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Original article

Synthesis and biological evaluation of 1,9-disubstituted β-carbolines as potent DNA intercalating and cytotoxic agents

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ABSTRACT

A series of novel 1,9-disubstituted β-carbolines was designed, synthesized and evaluated as cytotoxic and DNA intercalating agents. Compounds **7b**, **7c**, **8b** and **8c** exhibited the most potent cytotoxic activities with IC_{50} values of lower than 20 μM against ten human tumor cell lines. The results indicated that (1) the 3-chlorobenzyl and 3-phenylpropyl substituents in position-9 of β-carboline nucleus were the suitable pharmacophoric group giving rise to significant antitumor agents; (2) the length of the alkylamino side chain moiety affected their cytotoxic potencies, and three CH_2 units were more favorable. In addition, these compounds were found to exhibit remarkable DNA intercalating effects.

1. Introduction

DNA is one of the most important pharmacological targets of many drugs currently in clinical use. Small molecules that bind genomic DNA have proven to be effective antitumor, antivirus and antibacterial therapeutic agents. It is well-known that small molecules can interact with DNA through multiple modes including (i) intercalation; (ii) surface binding; (iii) minor groove binding and (iv) major groove binding. DNA intercalating agents form an important class of drugs in antitumor therapy [1]. To investigate the interaction of such agents with DNA, in particular, effects of structural requirement of them on the interaction, could offer novel insights into the design of new DNA targeting antitumor drugs [2].

The β-carboline alkaloids are a large group of natural and synthetic indole alkaloids associated with a broad spectrum of biochemical effects and pharmaceutical properties [3]. Recent reports [4–12] have pointed out β-carbolines as a new class of potential antitumor agents, which were discovered to exert their antitumor effects through multiple mechanisms of action, such as intercalating into DNA [5,10,13], inhibiting topoisomerase I and II [7,14,15], CDK [16–18], MK-2 [19,20], PLK1 [21], kinesin Eg5 [22] and IKK [19].

To promote the antitumor activity and DNA intercalating potency of such compounds, previous attempt [5,11] were focused on incorporating substituents into position-1 and 3 of β-carboline nucleus. The introduction of amino group into position-1 or the presence of a flexible amino side chain in position-3 of β-carboline nucleus was proved to improve the affinity of the intercalator for the DNA molecule, consequently to a greater cytotoxic activity. We have ever reported that a series of 9-alkyl or arylated alkyl substituted harmine derivatives as efficient DNA intercalating and potent cytotoxic agents [23]. In addition, our previous investigation [24–29] 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 was 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-9 of β-carboline nucleus played a vital role in determining their antitumor potencies, and the n-butyl and phenylpropyl substituents in position-9 was suitable pharmacophoric group giving rise to some potent antitumor agents.

More recently, our group reported that β-carbolines, bearing a flexible alkylamino side chain at position-3 and a alkyl or arylated alkyl substituent at position-9, exhibited significant antitumor

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activities *in vitro*, and the N^9-arylated alkyl substituted β-carbolines represented the most interesting cytotoxic activities [31,32]. In addition, previous literature described that the complex polycyclic ring system at postion-1 of β-carboline nucleus in manzamine A can be replaced with simpler amino substituents to provide active compounds [11].

In our continued efforts for simple but efficient antitumor and DNA intercalating agents, we report here, the molecular design and chemical synthesis of a series of novel β-carbolines bearing a flexible alkylamino side chain at position-1 and a alkyl or arylated alkyl substituent at position-9, respectively. These compounds were expected to intercalate into DNA more strongly and to exhibit efficient medical and biological use due to the improved water solubility and the increased flexibility of the aminoalkyl side chains.

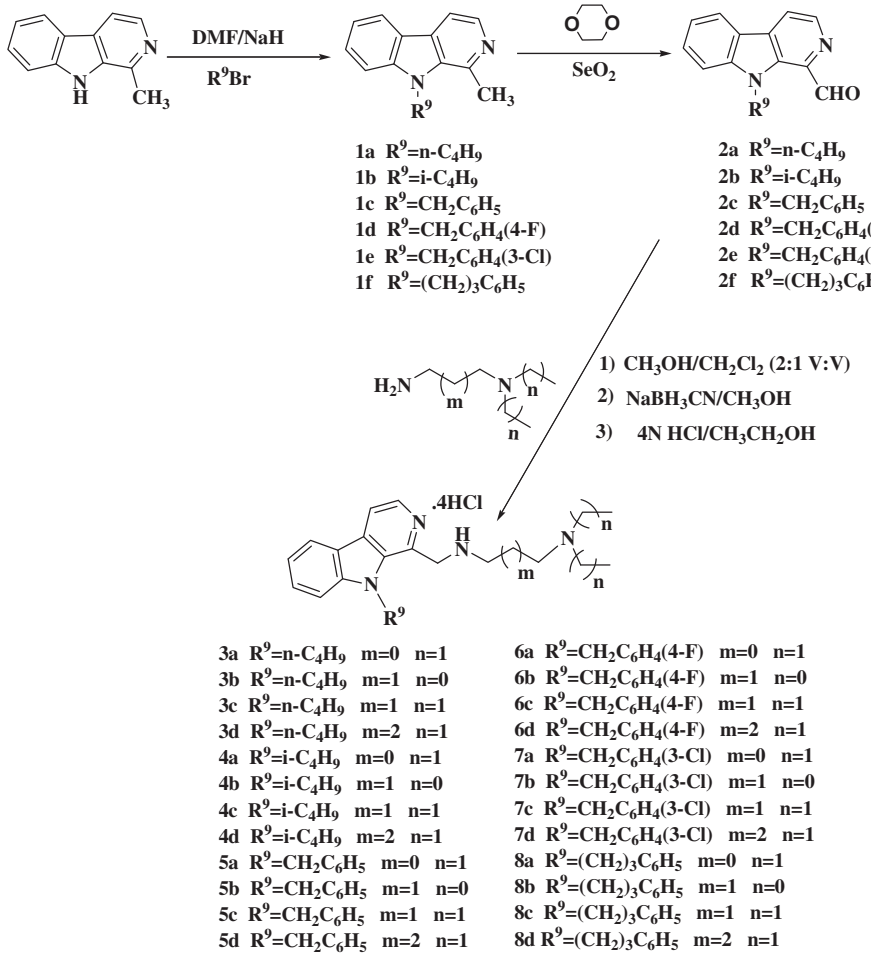
2. Chemistry

The synthetic routes of novel β-carbolines **3a**–**d**, **4a**–**d**, **5a**–**d**, **6a**–**d, 7a**–**d** and **8a**–**d** are outlined in Scheme 1. The 1-methyl β-carboline, prepared by the condensation of L-tryptophan with acetaldehyde in acid solution and followed by aromatization, oxidation and decarboxylation in a single step through the action of potassium dichromate according to the method previously described by Synder et al. [30], was alkylated or arylated by the action of sodium hydride in anhydrous DMF followed by the addition of the relevant appropriate alkylating and arylating agents to afford intermediates **1a**–**f** [24–26] in 65–76% yield. The methyl group in position-1 of compounds **1a**–**f** was oxidized to its corresponding carboxaldehyde by SeO_2 in anhydrous dioxane to provide compounds **2a**–**f** in 62–78% yield. The reaction of compounds **2a**–**f** 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 **3a**–**d**, **4a**–**d**, **5a**–**d**, **6a**–**d, 7a**–**d** and **8a**–**d** (Table 1) in 35–65% yield. The chemical structures of all the synthesized new compounds were characterized by MS, IR, ^{1}H NMR, and ^{13}C NMR spectra.

3. Results and discussion

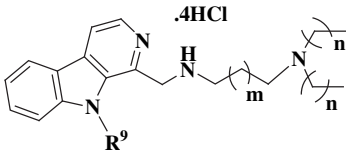
3.1. Cytotoxic activity in vitro

The cytotoxic potencies of novel β-carbolines bearing a N,N-dialkylaminoalkylamino moiety against a panel of human tumor cell lines were investigated and compared with the reference drugs cisplatin, paclitaxel and adriamycin. The tumor cell line panel consisted of renal carcinoma (769-P, 786-0 and OS-RC-2), epidermoid carcinoma of the nasopharynx (KB), gastric carcinoma (BGC-823), liver carcinoma (HepG2), melanoma (A375), colon carcinoma (HT-29), prostate carcinoma (22RV1) and breast carcinoma (MCF-7). As shown in Table 1, compounds **3a**–**d, 4a**–**d, 5a**–**d** and **6a**–**d** only demonstrated moderate cytotoxic activities, whereas compounds **7a**–**d** and **8a**–**d**, bearing a 3-chlorobenzyl and 3-phenylpropyl substituents in position-9 of β-carboline nucleus, respectively, exhibited significant cytotoxic potencies. Of all 9-(3-



Scheme 1. Synthesis of β-carbolines **3a**–**d**, **4a**–**d**, **5a**–**d**, **6a**–**d**, **7a**–**d** and **8a**–**d**.

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



Compd	R^9	m	n	769-P[b]	KB	BGC-823	786–0	HepG2	A375	HT-29	OS-RC-2	22RV1	MCF-7
3a	n-C_4H_9	0	1	43.7	>100	>100	>100	>100	31.3	>100	31.9	112	59.6
3b	n-C_4H_9	1	0	75.6	53.3	41.0	22.4	26.2	52.9	10.1	31.3	17.2	66.2
3c	n-C_4H_9	1	1	14.9	61.7	40.0	56.7	27.1	54.4	41.4	26.4	40.3	75.8
3d	n-C_4H_9	2	1	69.2	64.4	32.6	19.9	20.4	56.9	9.3	14.8	12.6	55.6
4a	i-C_4H_9	0	1	25.4	73.0	99.2	28.7	>100	84.6	8.4	50.9	16.5	96.0
4b	i-C_4H_9	1	0	>100	62.6	44.7	20.2	28.3	82.9	12.1	21.3	17.9	72.2
4c	i-C_4H_9	1	1	>100	92.8	72.8	33.5	53.8	68.3	17.0	47.0	23.0	>100
4d	i-C_4H_9	2	1	>100	74.6	56.2	18.6	27.2	75.8	19.5	26.3	19.3	83.1
5a	$CH_2C_6H_5$	0	1	34.9	>100	>100	68.6	59.5	28.0	63.8	32.5	73.2	62.3
5b	$CH_2C_6H_5$	1	0	15.1	44.2	41.0	39.3	23.5	26.9	32.7	12.5	24.5	53.6
5c	$CH_2C_6H_5$	1	1	19.2	60.0	66.9	39.6	22.0	25.8	33.4	22.5	34.3	51.6
5d	$CH_2C_6H_5$	2	1	71.7	39.9	33.8	16.7	27.0	56.2	12.3	20.4	12.8	58.2
6a	$CH_2C_6H_4$(p-F)	0	1	23.9	>100	>100	65.5	61.1	25.2	58.8	34.6	65.9	58.8
6b	$CH_2C_6H_4$(p-F)	1	0	11.7	32.5	28.8	30.1	20.0	28.6	27.8	10.9	29.9	44.2
6c	$CH_2C_6H_4$(p-F)	1	1	19.6	32.9	34.6	36.3	22.8	34.5	29.5	18.1	32.9	53.1
6d	$CH_2C_6H_4$(p-F)	2	1	13.3	30.0	29.7	17.7	20.7	51.8	18.8	12.6	19.1	52.0
7a	$CH_2C_6H_4$(m-Cl)	0	1	26.7	57.9	38.9	28.1	16.4	14.6	27.0	29.8	28.6	17.2
7b	$CH_2C_6H_4$(m-Cl)	1	0	24.5	14.0	12.0	7.5	9.3	13.1	6.1	10.2	6.1	14.7
7c	$CH_2C_6H_4$(m-Cl)	1	1	11.4	12.4	10.8	9.3	18.0	11.9	5.7	10.1	5.7	17.6
7d	$CH_2C_6H_4$(m-Cl)	2	1	28.5	10.3	9.3	8.8	7.2	14.6	5.6	9.9	5.9	20.8
8a	$(CH_2)_3C_6H_5$	0	1	14.8	9.2	14.7	18.2	10.6	14.7	16.3	8.1	21.9	25.7
8b	$(CH_2)_3C_6H_5$	1	0	1.7	3.2	5.9	4.4	4.6	18.1	3.9	11.1	5.3	17.7
8c	$(CH_2)_3C_6H_5$	1	1	2.4	3.1	7.5	5.0	3.5	11.6	3.7	10.0	6.9	16.6
8d	$(CH_2)_3C_6H_5$	2	1	12.4	9.7	20.7	10.0	8.5	4.7	3.7	51.1	10.2	27.4
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	<1.3	3.1	<1.3	17.2	4.2	<1.3	<1.3	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, 786-0 and OS-RC-2), epidermoid carcinoma of the nasopharynx (KB), gastric carcinoma (BGC-823), 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.

chloro)benzyl and 9-(3-phenyl)propyl substituted β-carboline derivatives, compounds **7b**, **7c**, **8b** and **8c**, having a N,N-diethylamino propylamino moiety in position-1, were found to be the most potent cytotoxic agents with IC_{50} value of lower than 20 μM against 10 human tumor cell lines investigated. These results indicated that (1) the 3-chlorobenzyl and 3-phenylpropyl substituents in position-9 of β-carboline nucleus were the suitable pharmacophoric group giving rise to potent antitumor agents; (2) the length of the alkylamino side chain moiety also affected their cytotoxic potencies, and three CH_2 units were more favorable.

3.2. UV spectral absorbance

The interaction of the selected compounds **3**–**8c** with calf thymus DNA (CT-DNA) was examined by UV spectroscopy. Fig. 1 illustrates the absorption spectra of compounds **3**–**8c** 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. These results suggested that these compounds had a significant interaction with CT-DNA double helix.

3.3. DNA thermal denaturation studies

Melting temperature (T_m) measurements were deployed to evaluate relative affinity for DNA of compounds **3**–**8c**. The T_m of CT-DNA in the presence and absence of compounds **3**–**8c** were obtained from melting curves (not shown) and the results of T_m analysis performed with CT-DNA were shown in Fig. 2. CT-DNA which melt at a low temperature (53.2 °C in PE buffer) afforded a sensitive determination of the DNA binding capacity of the studied molecules. As indicated in Fig. 2, compound **3c**, bearing a N,N-diethylamino propylamino group and n-butyl subsituent in position-1 and 9, respectively, markedly 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 19.2 °C. Whereas, 9-isobutyl (compound **4c**) and 9-benzyl (compound **5c**) substituted β-carboline congeners exhibited weaker effects on CT-DNA thermal stability with ΔT_m value of 16.1 and 14.8 °C, respectively. Unexpectedly, replacement of the n-butyl substituent in position-9 of compound **3c** with 4-fluorobenzyl group afforded compound **6c**, which displayed the poorest DNA binding capacity with ΔT_m value of 8.1 °C. Interestingly, compounds **7c** and **8c**, bearing 3-chlorobenzyl and 3-phenylpropyl group in position-9, respectively, were found to be the most potent compounds with ΔT_m value of 19.9 and 21.5 °C, respectively. The results suggested that (1) such compounds could significantly stabilize the double helix of CT-DNA; (2) the introduction of appropriate substituent into position-9 of β-carboline nucleus played a vital role in determining their DNA binding potency, and 3-chlorobenzyl and 3-phenylpropyl group were more favorable.

3.4. Fluorescence studies

β-Carboline alkaloids present intrinsic fluorescence properties which could be used to evaluate their DNA binding potencies. The

effect of CT-DNA on the fluoresence intensity of the selected β-carbolines **3**–**8c** was determined by the use of fluoresence spectroscopy. From the titration of compounds **3**–**8c** (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

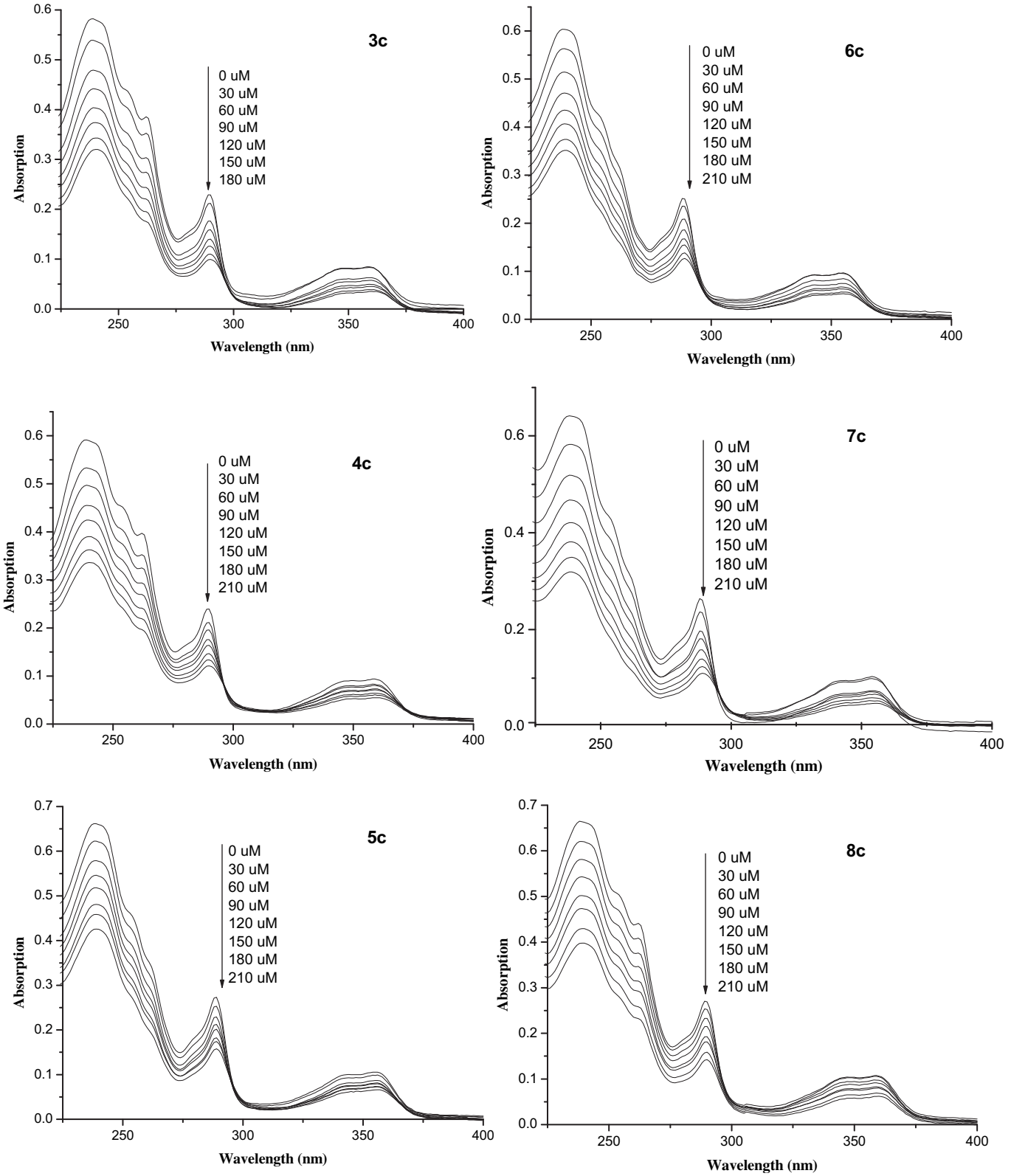


Fig. 1. Absorption spectra for compounds **3**–**8c** in 1 ml PE buffer (1 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.4) at different molarities of CT-DNA: top curve (0.0) and bottom curve (210 μM) were recorded in quartz cells (10 mm path length) by a UV–visible spectrophotometer at room temperature.

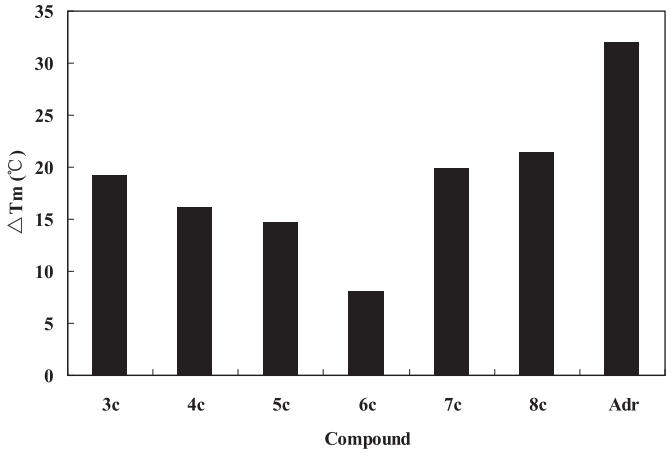


Fig. 2. Variation of the $\triangle T_m$ of the complexes between the tested compounds and CT-DNA. Melting temperature measurements were performed in PE buffer (1 mM Na_2HPO_4, 0.1 mM EDTA) at pH 7.4 with a drug/DNA ration of 0.2. Adr is abbreviation of adriamycin (Doxorubicin Hydrochloride).

fluorescence quenching of all investigated compounds, a slight bathochromic shift was also observed. These results suggested that such β-carbolines could effectively interact with DNA.

4. Conclusion

With the aim of elucidating the structure–activity relationship studies and probing the structural requirement for the potent antitumor activity of β-carbolines, a series of novel 1-(N,N-dialkylaminoalkylamino)- and 9-alkyl substituted β-carbolines has been synthesized and evaluated as potent cytotoxic and DNA intercalating agents. Their cytotoxic potencies and DNA intercalating capacities evidenced the importance of the length of N,N-dialkylaminoalkylamino group in position-1 and the nature of alkyl substituent in position-9 of β-carboline nucleus. The 3-chlorobenzyl and 3-phenylpropy substituents in position-9 were the suitable pharmacophore giving rise to potent antitumor agents and the optimal length of the alkylamino side chain moiety were three CH_2 units.

An overview of the cytotoxic activities data of all new synthesized β-carbolines bearing a flexible alkylamino side chain at position-1 and of the earlier reported β-carbolines bearing a flexible alkylamino side chain at position-3 [31,32] clearly indicated that (i) the N^9-arylated alkyl substituted β-carbolines represented the most interesting cytotoxic activities; (ii) the optimal length of the alkylamino side chain moiety were three CH_2 units and (iii) their cytotoxic potencies had no difference between β-carbolines bearing a flexible alkylamino side chain at C-1 and C-3.

An attempt to include all of the 1,9-disubstituted β-carbolines into a correlation of cytotoxic potency with $\triangle T_m$ values was unsuccessful, but this result has been observed for other DNA binding agents. Because the DNA binding process is complicated and dependent on many structural factors, undoubtedly, a more detailed molecular mechanics study on the DNA binding of these compounds is needed.

5. Experimental protocols

5.1. Reagents and general procedures

All solvents were of reagent grade and, when necessary, were purified and dried by standard methods. Reactions and products were routinely monitored by thin-layer chromatography (TLC) on silica gel (Kieselgel 60 F_{254}, Merck). 9-n-Butyl-1-methyl-β-carboline (**1a**) [26], 9-benzyl-1-methyl-β-carboline (**1c**) [26] and 9-(3-phenyl)propyl-1-methyl-β-carboline (**1f**) [26] are known compounds. NMR spectra were recorded on a Varian Mercury-Plus 300 spectrometer, 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 TMS and coupling constants (J) are given in Hertz. Mass spectra were obtained from VG ZAB-HS and LCMS-2010A. High-resolution mass spectrometry (HRMS); 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}.

5.2. General procedures for the synthesis of 9-alkyl substituted β-carbolines *1b*, *1d* and *1e*

A mixture of 1-methyl-β-carboline (1.82 g, 10 mmol) and anhydrous DMF (50 ml) was stirred at room temperature until clear, and then 60% NaH (0.6 g, 15 mmol) and halogenated alkane (15 mmol) were added. The mixture was stirred at room temperature. After completion of the reaction as indicated by TLC, the solution was poured into H_2O (50 ml) and extracted with ethyl acetate. The organic phase was washed with water and brine, then dried over anhydrous sodium sulfate, filtered and evaporated. The resulting oil was purified by silica column chromatography with ethyl acetate as the eluent. Upon recrystallization, white solid were obtained.

5.2.1. 9-Isobutyl-1-methyl-β-carboline (*1b*)

Yield 65%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.30 (d, J = 5.1 Hz, 1H, H-3), 8.08 (d, J = 7.8 Hz, 1H, H-4), 7.82 (d, J = 5.1 Hz, 1H, H-8), 7.22–7.57 (m, 3H, H-5, H-6, H-7), 4.33 (d, J = 7.5 Hz, 2H, NC***H***$_2$CH(CH_3)$_2$), 3.03 (s, 3H, C***H***$_3$), 2.22–2.31(m, 1H, NCH_2C***H***(CH_3)$_2$), 0.94 (s, 3H, CH(C***H***$_3$)$_2$), 0.92 (s, 3H, CH(C***H***$_3$)$_2$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 142.0, 141.4, 138.1, 135.4, 129.1, 127.9, 121.4, 121.2, 119.6, 113.0, 110.6, 51.9, 30.9, 24.2(d), 20.5; FAB-MS m/z (M + 1) 239.

5.2.2. 9-(4-Fluorobenzyl)-1-methyl-β-carboline (*1d*)

Yield 72%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.33 (d, J = 5.1 Hz, 1H, H-3), 8.13 (d, J = 7.8 Hz, 1H, H-4), 7.86 (d, J = 5.1 Hz, 1H, H-8), 7.50–7.55 (m, 1H, H-7), 7.25–7.34 (m, 2H, H-5, H-6), 6.96 (d, 4H, J = 7.2 Hz, Ph-***H***), 5.75 (s, 2H, NC***H***$_2$-4-fluorophenyl), 2.87 (s, 3H, C***H***$_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 163.8, 160.5, 141.8, 141.6, 138.7, 135.5, 133.8, 129.2, 128.6, 127.2, 127.1, 121.7, 121.5, 120.3, 116.2, 115.9, 113.1, 109.8, 47.7, 23.5(d); FAB-MS m/z (M + 1) 305.

5.2.3. 9-(3-Chlorobenzyl)-1-methyl-β-carboline (*1e*)

Yield 76%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.34 (d, J = 5.1 Hz, 1H, H-3), 8.14–8.16 (m, 1H, H-4), 7.86 (d, J = 5.1 Hz, 1H, H-8), 7.50–7.56 (m, 1H, H-7), 7.15–7.32 (m, 3H, H-5, H-6, Ph-***H***), 7.03 (s, 1H, Ph-***H***), 6.81 (d, J = 7.2 Hz, 2H, Ph-***H***), 5.74 (s, 2H, C***H***$_2$), 2.87 (s, 3H, C***H***$_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 141.8, 141.5, 140.3, 138.8, 135.5, 135.1, 130.5, 129.3, 128.7, 128.0, 125.8, 123.7, 121.7, 121.6, 120.4, 113.2, 109.8, 47.9, 23.5(d); FAB-MS m/z (M + 1) 321.

5.3. General procedures for the synthesis of 9-alkyl substituted β-carboline-1-carboxaldehydes ***2a–f***

To a solution of **1a–f** (5 mmol) in dioxane (100 ml) was added SeO_2 (10 mmol). The suspension was refluxed for 2 h. After completion of the reaction as indicated by TLC, the mixture was cooled and filtered through Celite. The filtrate was evaporated under reduced pressure. The residue was crystallized from acetone or acetone–petroleum ether to afford white solid.

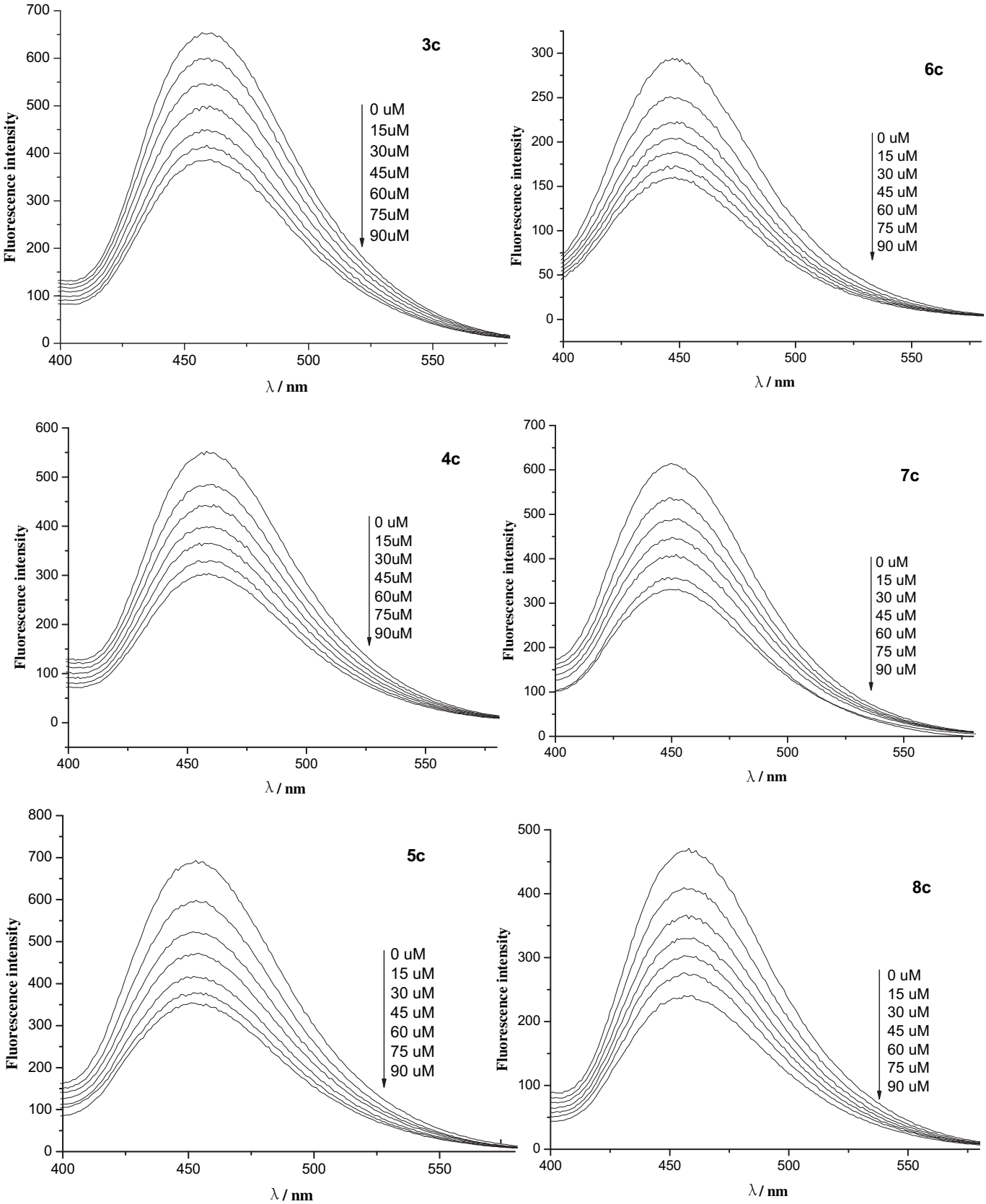


Fig. 3. Fluorescence spectra of the various β-carbolines upon incubation with graded concentration of CT-DNA. A fixed concentration of the various β-carbolines (10 μM) was incubated with increasing concentration of CT-DNA from 0 to 90 μM in PE buffer (1 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.4).

*5.3.1. 9-n-Butyl-β-carboline-1-carboxaldehyde (**2a**)*

Yield 65%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.31 (s, 1H, C***H***O); 8.63 (d, *J* = 4.8 Hz, 1H, H-3); 8.13–8.17 (m, 2H, H-4, H-8); 7.62–7.68 (m, 1H, H-7); 7.53 (d, *J* = 8.4 Hz, 1H, H-5); 7.24–7.32 (m, 1H, H-6); 4.86 (t, *J* = 7.5 Hz, 2H, NC***H***$_2$CH$_2$CH$_2$CH$_3$); 1.70–1.80 (m, 2H, NCH$_2$C***H***$_2$CH$_2$CH$_3$); 1.26–1.41 (m, 2H, NCH$_2$CH$_2$C***H***$_2$CH$_3$); 0.93 (t, *J* = 7.2 Hz, 3H, NCH$_2$CH$_2$CH$_2$C***H***$_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 193.9, 142.5, 138.3, 137.7, 135.1, 132.5, 129.3, 121.4, 120.9, 120.8, 118.6, 110.7, 46.8, 32.1, 20.3, 14.2; FAB-MS *m*/*z* (M + 1) 253.

*5.3.2. 9-Isobutyl-β-carboline-1-carboxaldehyde (**2b**)*

Yield 62%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.31 (s, 1H, C***H***O), 8.63 (d, *J* = 4.8 Hz, 1H, H-3), 8.14–8.19 (m, 2H, H-4, H-8), 7.31–7.66 (m, 3H, H-5, H-6, H-7), 4.78 (t, *J* = 7.5 Hz, 2H, NC***H***$_2$CH(CH$_3$)$_2$), 2.04–2.17 (m, 1H, NCH$_2$C***H***(CH$_3$)$_2$), 0.83 (s, 3H, C***H***$_3$), 0.80 (s, 3H, C***H***$_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 193.9, 142.9, 138.3, 138.0, 135.3, 132.5, 129.2, 121.4, 120.8, 118.7, 111.3, 109.9, 53.4, 30.0, 29.4, 20.1; FAB-MS *m*/*z* (M + 1) 253.

*5.3.3. 9-Benzyl-β-carboline-1-carboxaldehyde (**2c**)*

Yield 78%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.20 (s, 1H, C***H***O), 8.65 (d, *J* = 5.1 Hz, 1H, H-3), 8.18–8.23 (m, 2H, H-4, H-8), 7.34–7.63 (m, 3H, H-5, H-6, H-7), 7.16–7.19 (m, 3H, Ph-***H***), 6.92–6.95 (m, 2H, Ph-***H***), 6.18 (s, 2H, NC***H***$_2$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 193.9, 143.0, 138.9, 138.1, 137.7, 135.8, 132.8, 129.7, 128.8, 127.4, 126.3, 121.6, 121.3, 121.1, 118.8, 111.2, 50.4; FAB-MS *m*/*z* (M + 1) 287.

*5.3.4. 9-(4-Fluorobenzyl)-β-carboline-1-carboxaldehyde (**2d**)*

Yield 72%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.20 (s, 1H, C***H***O), 8.66 (d, *J* = 5.1 Hz, 1H, H-3), 8.17–8.23 (m, 2H, H-4, H-8), 7.35–7.64 (m, 3H, H-5, H-6, H-7), 6.84–6.95 (m, 4H, 4-fluorophenyl-***H***), 6.14 (s, 2H, NC***H***$_2$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 194.0, 163.6, 160.4, 142.8, 139.0, 138.0, 135.6, 133.5, 132.8, 129.8, 128.1, 128.0, 121.6, 121.4, 121.2, 118.9, 115.8, 115.5, 111.1, 49.8; FAB-MS *m*/*z* (M + 1) 305.

*5.3.5. 9-(3-Chlorobenzyl)-β-carboline-1-carboxaldehyde (**2e**)*

Yield 76%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.20 (s, 1H, C***H***O), 8.67 (d, *J* = 4.8 Hz, 1H, H-3), 8.18–8.23 (m, 2H, H-4, H-8), 7.58–7.64 (m, 1H, H-7), 7.36–7.44 (m, 2H, H-5, H-6), 7.09–7.17 (m, 2H, 3-chlorophenyl-***H***), 6.81–6.97 (m, 2H, 3-chlorophenyl-***H***), 6.16 (s, 2H, NC***H***$_2$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 194.1, 142.7, 140.0, 139.1, 138.0, 135.7, 134.7, 132.8, 130.1, 129.9, 127.7, 126.5, 124.5, 121.6, 121.5, 121.2, 118.9, 111.0, 50.1; FAB-MS *m*/*z* (M + 1) 321.

*5.3.6. 9-(3-Phenylpropyl)-β-carboline-1-carboxaldehyde (**2f**)*

Yield 68%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.30 (s, 1H, C***H***O), 8.62 (d, *J* = 4.8 Hz, 1H, H-3), 8.12–8.16 (m, 2H, H-4, H-8), 7.58–7.63 (m, 1H, H-7), 7.13–7.37 (m, 7H, H-5, H-6, Ph-***H***), 4.89 (t, *J* = 7.8 Hz, 2H, NC***H***$_2$CH$_2$CH$_2$Ph), 2.71 (t, *J* = 7.5 Hz, 2H, NCH$_2$CH$_2$C***H***$_2$Ph), 2.07–2.18 (m, 2H, NCH$_2$C***H***$_2$CH$_2$Ph). ^{13}C NMR (75 MHz, $CDCl_3$): δ 194.0, 142.3, 141.3, 138.4, 137.7, 135.1, 132.5, 129.4, 128.6, 128.5(2C), 126.2, 121.5, 120.9, 118.7, 110.6, 46.5, 33.1, 31.5; FAB-MS *m*/*z* (M + 1) 315.

*5.4. General procedures for the synthesis of β-carbolines **3a–d**, **4a–d**, **5a–d**, **6a–d**, **7a–d** and **8a–d***

A mixture of β-carboline-1-carboxaldehydes (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 4N 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 remove the residual ethanol.

*5.4.1. 1-[N-(2-diethylamino)-ethyl]-methylamino-9-n-butyl-β-carboline hydrochloride salt (**3a**)*

Yield 40%; IR (KBr, cm^{-1}) *v*: 2947, 2792, 2581, 1623, 1494, 1443, 1389, 1292, 1198, 1134, 1026, 758; ^{1}H NMR (300 MHz, DMSO-d_6 + D_2O): δ 8.41 (d, *J* = 5.1 Hz, 1H, H-3), 8.30–8.33 (m, 2H, H-4, H-8), 7.78 (d, *J* = 8.1 Hz, 1H, H-5), 7.64–7.69 (m, 1H, H-7), 7.33 (t, *J* = 7.5 Hz, 1H, H-6), 4.99 (s, 2H, C***H***$_2$NHCH$_2$CH$_2$N), 4.56 (t, *J* = 7.2 Hz, 2H, NC***H***$_2$CH$_2$CH$_2$CH$_3$), 3.64–3.66 (m, 4H, NHC***H***$_2$C***H***$_2$N), 3.18–3.25 (m, 4H, N(C***H***$_2$CH$_3$)$_2$), 1.70–1.80 (m, 2H, NCH$_2$C***H***$_2$CH$_2$CH$_3$), 1.25–1.37 (m, 8H, NCH$_2$CH$_2$C***H***$_2$CH$_3$, N(CH$_2$C***H***$_3$)$_2$), 0.89 (t, *J* = 7.2 Hz, 3H, NCH$_2$CH$_2$CH$_2$C***H***$_3$); ^{13}C NMR (75 MHz, D_2O): δ 144.3, 135.1, 132.6, 132.4, 130.8, 129.7, 122.5, 121.9, 118.6, 116.9, 110.9, 49.2, 48.3, 46.7, 45.4, 43.5, 32.2, 19.8, 13.5, 8.6; ESI-MS *m*/*z*: 353.4 (M + 1)$^+$.

*5.4.2. 1-[N-(3-dimethylamino)-propyl]-methylamino-9-n-butyl-β-carboline hydro-chloride salt (**3b**)*

Yield 48%; IR (KBr, cm^{-1}) *v*: 3013, 2956, 2869, 2611, 1625, 1524, 1462, 1382, 1338, 1243, 1170, 1058, 774, 752; ^{1}H NMR (300 MHz, DMSO-d_6 + D_2O): δ 8.49 (d, *J* = 5.4 Hz, 1H, H-3), 8.43 (d, *J* = 5.4 Hz, 1H, H-4), 8.37 (d, *J* = 8.1 Hz, 1H, H-8), 7.82 (d, *J* = 8.4 Hz, 1H, H-5), 7.70 (t, *J* = 7.5 Hz, 1H H-7,), 7.36 (t, *J* = 7.2 Hz, 1H, H-6), 4.95 (s, 2H, C***H***$_2$NHCH$_2$CH$_2$CH$_2$N), 4.60 (t, *J* = 6.6 Hz, 2H, NC***H***$_2$CH$_2$CH$_2$CH$_3$), 3.32 (t, *J* = 7.2 Hz, 2H, NHC***H***$_2$CH$_2$CH$_2$N), 3.22 (t, *J* = 7.2 Hz, 2H, NHCH$_2$CH$_2$C***H***$_2$N), 2.76 (s, 6H, N(C***H***$_3$)$_2$), 2.18–2.27 (m, 2H, NHCH$_2$C***H***$_2$CH$_2$N), 1.69–1.79 (m, 2H, NCH$_2$C***H***$_2$CH$_2$CH$_3$), 1.31–1.40 (m, 2H, NCH$_2$CH$_2$C***H***$_2$CH$_3$), 0.89 (t, *J* = 7.2 Hz, 3H, NCH$_2$CH$_2$CH$_2$C***H***$_3$); ^{13}C NMR (75 MHz, D_2O): δ 144.7, 136.3, 133.4, 132.7, 131.1, 126.1, 122.7, 122.0, 118.7, 117.7, 110.9, 54.5, 45.9 (2C), 45.4, 43.3, 31.8, 21.8, 19.8, 13.5; ESI-MS *m*/*z*: 339.4 (M + 1)$^+$.

*5.4.3. 1-[N-(3-diethylamino)-propyl]-methylamino-9-n-butyl-β-carboline hydro-chloride salt (**3c**)*

Yield 46%; IR (KBr, cm^{-1}) *v*: 2958, 2685, 1624, 1496, 1461, 1383, 1337, 1201, 1135, 1044, 758; ^{1}H NMR (300 MHz, DMSO-d_6 + D_2O): δ 8.49 (d, *J* = 5.1 Hz, 1H, H-3), 8.42 (d, *J* = 5.4 Hz, 1H, H-4), 8.36 (d, *J* = 8.1 Hz, 1H, H-8), 7.81 (d, *J* = 8.4 Hz, 1H, H-5), 7.70 (t, *J* = 7.5 Hz, 1H, H-7), 7.35 (t, *J* = 7.2 Hz, 1H, H-6), 4.94 (s, 2H, C***H***$_2$NHCH$_2$CH$_2$CH$_2$N), 4.59 (t, *J* = 6.9 Hz, 2H, NC***H***$_2$CH$_2$CH$_2$CH$_3$), 3.31 (t, *J* = 6.9 Hz, 2H, NHC***H***$_2$CH$_2$CH$_2$N), 3.20 (t, *J* = 7.5 Hz, 2H, NHCH$_2$CH$_2$C***H***$_2$N), 3.08–3.15 (m, 4H, N(C***H***$_2$CH$_3$)$_2$), 2.16–2.26 (m, 2H, NHCH$_2$C***H***$_2$CH$_2$N), 1.69–1.79 (m, 2H, NCH$_2$C***H***$_2$CH$_2$CH$_3$), 1.30–1.37 (m, 2H, NCH$_2$CH$_2$C***H***$_2$CH$_3$), 1.23 (t, *J* = 7.2 Hz, 6H, N(CH$_2$C***H***$_3$)$_2$), 0.88 (t, *J* = 7.2 Hz, 3H, NCH$_2$CH$_2$CH$_2$C***H***$_3$); ^{13}C NMR (75 MHz, DMSO-d_6 + D_2O): δ 144.2, 133.9, 133.9, 133.2, 131.9, 131.5, 123.6, 122.0, 120.0, 117.6, 111.8, 48.6, 47.1, 46.4, 45.6 (2C), 32.6, 21.0, 20.3, 14.6, 9.3; ESI-MS *m*/*z*: 367.4 (M + 1)$^+$.

*5.4.4. 1-[N-(4-diethylamino)-butyl]-methylamino-9-n-butyl-β-carboline hydro-chloride salt (**3d**)*

Yield 50%; IR (KBr, cm^{-1}) *v*: 2958, 2693, 1625, 1545, 1496, 1462, 1337, 1296, 1201, 1136, 1040, 759; ^{1}H NMR (300 MHz, DMSO-d_6 + D_2O): δ 8.63 (d, *J* = 5.4 Hz, 1H, H-3), 8.57 (d, *J* = 5.4 Hz, 1H, H-4), 8.39 (d, *J* = 8.1 Hz, 1H, H-8), 7.91 (t, *J* = 7.5 Hz, 1H, H-7), 7.84 (d, *J* = 8.4 Hz, 1H, H-5), 7.53 (t, *J* = 7.2 Hz, 1H, H-6), 5.09 (s, 2H, C***H***$_2$NHCH$_2$CH$_2$CH$_2$CH$_2$N), 4.62 (t, *J* = 7.5 Hz, 2H, NC***H***$_2$CH$_2$CH$_2$CH$_3$), 3.47 (t, *J* = 7.5 Hz, 2H, NHC***H***$_2$CH$_2$CH$_2$CH$_2$N), 3.23–3.31 (m, 6H,

NHCH$_2$CH$_2$CH$_2$C***H***$_2$N), 1.91–1.97 (m, 4H, NHCH$_2$C***H***$_2$C***H***$_2$CH$_2$N), 1.81–1.87 (m, 2H, NCH$_2$C***H***$_2$CH$_2$CH$_3$), 1.38–1.42 (m, 2H, NCH$_2$CH$_2$C***H***$_2$CH$_3$), 1.34 (t, J = 7.5 Hz, 6H, N(CH$_2$C***H***$_3$)$_2$), 0.94 (t, J = 7.5 Hz, 3H, NCH$_2$CH$_2$CH$_2$C***H***$_3$); ^{13}C NMR (75 MHz, D$_2$O): δ 144.9, 136.4, 133.6, 132.8, 131.3, 126.4, 122.9, 122.2, 118.9, 117.8, 111.1, 51.2, 48.4, 47.7, 45.9, 45.5, 31.8, 23.3, 21.1, 19.9, 13.5, 8.7; ESI-MS m/z: 381.4 (M + 1)$^+$.

*5.4.5. 1-[N-(2-diethylamino)-ethyl]-methylamino-9-isobutyl-β-carboline hydro-chloride salt (**4a**)*

Yield 55%; CH_2Cl_2/CH_3OH, 95:5; 2963, 2672, 1623, 1497, 1464, 1384, 1335, 1135, 1042, 777; ^{1}H NMR (300 MHz, DMSO-d_6 + D$_2$O): δ 8.45 (d, J = 5.4 Hz, 1H, H-3), 8.32–8.36 (m, 2H, H-4, H-8), 7.83 (d, J = 8.7 Hz, 1H, H-5), 7.63–7.69 (m, 1H, H-7), 7.33 (t, J = 7.8 Hz, 1H, H-6), 4.96 (s, 2H, C***H***$_2$NHCH$_2$CH$_2$N), 4.42 (d, J = 7.5 Hz, 2H, NC***H***$_2$CH(CH$_3$)$_2$), 3.66–3.68 (m, 4H, NHC***H***$_2$C***H***$_2$N), 3.18–3.25 (m, 4H, N(C***H***$_2$CH$_3$)$_2$), 2.11–2.20 (m, 1H, NCH$_2$C***H***(CH$_3$)$_2$), 1.28 (t, J = 7.2 Hz, 6H, N(CH$_2$C***H***$_3$)$_2$), 0.86 (d, J = 6.6 Hz, 6H, NCH$_2$CH(C***H***$_3$)$_2$); ^{13}C NMR (75 MHz, D$_2$O,): δ 145.0, 135.8, 133.1, 132.4, 130.0, 129.4, 122.6, 122.0, 118.6, 117.3, 111.6, 52.0, 48.5, 48.3, 46.6, 43.3, 30.1, 19.4, 8.6; ESI-MS m/z: 353.4 (M + 1)$^+$.

*5.4.6. 1-[N-(3-dimethylamino)-propyl]-methylamino-9-isobutyl-β-carboline hydro-chloride salt (**4b**)*

Yield 36%; IR (KBr, cm^{-1}) v: 3049, 2957, 2673, 2485, 1623, 1459, 1338, 1289, 1207, 1139, 1045, 749; ^{1}H NMR (300 MHz, DMSO-d_6 + D$_2$O): δ 8.50 (d, J = 5.4 Hz, 1H, H-3), 8.42 (d, J = 5.4 Hz, 1H, H-4), 8.37 (d, J = 7.8 Hz, 1H, H-8), 7.86 (d, J = 8.7 Hz, 1H, H-5), 7.69 (t, J = 7.5 Hz, 1H, H-7), 7.35 (t, J = 7.2 Hz, 1H, H-6), 4.91 (s, 2H, C***H***$_2$NHCH$_2$CH$_2$CH$_2$N), 4.44 (d, J = 7.2 Hz, 2H, NC***H***$_2$CH(CH$_3$)$_2$), 3.32 (t, J = 7.2 Hz, 2H, NHC***H***$_2$CH$_2$CH$_2$N), 3.23 (t, J = 7.5 Hz, 2H, NHCH$_2$CH$_2$C***H***$_2$N), 2.77 (s, 6H, N(C***H***$_3$)$_2$), 2.20–2.28 (m, 2H, NHCH$_2$C***H***$_2$CH$_2$N), 2.11–2.18 (m, 1H, NCH$_2$C***H***(CH$_3$)$_2$), 0.87 (d, J = 6.6 Hz, 6H, NCH$_2$CH(C***H***$_3$)$_2$); ^{13}C NMR (75 MHz, D$_2$O): δ 145.3, 136.3, 133.8, 132.5, 131.6, 126.7, 122.9, 122.1, 118.9, 117.8, 111.8, 54.5, 52.1, 46.3, 45.9, 43.3, 30.0, 21.8, 19.6; ESI-MS m/z: 339.4 (M + 1)$^+$.

*5.4.7. 1-[N-(3-diethylamino)-propyl]-methylamino-9-isobutyl-β-carboline hydro-chloride salt (**4c**)*

Yield 39%; IR (KBr, cm^{-1}) v: 2926, 2685, 1624, 1495, 1463, 1385, 1335, 1289, 1137, 1044, 758; ^{1}H NMR (300 MHz, DMSO-d_6 + D$_2$O): δ 8.49 (d, J = 5.4 Hz, 1H, H-3), 8.42 (d, J = 5.4 Hz, 1H, H-4), 8.36 (d, J = 7.5 Hz, 1H, H-8), 7.85 (d, J = 8.4 Hz, 1H, H-5), 7.65–7.71 (m, 1H, H-7), 7.34 (t, J = 7.2 Hz, 1H, H-6), 4.90 (s, 2H, C***H***$_2$NHCH$_2$CH$_2$CH$_2$N), 4.43 (d, J = 7.5 Hz, 2H, NC***H***$_2$CH(CH$_3$)$_2$), 3.31 (t, J = 7.2 Hz, 2H, NHC***H***$_2$CH$_2$CH$_2$N), 3.20 (t, J = 7.5 Hz, 2H, NHCH$_2$CH$_2$C***H***$_2$N), 3.07–3.15 (m, 4H, N(C***H***$_2$CH$_3$)$_2$), 2.19–2.27 (m, 2H, NHCH$_2$C***H***$_2$CH$_2$N), 2.10–2.17 (m, 1H, NCH$_2$C***H***(CH$_3$)$_2$), 1.24 (t, J = 7.2 Hz, 6H, N(CH$_2$C***H***$_3$)$_2$), 0.86 (d, J = 6.3 Hz, 6H, NCH$_2$CH(C***H***$_3$)$_2$); ^{13}C NMR (75 MHz, DMSO-d_6 + D$_2$O): δ 144.5, 134.1, 133.9, 133.5, 132.1, 131.4, 123.4, 121.8, 120.0, 117.3, 112.4, 52.1, 48.6, 47.1, 46.7, 45.6, 30.7, 21.0, 20.5, 9.3; ESI-MS m/z: 367.4 (M + 1)$^+$.

*5.4.8. 1-[N-(4-diethylamino)-butyl]-methylamino-9-isobutyl-β-carboline hydro-chloride salt (**4d**)*

Yield 40%; IR (KBr, cm^{-1}) v: 2928, 2677, 1623, 1550, 1461, 1335, 1211, 1138, 1041, 767; ^{1}H NMR (300 MHz, DMSO-d_6 + D$_2$O): δ 8.48 (d, J = 5.4 Hz, 1H, H-3), 8.39 (d, J = 5.4 Hz, 1H, H-4), 8.35 (d, J = 7.8 Hz, 1H, H-8), 7.85 (d, J = 8.1 Hz, 1H, H-5), 7.67 (t, J = 8.1 Hz, 1H, H-7), 7.34 (t, J = 7.2 Hz, 1H, H-6), 4.87 (s, 2H, C***H***$_2$NHCH$_2$CH$_2$CH$_2$CH$_2$N), 4.42 (d, J = 7.5 Hz, 2H, NC***H***$_2$CH(CH$_3$)$_2$), 3.22 (t, J = 6 Hz, 2H, NHC***H***$_2$CH$_2$CH$_2$CH$_2$N), 3.05–3.12 (m, 6H, NHCH$_2$CH$_2$CH$_2$C***H***$_2$N), 2.07–2.20(m, 1H, NCH$_2$C***H***(CH$_3$)$_2$), 1.81 (br s, 4H, NHCH$_2$C***H***$_2$C***H***$_2$CH$_2$N), 1.21 (t, J = 7.2 Hz, 6H, N(CH$_2$C***H***$_3$)$_2$), 0.86 (d, J = 6.3 Hz, 6H, NCH$_2$CH(C***H***$_3$)$_2$); ^{13}C NMR (75 MHz, D$_2$O): δ 145.3, 136.1, 134.0, 132.4, 132.1, 127.4, 122.9, 122.2, 119.2, 117.7, 111.9, 52.2, 51.2, 48.3, 47.8, 46.5, 30.1, 23.4, 21.2, 19.5, 8.7; ESI-MS m/z: 381.4 (M + 1)$^+$.

*5.4.9. 1-[N-(2-diethylamino)-ethyl]-methylamino-9-benzyl-β-carboline hydro-chloride salt (**5a**)*

Yield 45%; IR (KBr, cm^{-1}) v: 2977, 2660, 1624, 1495, 1460, 1389, 1336, 1297, 1134, 1025, 753; ^{1}H NMR (300 MHz, DMSO-d_6 + D$_2$O): δ 8.46 (d, J = 5.1 Hz, 1H, H-3), 8.39 (br s, 1H, H-4), 8.37 (br s, 1H, H-8), 7.72 (d, J = 8.4 Hz, 1H, H-5), 7.63 (t, J = 7.2 Hz, 1H, H-7), 7.35 (t, J = 7.2 Hz, 1H, H-6), 7.22–7.29 (m, 3H, Ph-***H***), 6.96–6.99 (m, 2H, Ph-***H***), 5.93 (s, 2H, NC***H***$_2$-Ph), 4.79 (s, 2H, C***H***$_2$NHCH$_2$CH$_2$N), 3.45–3.49 (m, 4H, NHC***H***$_2$C***H***$_2$N), 3.12–3.19 (m, 4H, N(C***H***$_2$CH$_3$)$_2$), 1.23 (t, J = 7.2 Hz, 6H, C***H***$_3$); ^{13}C NMR (75 MHz, DMSO-d_6 + D$_2$O): δ 143.6, 138.1, 135.6, 134.2, 133.3, 132.4, 131.1, 129.7, 128.3, 126.3, 123.2, 121.9, 120.6, 117.0, 111.7, 48.7, 47.8, 47.5, 46.9, 42.1, 9.4; ESI-MS m/z: 387.4 (M + 1)$^+$.

*5.4.10. 1-[N-(3-dimethylamino)-propyl]-methylamino-9-benzyl-β-carboline hydro-chloride salt (**5b**)*

Yield 65%; IR (KBr, cm^{-1}) v: 2976, 2661, 1624, 1494, 1458, 1336, 1297, 1199, 1134, 1027, 749; ^{1}H NMR (300 MHz, DMSO-d_6 + D$_2$O): δ 8.46 (d, J = 5.4 Hz, 1H, H-3), 8.36–8.39 (m, 2H, H-4, H-8), 7.75 (d, J = 8.4 Hz, 1H, H-5), 7.61–7.67 (m, 1H, H-7), 7.36 (t, J = 7.5 Hz, 1H, H-6), 7.20–7.31 (m, 3H, Ph-***H***), 6.94–6.97 (m, 2H, Ph-***H***), 5.92 (s, 2H, NC***H***$_2$-Ph), 4.69 (s, 2H, C***H***$_2$NHCH$_2$CH$_2$CH$_2$N), 3.05–3.15 (m, 4H, NHC***H***$_2$CH$_2$C***H***$_2$N), 2.79 (s, 6H, C***H***$_3$), 2.02–2.12 (m, 2H, NHCH$_2$C***H***$_2$CH$_2$N); ^{13}C NMR (75 MHz, D$_2$O): δ 144.8, 136.3, 135.5, 134.0, 132.7, 132.5, 129.6, 128.5, 127.1, 125.6, 123.1, 122.5, 119.1, 117.9, 110.4, 54.3, 48.4, 45.6 (2C), 43.3, 21.5; ESI-MS m/z: 373.4 (M + 1)$^+$.

*5.4.11. 1-[N-(3-diethylamino)-propyl]-methylamino-9-benzyl-β-carboline hydro-chloride salt (**5c**)*

Yield 42%; IR (KBr, cm^{-1}) v: 2977, 2661, 1624, 1495, 1459, 1336, 1297, 1199, 1134, 1026, 751; ^{1}H NMR (300 MHz, DMSO-d_6 + D$_2$O): δ 8.45 (d, J = 5.1 Hz, 1H, H-3), 8.35 (m, 2H, H-4, H-8), 7.73 (d, J = 8.4 Hz, 1H, H-5), 7.63 (t, J = 7.5 Hz, 1H, H-7), 7.35 (t, J = 7.5 Hz, 1H, H-6), 7.22–7.30 (m, 3H, Ph-***H***), 6.93–6.95 (m, 2H, Ph-***H***), 5.91 (s, 2H, NC***H***$_2$-Ph), 4.68 (s, 2H, C***H***$_2$NHCH$_2$CH$_2$CH$_2$N), 3.04–3.13 (m, 8H, NHC***H***$_2$CH$_2$C***H***$_2$N(C***H***$_2$CH$_3$)$_2$), 2.00–2.10 (m, 2H, NHCH$_2$C***H***$_2$CH$_2$N), 1.20 (t, J = 7.5 Hz, 6H, C***H***$_3$); ^{13}C NMR (75 MHz, D$_2$O): δ 144.0, 136.2, 134.6, 134.1, 133.9, 131.8, 129.6, 129.1, 128.4, 125.65, 122.7, 122.0, 119.5, 117.1, 110.3, 48.6, 48.2, 47.9, 46.6, 45.4, 21.0, 8.7; ESI-MS m/z: 401.4 (M + 1)$^+$.

*5.4.12. 1-[N-(4-diethylamino)-butyl]-methylamino-9-benzyl-β-carboline hydro-chloride salt (**5d**)*

Yield 61%; IR (KBr, cm^{-1}) v: 2941, 2677, 1624, 1459, 1338, 1199, 1038, 746; ^{1}H NMR (C300 MHz, DMSO-d_6 + D$_2$O): δ 8.55–8.60 (m, 2H, H-3, H-4), 8.47 (d, J = 7.8 Hz, 1H, H-8), 7.80 (d, J = 8.4 Hz, 1H, H-5), 7.70 (t, J = 7.5 Hz, 1H, H-7), 7.40 (t, J = 7.5 Hz, 1H, H-6), 7.23–7.29 (m, 3H, Ph-***H***), 7.01 (d, J = 6.3 Hz, 2H, Ph-***H***), 6.01 (s, 2H, NC***H***$_2$-Ph), 4.73 (s, 2H, C***H***$_2$NHCH$_2$CH$_2$CH$_2$CH$_2$N), 3.00–3.10 (m, 8H, NHC***H***$_2$CH$_2$CH$_2$C***H***$_2$N(C***H***$_2$C***H***$_3$)$_2$), 1.72 (br s, 4H, NHCH$_2$C***H***$_2$C***H***$_2$CH$_2$N), 1.21 (t, J = 7.2 Hz, 6H, C***H***$_3$); ^{13}C NMR (75 MHz, DMSO-d_6 + D$_2$O): δ 143.9, 138.0, 135.3, 134.3, 132.9, 132.8, 131.5, 129.7, 128.3, 126.2, 123.4, 122.0, 120.4, 117.1, 111.6, 50.6, 48.7, 47.3, 46.9, 46.7, 23.4, 21.1, 9.3; ESI-MS m/z: 415.4 (M + 1)$^+$.

*5.4.13. 1-[N-(2-diethylamino)-ethyl]-methylamino-9-(4-fluorobenzyl)-β-carboline hydrochloride salt (**6a**)*

Yield 60%; IR (KBr, cm^{-1}) v: 2976, 2662, 1624, 1494, 1457, 1337, 1297, 1246, 1199, 1134, 1029, 749; ^{1}H NMR (300 MHz, DMSO-d_6 + D$_2$O): δ 8.45 (d, J = 5.1 Hz, 1H, H-3), 8.35–8.39 (m, 2H, H-4, H-8), 7.72 (d, J = 8.4 Hz, 1H, H-5), 7.63 (t, J = 7.5 Hz, 1H, H-7), 7.36 (t, J = 7.5 Hz, 1H, H-6), 7.08–7.14 (m, 2H, 4-fluorophenyl-***H***), 6.99–7.04 (m, 2H, 4-fluorophenyl-***H***), 5.91 (s, 2H, NC***H***$_2$-4-fluorophenyl), 4.79

(s, 2H, C***H***$_2$NHCH$_2$CH$_2$N), 3.49 (m, 4H, NHC***H***$_2$C***H***$_2$N) 3.13–3.20 (m, 4H, N(C***H***$_2$CH$_3$)$_2$), 1.24 (t, J = 7.2 Hz, 6H, C***H***$_3$); ^{13}C NMR (75 MHz, D$_2$O): δ 163.5, 160.2, 144.1, 134.8, 133.3, 133.1, 132.1, 131.0, 127.5, 127.4, 122.8, 122.2, 119.2, 116.9, 116.1, 115.8, 110.4, 49.4, 48.2, 47.9, 46.8, 43.1, 8.6; ESI-MS m/z: 405.4 (M + 1)$^+$.

*5.4.14. 1-[N-(3-dimethylamino)-propyl]-methylamino-9-(4-fluorobenzyl)-β-carboline hydrochloride salt (**6b**)*

Yield 35%; IR (KBr, cm^{-1}) v: 2950, 2692, 1625, 1547, 1505, 1466, 1384, 1332, 1290, 1222, 1158, 1130, 1047, 1019, 751; ^{1}H NMR (300 MHz, DMSO-d_6 + D$_2$O): δ 8.49 (d, J = 5.1 Hz, 1H, H-3), 8.38–8.42 (m, 2H, H-4, H-8), 7.77 (d, J = 8.4 Hz, 1H, H-5), 7.63–7.69 (m, 1H, H-7), 7.37 (t, J = 7.2 Hz, 1H, H-6), 7.08–7.14 (m, 2H, 4-fluorophenyl-***H***), 6.99–7.04 (m, 2H, 4-fluorophenyl-***H***), 5.93 (s, 2H, NC***H***$_2$-4-fluorophenyl), 4.70 (s, 2H, C***H***$_2$NHCH$_2$CH$_2$CH$_2$N), 3.11–3.18 (m, 4H, NHC***H***$_2$CH$_2$C***H***$_2$N), 2.74 (s, 6H, C***H***$_3$), 2.06–2.16 (m, 2H, NHCH$_2$C***H***$_2$CH$_2$N); ^{13}C NMR (75 MHz, DMSO-d_6 + D$_2$O): δ 163.7, 160.4, 144.1, 134.5, 134.3, 134.0, 133.7, 132.0, 131.9, 128.6, 128.5, 123.6, 122.3, 120.4, 117.6, 116.7, 116.4, 111.7, 54.2, 48.2, 46.3, 45.4, 42.8, 21.5; ESI-MS m/z: 391.4 (M + 1)$^+$.

*5.4.15. 1-[N-(3-diethylamino)-propyl]-methylamino-9-(4-fluorobenzyl)-β-carboline hydrochloride salt (**6c**)*

Yield 40%; IR (KBr, cm^{-1}) v: 2944, 2683, 1625, 1506, 1465, 1385, 1336, 1290, 1224, 1159, 1134, 1045, 754; ^{1}H NMR (300 MHz, DMSO-d_6 + D$_2$O): δ 8.44 (d, J = 5.1 Hz, 1H, H-3), 8.31–8.37 (m, 2H, H-4, H-8), 7.72 (d, J = 8.4 Hz, 1H, H-5), 7.63 (t, J = 7.5 Hz, 1H, H-7), 7.35 (t, J = 7.5 Hz, 1H, H-6), 7.09–7.13 (m, 2H, 4-fluorophenyl-***H***), 6.96–7.00 (m, 2H, 4-fluorophenyl-***H***), 5.89 (s, 2H, NC***H***$_2$-4-fluorophenyl), 4.68 (s, 2H, C***H***$_2$NHCH$_2$CH$_2$CH$_2$N), 3.05–3.14 (m, 8H, NHC***H***$_2$CH$_2$C***H***$_2$N(C***H***$_2$CH$_3$)$_2$), 2.03–2.09 (m, 2H, NHCH$_2$C***H***$_2$CH$_2$N), 1.21 (t, J = 7.2 Hz, 6H, C***H***$_3$); ^{13}C NMR (75 MHz, D$_2$O): δ 163.5, 160.3, 144.3, 135.6, 134.0, 133.3, 132.3, 131.7, 128.0, 127.6, 127.5, 122.9, 122.3, 119.4, 117.6, 116.3, 116.0, 110.4, 48.7, 47.9, 47.6, 46.1, 45.7, 21.1, 8.7; ESI-MS m/z: 419.4 (M + 1)$^+$.

*5.4.16. 1-[N-(4-diethylamino)-butyl]-methylamino-9-(4-fluorobenzyl)-β-carboline hydrochloride salt (**6d**)*

Yield 38%; IR (KBr, cm^{-1}) v: 2946, 2683, 1625, 1504, 1462, 1337, 1221, 752; ^{1}H NMR (300 MHz, DMSO-d_6 + D$_2$O): δ 8.51 (br s, 2H, H-3, H-4), 8.37 (d, J = 7.8 Hz, 1H, H-8), 7.63–7.74 (m, 2H, H-5, H-7), 7.36 (t, J = 7.5 Hz, 1H, H-6), 7.00–7.10 (m, 4H, 4-fluorophenyl-***H***), 5.92 (s, 2H, NC***H***$_2$-4-fluorophenyl), 4.71 (s, 2H, C***H***$_2$NHCH$_2$CH$_2$CH$_2$CH$_2$N), 3.00–3.10 (m, 8H, NHC***H***$_2$CH$_2$CH$_2$C***H***$_2$N(C***H***$_2$CH$_3$)$_2$), 1.72 (br s, 4H, NHCH$_2$C***H***$_2$C***H***$_2$CH$_2$N), 1.81 (t, J = 6.9 Hz, 6H, C***H***$_3$); ^{13}C NMR (75 MHz, DMSO-d_6 + D$_2$O): δ 163.6, 160.4, 143.6, 135.6, 134.1, 132.8, 131.4, 128.4, 128.3, 123.3, 122.1, 120.5, 117.0, 116.7, 116.4, 111.4, 50.8, 48.0, 47.4, 47.0 (2C), 23.4, 21.1, 9.3; ESI-MS m/z: 433.4 (M + 1)$^+$.

*5.4.17. 1-[N-(2-diethylamino)-ethyl]-methylamino-9-(3-chlorobenzyl)-β-carboline hydrochloride salt (**7a**)*

Yield 47%; IR (KBr, cm^{-1}) v: 2971, 2649, 1623, 1465, 1434, 1336, 1195, 772; ^{1}H NMR (300 MHz, DMSO-d_6 + D$_2$O): δ 8.43–8.49 (m, 2H, H-3, H-4), 8.29 (d, J = 7.8 Hz, 1H, H-8), 7.55–7.63 (m, 2H, H-5, H-7), 7.29–7.34 (m, 1H, H-6), 7.17–7.25 (m, 2H, 3-chlorophenyl-***H***), 6.89–6.93 (m, 2H, 3-chlorophenyl-***H***), 5.88 (s, 2H, NC***H***$_2$-3-chlorophenyl), 4.77 (s, 2H, C***H***$_2$NHCH$_2$CH$_2$N), 3.47–3.54 (m, 4H, NHC***H***$_2$C***H***$_2$N), 3.13–3.20 (m, 4H, NHCH$_2$CH$_2$N(C***H***$_2$CH$_3$)$_2$), 1.21 (d, J = 7.2 Hz, 6H, C***H***$_3$); ^{13}C NMR (75 MHz, DMSO-d_6 + D$_2$O): δ 143.4, 140.1, 135.3, 134.3, 133.9, 132.9 (2C), 131.6 (2C), 128.4, 125.9, 124.9, 123.2, 122.3, 120.4, 117.0, 111.3, 48.1, 47.6, 47.5, 42.3, 9.3; ESI-MS m/z: 421.1 (M + 1)$^+$.

*5.4.18. 1-[N-(3-dimethylamino)-propyl]-methylamino-9-(3-chlorobenzyl)-β-carboline hydrochloride salt (**7b**)*

Yield 41%; IR (KBr, cm^{-1}) v: 2969, 2648, 1622, 1463, 1336, 1196, 1038, 771; ^{1}H NMR (300 MHz, DMSO-d_6 + D$_2$O): δ 8.47 (d, J = 5.4 Hz, 1H, H-3), 8.37–8.39 (m, 2H, H-4, H-8), 7.70 (d, J = 8.4 Hz, 1H, H-5), 7.64 (t, J = 8.1 Hz, 1H, H-7), 7.36 (t, J = 6.9 Hz, 1H, H-6), 7.28–7.29 (m, 2H, 3-chlorophenyl-***H***), 6.98 (s, 1H, 3-chlorophenyl-***H***), 6.83–6.87 (m, 1H, 3-chlorophenyl-***H***), 5.92 (s, 2H, NC***H***$_2$-3-chlorophenyl), 4.68 (s, 2H, C***H***$_2$NHCH$_2$CH$_2$CH$_2$N), 3.07–3.14 (m, 4H, NHC***H***$_2$CH$_2$C***H***$_2$N), 2.73 (s, 6H, C***H***$_3$), 2.01–2.11 (m, 2H, NHCH$_2$C***H***$_2$CH$_2$N); ^{13}C NMR (75 MHz, DMSO-d_6 + D$_2$O): δ 144.2, 140.1, 134.4, 134.3, 134.2, 133.9, 132.2, 131.6, 131.1, 128.4, 126.3, 125.0, 123.8, 122.5, 120.1, 118.0, 111.7, 54.1, 48.4, 45.9, 45.4, 42.8, 21.5; ESI-MS m/z: 407.4 (M + 1)$^+$.

*5.4.19. 1-[N-(3-diethylamino)-propyl]-methylamino-9-(3-chlorobenzyl)-β-carboline hydrochloride salt (**7c**)*

Yield 55%; IR (KBr, cm^{-1}) v: 2971, 2927, 2881, 2752, 2534, 2447, 1623, 1562, 1468, 1432, 1341, 1288, 1195, 1137, 1092, 1040, 772; ^{1}H NMR (300 MHz, DMSO-d_6 + D$_2$O): δ 8.66–8.67 (d, J = 5.7 Hz, 1H, H-3), 8.60 (d, J = 5.7 Hz, 1H, H-4), 8.49 (d, J = 7.8 Hz, 1H, H-8), 7.80 (d, J = 8.4 Hz, 1H, H-5), 7.71 (t, J = 7.8 Hz, 1H, H-7), 7.41 (t, J = 7.5 Hz, 1H, H-6), 7.28–7.29 (m, 2H, 3-chlorophenyl-***H***), 7.14 (s, 1H, 3-chlorophenyl-***H***), 6.93 (t, J = 3.9 Hz, 1H, 3-chlorophenyl-***H***), 6.06 (s, 2H, NC***H***$_2$-3-chlorophenyl), 4.77 (s, 2H, C***H***$_2$NHCH$_2$CH$_2$CH$_2$N), 3.16–3.27 (m, 4H, NHC***H***$_2$CH$_2$C***H***$_2$N), 3.05–3.13 (m, 4H, NHCH$_2$CH$_2$CH$_2$N(C***H***$_2$CH$_3$)$_2$), 2.13–2.23 (m, 2H, NHCH$_2$C***H***$_2$CH$_2$N), 1.22 (t, J = 7.2 Hz, 6H, C***H***$_3$); ^{13}C NMR (75 MHz, DMSO-d_6 + D$_2$O): δ 144.0, 140.4, 134.8, 134.4, 134.3, 133.5, 132.3, 131.8, 131.6, 128.3, 126.3, 125.0, 123.6, 122.3, 120.4, 117.5, 111.7, 48.5, 48.3, 47.1, 46.4, 45.5, 20.9, 9.3; ESI-MS m/z: 435.4 (M + 1)$^+$.

*5.4.20. 1-[N-(4-diethylamino)-butyl]-methylamino-9-(3-chlorobenzyl)-β-carboline hydrochloride salt (**7d**)*

Yield 50%; IR (KBr, cm^{-1}) v: 2939, 2671, 1624, 1464, 1337, 1197, 1041, 770; ^{1}H NMR (300 MHz, DMSO-d_6 + D$_2$O): δ 8.55 (br s, 2H, H-3, H-4), 8.43 (d, J = 7.8 Hz, 1H, H-8), 7.75 (d, J = 8.4 Hz, 1H, H-5), 7.69 (t, J = 6.9 Hz, 1H, H-7), 7.39 (t, J = 7.2 Hz, 1H, H-6), 7.28–7.30 (m, 2H, 3-chlorophenyl-***H***), 7.09 (s, 1H, 3-chlorophenyl-***H***), 6.90–6.91 (m, 1H, 3-chlorophenyl-***H***), 6.00 (s, 2H, NC***H***$_2$-3-chlorophenyl), 4.72 (s, 2H, C***H***$_2$NHCH$_2$CH$_2$CH$_2$CH$_2$N), 3.01–3.09 (m, 8H, NHC***H***$_2$CH$_2$CH$_2$C***H***$_2$N(C***H***$_2$CH$_3$)$_2$), 1.73 (br s, 4H, NHCH$_2$C***H***$_2$C***H***$_2$CH$_2$N), 1.20 (t, J = 6.9 Hz, 6H, C***H***$_3$); ^{13}C NMR (75 MHz, DMSO-d_6 + D$_2$O): δ 143.5, 140.6, 135.7, 134.3, 134.2, 133.1, 132.7, 131.7, 131.4, 128.3, 126.2, 124.9, 123.3, 122.1, 120.5, 117.0, 111.5, 50.7, 48.2, 47.4, 46.9 (2C), 23.4, 21.1, 9.3; ESI-MS m/z: 449.4 (M + 1)$^+$.

*5.4.21. 1-[N-(2-diethylamino)-ethyl]-methylamino-9-(3-phenylpropyl)-β-carboline hydrochloride salt (**8a**)*

Yield 56%; IR (KBr, cm^{-1}) v: 2932, 2615, 1622, 1571, 1490, 1451, 1386, 1336, 1208, 1135, 1042, 773; ^{1}H NMR (500 MHz, D$_2$O): δ 8.18 (d, J = 7.5 Hz, 1H, H-3), 8.12 (d, J = 7.5 Hz, 1H, H-4), 7.89 (d, J = 10 Hz, 1H, H-8), 7.52 (t, J = 9.5 Hz, 1H, H-7), 7.13–7.19 (m, 5H, H-5, H-6, Ph-***H***), 6.95 (d, J = 8.5 Hz, 2H, Ph-***H***), 4.13 (s, 2H, C***H***$_2$NHCH$_2$CH$_2$N), 4.03 (t, J = 9.5 Hz, 2H, NC***H***$_2$CH$_2$CH$_2$Ph), 3.37 (t, J = 8.5 Hz, 2H, NHC***H***$_2$CH$_2$N), 3.24–3.27 (m, 4H, N(C***H***$_2$CH$_3$)$_2$), 3.10 (t, J = 8.5 Hz, 2H, NHCH$_2$C***H***$_2$N), 2.48 (t, J = 8 Hz, 2H, NCH$_2$CH$_2$C***H***$_2$Ph), 1.74–1.79 (m, 2H, NCH$_2$C***H***$_2$CH$_2$Ph), 1.30 (t, J = 9 Hz, 6H, C***H***$_3$); ^{13}C NMR (125 MHz, D$_2$O): δ 143.8, 140.7, 134.5, 134.1, 132.1, 131.9, 129.0, 128.7, 128.5, 126.4, 122.6, 121.9, 119.1, 116.3, 110.7, 50.1, 47.8, 46.6, 44.3, 43.2, 31.7, 30.6, 8.1; ESI-MS m/z: 415.4 (M + 1)$^+$.

*5.4.22. 1-[N-(3-dimethylamino)-propyl]-methylamino-9-(3-phenylpropyl)-β-carboline hydrochloride salt (**8b**)*

Yield 40%; IR (KBr, cm^{-1}) v: 2924, 2684, 1625, 1458, 1383, 1338, 1052, 752; ^{1}H NMR (500 MHz, DMSO-d_6 + D$_2$O): δ 8.47 (d, J = 5.4 Hz, 1H, H-3), 8.34–8.40 (m, 2H, H4, H-8), 7.65–7.74 (m, 2H, H-5, H-7), 7.32–7.38 (m, 1H, H-6), 7.16–7.27 (m, 5H, Ph-***H***), 4.86 (s, 2H, C***H***$_2$NHCH$_2$CH$_2$CH$_2$N), 4.62 (t, J = 7.5 Hz, 2H, NC***H***$_2$CH$_2$CH$_2$Ph),

3.19–3.25 (m, 4H, NHC***H***$_2$CH$_2$C***H***$_2$N), 2.69–2.77 (m, 8H, C***H***$_3$, NCH$_2$CH$_2$C***H***$_2$Ph), 2.16–2.26 (m, 2H, NCH$_2$C***H***$_2$CH$_2$Ph), 2.02–2.10 (m, 2H, NCH$_2$C***H***$_2$CH$_2$Ph); ^{13}C NMR (100 MHz, D$_2$O): δ 144.5, 140.5, 136.2, 133.5, 132.5, 131.7, 128.7, 128.3, 126.8, 126.4, 123.0, 122.2, 119.4, 117.7, 111.0, 54.2, 46.0, 45.2, 44.4, 42.9, 31.8, 29.8, 21.5; ESI-MS m/z: 401.4 $(M + 1)^+$

5.4.23. 1-[N-(3-diethylamino)-propyl]-methylamino-9-(3-phenylpropyl)-β-carboline hydrochloride salt (**8c**)

Yield 55%; IR (KBr, cm^{-1}) ν: 2933, 2669, 1624, 1493, 1457, 1385, 1337, 1042, 753; ^{1}H NMR (500 MHz, D$_2$O): δ 8.45–8.49 (m, 2H H-3, H-4), 8.30 (d, J = 8 Hz, 1H, H-8), 7.84 (t, J = 7 Hz, 1H, H-7), 7.65 (d, J = 8.5 Hz, 1H, H-5), 7.49 (t, J = 8 Hz, 1H, H-6), 7.25–7.32 (m, 3H, Ph-***H***), 7.12 (d, J = 6.5 Hz, 2H, Ph-***H***), 4.65 (s, 2H, C***H***$_2$NHCH$_2$CH$_2$CH$_2$N), 4.49 (t, J = 7.5 Hz, 2H, NC***H***$_2$CH$_2$CH$_2$Ph), 3.27–3.33 (m, 6H, NHC***H***$_2$CH$_2$CH$_2$N(C***H***$_2$CH$_3$)$_2$), 3.13 (t, J = 7.5 Hz, 2H, NHCH$_2$CH$_2$C***H***$_2$N), 2.71 (t, J = 7 Hz, 2H, NCH$_2$CH$_2$C***H***$_2$Ph), 2.13–2.24 (m, 4H, NCH$_2$C***H***$_2$CH$_2$Ph, NHCH$_2$C***H***$_2$CH$_2$N), 1.35 (t, J = 7 Hz, 6H, C***H***$_3$); ^{13}C NMR (125 MHz, D$_2$O): δ 144.4, 140.6, 135.8, 133.5, 132.3, 131.9, 128.7, 128.4, 127.2, 126.4, 122.9, 122.1, 119.5, 117.5, 110.9, 48.4, 47.7, 46.2, 45.3, 44.4, 31.8, 29.8, 21.0, 8.3; ESI-MS m/z: 429.4 $(M + 1)^+$.

5.4.24. 1-[N-(4-diethylamino)-butyl]-methylamino-9-(3-phenylpropyl)-β-carboline hydrochloride salt (**8d**)

Yield 59%; IR (KBr, cm^{-1}) ν: 2938, 2667, 1625, 1455, 1337, 1292, 1143, 1041, 762; ^{1}H NMR (300 MHz, DMSO-d_6 + D$_2$O): δ 8.43–8.48 (m, 2H, H-3, H-4), 8.31 (d, J = 7.8 Hz, 1H, H-8), 7.63–7.70 (m, 2H, H-5, H-7), 7.31–7.36 (m, 1H, H-6), 7.11–7.22 (m, 5H, Ph-***H***), 4.77 (s, 2H, C***H***$_2$NHCH$_2$CH$_2$CH$_2$CH$_2$N), 4.56 (t, J = 6.9 Hz, 2H, NHC***H***$_2$CH$_2$CH$_2$Ph), 3.05–3.12 (m, 8H, NHC***H***$_2$CH$_2$CH$_2$C***H***$_2$N (C***H***$_2$CH$_3$)$_2$), 2.65 (t, J = 7.2 Hz, 2H, NCH$_2$CH$_2$C***H***$_2$Ph), 1.97–2.06 (m, 2H, NCH$_2$C***H***$_2$CH$_2$Ph), 1.77 (br s, 4H, NHCH$_2$C***H***$_2$C***H***$_2$CH$_2$N), 1.19 (t, J = 7.2 Hz, 6H, C***H***$_3$); ^{13}C NMR (75 MHz, DMSO-d_6 + D$_2$O): δ 143.5, 141.4, 134.5, 133.6, 133.0, 132.0, 131.4, 129.1, 129.0, 126.7, 123.2, 121.9, 120.3, 117.0, 111.4, 51.0, 47.7, 47.2 (2C), 45.2, 32.7, 31.9, 23.5, 21.2, 9.3; ESI-MS m/z: 443.5 $(M + 1)^+$.

5.5. Cytotoxicity assays in vitro

Cytotoxicity assays *in vitro* were carried out using 96 microtitre plate cultures and MTT staining according to the procedures described in our previous report [24]. 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, 786-0 and OS-RC-2), epidermoid carcinoma of the nasopharynx (KB), gastric carcinoma (BGC-823), 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.

5.6. 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. [5] 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 path length 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 **3**–**8c**, 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 25ul syringe and adequate Teflon tubing.

5.7. Determination of ΔT_m

T_m measurements were performed using a Shimadzu UV 2501PC Spectrometer and following the methods described by Xiao et al. [5] 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.

5.8. Fluorescence spectroscopy

Fluorescence spectral measurement 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, 15, 30, 45, 60, 75 and 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.

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