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Synthesis of novel β-carbolines with efficient DNA-binding capacity and potent cytotoxicity

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ABSTRACT

A series of water-soluble β-carbolines, bearing a flexible amino side chain, was prepared and evaluated in vitro against a panel of human tumor cell lines. The N^9-arylated alkyl substituted β-carbolines represented the most interesting cytotoxic activities, and compound **7b** was found to be the most potent antitumor agent with IC_{50} values lower than 10 μM against eight human tumor cell lines. The results confirmed that the N^9-arylated alkyl substituents of β-carboline nucleus played an important role in the modulation of the cytotoxic potencies. In addition, these compounds were found to exhibit significant DNA-binding affinity.

The β-carboline alkaloids have been characterized as a class of potential antitumor agents, which was discovered to function their antitumor activity through multiple mechanisms, such as intercalating into DNA,[1] inhibiting topoisomerase I and II,[2] CDK,[3] MAPKAP-K2,[4] MK-2[5] and kinesin Eg5.[6] We also observed that the ability of β-carbolines to act as DNA intercalating agents and topoisomerase I inhibitors was related to their potent antitumor activities,[7] and some β-carbolines can induce apoptosis in HepG2 cells and down-regulate the expression of *Bcl-2* gene and upregulate the expression of death receptor *Fas* without altering the level of Bax and P53.[8]

Recently, our group described the syntheses of numerous β-carboline derivatives bearing various substituents at position-1, 2, 3, 7 and 9 of β-carboline nucleus and evaluated their antitumor activities in vitro[9–15] and in vivo.[9,11] The structure–activity relationships (SARs) analysis revealed that the introduction of appropriate substituents into position-3 and 9 of β-carboline nucleus facilitated the antitumor activity; and the *n*-butyl, benzyl or phenylpropyl substituents at position-9 were optimal 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. The design of substituents at position-9 of β-carboline ring was based on the previous SARs analysis,[16] and the choice of the amino substituents was limited to diethylaminoethylamino, diethylaminopropylamino and diethylaminobutylamino moiety. The focus of this investigation was to probe the optimal structural requirement of these compounds with regard to antitumor activities, and further develop new antitumor β-carbolines with improved water solubility and bioavailability.

The synthetic routes of novel β-carbolines **1a**, **2a–c**, **3a–c, 4a–c, 5a–c, 6a–c** and **7a–c** were outlined in Scheme 1. 3-Carboxaldehyde-β-carbolines **1–7** 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.[9–11] The reaction of 3-carboxaldehyde-β-carbolines **1–7** with the corresponding diamines to form schiff bases took place readily at room temperature in good yield. The crude schiff bases were reduced with $NaBH_3CN$ in anhydrous methanol to give the target β-carbolines **1a**, **2a–c**, **3a–c, 4a–c, 5a–c, 6a–c** and **7a–c** in 30–65% yields.[17] The chemical structures of all the synthesized novel compounds were characterized by MS, HRMS, IR, ^1H NMR and ^{13}C NMR spectra.

The cytotoxic potential of all newly synthesized β-carboline derivatives was evaluated in vitro against a panel of human tumor cell lines according to procedures described in our previous reports.[9] As predicted, all compounds showed significantly improved water solubility (more than 500 mg/ml). 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

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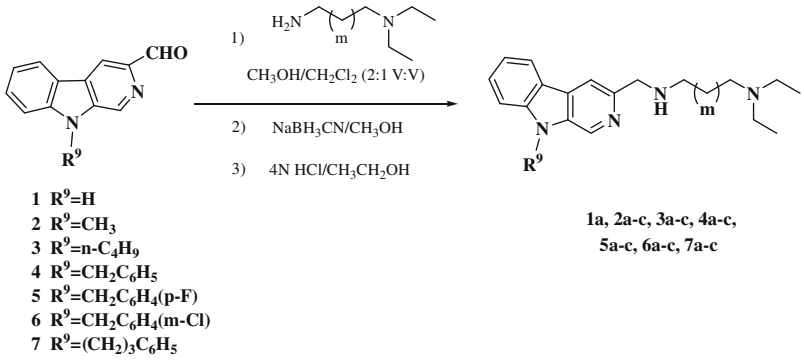


Scheme 1. Synthesis of β-carboline derivatives.

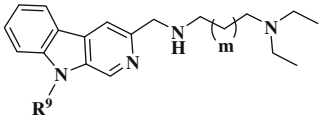
(A375), colon carcinoma (HT-29), prostate carcinoma (22RV1) and breast carcinoma (MCF-7). The results were summarized in Table 1.

As shown in Table 1, compound **1a** without substituent at position-9 and compounds **2a–c** bearing a methyl substituent at position-9 of β-carboline core only exhibited weak cytotoxic activities with IC_{50} values of more than 50 μM. Whereas, compounds **3a–c**, **4a–c**, **5a–c**, **6a–c** and **7a–c**, bearing N^9-*n*-butyl, benzyl, 4-fluorobenzyl, 3-chlorobenzyl and 3-phenylpropyl substituents, respectively, all exhibited excellent cytotoxic activities against most of human tumor cell lines with IC_{50} values of lower than 10 μM. As a whole, 769-P, KB, BGC-823,786-0, HepG2, HT-29 and 22RV1 cell lines were more sensitive to such compounds than A375, OS-RC-2 and MCF-7 cell lines.

Of all 9-substituted β-carbolines, the N^9-arylated alkyl substituted compounds **4a–c**, **5a–c**, **6a–c** and **7a–c** exhibited more potent cytotoxic activities than the N^9-alkylated substituted compounds **2a–c** and **3a–c**. The N^9-(3-phenyl)propyl substituted β-carbolines **7a–c** represented the most interesting cytotoxic activities, and compound **7b** was found to be the most potent cytotoxic agent with IC_{50} values lower than 10 μM against eight human tumor cell lines. These results suggested that the arylated alkyl substituents might be a favorable group to exploit in searching for new antitumor leading compounds. Interestingly, compounds **3b**, **4b**, **5b**, **6b** and **7b,** bearing a diethylaminopropylamino moiety, all displayed more potent cytotoxic activity than those compounds having a diethylaminoethylamino or diethylaminobutylamino substituents. The results suggested that the length of the alkylamino side chain moiety also affected their cytotoxic potencies, and three CH_2 units were more favorable.

The interaction of compounds **1a** and **7b** with calf thymus DNA (CT-DNA) was examined by UV–vis spectroscopy.[7] Figure 1 illus-

Table 1
Cytotoxicity of β-carboline derivatives in vitro[c] (IC_{50},[a] μM)



Compd	R^9	*m*	769-P[b]	KB	BGC-823	786-0	HepG2	A375	HT-29	OS-RC-2	22RV1	MCF-7
1a	H	1	75.3	>100	>100	>100	93.7	>100	>100	67.4	>100	>100
2a	CH_3	0	>100	>100	75.7	>100	>100	>100	41.1	>100	>100	>100
2b	CH_3	1	>100	59.7	>100	87.6	96.1	>100	35.7	>100	>100	>100
2c	CH_3	2	>100	70.6	>100	>100	>100	>100	33.1	>100	>100	>100
3a	*n*-C_4H_9	0	7.4	13.7	20.2	37.6	10.1	75.9	7.8	>100	25.7	92.8
3b	*n*-C_4H_9	1	2.2	6.2	5.9	19.5	4.6	44.2	2.6	35.7	8.8	64.2
3c	*n*-C_4H_9	2	8.0	10.2	23.4	14.1	8.0	15.2	27.4	71.7	13.5	23.4
4a	$CH_2C_6H_5$	0	2.0	8.2	9.7	14.4	13.1	39.1	5.2	>100	15.9	68.1
4b	$CH_2C_6H_5$	1	1.5	4.9	2.8	6.4	4.4	17.5	3.7	14.5	7.3	32.5
4c	$CH_2C_6H_5$	2	31.0	8.2	29.3	6.2	3.0	16.2	27.0	55.0	8.9	13.4
5a	$CH_2C_6H_4$(*p*-F)	0	8.8	37.1	10.9	13.1	9.1	35.0	36.2	93.6	9.0	43.6
5b	$CH_2C_6H_4$(*p*-F)	1	8.5	33.1	2.6	6.1	8.0	13.7	10.2	13.6	3.6	31.8
5c	$CH_2C_6H_4$(*p*-F)	2	2.9	6.5	9.4	5.8	2.5	6.5	10.1	31.7	9.6	20.2
6a	$CH_2C_6H_4$(*m*-Cl)	0	19.3	21.6	37.9	47.6	17.7	22.7	19.7	52.3	58.5	42.2
6b	$CH_2C_6H_4$(*m*-Cl)	1	14.3	7.8	8.1	13.0	11.7	14.7	9.3	8.0	8.4	17.4
6c	$CH_2C_6H_4$(*m*-Cl)	2	8.2	4.2	9.8	19.4	10.8	12.6	12.2	6.7	8.6	19.8
7a	$(CH_2)_3C_6H_5$	0	7.3	9.7	14.2	11.2	7.2	5.9	4.2	43.9	8.2	27.7
7b	$(CH_2)_3C_6H_5$	1	5.1	4.8	7.4	8.3	3.8	8.9	2.6	13.1	7.7	13.8
7c	$(CH_2)_3C_6H_5$	2	10.9	6.1	8.8	9.2	3.4	5.8	6.4	27.7	7.6	9.1
	Cisplatin		19.2	4.6	13.4	4.9	16.0	9.4	85.7	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
	Adriamycin		11.5	0.6	<1.0	3.1	<1.0	17.2	4.2	<1.0	<1.0	5.1

[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.

trates the absorption spectra of compounds **1a** and **7b** in the PE buffer (1 mM Na_2HPO_4, 0.1 mM 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 hypochromism. The results indicated that such compounds possessed an effective interaction with CT-DNA double helix.

In order to confirm the DNA-binding ability of those compounds, we investigated the stabilization of the DNA helix by the selected β-carbolines **1a**, **2b**, **3b**, **4b** and **7b** 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 **7b** were obtained from melting curves (not shown) and the results of T_m analysis performed with CT-DNA are shown in Figure 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 Figure 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 12.6 °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 24.5 and 20.6 °C, respectively. Whereas 9-benzyl (compound **4b**) and 9-phenylpropyl (compound **7b**) substituted β-carboline congeners exhibited more weaker effect on CT-DNA thermal stability with ΔT_m value of 15.6 and 18.8 °C, respectively. The results suggested that (1) these compounds could significantly stabilize the double helix of CT-DNA; (2) the introduction of substituent into position-9 of β-carboline nucleus facilitated the intercalating potency, and short alkyl group was superior to arylated aklyl substituent. Because the cytotoxic effects of such compounds on human tumor cell lines were involved in absorption, metabolism and bioclearance, there were no correlation between their cytotoxic activities and DNA-binding potencies.

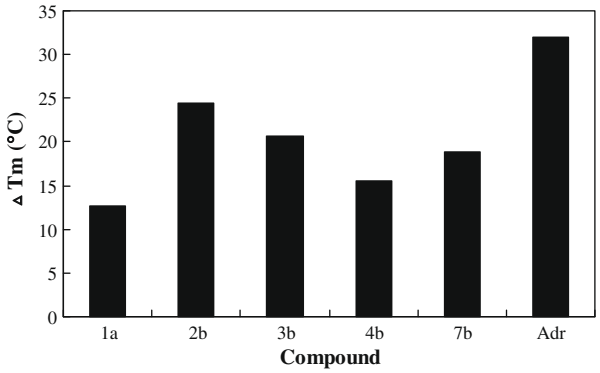


Figure 2. Variation of the ΔT_m of the complexes between the tested compounds and CT-DNA. Melting temperature measurements were performed in PE buffer (1 mM Na_2HPO_4, 0.1 mM EDTA) at pH 7.4 with a drug/DNA ratio of 0.2. Adr is abbreviation of adriamycin (doxorubicin hydrochloride).

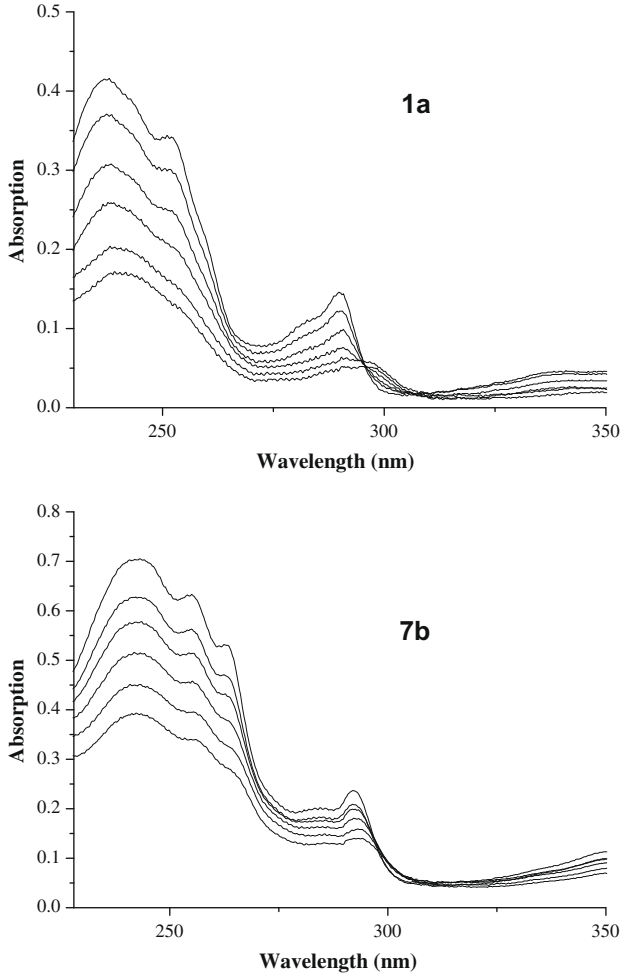


Figure 1. Absorption spectra for compounds **1a** and **7b** in 1 mL PE buffer (1 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.4) at different molarities of CT-DNA: top curve (0.0) and bottom curve (0.2 mM) were recorded in quartz cells (10 mm path length) by a UV spectrophotometer at room temperature.

In addition, the effects of CT-DNA on the fluorescence intensity of compounds **1a** and **7b** were also determined by the use of fluorescence spectroscopy. In the determinations, a solution of compounds **1a** and **7b** in PE buffer (1 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.4) was titrated with 10 μl of solution containing serial concentrations (0, 40, 80, 120, 160, 200, 240, 280, 320 μM) of CT-DNA in PE buffer. The changes in fluorescence intensities (fluorescence quenching) of **1a** and **7b** were recorded on a Shimadzu RF-5310PC spectrofluorometer at a fluorescence excitation wavelength of 372 nm. Figure 3 illustrates the typical course of the fluorescence quenching of **1a** and **7b**, the emission maxima undergone a slight red shift after binding to increasing concentration of CT-DNA. The gradual decrease of the fluorescence intensities indicated a marked quenching upon binding to CT-DNA. These data implied that these compounds could significantly interact with DNA.

In summary, a series of water-soluble β-carbolines bearing a flexible amino side chain described in this Letter were proved to be significantly cytotoxic activities. The N^9-arylated alkyl substituted β-carbolines represented the most interesting cytotoxic activities. These results confirmed that the N^9-arylated alkyl substituents of β-carboline nucleus played an important role in the modulation of the cytotoxic potencies. On the basis of the significant spectral changes (red-shift and hypochromism), ΔT_m value and fluorescence quenching effects, these compounds revealed a significantly DNA-binding potency. However, different DNA binding modes can account for the spectral perturbation and it is impossible to distinguish the mode of interaction of a drug with DNA from absorption measurements alone and DNA intercalation and groove binding can lead to similar and important spectral variation. Undoubtedly, further investigation on the DNA binding mechanisms of this class of β-carbolines is needed.

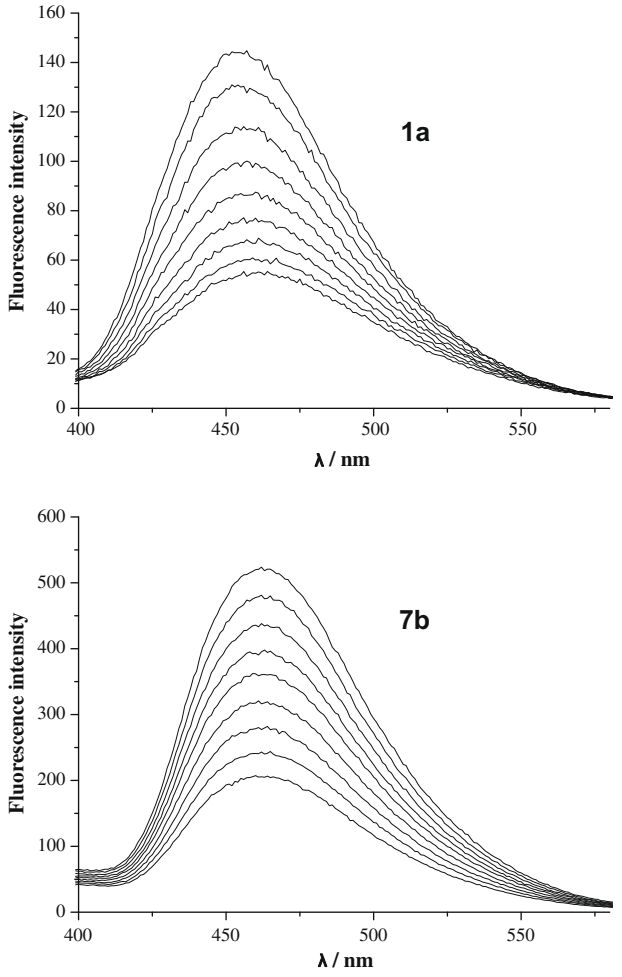


Figure 3. Fluorescence spectra of the selected compounds **1a** and **7b** upon incubation with graded concentration of CT-DNA. A fixed concentration of compounds **1a** and **7b** (10 μM) was incubated with increasing concentration of CT-DNA from 0 to 90 μM in PE buffer (1 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.4).

Acknowledgments

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17. *General procedure for the preparation of β-carbolines* **1a, 2a–c, 3a–c, 4a–c, 5a–c, 6a–c** *and* **7a–c**. A mixture of 3-carboxaldehyde-β-carboline (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 above-mentioned 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. The solid were dried in vacuo at 100 °C for 3 days to give the target compounds **1a, 2a–c, 3a–c, 4a–c, 5a–c, 6a–c** and **7a–c** in 30–65% yields. Compound **7b**: Yield 56%, IR (KBr, cm^{-1}) *v*: 2940, 2626, 1633, 1508, 1459, 1378, 1342, 755; ^1H NMR (500 MHz, D_2O): δ 8.75 (s, 1H), 8.46 (s, 1H), 8.08–8.10 (d, *J* = 10 Hz, 1H), 7.61 (t, *J* = 9.5 Hz, 1H), 7.27–7.33 (m, 2H), 6.96–6.98 (m, 3H), 6.76–6.78 (m, 2H), 4.72 (s, 2H), 4.19 (t, *J* = 8.5 Hz, 2H), 3.34 (t, *J* = 10 Hz, 2H), 3.32–3.28 (m, 6H), 2.43 (t, *J* = 8.5 Hz, 2H), 2.19–2.27 (m, 2H), 1.98–2.05 (m, 2H), 1.27 (t, *J* = 9 Hz, 6H); ^{13}C NMR (125 MHz, D_2O): δ 144.0, 140.7, 135.0, 133.1, 132.4, 131.2, 128.4, 128.1, 126.3, 126.1, 123.3, 122.2, 119.4, 118.9, 111.1, 48.6, 48.2, 47.8, 45.0, 43.4, 32.2, 28.8, 21.0, 8.4; HRMS (EI) calcd for $C_{28}H_{36}N_4$: 428.2934, found: 428.2928.