.8, 42.9, 31.9, 21.3, 18.0; ESI-MS m/z: 311.4 $(M+1)^+$.

3-[*N*-(3-Diethylamino)-propyl]-aminomethyl-1,9-dimethyl-β-carboline Hydrochloride Salt (**2c**): Yield 75%; IR (KBr, cm^{-1}) ν: 2951, 2741, 1630, 1469, 1386, 1344, 1254, 1136, 1045, 768; ^{1}H-NMR (500 MHz, D_2O) δ: 8.36 (s, 1H), 7.94 (d, J=8.0 Hz, 1H), 7.56—7.60 (m, 1H), 7.34 (d, J=8.5 Hz, 1H), 7.22 (t, J=7.5 Hz, 1H), 4.72 (s, 2H), 3.85 (s, 3H), 3.35 (t, J=8.0 Hz, 2H), 3.21—3.28 (m, 6H), 3.03 (s, 3H), 2.20—2.25 (m, 2H), 1.27 (t, J=7.5 Hz, 6H); ^{13}C-NMR (100 MHz, D_2O+dioxane) δ: 144.2, 140.1, 133.4, 132.1, 131.8, 129.9, 122.2, 121.9, 118.4, 117.2, 110.4, 48.4, 47.6, 47.3, 44.9, 32.0, 20.8, 18.0, 8.2; ESI-MS m/z: 339.4 $(M+1)^+$.

3-[*N*-(4-Diethylamino)-butyl]-aminomethyl-1,9-dimethyl-β-carboline Hydrochloride Salt (**2d**): Yield 75%; IR (KBr, cm^{-1}) ν: 2952, 2139, 1629, 1469, 1386, 1027, 766; ^{1}H-NMR (300 MHz, D_2O) δ: 8.29 (s, 1H), 7.92 (d, J=7.8 Hz, 1H), 7.55 (t, J=7.2 Hz, 1H), 7.32 (d, J=8.7 Hz, 1H), 7.18 (t, J=7.2 Hz, 1H), 4.61 (s, 2H), 3.84 (s, 3H), 3.21 (t, J=6.3 Hz, 2H), 3.11—3.16 (m, 6H), 2.99 (s, 3H), 1.75—1.77 (m, 4H), 1.18 (t, J=8.0 Hz, 7.2H); ^{13}C-NMR (100 MHz, D_2O+dioxane) δ: 144.0, 139.9, 133.2, 131.9, 131.7, 130.2, 122.1, 121.8, 118.2, 117.1, 110.2, 50.9, 47.4 (2C), 47.1, 31.9, 22.8, 20.7, 18.0, 8.3; ESI-MS m/z: 353.4 $(M+1)^+$.

3-[*N*-(2-Diethylamino)-ethyl]-aminomethyl-9-*n*-butyl-1-methyl-β-carboline Hydrochloride Salt (**3a**): Yield 55%; IR (KBr, cm^{-1}) ν: 2969, 2619, 1629, 1462, 1385, 1339, 1253, 1137, 1058, 1026, 755; ^{1}H-NMR (500 MHz, D_2O) δ: 8.62 (s, 1H), 8.34 (d, J=8.0 Hz, 1H), 7.88 (t, J=7.5 Hz, 1H), 7.80 (d, J=8.5 Hz, 1H), 7.52 (t, J=7.5 Hz, 1H), 4.84 (s, 2H), 4.64 (t, J=7.5 Hz, 2H), 3.74—3.77 (m, 2H), 3.65—3.68 (m, 2H), 3.37—3.42 (m, 4H), 3.27 (s, 3H), 1.82—1.88 (m, 2H), 1.43—1.46 (m, 2H), 1.40 (t, J=7.5 Hz, 6H), 0.95 (t, J=7.5 Hz, 3H); ^{13}C-NMR (100 MHz, D_2O) δ: 144.4, 139.8, 133.2, 133.0, 132.1, 130.2, 122.6, 122.2, 119.0, 117.3, 111.1, 48.3, 47.8, 47.0, 44.9, 41.9, 32.4, 19.4, 18.1, 13.0, 8.3; ESI-MS m/z: 367.4 $(M+1)^+$.

3-[*N*-(3-Dimethylamino)-propyl]-aminomethyl-9-*n*-butyl-1-methyl-β-carboline Hydrochloride Salt (**3b**): Yield 42%; CH_2Cl_2/CH_3OH/NH_4OH, 95 : 5 : 1; IR (KBr, cm^{-1}) ν: 2957, 2727, 1625, 1536, 1466, 1382, 1340, 1252, 1133, 1058, 764; ^{1}H-NMR (500 MHz, D_2O) δ: 8.57 (s, 1H), 8.24 (d, J=8.5 Hz, 1H), 7.81 (t, J=7.5 Hz, 1H), 7.70 (d, J=8.5 Hz, 1H), 7.45 (t, J=7.5 Hz, 1H), 4.84 (s, 2H), 4.53 (t, J=7.5 Hz, 2H), 3.42—3.45 (m, 2H), 3.35—3.38 (m, 2H), 3.22 (s, 3H), 2.99 (s, 6H), 2.30—2.37 (m, 2H), 1.75—1.81 (m, 2H), 1.37—1.41 (m, 2H), 0.93 (t, J=7.5 Hz, 3H); ^{13}C-NMR (100 MHz, D_2O) δ: 144.1, 139.5, 132.9, 132.8, 132.1, 130.0, 122.5, 122.1, 118.7, 117.4, 110.9, 54.3, 47.2, 44.9 (2C), 43.0, 32.4, 21.3, 19.3, 18.0, 13.1; ESI-MS m/z: 353.4 $(M+1)^+$.

3-[*N*-(3-Diethylamino)-propyl]-aminomethyl-9-*n*-butyl-1-methyl-β-carboline Hydrochloride Salt (**3c**): Yield 54%; CH_2Cl_2/CH_3OH/NH_4OH, 95 : 5 : 1; IR (KBr, cm^{-1}) ν: 2960, 2676, 1624, 1570, 1468, 1383, 1340, 1254, 1207, 1136, 1033, 755; ^{1}H-NMR (500 MHz, D_2O) δ: 8.63 (s, 1H), 8.36 (d, J=8.0 Hz, 1H), 7.89 (t, J=7.5 Hz, 1H), 7.81 (d, J=8.5 Hz, 1H), 7.53 (t, J=7.0 Hz, 1H), 4.83 (s, 2H), 4.66 (t, J=7.5 Hz, 2H), 3.43 (t, J=7.5 Hz, 2H), 3.30—3.36 (m, 6H), 3.27 (s, 3H), 2.27—2.33 (m, 2H), 1.83—1.89 (m, 2H), 1.39—1.47 (m, 2H), 1.36 (t, J=7.0 Hz, 6H), 0.96 (t, J=7.5 Hz, 3H); ^{13}C-NMR (100 MHz, D_2O) δ: 144.2, 139.6, 132.9, 132.8, 132.1, 130.1, 122.5, 122.1, 118.8, 117.4, 111.0, 48.5, 47.7, 47.3, 45.0, 44.9, 32.4, 20.9, 19.4, 18.0, 13.1, 8.3; ESI-MS m/z: 381.4 $(M+1)^+$.

3-[*N*-(4-Diethylamino)-butyl]-aminomethyl-9-*n*-butyl-1-methyl-β-carboline Hydrochloride Salt (**3d**): Yield 60%; CH_2Cl_2/CH_3OH/NH_4OH, 95 : 5 : 1; IR (KBr, cm^{-1}) ν: 2968, 2777, 1669, 1628, 1546, 1452, 1381, 1341, 1143, 1038, 774; ^{1}H-NMR (500 MHz, D_2O) δ: 8.47 (s, 1H), 8.03 (d, J=8.0 Hz, 1H), 7.64 (t, J=7.5 Hz, 1H), 7.47 (d, J=8.5 Hz, 1H), 7.27 (t, J=7.5 Hz, 1H), 4.79 (s, 2H), 4.29 (t, J=7.5 Hz, 2H), 3.39 (t, J=7.0 Hz, 2H), 3.23—3.29 (m, 6H), 3.10 (s, 3H), 1.88—1.97 (m, 4H), 1.57—1.63 (m, 2H), 1.26—1.34 (m, 8H), 0.86 (t, J=7.0 Hz, 3H); ^{13}C-NMR (125 MHz, D_2O) δ: 144.5, 139.9, 133.2, 133.1, 132.4, 130.7, 122.9, 122.4, 119.1, 117.6, 111.2, 51.4, 47.9 (2C), 47.5, 45.2, 32.7, 23.3, 21.2, 19.7, 18.4, 13.4, 8.7; ESI-MS m/z: 395.4 $(M+1)^+$.

3-[*N*-(2-Diethylamino)-ethyl]-aminomethyl-9-benzyl-1-methyl-β-carboline Hydrochloride Salt (**4a**): Yield 48%; IR (KBr, cm^{-1}) ν: 2739, 1627, 1462, 1388, 1344, 1261, 1203, 1135, 1025, 765; ^{1}H-NMR (500 MHz, D_2O) δ: 8.66 (s, 1H), 8.15 (d, J=8.0 Hz, 1H), 7.37 (t, J=8.0 Hz, 1H), 7.19 (t, J=7.5 Hz, 1H), 7.13 (d, J=8.5 Hz, 1H), 6.98—7.00 (m, 3H), 6.70—6.72 (m, 2H), 5.52 (s, 2H), 4.89 (s, 2H), 3.81—3.84 (m, 2H), 3.68—3.71 (m, 2H), 3.33—3.38 (m, 4H), 2.84 (s, 3H), 1.34 (t, J=7.5 Hz, 6H); ^{13}C-NMR (125 MHz, D_2O) δ: 144.3, 139.8, 136.1, 133.3, 133.1, 132.1, 130.6, 128.8, 127.6, 125.1, 122.8, 122.3, 118.9, 117.5, 110.5, 48.1, 47.7, 47.4, 46.8, 41.8, 17.6, 8.1; ESI-MS m/z: 401.4 $(M+1)^+$.

3-[*N*-(3-Dimethylamino)-propyl]-aminomethyl-9-benzyl-1-methyl-β-carboline Hydrochloride Salt (**4b**): Yield 44%; CH_2Cl_2/CH_3OH/NH_4OH, 95 : 5 : 1; IR (KBr, cm^{-1}) ν: 2952, 2693, 1623, 1538, 1465, 1340, 1260, 1209, 760; ^{1}H-NMR (500 MHz, D_2O) δ: 8.57 (s, 1H), 8.20 (d, J=8.0 Hz, 1H), 7.52—7.55 (m, 1H), 7.29—7.34 (m, 2H), 7.12—7.13 (m, 3H), 6.79—6.80 (m, 2H), 5.66 (s, 2H), 4.75 (s, 2H), 3.34—3.37 (m, 2H), 3.26—3.29 (m, 2H), 2.90 (s, 6H), 2.87 (s, 3H), 2.22—2.29 (m, 2H); ^{13}C-NMR (100 MHz, D_2O+1,4-dioxane) δ: 144.7, 140.3, 136.6, 133.8, 133.5, 132.5, 131.1, 129.2, 128.1, 125.5, 123.1, 122.7, 119.4, 117.7, 111.0, 54.4, 48.0, 47.5, 44.9, 43.0, 21.5, 17.9; ESI-MS m/z: 387.4 $(M+1)^+$.

3-[*N*-(3-Diethylamino)-propyl]-aminomethyl-9-benzyl-1-methyl-β-carboline Hydrochloride Salt (**4c**): Yield 52%; IR (KBr, cm^{-1}) ν: 2949, 2764, 1627, 1463, 1384, 1346, 1135, 1045, 749; ^{1}H-NMR (500 MHz, D_2O) δ: 8.70 (s, 1H), 8.37 (d, J=8.0 Hz, 1H), 7.73 (t, J=7.5 Hz, 1H), 7.54 (d, J=8.5 Hz, 1H), 7.48 (t, J=7.5 Hz, 1H), 7.28—7.29 (m, 3H), 6.95—6.96 (m, 2H), 5.85 (s, 2H), 4.85 (s, 2H), 3.45 (t, J=7.5 Hz, 2H), 3.29—3.36 (m, 6H), 3.01 (s, 3H), 2.28—2.35 (m, 2H), 1.35 (t, J=7.5 Hz, 6H); ^{13}C-NMR (125 MHz, D_2O) δ: 145.2, 140.6, 137.0, 134.2, 133.9, 132.8, 131.4, 129.5, 128.3, 125.7, 123.4, 123.0, 119.7, 117.9, 111.3, 48.8, 48.3, 48.0, 47.8, 45.3, 21.2, 18.2, 8.6; ESI-MS m/z: 415.4 $(M+1)^+$.

3-[*N*-(4-Diethylamino)-butyl]-aminomethyl-9-benzyl-1-methyl-β-carboline Hydrochloride Salt (**4d**): Yield 40%; IR (KBr, cm^{-1}) *v*: 2953, 2733, 1627, 1463, 1395, 1345, 1039, 764; ^{1}H-NMR (500 MHz, D_2O) δ: 8.67 (s, 1H), 8.30 (d, *J*=8.0 Hz, 1H), 7.61 (t, *J*=7.0 Hz, 1H), 7.37—7.41 (m, 2H), 7.19—7.20 (m, 3H), 6.87—6.89 (m, 2H), 5.73 (s, 2H), 4.82 (s, 2H), 3.40 (t, *J*=7.0 Hz, 2H), 3.23—3.29 (m, 6H), 2.96 (s, 3H), 1.87—1.98 (m, 2H), 1.32 (t, *J*=7.0 Hz, 6H); ^{13}C-NMR (100 MHz, D_2O+dioxane) δ: 144.6, 140.0, 136.5, 133.6, 133.4, 132.4, 131.2, 129.1, 127.9, 125.4, 123.0, 122.6, 119.2, 117.6, 110.8, 50.9, 47.9, 47.4 (2C), 47.2, 22.9, 20.8, 17.8, 8.3; ESI-MS *m/z*: 429.4 $(M+1)^+$.

3-[*N*-(2-Diethylamino)-ethyl]-aminomethyl-9-(4-fluoro)benzyl-1-methyl-β-carboline Hydrochloride Salt (**5a**): Yield 52%; IR (KBr, cm^{-1}) *v*: 2763, 2615, 1621, 1505, 1468, 1387, 1255, 1217, 1052, 758; ^{1}H-NMR (400 MHz, D_2O) δ: 8.62 (s, 1H), 8.17 (d, *J*=8.0 Hz, 1H), 7.53 (t, *J*=7.2 Hz, 1H), 7.27—7.32 (m, 2H), 6.74—6.82 (m, 4H), 5.62 (s, 2H), 4.84 (s, 2H), 3.73—3.77 (m, 2H), 3.61—3.65 (m, 2H), 3.27—3.35 (m, 4H), 2.88 (s, 3H), 1.31 (t, *J*=7.2 Hz, 1H); ^{13}C-NMR (100 MHz, D_2O) δ: 163.1, 160.7, 144.5, 140.2, 133.6, 133.5, 132.5, 132.3, 131.0, 127.3, 127.2, 123.0, 122.7, 119.3, 117.7, 115.8, 115.6, 110.8, 48.3, 47.8, 47.4, 46.9, 41.9, 17.8, 8.3; ESI-MS *m/z*: 419.4 $(M+1)^+$.

3-[*N*-(3-Dimethylamino)-propyl]-aminomethyl-9-(4-fluoro)benzyl-1-methyl-β-carboline Hydrochloride Salt (**5b**): Yield 45%; IR (KBr, cm^{-1}) *v*: 2956, 2652, 1626, 1507, 1466, 1384, 1338, 1220, 1157, 1056, 752; ^{1}H-NMR (500 MHz, D_2O) δ: 8.62 (s, 1H), 8.30 (d, *J*=10.0 Hz, 1H), 7.67—7.71 (m, 1H), 7.49 (d, *J*=10.5 Hz, 1H), 7.42 (t, *J*=9 Hz, 1H), 6.87—6.96 (m, 4H), 5.78 (s, 2H), 4.78 (s, 2H), 3.37 (t, *J*=9.5 Hz, 2H), 3.27—3.31 (m, 2H), 2.96 (s, 3H), 2.92 (s, 6H), 2.23—2.31 (m, 2H); ^{13}C-NMR (125 MHz, D_2O) δ: 163.2, 160.8, 144.8, 140.3, 133.9, 133.7, 132.5, 131.1, 127.3, 127.2, 123.1, 122.7, 119.5, 117.6, 115.9, 115.7, 111.0, 54.3, 47.5, 44.8, 42.9, 21.3, 17.9; ESI-MS *m/z*: 405.4 $(M+1)^+$.

3-[*N*-(3-Diethylamino)-propyl]-aminomethyl-9-(4-fluoro)benzyl-1-methyl-β-carboline Hydrochloride Salt (**5c**): Yield 56%; IR (KBr, cm^{-1}) *v*: 2943, 1624, 1546, 1509, 1471, 1383, 1222, 1160, 1056, 758; ^{1}H-NMR (400 MHz, D_2O) δ: 8.60 (s, 1H), 8.22 (d, *J*=8.0 Hz, 1H), 7.58 (t, *J*=7.2 Hz, 1H), 7.31—7.38 (m, 2H), 6.80—6.87 (m, 4H), 5.66 (s, 2H), 4.78 (s, 2H), 3.38 (t, *J*=8.0 Hz, 2H), 3.21—3.30 (m, 6H), 2.90 (s, 3H), 2.21—2.29 (m, 2H), 1.27 (t, *J*=6.8 Hz, 6H); ^{13}C-NMR (100 MHz, D_2O) δ: 163.1, 160.7, 144.6, 140.2, 133.7, 133.5, 132.4, 131.2, 127.3, 127.2, 123.0, 122.7, 119.4, 117.7, 115.9, 115.7, 110.9, 48.5, 47.7, 47.5, 47.4, 45.0, 20.9, 17.9, 8.3; ESI-MS *m/z*: 433.4 $(M+1)^+$.

3-[*N*-(4-Diethylamino)-butyl]-aminomethyl-9-(4-fluoro)benzyl-1-methyl-β-carboline Hydrochloride Salt (**5d**): Yield 50%; IR (KBr, cm^{-1}) *v*: 2951, 2772, 1626, 1505, 1465, 1385, 1344, 1220, 1153, 1045, 761; ^{1}H-NMR (500 MHz, D_2O) δ: 8.65 (s, 1H), 8.12 (d, *J*=8.0 Hz, 1H), 7.36 (t, *J*=7.5 Hz, 1H), 7.12—7.16 (m, 2H), 6.69—6.72 (m, 2H), 6.63 (t, *J*=8.5 Hz, 2H), 5.49 (s, 2H), 4.82 (s, 2H), 3.40 (t, *J*=7.0 Hz, 2H), 3.19—3.24 (m, 6H), 2.86 (s, 3H), 1.87—1.95 (m, 4H), 1.27 (t, *J*=7.0 Hz, 6H); ^{13}C-NMR (100 MHz, D_2O+dioxane) δ: 162.9, 160.5, 144.2, 139.8, 133.4, 132.3 (2C), 131.6, 127.4, 127.3, 123.1, 122.6, 119.2, 117.8, 115.8, 115.6, 110.6, 51.1, 47.6, 47.5, 47.4, 47.2, 23.1, 21.0, 17.9, 8.5; ESI-MS *m/z*: 447.4 $(M+1)^+$.

3-[*N*-(2-Diethylamino)-ethyl]-aminomethyl-9-(3-phenyl)propyl-1-methyl-β-carboline Hydrochloride Salt (**6a**): Yield 64%; IR (KBr, cm^{-1}) *v*: 2931, 2669, 1621, 1494, 1435, 1384, 1337, 1247, 1139, 1053, 1024, 777, 753; ^{1}H-NMR (500 MHz, D_2O) δ: 8.52 (s, 1H), 8.27 (d, *J*=8.0 Hz, 1H), 7.83 (t, *J*=7.5 Hz, 1H), 7.59 (d, *J*=8.5 Hz, 1H), 7.49 (t, *J*=7.0 Hz, 1H), 7.21—7.27 (m, 3H), 7.11 (d, *J*=8.5 Hz, 2H), 4.79 (s, 2H), 4.54 (t, *J*=7.0 Hz, 2H), 3.70—3.74 (m, 2H), 3.64—3.68 (m, 2H), 3.37—3.42 (m, 4H), 3.02 (s, 3H), 2.73 (t, *J*=6.5 Hz, 2H), 2.15—2.21 (m, 2H), 1.40 (t, *J*=7.5 Hz, 6H); ^{13}C-NMR (100 MHz, D_2O) δ: 143.7, 140.5, 139.3, 132.8, 132.7, 132.0, 130.3, 128.4, 128.2, 126.2, 122.6, 122.2, 118.9, 117.3, 110.4, 48.3, 47.6, 46.9, 44.0, 42.0, 31.8, 31.2, 17.7, 8.3; ESI-MS *m/z*: 429.4 $(M+1)^+$.

3-[*N*-(3-Dimethylamino)-propyl]-aminomethyl-9-(3-phenyl)propyl-1-methyl-β-carboline Hydrochloride Salt (**6b**): Yield 55%; IR (KBr, cm^{-1}) *v*: 2936, 2711, 2407, 1632, 1508, 1454, 1381, 1336, 1056, 1026, 751; ^{1}H-NMR (500 MHz, D_2O) δ: 8.68 (s, 1H), 8.31 (d, *J*=8.0 Hz, 1H), 7.85 (t, *J*=7.0 Hz, 1H), 7.55 (t, *J*=7.5 Hz, 1H), 7.49 (d, *J*=8.5 Hz, 1H), 7.35—7.41 (m, 3H), 7.20—7.22 (m, 2H), 5.01 (s, 2H), 4.44 (t, *J*=7.5 Hz, 2H), 3.64 (t, *J*=7.5 Hz, 2H), 3.54—3.59 (m, 2H), 3.20 (s, 6H), 3.10 (s, 3H), 2.78 (t, *J*=7.5 Hz, 2H), 2.52—2.58 (m, 2H), 2.11—2.17 (m, 2H); ^{13}C-NMR (75 MHz, D_2O) δ: 143.9, 140.6, 139.3, 132.8, 132.1, 130.4, 128.6, 128.4, 126.3, 122.9, 122.3, 119.0, 117.7, 110.5, 54.6, 47.4, 45.3, 44.3, 43.3, 32.1, 31.6, 21.7, 18.0; ESI-MS *m/z*: 415.4 $(M+1)^+$.

3-[*N*-(3-Diethylamino)-propyl]-aminomethyl-9-(3-phenyl)propyl-1-methyl-β-carboline Hydrochloride Salt (**6c**): Yield 55%; IR (KBr, cm^{-1}) *v*: 2943, 2730, 1623, 1536, 1498, 1470, 1385, 1340, 1304, 1057, 758; ^{1}H-NMR (500 MHz, D_2O) δ: 8.39 (s, 1H), 8.12 (d, *J*=8.0 Hz, 1H), 7.66—7.69 (m, 1H), 7.40 (d, *J*=8.5 Hz, 1H), 7.35 (t, *J*=7.0 Hz, 1H), 7.12—7.19 (m, 3H), 7.02 (t, *J*=7.0 Hz, 2H), 4.69 (s, 2H), 4.33 (t, *J*=7.5 Hz, 2H), 3.32 (t, *J*=7.5 Hz, 2H), 3.21—3.27 (m, 6H), 2.86 (s, 3H), 2.61 (t, *J*=7.0 Hz, 2H), 2.18—2.24 (m, 2H), 1.98—2.04 (m, 2H), 1.26 (t, *J*=7.0 Hz, 6H); ^{13}C-NMR (100 MHz, D_2O) δ: 143.9, 140.6, 139.4, 132.9, 132.8, 132.0, 130.4, 128.5, 128.2, 126.2, 122.7, 122.2, 119.0, 117.4, 110.6, 48.5, 47.7, 47.3, 45.0, 44.1, 31.8, 31.2, 20.8, 17.8, 8.3; ESI-MS *m/z*: 443.4 $(M+1)^+$.

3-[*N*-(4-Diethylamino)-butyl]-aminomethyl-9-(3-phenyl)propyl-1-methyl-β-carboline Hydrochloride Salt (**6d**): Yield 62%; IR (KBr, cm^{-1}) *v*: 2945, 2733, 1626, 1464, 1387, 1345, 1033, 761; ^{1}H-NMR (500 MHz, D_2O) δ: 8.46 (s, 1H), 8.17 (d, *J*=8.0 Hz, 1H), 7.73 (t, *J*=7.5 Hz, 1H), 7.39—7.45 (m, 2H), 7.19—7.24 (m, 3H), 7.05—7.07 (d, *J*=7.0 Hz, 2H), 4.75 (s, 2H), 4.37 (t, *J*=7.0 Hz, 2H), 3.36 (t, *J*=7.0 Hz, 2H), 3.22—3.29 (m, 6H), 2.92 (s, 3H), 2.66 (t, *J*=6.5 Hz, 2H), 2.02—2.08 (m, 2H), 1.89—1.93 (m, 4H), 1.33 (t, *J*=7.5 Hz, 6H); ^{13}C-NMR (300 MHz, DMSO-d_6) δ: 144.4, 141.5, 139.9, 133.8, 133.6, 132.4, 132.2, 129.0 (2C), 126.7, 123.3, 122.5, 119.8, 118.2, 112.1, 50.5, 46.7 (3C), 44.9, 32.8, 23.6, 21.1, 18.4, 9.3; ESI-MS *m/z*: 457.5 $(M+1)^+$.

Cytotoxicity Assays *in Vitro* Cytotoxicity assays *in vitro* were carried out using 96 microtitre plate cultures and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) staining according to the procedures described in our previous report.[22] Briefly, cells were grown in RPMI-1640 medium containing 10% (v/v) fetal calf serum and 100 U/ml penicillin and 100 U/ml streptomycin. Cultures were propagated at 37 °C in a humified atmosphere containing 5% CO_2. Cell lines were obtained from Shanghai Cell Institute, Chinese Academy of Science. Drug stock solutions were prepared in pure water. The human tumor cell line panel consisted of renal carcinoma (769-P), epidermoid carcinoma of the nasopharynx (KB), gastric carcinoma (BGC-823), renal carcinoma (786-0 and OS-RC-2), liver carcinoma (HepG2), melanoma (A375), colon carcinoma (HT-29), prostate carcinoma (22RV1) and breast carcinoma (MCF-7). In all of these experiments, three replicate wells were used to determine each point.

DNA Binding Studies The interaction of the selected β-carboline derivatives with CT-DNA was studied by UV spectrometry following the methods described by Xiao *et al.*[11] with some modification. Measurements were taken in PE buffer (1 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.4) in a 1 cm pathlength quartz cuvette at room temperature using a Shimadzu UV 2501PC Spectrometer. The cuvette initially held 0.75 ml of a 20 μM solution of compounds **1a**, **2b**, **3b**, **4b** and **6b**, respectively, and then was progressively titrated by increasing amounts of CT-DNA to obtain the spectrum of fully bound drugs in the presence of a large excess of DNA by means of a dispenser equipped with a 25 μl syringe and adequate Teflon tubing.

Determination of ΔT_m T_m measurements were performed using a Shimadzu UV 2501PC Spectrometer and following the methods described by Xiao *et al.*[11] with slight modification. Experiments were carried out in PE buffer (1 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.4) in a thermostatically controlled cell hold, and the quartz cuvette (1 cm path length) was heated by circulating water at a heating rate of 0.5 °C/min from 25 to 95 °C. Doxorubicin Hydrochloride was used as standards. In all cases, the ratio of compound to CT-DNA is 0.2.

Fluorescence Spectroscopy Fluorescence spectral measurement[16,32] was recorded using 10 μM of the fluorescent drugs incubated in 1 ml of PE buffer in the presence or absence of increasing concentrations of CT-DNA (0, 10, 20, 30, 40, 50, 60, 70, 80, 90 μM) in a quartz cuvette of 10 mm path length. The corresponding changes in the fluorescence intensity of the selected compounds were observed on a Shimadzu RF-5310PC spectrofluorometer at a fluorescence excitation wavelength of 372 nm.

Acknowledgements We are thankful for the financial support of this work by Xinjiang Huashidan Pharmaceutical Co., Ltd.

References

1) Cao R., Peng W., Wang Z., Xu A., *Curr. Med. Chem.*, **14**, 479—500 (2007).
2) Cain M., Weber R. W., Guzman F., Cook J. M., Barker S. A., Rice K. C., Crawley J. N., Paul S. M., Skolnick P., *J. Med. Chem.* , **25**, 1081—1091 (1982).
3) Hollinshead S. P., Trudell M. L., Skolnick P., Cook J. M., *J. Med. Chem.*, **33**, 1062—1069 (1990).
4) Allen M. S., LaLoggia A. J., Dorn L. J., Martin M. J., Costantino G., Hagen T. J., Koehler K. F., Skolnick P., Cook J. M., *J. Med. Chem.*, **35**, 4001—4010 (1992).

5) Cox E. D., Diaz-Arauzo H., Huang Q., Reddy M. S., Ma C., Harris B., McKernan R., Skolnick P., Cook J. M., *J. Med. Chem.*, **41**, 2537—2552 (1998).
6) Grella B., Teitler M., Smith C., Herrick-Davis K., Glennon R. A., *Bioorg. Med. Chem. Lett.*, **13**, 4421—4425 (2003).
7) Audia J. E., Evrard D. A., Murdoch G. R., Droste J. J., Nissen J. S., Schenck K. W., Fludzinski P., Lucaites V. L., Nelson D. L., Cohen M. L., *J. Med. Chem.*, **39**, 2773—2780 (1996).
8) Husbands S. M., Glennon R. A., Gorgerat S., Gough R., Tyacke R., Crosby J., Nutt D. J., Lewis J. W., Hudson A. L., *Drug Alcohol Depend.*, **64**, 203—208 (2001).
9) Miralles A., Esteban S., Sastre-Coll A., Moranta D., Asensio V. J., Garcia-Sevilla J. A., *Eur. J. Pharmacol.*, **518**, 234—242 (2005).
10) Ishida J., Wang H. K., Bastow K. F., Hu C. Q., Lee K. H., *Bioorg. Med. Chem. Lett.*, **9**, 3319—3324 (1999).
11) Xiao S., Lin W., Wang C., Yang M., *Bioorg. Med. Chem. Lett.*, **11**, 437—441 (2001).
12) Shen Y. C., Chen C. Y., Hsieh P. W., Duh C. Y., Lin Y. M., Ko C. L., *Chem. Pharm. Bull.*, **53**, 32—36 (2005).
13) Deveau A.M., Labroli M. A., Dieckhaus C. M., Barthen M. T., Smith K. S., Macdonald T. L., *Bioorg. Med. Chem. Lett.*, **11**, 1251—1255 (2001).
14) Zhao M., Bi L., Wang W., Wang C., Baudy-Floc'h M., Ju J., Peng S., *Bioorg. Med. Chem.*, **14**, 6998—7010 (2006).
15) Formagio A. S. N., Dusman L. T. D., Foglio M. A., Madjarof C., Carvalho J. E., Costa W. F., Cardoso F. P., Sarragiotto M. H., *Bioorg. Med. Chem.*, **16**, 9660—9667 (2008).
16) Wu J., Zhao M., Qian K., Lee K.-H., Morris-Natschke S., Peng S., *Eur. J. Med. Chem.*, **44**, 4153—4161 (2009).
17) Boursereau Y., Coldham I., *Bioorg. Med. Chem. Lett.*, **14**, 5841—5844 (2004).
18) Hayashi K., Nagao M., Sugimura T., *Nucleic Acids Res.*, **4**, 3679—3685 (1977).
19) Song Y., Wang J., Teng S. F., Kesuma D., Deng Y., Duan J., Wang J. H., Qi R. Z., Sim M. M., *Bioorg. Med. Chem. Lett.*, **12**, 1129—1132 (2002).
20) Song Y., Kesuma D., Wang J., Deng Y., Duan J., Wang J. H., Qi R. Z., *Biochem. Biophys. Res. Commun.*, **317**, 128—132 (2004).
21) Li Y., Liang F., Jiang W., Yu F., Cao R., Ma Q., Dai X., Jiang J., Wang Y., Si S., *Cancer Biol. Ther.*, **6**, 1193—1199 (2007).
22) Castro A. C., Dang L. C., Soucy F., Grenier L., Mazdiyasni H., Hottelet M., Parent L., Pien C., Palombella V., Adams J., *Bioorg. Med. Chem. Lett.*, **13**, 2419—2422 (2003).
23) Trujillo J. I., Meyers M. J., Anderson D. R., Hegde S., Mahoney M. W., Vernier W. F., Buchler I. P.,. Wu K. K, Yang S., Hartmann S. J., Reitz D. B., *Bioorg. Med. Chem. Lett.*, **17**, 4657—4663 (2007).
24) Zhang J., Li Y., Guo L., Cao R., Zhao P., Jiang W., Ma Q., Yi H., Li Z., Jiang J., Wu J., Wang Y., Si S., *Cancer Biol. Ther.*, **8**, 2374—2383 (2009).
25) Barsanti P. A., Wang W., Ni Z., Duhl D., Brammeier N., Martin E., Bussiere O., Walter A. O., *Bioorg. Med. Chem. Lett.*, **20**, 157—160 (2010).
26) Cao R., Chen Q., Hou X., Chen H., Guan H., Ma Y., Peng W., Xu A., *Bioorg. Med. Chem.*, **12**, 4613—4623 (2004).
27) Cao R., Peng W., Chen H., Hou X., Guan H., Chen Q., Ma Y., Xu A., *Eur. J. Med. Chem.*, **40**, 249—257 (2005).
28) Cao R., Chen H., Peng W., Ma Y., Hou X., Guan H., Liu X., Xu A., *Eur. J. Med. Chem.*, **40**, 991—1001 (2005).
29) Guan H., Chen H., Peng W., Ma Y., Cao R., Liu X., Xu A., *Eur. J. Med. Chem.*, **41**, 1167—1179 (2006).
30) Cao R., Yi W., Wu Q., Guan X., Feng M., Ma C., Chen Z., Song H., Peng W., *Bioorg. Med. Chem. Lett.*, **18**, 6558—6561 (2008).
31) Wu Q., Cao R., Feng M., Guan X., Ma C., Liu J., Song H., Peng W., *Eur. J. Med. Chem.*, **44**, 533—540 (2009).
32) Lemster T., Pindur U., Lenglet G., Depauw S., Dassi C., David-Cordonnier M., *Eur. J. Med. Chem.*, **44**, 3235—3252 (2009).
33) Tan C., Liu J., Chen L., Shi S., Ji L., *J. Inorg. Biochem.*, **102**, 1644—1653 (2008).