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Original article

Design of β-carboline derivatives as DNA-targeting antitumor agents

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Abstract

This research studied the structure-activity relationship of β-carboline derivatives as antitumor agents, in which 41 synthesized compounds and their cytotoxicity to tumor and normal cell lines were assayed. It was proved that substituent in position-9 of the β-carboline ring could reinforce the DNA intercalating ability and consequently cytotoxicity to tumor cell lines, and the amidation of amino group at the end of the DNA targeting side chain in position-3 could cripple the DNA intercalating activity of these compounds, which resultingly initiated the cytotoxic selectivity to tumor cell lines rather than to normal ones. Furthermore, the S and G2-M arrest induced by these compounds confirmed that they could target DNA and lead to DNA destructions in Hela cells. In short, this study may provide a framework to design a novel antitumor drug that could surpass Adriamycin.

Keywords: Harmine; β-carboline; DNA intercalator; Prodrug

1. Introduction

Researches on antitumor alkaloids isolated from plants have been actively explored in the last thirty years, in which the antitumor effect of the naturally occurring β-carboline derivatives have been noticed recently after an intensive concentration on their high affinity to 5-HT [1] and benzodiazepine receptors [2–5] that causes prominent CNS (central nervous system) effects. As for the antitumor activity, harmine, known as one of the β-carboline alkaloids isolated from *Peganum harmala* L., has been shown to have strong cytotoxicity to tumor cell lines in vitro [6]. Recently it is discovered that β-carboline derivatives may function their antitumor activity through multiple mechanisms, such as intercalating into DNA [9], inhibiting Topoisomerase I and II [8,9], CDK [10,11] and IκK (IκB kinase complex) [12]. Our previous study has demonstrated that they can downregulate Bcl-2 and upregulate death receptor Fas without dependence on P53 [13], and exhibit photosensitivity [14]. Thus far, series of structure modification on β-carboline have been carried out, with an expected enhancement either in antitumor activity or affinity to 5-HT or benzodiazepine receptors, which could be served as an inspiration for rational drug design in this research.

In the β-carboline ring system, a methyl group in position-1 is desirable to reduce the binding of the compound to the benzodiazepine receptor [4,5], and among all the substituents added to position-1 in our previous research, a methyl group achieved the lowest IC_{50} to tumor cell lines [15]. The 2-substituted β-carboline derivatives might be one of the metabolic forms of β-carboline in vivo that may lead to distinct neurotoxic effects [16]. A DNA targeting side chain in position-3 could reinforce the DNA intercalating ability of the compound hence boost the cytotoxicity to tumor cell lines in vitro [7], implying that for further chemical modification to increase antitumor activity, position-3 would present a great potential to be worked on. Hydrogen atoms in position-4, 5, 6, 7 and 8 are with the similar chemical activity and consequently difficult to control the substitution reaction when the cyclization of β-carboline ring in this research underwent a Pictet–Spengler reaction from L-tryptophan. Moreover, substituent in position-

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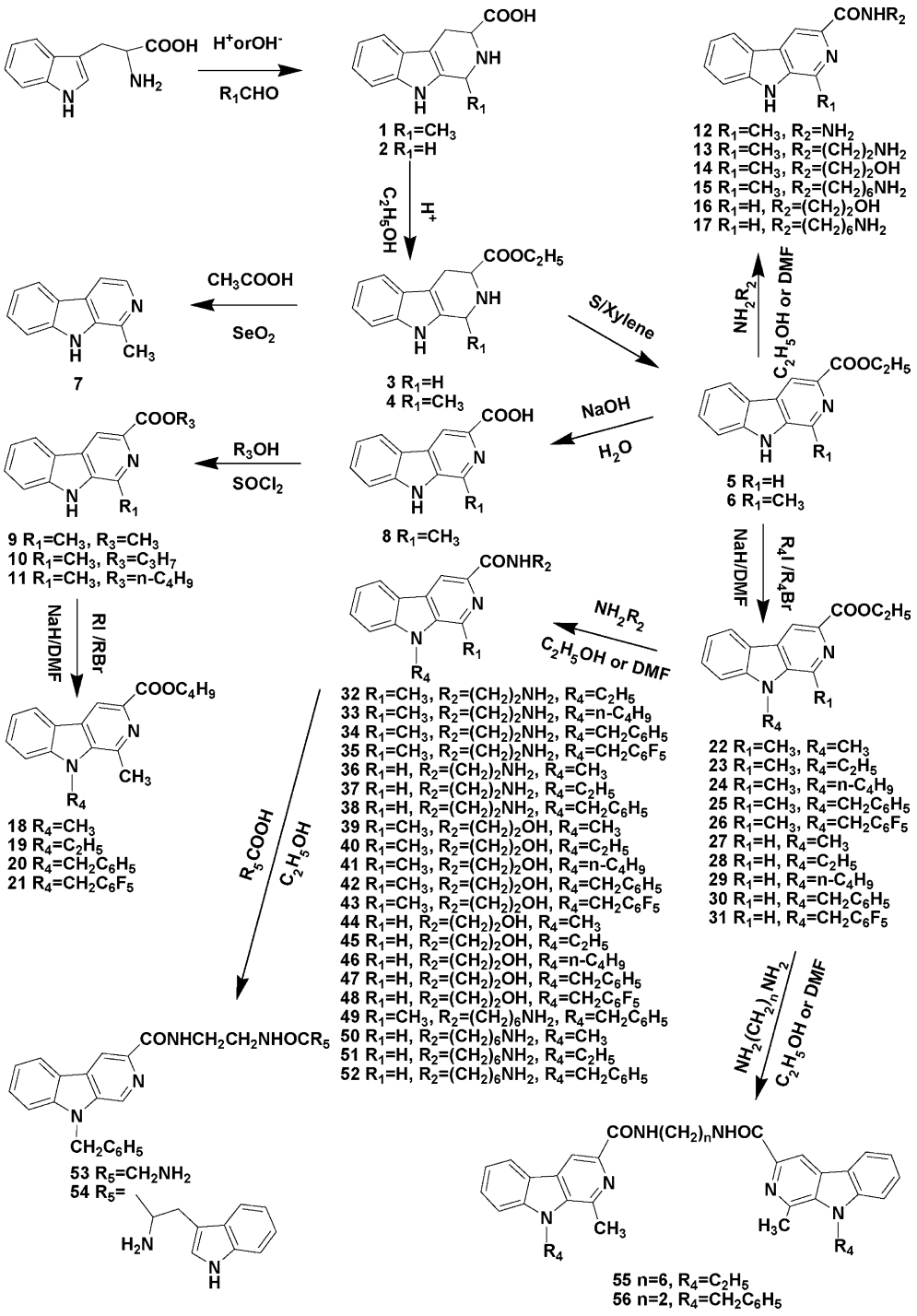

Scheme 1. Design and synthesis of β-carboline derivatives.

6 would impair the CDK (cyclin-dependent kinases) inhibition activity [10]. In terms of position-7 and 9, our previous research has demonstrated that the methoxyl group in position-7 could bring up remarkable neurotoxicity in mice, and substituent in position-9 could greatly enhance the antitumor activity both in vitro and in vivo [15,17].

Here we report our further chemical modification on β-carboline derivatives aiming at better understanding of chemical biology of these newly synthesized compounds. Firstly the intact β-carboline ring was reserved with a methyl group in position-1 to reduce the binding to benzodiazepine receptor, then different carboxylate or carboxamide chains were added to position-3 in pursuit of the descent substituents in position-3 that could perform strong cytotoxicity to tumor cell lines. In succession, combining the substituent in position-3 obtained above and the methyl group in position-1, different alkyl and aryl groups were added to position-9 in order to boost the antitumor activity.

2. Chemistry and biological results

2.1. Influence of the side chain in position-3 on cytotoxicity

The design and synthesis of β-carboline derivatives were displayed as Scheme 1. Proper substituents in position-3 were obtained based on the comparison in cytotoxicity of those 3-substituted 1-methyl-β-carboline derivatives (Compounds **6**–**15**) listed in Table 1, in which the butyl carboxylate side chain (Compound **11**) showed the lowest IC_{50} and was then considered as the most appropriate substituent in position-3. On the other hand, the side chains in position-3 of compounds **13** and **15** have been proved to be DNA targeting due to the hydrogen bonding between the amino group at the end of the side chain and DNA bases, which was supported by the computer-modeling result [7]. For the theory of isosterism, the replacement of this amino group to hydroxyl group (Compound **14**) could possibly reserve the DNA targeting function of the side chain. Then for further chemical modification, importing substituents to position-9 to compounds **11**, **13**, **14** and **15** may help understanding the relationship between DNA intercalating ability and the reinforced cytotoxicity induced by adding groups to position-9 [17].

2.2. Methyl group in position-1 does not influence cytotoxicity

Methyl group in position-1 is supposed to hinder the compound from binding to benzodiazepine receptor [4,5]. Since substituents in position-9 can also cripple the affinity to benzodiazepine receptor [4,5], this methyl group might be futile after

Table 1
Cytotoxicity of β-carboline derivatives to tumor and normal cell lines

Comp.	IC_{50} (μmol/ml)							
	Bel7402	Hela	C6	Lovo	PLA801	BGC823	HFL-1	VSMC
Harmine	0.0260	0.0640	0.0713	0.0704	0.0482	0.0725	0.0623	0.0552
6	>3.0	>3.0	>3.0	1.57	>3.0	>3.0		
7	0.244	0.338	0.146	0.535	0.371	0.250		
9	0.233	0.197	0.114	0.085	0.160	0.125		
10	>3.0	2.93	0.503	0.157	>3.0	2.63		
11	0.114	0.0769	0.0841	0.0274	0.0874	0.0635		
12	0.496	0.525	0.405	0.235	1.38	0.852		
13	1.96	0.405	1.43	0.370	0.752	0.315		
14	0.594	0.243	0.124	0.379	0.333	0.342		
15	0.456	0.168	0.305	0.342	0.266	0.213		
16	0.560	0.237	0.532	0.00942	0.817	0.137		
17	0.251	0.141	0.144	1.44	1.36	0.987		
18	0.306	0.334	0.523	0.0607	0.261	0.209		
19	0.359	0.268	0.438	0.225	0.437	0.174		
20	0.0999	0.548	0.104	0.244	0.262	0.577		
21	>3.0	0.0492	1.43	0.0508	0.593	0.429		
32	0.0160	0.0158	0.0204	0.0477	0.0661	0.0419	0.0411	0.0380
33	0.0298	0.00680	0.0114	0.0308	0.0431	0.0220	0.0471	0.0326
34	0.00560	0.0117	0.00610	0.0114	0.0597	0.0402	0.0380	0.0256
35	0.0336	0.0190	0.0229	0.00699	0.0232	0.0206		
36	0.0138	0.0339	0.0432	0.0670	0.0823	0.0497		
37	0.0293	0.0318	0.0212	0.0127	0.0384	0.0163		
38	0.00714	0.0159	0.0275	0.0204	0.0346	0.0313		
39	0.0335	0.190	0.0229	0.00696	0.0231	0.0205		
40	0.123	0.238	0.128	1.315	0.0287	0.0465		
41	0.174	0.197	0.125	0.0711	0.146	0.148		
42	>3.0	>3.0	>3.0	>3.0	>3.0	>3.0		
43	0.109	0.0335	0.366	0.805	0.113	0.893		
44	0.108	0.0990	0.0368	0.114	0.0570	0.0638		
45	0.0328	0.0315	0.0226	0.192	0.146	0.0761		
46	0.121	0.362	0.0933	0.0941	1.14	0.0706		
47	0.0268	0.0154	0.0671	0.00821	0.0239	0.0562		
48	>3.0	>3.0	0.322	0.846	1.73	1.68		
49	0.418	0.332	0.383	0.424	0.515	0.377		
50	0.422	0.706	0.150	0.120	0.131	0.111		
51	0.235	0.0660	0.0421	0.0864	0.0942	0.161		
52	0.0191	0.00953	0.00648	0.0162	0.0347	0.0265		
53	>3.0	>3.0	>3.0	>3.0	>3.0	>3.0		
54	>3.0	>3.0	>3.0	>3.0	>3.0	>3.0		
55	0.0354	0.0575	0.0109	0.0275	0.0389	0.0376	0.0269	0.0821
56	0.00583	0.00989	0.00544	0.00923	0.00957	0.0132	0.00881	0.0630

Bel7402, Hela, C6, Lovo, PLA801and BGC823 were chosen as tumor cell lines and HFL-1 and VSMC as normal ones.

substituents are added to position-9. Compare the cytotoxicity of compounds **14**–**16** and **15**–**17**, Table 1 showed that the omission of the methyl group in position-1 did not have a strong influence on cytotoxicity, neither the solubility, suggesting that this methyl group might not have a strong influence on DNA affinity, which would be validated later in the DNA melting temperature assay. Based on this result, 1,3,9-trisubstituted or 3,9-bisubstituted β-carboline derivatives could be synthesized with respect that they might share similar antitumor activity and affinity to benzodiazepine receptor.

2.3. Substituent in position-9 could boost cytotoxicity by enhancing DNA intercalating ability

9-Substituted β-carboline derivatives containing a proper side chain in position-3 obtained in 2.1 with or without a methyl group in position-1 might show decent antitumor activity. Following this proposal, different substituents were added to the position-9 of compound **11**, **13**, **14** and **15** to boost the cytotoxicity. Firstly, series of 9-substituted β-carboline in the form of butyl carboxylates (compound **18**–**21**) were synthesized. Phase transfer catalysis was attempted to import a substituent to position-9, but it was found that the carboxylate bond in position-3 was instable in the basic water phase (50% NaOH solution) in the company of a hydrolysis process in position-3. Then the nucleophilic substitution reaction was carried out in a less basic DMF medium. However, Table 1 (compound **18**–**21**) showed that substituent in position-9 did not enhance the cytotoxicity as our previous research did [15, 17] but *J*eopardized it instead, which could be accounted for a decrease in the solubility of these compounds in water.

When importing alkyl or aryl groups to position-9 of compound **13**, some 9-substituted intermediates (compound **22**–**31**) were synthesized in advance [15] due to the instability of the carboxamide bond of compound **13** in the basic DMF medium for nucleophilic substitution reaction. Groups that added to position-9 of compound **13** brought up much stronger cytotoxicity to tumor cell lines (compound **32**–**38**, Table 1) than that before substitution, in which both *n*-butyl group (compound **33**) and benzyl group (compound 34) seemed to be favorable for the high cytotoxicity. The DNA thermal denaturation assay (Fig. 1) showed that the ethyl and *n*-butyl group in compound

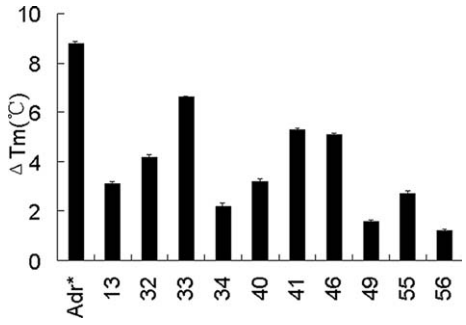

Fig. 1. Effect of binding by β-carboline derivatives on the thermal stability of the CT-DNA. *Adr is short for Adriamycin. Results were expressed as mean ± S.D. ($N = 4$).

32 and **33**, respectively, had a stronger DNA intercalating ability than compound **13** ($P < 0.005$, $P < 0.005$, respectively), while the benzyl group in compound **34** showed the reversed effect ($P < 0.01$), suggesting that proper substituents like ethyl and *n*-butyl but not benzyl groups in position-9 were favorable for the affinity to DNA, which was reserved as the possible reason for the enhancement in cytotoxicity induced by substituents in position-9. Nevertheless, Fig. 1 demonstrated that compound **33** had a much stronger ability to increase the denaturation temperature of calf thymus DNA (CT-DNA) than **34** ($P < 0.005$) when they shared similar cytotoxicity to tumor cell lines, implying that besides intercalating into DNA, these compounds may have other antitumor mechanisms, such as those mentioned in the Introduction. And the tested compounds showed no obvious selectivity in these tumor cell lines, in which only PLA-801 reacted relatively less sensitive, indicating that these compounds could be used as broad-spectrum antitumor drugs. When up to 50% tumor cell lines bearing mutant or deleted p53 gene exhibit drug resistance to the antitumor drugs that are dependent on the p53 pathway, β-carboline derivatives seem to work universally in respect that they do not alter the level of P53 [13].

Different groups were added to the position-9 of compound **14**. The target compounds (compound **39**–**48**) showed strong cytotoxicity (Table 1) but weaker than the 9-substituted β-carboline derivatives with a short DNA targeting side chain did (compound **32**–**38**). Now that compound **40** and **41** also performed a weaker DNA intercalating capability in the DNA thermal denaturation assay than **32** and **33** did ($P < 0.005$, $P < 0.005$, respectively, Fig. 1), respectively, thus, it could be concluded that the replacement of hydroxyl group to amino group at the end of the side chain in position-3 would lead to a decline in the affinity to DNA and cytotoxicity. As an explanation for this result, the hydroxyl group with strong electronegativity is prone to forming intra- or inter-molecule hydrogen bonding that is thermodynamically more stable than the one with DNA base; therefore it would *J*eopardize the formation of hydrogen bonding between the hydroxyl group and DNA base and consequently the DNA intercalating ability. This explanation was supported by the fact that the polarity of compound **13** is stronger than **14** (detected by thin layer chromatography at 30°C; ethyl acetate, methanol, and ethyl acetate/methanol/glacial acid = 5:3:1 were used as the developing systems). At the same time, Fig. 1 showed that compound **41** and **46** shared similar DNA affinity, which validated the proposal in 2.2 that the methyl group in position-1 did not have a strong influence on DNA intercalating ability hence cytotoxicity. However, compound **42** completely lost the cytotoxicity to tumor cell lines, owing to a drastic decline in solubility in water.

As for the 9-substituted β-carboline with a long DNA targeting side chain in position-3 (compound **49**–**52**, Table 1), they did not have a stronger cytotoxicity than those with a short DNA targeting side chain (compound **32**–**38**), indicating that the long one did not work as well as the short one did. The computer-modeling result has shown that β-carboline with a

medium-length side chain (–$CONHCH_2CH_2CH_2CH_2NH_2$) in position-3 fits the DNA base plane by the square, so that the steric hindrance between two DNA strands might constrain the lengthening of the side chain, accordingly β-carboline with a long DNA targeting side chain would have a decline in the DNA intercalating ability [7], as observed in Fig. 1 that compound **49** had a lower ΔT_m value than compound **34** ($P < 0.005$).

2.4. Failure in hydrogen bonding could decrease DNA affinity and cytotoxicity to normal cell lines but not tumor ones

Among all the synthesized compounds above, 9-substituted β-carboline with a short DNA targeting side chain in position-3 (compounds **32**–**38**) represented the lowest IC_{50} to tumor cell lines. But a problem subsequently comes as these compounds (compounds **32**–**34**, Table 1) are also highly cytotoxic to normal cell lines (HFL-1 and VSMC). And a proposed solution for this problem is the amidation of the amino group at the end of the DNA targeting side chain in position-3, which was supposed to bind to DNA base with hydrogen bonding [7]. The amidation might lead to a decrease in the affinity of the compound to DNA, but under the hydrolysis effect of amidase in tumor cells, which is generally accepted to be more active than the normal ones and commonly involved in prodrug design [18], this functional amino group could be released. As a result, the amidation product would act as a prodrug and regain the DNA intercalating ability in tumor cells but with a weak effect on normal cells in theory. In terms of choosing amidation agents, the preferred ones would be amino acids, because they might involve the β-carboline molecule into the metabolic pathway of amino acids. But when the amino acid was imported, the free amino group that came along with amino acid molecule might still bind to DNA base with hydrogen bonding; therefore, as an attempt, tryptophan was introduced as the amidation agent to prevent the hydrogen bonding owing to the steric hindrance caused by the large indole ring (compound **54**). However, the hydrochloride salt solutions of compounds **53** and **54** were found to be rather instable with a hydrolysis process in the carboxamide bond between the amino acid molecule and β-carboline side chain (detected by thin layer chromatography), then the alkaloid forms of **53** and **54** were used for the drug screening. But disappointingly, both of **53** and **54** lost their cytotoxicity to tumor cell lines by reason of their weak solubility in water (Table 1).

Considering that the carboxyl group in the alkyl chain of amino acid might result in the instability of the carboxamide bond in the hydrochloride salt solution, an aryl carboxyl group with less chemical activity would be favorable for the amidation reaction. Then the β-carboline carboxylic acids were selected as the amidation agents to construct the dimer of β-carboline, which contained two identical carboxamide bonds that could be hydrolyzed into the same DNA intercalator (compounds **55** and **56**). The DNA thermal denaturation assay did show that after amidation of the amino group at the end of the side chain in position-3, compound **56** performed a weaker DNA intercalating ability than **34** did ($P < 0.005$, Fig. 1), suggesting that the amidation successfully impair the hydrogen bonding with DNA. However, this electronegative amino group might endow the compound with the affinity to many biological molecules; now that only the binding to DNA has been proved [7], we discuss the DNA intercalating activity here without consideration of other possible interactions between this amino group and other biological molecules. Compound **55** and **56** were both stable in their hydrochloride salt solutions and showed strong cytotoxicity in Table 1, while whether they would have a weak effect on normal cell lines would be tested in the next step.

To detect the cytotoxic selectivity of these compounds to tumor and normal cell lines, the normal cell lines selected were HFL-1, which has normal human genome but proliferates as fast as tumor cell lines do, and VSMC, which is vascular smooth muscle cells isolated from rats and barely proliferates in vitro. Table 1 showed that compounds **32**, **33** and **34** performed strong cytotoxicity both to tumor and normal cell lines, suggesting the DNA targeting side chain worked effectively in all these cell lines. While the IC_{50} of the β-carboline dimers **55** and **56** to VSMC was 7.5 and 11.6 times higher than that to C6, respectively, demonstrating that the amidation of the functional amino group at the end of the DNA targeting side chain was a practicable method to make compounds into prodrugs, which could selectively act on tumor cells but with a weak effect on normal cells.

2.5. β-carboline derivatives could induce an S and G2-M arrest in Hela cells

Results of cell cycle analysis by flow cytometry were displayed in Fig. 2. At the concentration of 5 μM, no clear cell cycle arrest had occurred, but up to 78.22% and 81.04% apoptosis had achieved after the incubation with compound **33** for 48 and 72 hours, respectively, when our previous research has demonstrated that β-carboline derivatives induce cell death via apoptosis [13]. After Hela cells were treated with compound **33** for 24 hours, the G2-M arrest had been triggered (Fig. 2E). And after treated for 48 and 72 hours, a clear G2-M arrest could be observed (Fig. 2F, G), which could be accounted for the DNA damage induced by compound **33** that inhibited mitosis. Meanwhile, a distinct S phase arrest was also observed, which could be caused by the inhibition to Topoisomerase I and II (our unreported data) at the beginning of DNA synthesis (Fig. 2E). However, when compound **33** effectively intercalates into DNA, it might lead to the DNA structural instability hence DNA strand breaks like Adriamycin does [19,20]; as a result, cells that could not reach 2N genome after duplication were negatively selected at the G2 cell cycle checkpoint due to the inactivation of CDC-2 [21], and consequently stuck at the S phase (Fig. 2F, G). Since β-carboline derivatives do not alter the level of P53 [13], cells might waltz through the p53-led G1 checkpoint into the S phase [22]; after all, no obvious G0-G1 arrest was induced by compound 33. The DNA binding property of β-carboline derivatives has been studied well in a cell-

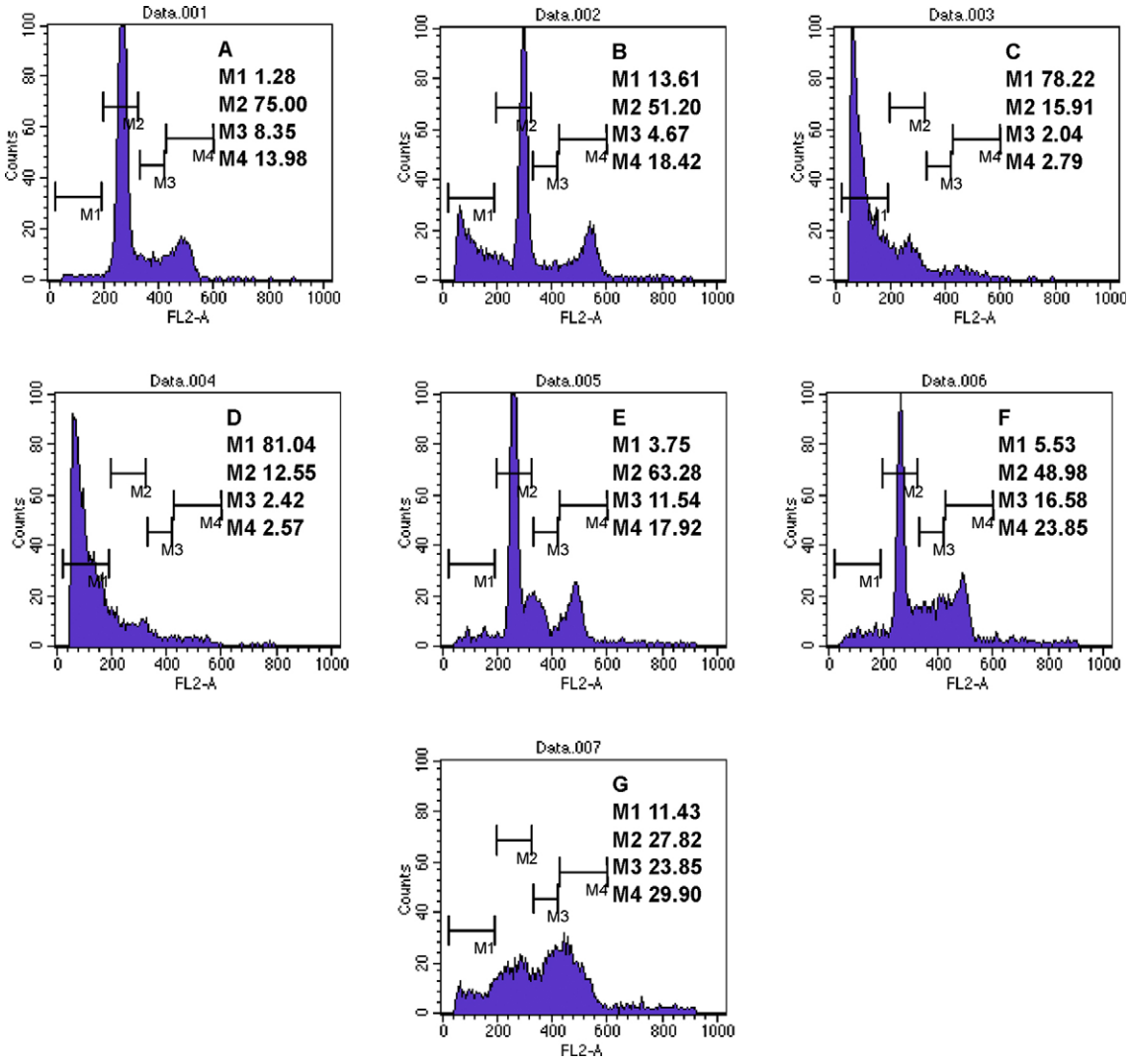

Fig. 2. Cell cycle analysis of Hela by flow cytometry. A, control; B, C and D, Cells were treated with compound **33** at the concentration of 5 μM after incubated for 24, 48 and 72 hours, respectively; E, F and G, Cells were treated with compound **33** at the concentration of 1.25 μM after incubated for 24, 48 and 72 hours, respectively. M1, M2, M3 and M4 showed the "sub-G1", "G0-G1", "S" and "G2-M" peaks, respectively. Percentages of gated cells in each phase were displayed.

free system [7], yet it is still not convincing that it reflects the practical situation in cells. However, this result can be evidence that β-carboline derivatives can induce cellular response as the consequences of compounds binding to DNA.

3. Conclusion

In the β-carboline ring, the methyl group in position-1 can impair the binding of the compound to benzodiazepine receptor but not the DNA intercalating ability, cytotoxicity to tumor cell lines or solubility in water. Though the β-carboline plane alone can intercalate into DNA with van der Waals interactions, the short DNA targeting side chain ($-CONHCH_2CH_2NH_2$) in position-3 is responsible for the hydrogen bonding with DNA base. Replacement of the amino group at the end of the DNA targeting side chain by hydroxyl group or the lengthening the side chain would lead to a weakened cytotoxicity due to a decline in the DNA intercalating ability, because the hydroxyl group is prone to forming an intra- or inter-molecule hydrogen bond and the lengthening of the side chain would be constrained by the steric hindrance between two DNA strands. However, the amidation of this functional amino group could endow the compound with the cytotoxic selectivity to tumor cell lines rather than to normal ones, due to the block out of hydrogen bonding to DNA, demonstrating that the failure in intercalating into DNA is closely related to the cytotoxicity to normal cell lines. While under the hydrolysis effect of amidase in tumor cells, which is more active than that in normal cells, this amino group can be released and regain the DNA intercalating. Proper substituents in position-9 will reinforce the binding of compound to DNA, increase cytotoxicity but decrease the solubility in water. While in some cases like Compound **34**, the decrease in DNA intercalating ability caused by groups in position-9 does not pull down cytotoxicity, suggesting that besides intercalating into DNA, these compounds may function antitumor activity via other mechanisms. In short, this research may provide some new inspirations for the design of DNA intercalators and prodrugs.

4. Experimental

4.1. Synthetic protocols

All reagents were purchased from commercial suppliers and dried and purified when necessary

4.1.1. Propyl-1-methyl-β-carboline-3-carboxylate (10)

Compound **8** (1.17 g, 5 mmol) was suspended in 1-propanol (50 ml), and then $SOCl_2$ (4 ml) was added drop-wise under ice bath. After refluxed for 4 h, the mixture was poured into water (200 ml) neutralized by $NaHCO_3$ and extracted with ethyl acetate (4 × 100 ml). The organic layer was dried with anhydrous sodium sulfate, removed by evaporation and the residue was recrystallized with ethanol twice. White crystals of **10** were obtained (0.56 g, 42%). m.p.: 220–221°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.67 (s, 1H, 9-H), 8.76 (s, 1H, 4-H), 8.18 (d, J = 8 Hz, 1H, 8-H), 7.62 (d, J = 8.5 Hz, 1H, 5-H), 7.56 (m, 1H, 7-H), 7.35 (m, 1H, 6-H), 4.39 (t, J = 6.5 Hz,COC**H**$_2$CH$_2$CH$_3$), 2.84 (s, 3H, 1- CH$_3$), 1.83 (m, 2H, COCH$_2$C**H**$_2$CH$_3$), 1.02 (t, J = 7.5 Hz, 3H, COCH$_2$CH$_2$C**H**$_3$); IR (KBr): 3237, 3069, 2969, 2884, 1911, 1704, 1453, 1388, 1348, 1260, 972, 742 cm^{-1}; UV:λ_{max} 356, 341, 307, 289, 249, 212 nm; FAB-MS (M + 1): [M + 1]$^+$ calcd for $C_{16}H_{16}N_2O_2$, 269; found 269; Analysis (calcd, found for $C_{16}H_{16}N_2O_2$): C (71.6, 71.6), H (6.0, 6.0), N (10.4, 10.4).

4.1.2. Butyl-1-methyl-β-carboline-3-carboxylate (11)

Prepared by the same procedure as compound **10** from **8** (1.17 g, 5 mmol) and 1-butanol (50 ml). White crystals of **11** were obtained (0.65 g, 46%). m.p.: 168–169°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.83 (s, 1H, 9-H), 8.76 (s, 1H, 4-H), 8.18 (d, J = 8 Hz, 1H, 8-H), 7.62 (d, J = 8.5 Hz, 1H, 5-H), 7.57 (m, 1H, 6-H), 7.35 (m, 1H, 7-H), 4.43 (t, J = 7 Hz, COC**H**$_2$CH$_2$CH$_2$CH$_3$), 2.81 (s, 3H, 1-CH$_3$), 1.77 (m, 2H, COCH$_2$C**H**$_2$CH$_2$CH$_3$), 1.45 (m, 2H, COCH$_2$CH$_2$C**H**$_2$CH$_3$), 0.94 (t, J = 7.5 Hz, 3H, COCH$_2$CH$_2$CH$_2$C**H**$_3$); IR (KBr): 3236, 2957, 2870, 1698, 1624, 1503, 1461, 1346, 1248, 735 cm^{-1}; UV:λ_{max} 352, 338, 317, 297, 250, 225, 212 nm; FAB-MS (M + 1): [M + 1]$^+$ calcd for $C_{17}H_{18}N_2O_2$, 283; found 283; Analysis (calcd, found for $C_{17}H_{18}N_2O_2$): C (72.3, 72.3), H (6.4, 6.5), N (9.9, 9.8).

4.1.3. 1-methyl-β-carboline-3-carboxazine (12)

Compound **6** (1.02 g, 4 mmol) was diluted and boiled in ethanol (60 ml), and then hydrazine hydrate (85%, 4 ml) was added drop-wise in 1 h. After heated for 4 h, the mixture was poured into water (200 ml) and extracted with ethyl acetate (4 × 100 ml). The organic layer was dried with anhydrous sodium sulfate, removed by evaporation and the residue was recrystallized with ethanol twice. White crystals of **12** were obtained (0.83 g, 86%) m.p.: 204–205°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.92 (s, 1H, 9-H), 8.82 (s, 1H, 4-H), 8.17 (d, J = 8 Hz, 1H, 8-H), 7.68 (d, J = 8.5 Hz, 1H, 5-H), 7.49 (m, 1H, 6-H), 7.32 (m, 1H, 7-H), 2.93 (s, 3H, 1-CH$_3$); IR (KBr): 3419, 3340, 3267, 3203, 3037, 1623, 1498, 1349, 1254, 732 cm^{-1}; UV:λ_{max} 346, 333, 275, 266, 244, 225, 215 nm; FAB-MS (M + 1): [M + 1]$^+$ calcd for $C_{13}H_{12}N_4O$, 241; found 241; Analysis (calcd, found for $C_{13}H_{12}N_4O$): C (65.0, 64.7), H (5.0, 5.4), N (23.3, 23.0).

4.1.4. N-(2-aminoethyl)-1-methyl-β-carboline-3-carboxamide (13)

Ethylenediamide (18 ml, 20.3 mmol) was heated at 80~90° C, then compound **6** (1.02 g, 4 mmol) diluted in ethanol (20 ml) was added drop-wise in 1 h. After heated for 48 h, the mixture was poured into water (200 ml) and extracted with ethyl acetate (5 × 100 ml). The organic layer was dried with anhydrous sodium sulfate, removed by evaporation and the residue was purified by silica column chromatography with methanol as the eluent to remove the side-product of dimer and excess ethylenediamide. After recrystallization with ethanol, white crystals of **13** were obtained (0.24 g, 22%). m. p.: 198–199°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.69 (s, 1H, 4-H), 8.62 (t, J = 6 Hz, 1H, CONH), 8.35 (d, J = 8 Hz, 1H, 8-H), 7.65 (d, J = 8.5 Hz, 1H, 5-H), 7.52 (m, 1H, 6-H), 7.31 (m, 1H, 7-H), 3.35 (m, 2H, NHC**H**$_2$CH$_2$NH$_2$), 3.07 (m, 2H, NHCH$_2$C**H**$_2$NH$_2$), 2.82 (s, 3H, 1- CH$_3$); IR (KBr): 3264, 3042, 2893, 1653, 1534, 1461, 1367, 1235, 752 cm^{-1}; UV: λ_{max} 344, 332, 298, 284, 248, 224, 212 nm; FAB-MS (M + 1): [M + 1]$^+$ calcd for $C_{15}H_{16}N_4O$, 269; found 269; Analysis (calcd, found for $C_{15}H_{16}N_4O$): C (67.2, 67.3), H (6.0, 5.7), N (20.9, 21.1).

4.1.5. N-(2-hydroxylethyl)-1-methyl-β-carboline-3-carboxamide (14)

A mixture of ethanolamide (8 ml, 133 mmol) and ethanol (40 ml) was heated at 80~90°C, then compound **6** (1.02 g, 4 mmol) diluted in ethanol (20 ml) was added drop-wise in 1 h. After heated for 8 h, the mixture was poured into water (200 ml) and extracted with ethyl acetate (5 × 100 ml). The organic layer was dried with anhydrous sodium sulfate, removed by evaporation and the residue was recrystallized with ethanol twice. White crystals of **14** were obtained (0.67 g, 62%). m.p.: 198–199°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.68 (s, 1H, 4-H), 8.61 (t, J = 6 Hz, 1H, CONH), 8.35 (d, J = 8Hz, 1H, 8-H), 7.64 (d, J = 8.5 Hz, 1H, 5-H), 7.58 (m, 1H, 6-H), 7.28 (m, 1H, 7-H), 4.89 (s, 1H, OH), 3.57 (m, 2H, NHCH$_2$C**H**$_2$OH), 3.46 (m, 2H, NHC**H**$_2$CH$_2$OH), 2.50 (s, 3H, 1- CH$_3$); IR (KBr): 3420, 3219, 2943, 1629, 1542, 1463, 1349, 1250, 741 cm^{-1}; UV:λ_{max} 346, 332, 300, 282, 247, 223, 212 nm; FAB-MS (M + 1): [M + 1]$^+$ calcd for $C_{15}H_{15}N_3O_2$, 270; found 270; Analysis (calcd, found for $C_{15}H_{15}N_3O_2$): C (66.9, 66.8), H (5.6, 5.8), N (15.6, 15.5).

4.1.6. N-(2-aminohexyl)-1-methyl-β-carboline-3-carboxamide (15)

1,6-Hexanediamide (3.48 g, 30 mmol) was diluted in DMF (30 ml) and boiled, and then compound **6** (1.016 g, 4 mmol) diluted in DMF (10 ml) was added drop-wise in 1 h. After heated for 48 h, the mixture was poured into water (200 ml)

and extracted with ethyl acetate (5 × 100 ml). The organic layer was dried with anhydrous sodium sulfate, removed by evaporation and the residue was purified by silica column chromatography with methanol as the eluent. After recrystallization with methanol, white crystals of **15** were obtained (0.21 g, 19%). m.p.: 240–241°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.92 (s, 1H, 4-H), 8.61 (t, J = 6 Hz, 1H, CONH), 8.35 (d, J = 8 Hz, 1H, 8-H), 7.74 (d, J = 8.5 Hz, 1H, 5-H), 7.62 (m, 1H, 6-H), 7.32 (m, 1H, 7-H), 3.35 (m, 2H, NHC**H**$_2$(CH$_2$)$_5$NH$_2$), 3.00 (m, 2H, NH(CH$_2$)$_5$C**H**$_2$NH$_2$), 2.98 (s, 3H, 1- CH$_3$), 1.55 (m, 2H, NHCH$_2$C**H**$_2$(CH$_2$)$_4$NH$_2$), 1.32–1.38 (m, 2H, CH$_2$ × 3); IR (KBr): 3339, 2932, 2854, 1645, 1544, 1453, 1395, 1370, 1366, 1264, 722 cm^{-1}; UV:λ_{max} 344, 330, 284, 252, 226, 212 nm; FAB-MS (M + 1): [M + 1]$^+$ calcd for $C_{19}H_{24}N_4O$, 325; found 325; Analysis (calcd, found for $C_{19}H_{24}N_4O$): C (70.4, 70.4), H (7.4, 7.3), N (17.3, 17.2).

4.1.7. N-(2-hydroxylethyl)-β-carboline-3-carboxamide (16)

Prepared by the same procedure as compound **14** from **5** (0.96 g, 4 mmol) and ethanolamide (8 ml, 133 mmol). White crystals of **16** were obtained (0.59 g, 58%). m.p.: 218–219°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.89 (s, 1H, 1-H), 8.85 (s, 1H, 4-H), 8.67 (t, J = 6 Hz, 1H, CONH), 8.40 (d, J = 8 Hz, 1H, 8-H), 7.65 (d, J = 8 Hz, 1H, 5-H), 7.58 (m, 1H, 6-H), 7.28 (m, 1H, 7-H), 4.87 (s, 1H, OH), 3.57 (m, 2H, NHCH$_2$C**H**$_2$OH), 3.45 (m, 2H, NHC**H**$_2$CH$_2$OH); IR (KBr): 3314, 3210, 3056, 2935, 1658, 1536, 1460, 1339, 1250, 732 cm^{-1}; UV:λ_{max} 351, 336, 289, 283, 269, 247, 227, 215 nm; FAB-MS (M + 1): [M + 1]$^+$ calcd for $C_{14}H_{13}N_3O_2$, 256; found 256; Analysis (calcd, found for $C_{14}H_{13}N_3O_2$): C (65.9, 65.9), H (5.1, 5.3), N (16.5, 16.4).

4.1.8. N-(2-aminohexyl)-β-carboline-3-carboxamide (17)

Prepared by the same procedure as compound **15** from **5** (0.96 g, 4 mmol) and 1,6-hexanediamide (3.48 g, 30 mmol). White crystals of **17** were obtained (0.25 g, 20%). m.p.: 192–193°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.99 (s, 1H, 4-H), 8.61 (t, J = 6 Hz, 1H, CONH), 8.35 (d, J = 8 Hz, 1H, 8-H), 7.72 (d, J = 8.5 Hz, 1H, 5-H), 7.62 (m, 1H, 6-H), 7.33 (m, 1H, 7-H), 3.42 (m, 2H, NHC**H**$_2$(CH$_2$)$_5$NH$_2$), 3.08 (m, 2H, NH(CH$_2$)$_5$C**H**$_2$NH$_2$), 2.98 (s, 3H, 1- CH$_3$), 1.55 (m, 2H, NHCH$_2$C**H**$_2$(CH$_2$)$_4$NH$_2$), 1.30–1.37 (m, 6H, CH$_2$×3); IR (KBr): 3342, 2924, 2854, 1650, 1533, 1450, 1385, 1370, 1365, 1254, 725 cm^{-1}; UV:λ_{max} 345, 330, 266, 230, 212 nm; FAB-MS (M + 1): [M + 1]$^+$ calcd for $C_{18}H_{22}N_4O$, 311; found 311; Analysis (calcd, found for $C_{18}H_{22}N_4O$): C (69.7, 69.7), H (7.1, 7.3), N (18.1, 18.2).

4.1.9. Butyl-9-methyl-1-methyl-β-carboline-3-carboxylate (18)

A mixture of **11** (1.13 g, 4 mmol) and anhydrous DMF (60 ml) was stirred at room temperature until clear, and then 60% NaH (0.3 g, 7.5 mmol) and iodomethane (0.25 ml, 3.75 mmol) were added. The resulting mixture was poured into water (200 ml) and extracted with ethyl acetate (4 × 100 ml). The organic layer was dried with anhydrous sodium sulfate, removed by evaporation and the residue was recrystallized with ethanol twice. White crystals of **18** were obtained (0.97 g, 82%). m.p.: 71–72°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.78 (s, 1H, 4-H), 8.22 (d, J = 7.5 Hz, 1H, 8-H), 7.62 (d, J = 8.5 Hz, 1H, 5-H), 7.58 (m, 1H, 6-H), 7.36 (m, 1H, 7-H), 4.46 (t, J = 6 Hz, 2H, COC**H**$_2$CH$_2$CH$_2$CH$_3$), 3.25 (s, 3H, 9-CH3), 2.95 (s, 3H, 1-CH$_3$), 1.83 (m, 2H, COCH$_2$C**H**$_2$CH$_2$CH$_3$), 1.51 (m, 2H, COCH$_2$CH$_2$C**H**$_2$CH$_3$), 1.00 (t, J = 7.5 Hz, 3H, COCH$_2$CH$_2$CH$_2$C**H**$_3$); IR (KBr): 3371, 2958, 2650, 1727, 1618, 1460, 1348, 1266, 757 cm^{-1}; UV:λ_{max} 350, 336, 299, 280, 248, 227, 213 nm; FAB-MS (M + 1): [M + 1]$^+$ calcd for $C_{18}H_{20}N_2O_2$, 297; found 297; Analysis (calcd, found for $C_{18}H_{20}N_2O_2$): C (73.0, 73.0), H (6.8, 6.5), N (9.5, 9.6).

4.1.10. Butyl-9-ethyl-1-methyl-β-carboline-3-carboxylate (19)

Prepared by the same procedure as compound **18** from **11** (1.13 g, 4 mmol), 60% NaH (0.3 g, 7.5 mmol) and iodoethane (1.6 ml, 19.2 mmol). White crystals of **19** were obtained (0.98 g, 79%). m.p.: 96–97°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.76 (s, 1H, 4-H), 8.22 (d, J = 8 Hz, 1H, 8-H), 7.66 (m, 1H, 5-H), 7.54 (m, 1H, 6-H), 7.37 (m, 1H, 7-H), 4.70 (m, 2H, 9- C**H**$_2$CH$_3$), 4.47 (t, J = 7Hz, 2H, COC**H**$_2$CH$_2$CH$_2$CH$_3$), 3.21 (s, 3H, 1-CH$_3$), 1.85 (m, 2H, COCH$_2$C**H**$_2$CH$_2$CH$_3$), 1.53 (m, 2H, COCH$_2$CH$_2$C**H**$_2$CH$_3$), 1.22 (t, J = 7.5Hz, 3H, 9- CH$_2$C**H**$_3$), 1.01 (t, J = 7.5 Hz, 3H, COCH$_2$CH$_2$CH$_2$C**H**$_3$); IR (KBr): 3438, 2960, 2601, 1728, 1620, 1557, 1452, 1381, 1344, 1240, 751 cm^{-1}; UV:λ_{max} 348, 335, 301, 276, 246, 227, 212 nm; FAB-MS (M + 1): [M + 1]$^+$ calcd for $C_{19}H_{22}N_2O_2$, 311; found 311; Analysis (calcd, found for $C_{19}H_{22}N_2O_2$): C (73.5, 73.5), H (7.1, 7.3), N (9.0, 8.9).

4.1.11. Butyl-9-benzyl-1-methyl-β-carboline-3-carboxylate (20)

Prepared by the same procedure as compound **18** from **11** (1.13 g, 4 mmol), 60% NaH (0.3 g, 7.5 mmol) and benzyl bromide (0.54 ml, 4.3 mmol). White crystals of **20** were obtained (0.87 g, 58%). m.p.: 112–113°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.79 (s, 1H, 4-H), 8.24 (d, J = 7.5 Hz, 1H, 8-H), 7.58 (m, 1H, 6-H), 7.42 (d, J = 8.5 Hz, 1H, 5-H), 7.37 (m, 1H, 7-H), 7.21–7.35 (m, 5H, 9- CH$_2$C$_6$**H**$_5$), 5.86 (s, 2H, 9-C**H**$_2$C$_6$H$_5$), 4.46 (t, J = 6 Hz, 2H, COC**H**$_2$CH$_2$CH$_2$CH$_3$), 2.96 (s, 3H, 1-CH$_3$), 1.85 (m, 2H, COCH$_2$C**H**$_2$CH$_2$CH$_3$), 1.53 (m, 2H, COCH$_2$CH$_2$C**H**$_2$CH$_3$), 1.01 (t, J = 7.5 Hz, 3H, COCH$_2$CH$_2$CH$_2$C**H**$_3$); IR (KBr): 3422, 3064, 2956, 1693, 1557, 1452, 1394, 1340, 1236, 728 cm^{-1}; UV:λ_{max} 346, 333, 303, 274, 245, 226, 211 nm; FAB-MS (M + 1): [M + 1]$^+$ calcd for $C_{24}H_{24}N_2O_2$, 373; found 373; Analysis (calcd, found for $C_{24}H_{24}N_2O_2$): C (77.4, 77.4), H (6.5, 6.8), N (7.5, 7.3).

4.1.12. Butyl-9-(2′,3′,4′,5′,6′-pentafluoro)benzyl-1-methyl-β-carboline-3-carboxylate (21)

Prepared by the same procedure as compound **18** from **11** (1.13 g, 4 mmol), 60% NaH (0.3 g, 7.5 mmol) and α-bromo-2,3,4,5,6-pentafluorotoluene (0.65 ml, 4.3 mmol). White crystals of **21** were obtained (1.33 g, 72%). m.p.: 120–121°C; ^{1}H NMR

(500 MHz, DMSO-d_6): δ 8.74 (s, 1H, 4-H), 8.20 (d, J = 8 Hz, 1H, 8-H), 7.58 (m, 1H, 6-H), 7.42 (d, J = 8.5 Hz, 1H, 5-H), 7.37 (m, 1H, 7-H), 6.01 (s, 2H, 9-C**H**$_2$C$_6$F$_5$), 4.47 (t, J = 6 Hz, 2H, COC**H**$_2$CH$_2$CH$_2$CH$_3$), 3.17 (s, 3H, 1-CH$_3$), 1.86 (m, 2H, COCH$_2$C**H**$_2$CH$_2$CH$_3$), 1.52 (m, 2H, COCH$_2$CH$_2$C**H**$_2$CH$_3$), 1.02 (t, J = 7.5 Hz, 3H, COCH$_2$CH$_2$CH$_2$C**H**$_3$); IR (KBr): 3425, 2961, 2873, 1725, 1695, 1557, 1503, 1451, 1391, 1345, 1220, 747 cm^{-1}; UV:λ_{max} 345, 332, 303, 274, 245, 226, 211 nm; FAB-MS (M + 1): [M + 1]$^+$ calcd for $C_{24}H_{19}N_2O_2F_5$, 463; found 463; Analysis (calcd, found for $C_{24}H_{19}N_2O_2F_5$): C (62.3, 62.3), H (4.1, 4.1), N (6.1, 6.0).

*4.1.13. N-(2-aminoethyl)-9-ethyl-1-methyl-β-carboline-3-carboxamide (**32**)*

Prepared by the same procedure as compound **13** from **23** (0.85 g, 3 mmol) and ethylenediamide (18 ml, 20.3 mmol). Light yellow crystals of **32** were obtained (0.19 g, 21%). m.p.: 163–165°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.68 (s, 1H, 4-H), 8.63 (t, J = 6Hz, 1H, CONH), 8.37 (d, J = 8 Hz, 1H, 8-H), 7.65 (d, J = 8.5 Hz, 1H, 5-H), 7.63 (m, 1H, 6-H), 7.31 (t, J = 14.5 Hz, 1H, 7-H), 4.69 (m, 2H, 9- C**H**$_2$CH$_3$), 3.35 (m, 2H, NHC**H**$_2$CH$_2$NH$_2$), 2.88 (s, 3H, 1- CH$_3$), 2.76 (m, 2H, NHCH$_2$C**H**$_2$NH$_2$), 1.39 (m, 3H, 9- CH$_2$C**H**$_3$); IR (KBr): 3353, 3053, 2925, 1642, 1529, 1450, 1346, 1238, 752 cm^{-1}; UV:λ_{max} 356, 340, 304, 273, 240, 202 nm; FAB-MS (M + 1): [M + 1]$^+$ calcd for $C_{17}H_{20}N_4O$, 297; found 297; Analysis (calcd, found for $C_{17}H_{20}N_4O$): C (68.9, 68.8), H (6.8, 6.9), N (18.9, 18.6).

*4.1.14. N-(2-aminoethyl)-9-n-butyl-1-methyl-β-carboline-3-carboxamide (**33**)*

Prepared by the same procedure as compound **13** from **24** (0.93 g, 3 mmol) and ethylenediamide (18 ml, 20.3 mmol). Light yellow crystals of **33** were obtained (0.26 g, 27%). m.p.: 144–145°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.67 (s, 1H, 4-H), 8.63 (t, J = 6 Hz, 1H, CONH), 8.37 (d, J = 7.5 Hz, 1H, 8-H), 7.75 (d, J = 8.5 Hz, 1H, 5-H), 7.63 (t, J = 15.5 Hz, 1H, 6-H), 7.31 (t, J = 15Hz, 1H, 7-H), 4.63 (t, J = 7.5Hz, 2H, 9- C**H**$_2$CH$_2$CH$_2$CH$_3$), 3.43 (m, 2H, NHC**H**$_2$CH$_2$NH$_2$), 2.89 (s, 3H, 1- CH$_3$), 2.77 (m, 2H, NHCH$_2$C**H**$_2$NH$_2$), 1.77 (m, 2H, 9-CH$_2$C**H**$_2$CH$_2$CH$_3$), 1.39 (m, 2H, 9- CH$_2$CH$_2$C**H**$_2$CH$_3$), 0.92 (t, J = 7.5Hz, 3H, 9- CH$_2$CH$_2$CH$_2$C**H**$_3$); IR (KBr): 3374, 3055, 2960, 2865, 1644, 1521, 1452, 1352, 1249, 752 cm^{-1}; UV: λ_{max} 356, 340, 304, 274, 240 nm; FAB-MS (M + 1): [M + 1]$^+$ calcd for $C_{19}H_{24}N_4O$, 325; found 325; Analysis (calcd, found for $C_{19}H_{24}N_4O$): C (70.2, 70.4), H (7.4, 7.8), N (17.3, 17.0).

*4.1.15. N-(2-aminoethyl)-9-benzyl-1-methyl-β-carboline-3-carboxamide (**34**)*

Prepared by the same procedure as compound **13** from **25** (1.03 g, 3 mmol) and ethylenediamide (18 ml, 20.3 mmol). Light yellow crystals of **34** were obtained (0.26 g, 24%). m.p.: 122–124°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.79 (s, 1H, 4-H), 8.63 (t, J = 6 Hz, 1H, CONH), 8.46 (d, J = 7.5 Hz, 1H, 8-H), 7.78 (d, J = 8.0 Hz, 1H, 5-H), 7.63 (m, 1H, 6-H), 7.36 (m, 1H, 7-H), 7.20–7.34 (m, 5H, 9-CH$_2$C$_6$**H**$_5$), 5.99 (s, 2H, 9-C**H**$_2$C$_6$H$_5$), 3.43 (m, 2H, NHC**H**$_2$CH$_2$NH$_2$), 3.34 (m, 2H, NHCH$_2$C**H**$_2$NH$_2$), 2.89 (s, 3H, 1- CH$_3$); IR (KBr): 3370, 3061, 2927, 2864, 1652, 1620, 1559, 1526, 1451, 1342, 731 cm^{-1}; UV:λ_{max} 386, 352, 339, 302, 275, 238, 206 nm; FAB-MS (M + 1): [M + 1]$^+$ calcd for $C_{22}H_{22}N_4O$, 359; found 359; Analysis (calcd, found for $C_{22}H_{22}N_4O$): C (73.7, 73.6), H (6.1, 6.4), N (15.6, 15.4).

*4.1.16. N-(2-aminoethyl)-9-(2′,3′,4′,5′,6′-pentafluoro)benzyl-1-methyl-β-carboline-3-carboxamide (**35**)*

Prepared by the same procedure as compound **13** from **26** (1.30 g, 3 mmol) and ethylenediamide (18 ml, 20.3 mmol). White crystals of **35** were obtained (0.31 g, 23%). m.p.: 228–229°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.99 (s, 1H, 4-H), 8.82 (d, J = 7.5 Hz, 1H, 8-H), 8.68 (t, J = 6Hz, 1H, CONH), 8.44 (d, J = 8.0 Hz, 1H, 5-H), 7.73 (m, 1H, 6-H), 7.36 (m, 1H, 7-H), 5.88 (s, 2H, 9-C**H**2C6F5), 3.52 (m, 2H, NHC **H**$_2$CH$_2$NH$_2$), 3.40 (m, 2H, NHCH$_2$C**H**$_2$NH$_2$), 2.50 (s, 3H, 1-CH$_3$); IR (KBr): 3226, 3061, 2927, 1659, 1613, 1548, 1501, 1470, 1351, 1275, 754 cm^{-1}; UV:λ_{max} 352, 337, 301, 277, 246, 202 nm; FAB-MS (M + 1): [M + 1]$^+$ calcd for $C_{22}H_{17}N_4OF_5$, 449; found 449; Analysis (calcd, found for $C_{22}H_{17}N_4OF_5$): C (58.9, 58.8), H (3.8, 3.6), N (12.5, 12.3).

*4.1.17. N-(2-aminoethyl)-9-methyl-β-carboline-3-carboxamide (**36**)*

Prepared by the same procedure as compound **13** from **27** (1.02 g, 4 mmol) and ethylenediamide (18 ml, 20.3 mmol). White crystals of **36** were obtained (0.23 g, 21%). m.p.: 105–106°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.02 (s, 1H, 1-H), 8.77 (s, 1H, 4-H), 8.63 (t, J = 6Hz, 1H, CONH), 8.37 (d, J = 8Hz, 1H, 8-H), 7.65 (d, J = 8.5Hz, 1H, 5-H), 7.62 (m, 1H, 6-H), 7.31 (t, J = 14.5Hz, 1H, 7-H), 4.15 (m, 2H, 9-C **H**$_3$), 3.56 (m, 2H, NHC**H**$_2$CH$_2$NH$_2$), 3.44 (m, 2H, NHCH$_2$C**H**$_2$NH$_2$); IR (KBr): 3260, 3048, 2882, 1661, 1538, 1460, 13492, 1227, 752 cm^{-1}; UV:λ_{max} 352, 339, 303, 273, 238, 202 nm; FAB-MS (M + 1): [M + 1]$^+$ calcd for $C_{15}H_{16}N_4O$, 269; found 269; Analysis (calcd, found for $C_{15}H_{16}N_4O$): C (67.2, 67.1), H (6.0, 5.8), N (20.9, 20.8).

*4.1.18. N-(2-aminoethyl)-9-ethyl-β-carboline-3-carboxamide (**37**)*

Prepared by the same procedure as compound **13** from **28** (0.80 g, 3 mmol) and ethylenediamide (18 ml, 20.3 mmol). White crystals of **37** were obtained (0.18 g, 21%). m.p.: 123–124°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.02 (s, 1H, 1-H), 8.68 (s, 1H, 4-H), 8.63 (t, J = 6 Hz, 1H, CONH), 8.36 (d, J = 8Hz, 1H, 8-H), 7.65 (d, J = 8.5Hz, 1H, 5-H), 7.63 (m, 1H, 6-H), 7.32 (t, J = 14.5 Hz, 1H, 7-H), 4.72 (m, 2H, 9- C **H**$_2$CH$_3$), 3.35 (m, 2H, NHC**H**$_2$CH$_2$NH$_2$), 2.83 (m, 2H, NHCH$_2$C**H**$_2$NH$_2$), 1.39 (m, 3H, 9- CH$_2$C**H**$_3$); IR (KBr): 3350, 3053, 2920, 1644, 1529, 1450, 1344, 1224, 751 cm^{-1}; UV:λ_{max} 356, 341, 304, 272, 239, 202 nm; FAB-MS (M + 1): [M + 1]$^+$ calcd for $C_{16}H_{18}N_4O$, 283; found 283; Analysis (calcd, found for $C_{16}H_{18}N_4O$): C (68.1, 68.0), H (6.4, 6.2), N (19.9, 19.8).

*4.1.19. N-(2-aminoethyl)-9-benzyl-β-carboline-3-carboxamide (**38**)*

Prepared by the same procedure as compound **13** from **30** (0.99 g, 3 mmol) and ethylenediamide (18 ml, 20.3 mmol). White crystals of **38** were obtained (0.23 g, 22%). m.p.: 122–124°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.03 (s, 1H, 1-H), 8.87 (s, 1H, 4-H), 8.63 (t, J = 6Hz, 1H, CONH), 8.44 (d, J = 7.5Hz, 1H, 8-H), 7.78 (d, J = 8.0Hz, 1H, 5-H), 7.63 (m, 1H, 6-H), 7.31 (m, 1H, 7-H), 7.21–7.32 (m, 5H, 9-$CH_2C_6\mathbf{H}_5$), 5.93 (s, 2H, 9- C$\mathbf{H}_2C_6H_5$), 3.43 (m, 2H, NHC $\mathbf{H}_2CH_2NH_2$), 3.34 (m, 2H, $NHCH_2C\mathbf{H}_2NH_2$); IR (KBr): 3367, 3055, 2926, 2864, 1662, 1623, 1585, 1526, 1494, 1462, 1333, 739, 698 cm^{-1}; UV:λ_{max} 386, 356, 341, 301, 273, 238, 206 nm; FAB-MS (M + 1): $[M + 1]^+$ calcd for $C_{21}H_{20}N_4O$, 345; found 345; Analysis (calcd, found for $C_{21}H_{20}N_4O$): C (73.3, 73.4), H (5.8, 5.6), N (16.3, 16.1).

*4.1.20. N-(2-hydroxylethyl)-9-methyl-1-methyl-β-carboline-3-carboxamide (**39**)*

Prepared by the same procedure as compound **14** from **22** (0.81 g, 3 mmol) and ethanolamide (8 ml, 133 mmol). White crystals of **39** were obtained (0.59 g, 69%). m.p.: 189–190°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.71 (s, 1H, 4-H), 8.63 (t, J = 6Hz, 1H, CONH), 8.36 (d, J = 8Hz, 1H, 8-H), 7.75 (d, J = 8.5Hz, 1H, 5-H), 7.65 (t, J = 7.5Hz, 1H, 6-H), 7.32 (m, 1H, 7-H), 4.21 (s, 3H, 9- CH_3), 3.58 (m, 2H, $NHCH_2C\mathbf{H}_2OH$), 3.45 (m, 2H, NHC$\mathbf{H}_2CH_2OH$), 2.83 (s, 3H, 1- CH_3); IR (KBr): 3356, 2930, 2862, 1635, 1538, 1458, 1368, 1337, 1279, 751 cm^{-1}; UV:λ_{max} 352, 338, 289, 248, 220, 213 nm; FAB-MS (M + 1): $[M + 1]^+$ calcd for $C_{16}H_{17}N_3O_2$, 284; found 284; Analysis (calcd, found for $C_{16}H_{17}N_3O_2$): C (67.8, 67.7), H (6.0, 5.8), N (14.8, 14.7).

*4.1.21. N-(2-hydroxylethyl)-9-ethyl-1-methyl-β-carboline-3-carboxamide (**40**)*

Prepared by the same procedure as compound **14** from **23** (0.85 g, 3 mmol) and ethanolamide (8 ml, 133 mmol). White crystals of **40** were obtained (0.64 g, 72%). m.p.: 174–175°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.71 (s, 1H, 4-H), 8.63 (t, J = 6Hz, 1H, CONH), 8.36 (d, J = 8Hz, 1H, 8-H), 7.75 (d, J = 8.5Hz, 1H, 5-H), 7.65 (t, J = 7.5Hz, 1H, 6-H), 7.32 (m, 1H, 7-H), 4.72(m, 2H, 9- C$\mathbf{H}_2CH_3$), 3.57 (m, 2H, $NHCH_2C\mathbf{H}_2OH$), 3.45 (m, 2H, NHC$\mathbf{H}_2CH_2OH$), 2.83 (s, 3H, 1- CH_3), 1.39 (t, J = 6Hz, 3H, 9- $CH_2C\mathbf{H}_3$); IR (KBr): 3396, 2933, 2875, 1647, 1534, 1453, 1375, 1340, 1231, 753 cm^{-1}; UV:λ_{max} 352, 339, 276, 248, 220, 202 nm; FAB-MS (M + 1): $[M + 1]^+$ calcd for $C_{17}H_{19}N_3O_2$, 298; found 298; Analysis (calcd, found for $C_{17}H_{19}N_3O_2$): C (68.7, 68.7), H (6.4, 6.2), N (14.1, 14.2).

*4.1.22. N-(2-hydroxylethyl)-9-n-butyl-1-methyl-β-carboline-3-carboxamide (**41**)*

Prepared by the same procedure as compound **14** from **24** (0.93 g, 3 mmol) and ethanolamide (8 ml, 133 mmol). White crystals of **41** were obtained (0.70 g, 72%). m.p.: 126–127°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.68 (s, 1H, 4-H), 8.57 (t, J = 6Hz, 1H, CONH), 8.36 (d, J = 8Hz, 1H, 8-H), 7.75 (d, J = 8.5Hz, 1H, 5-H), 7.62 (m, 1H, 6-H), 7.30 (m, 1H, 7-H), 4.79 (t, J = 5.5Hz, 1H, OH), 4.64 (t, J = 8Hz, 2H, 9- C$\mathbf{H}_2CH_2CH_2CH_3$), 3.57 (m, 2H, $NHCH_2C\mathbf{H}_2OH$), 3.45 (m, 2H, NHC$\mathbf{H}_2CH_2OH$), 3.06 (s, 3H, 1- CH_3), 1.77 (m, 2H, 9-$CH_2C\mathbf{H}_2CH_2CH_3$), 1.41 (m, 2H, 9- $CH_2CH_2C\mathbf{H}_2CH_3$), 0.93 (t, J = 7.5Hz, 3H, 9- $CH_2CH_2CH_2C\mathbf{H}_3$); IR (KBr): 3386, 3304, 3061, 2957, 2868, 1642, 1529, 1454, 1342, 1256, 751 cm^{-1}; UV:λ_{max} 356, 340, 304, 274, 264, 240, 230, 223 nm; FAB-MS (M + 1): $[M + 1]^+$ calcd for $C_{19}H_{23}N_3O_2$, 326; found 326; Analysis (calcd, found for $C_{19}H_{23}N_3O_2$): C (70.2, 70.1), H (7.1, 6.9), N (12.9, 12.7).

*4.1.23. N-(2-hydroxylethyl)-9-benzyl-1-methyl-β-carboline-3-carboxamide (**42**)*

Prepared by the same procedure as compound **14** from **25** (1.03 g, 3 mmol) and ethanolamide (8 ml, 133 mmol). White crystals of **42** were obtained (0.81 g, 75%). m.p.: 182–183°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.76 (s, 1H, 4-H), 8.56(t, J = 6Hz, 1H, CONH), 8.39 (d, J = 7.5Hz, 1H, 8-H), 7.70 (d, J = 8.5Hz, 1H, 5-H), 7.58 (m, 1H, 6-H), 7.34 (m, 1H, 7-H), 7.21–7.36 (m, 5H, 9-$CH_2C_6\mathbf{H}_5$), 5.98 (s, 2H, 9-C$\mathbf{H}_2C_6H_5$), 3.56 (m, 2H, $NHCH_2C\mathbf{H}_2OH$), 3.45 (m, 2H, NHC $\mathbf{H}_2CH_2OH$), 2.86 (s, 3H, 1- CH_3); IR (KBr): 3340, 2935, 2871, 1635, 1533, 1453, 1348, 1205, 743 cm^{-1}; UV:λ_{max} 352, 338, 276, 244, 228, 212 nm; FAB-MS (M + 1): $[M + 1]^+$ calcd for $C_{22}H_{21}N_3O_2$, 360; found 360; Analysis (calcd, found for $C_{22}H_{21}N_3O_2$): C (73.5, 73.5), H (5.8, 5.9), N (11.7, 11.7).

*4.1.24. N-(2-hydroxyl)-9-(2′,3′,4′,5′,6′-pentafluoro)benzyl-1-methyl-β-carboline-3-carboxamide (**43**)*

Prepared by the same procedure as compound **14** from **26** (1.30 g, 3 mmol) and ethanolamide (8 ml, 133 mmol). White crystals of **43** were obtained (1.02 g, 76%). m.p.: 212–213°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.71 (s, 1H, 4-H), 8.59 (t, J = 6Hz, 1H, CONH), 8.39 (d, J = 7.5Hz, 1H, 8-H), 7.61 m, 1H, 5-H), 7.57 (m, 1H, 6-H), 7.34 (m, 1H, 7-H), 5.97 (s, 2H, 9-C$\mathbf{H}_2C_6F_5$), 3.58 (m, 2H, $NHCH_2C\mathbf{H}_2OH$), 3.45 (m, 2H, NHC$\mathbf{H}_2CH_2OH$), 3.05 (s, 3H, 1- CH_3); IR (KBr): 3332, 2944, 1653, 1548, 1507, 1453, 1342, 1297, 1020, 748 cm^{-1}; UV:λ_{max} 352, 338, 276, 243, 228, 202 nm; FAB-MS (M + 1): $[M + 1]^+$ calcd for $C_{22}H_{16}N_3O_2F_5$, 450; found 450; Analysis (calcd, found for $C_{22}H_{16}N_3O_2F_5$): C (58.8, 58.8), H (3.6, 3.4), N (9.4, 9.5).

*4.1.25. N-(2-hydroxylethyl)-9-methyl-β-carboline-3-carboxamide (**44**)*

Prepared by the same procedure as compound **14** from **27** (1.02 g, 4 mmol) and ethanolamide (8 ml, 133 mmol). White crystals of **44** were obtained (0.80 g, 74%). m.p.: 162–163°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.03 (s, 1H, 1-H), 8.85 (s, 1H, 4-H), 8.64 (t, J = 6Hz, 1H, CONH), 8.42 (d, J = 8Hz, 1H, 8-H), 7.75 (d, J = 8.5Hz, 1H, 5-H), 7.67 (m, 1H, 6-H), 7.34 (m, 1H, 7-H), 4.79 (t, J = 5.5Hz, 1H, OH), 4.05 (s, 3H, 9- C$\mathbf{H}_3$), 3.57 (m, 2H, $NHCH_2C\mathbf{H}_2OH$), 3.44 (m, 2H, NHC$\mathbf{H}_2CH_2OH$);

IR (KBr): 3380, 2924, 2855, 1666, 1602, 1542, 1466, 1431, 1378, 1263, 750 cm^{-1}; UV:λ_{max} 358, 342, 277, 260, 248, 224, 214 nm; FAB-MS (M + 1): $[M + 1]^+$ calcd for $C_{15}H_{15}N_3O_2$, 270; found 270; Analysis (calcd, found for $C_{15}H_{15}N_3O_2$): C (66.9, 66.8), H (4.1, 4.4), N (15.6, 15.4).

4.1.26. N-(2-hydroxylethyl)-9-ethyl-β-carboline-3-carboxamide (45)

Prepared by the same procedure as compound **14** from **28** (0.80 g, 3 mmol) and ethanolamide (8 ml, 133 mmol). White crystals of **45** were obtained (0.65 g, 77%). m.p.: 214–216°C; ^1H NMR (500 MHz, DMSO-d_6): δ 9.03 (s, 1H, 1-H), 8.85 (s, 1H, 4-H), 8.63 (t, J = 6Hz, 1H, CONH), 8.42 (d, J = 8Hz, 1H, 8-H), 7.73 (d, J = 8.5Hz, 1H, 5-H), 7.67 (m, 1H, 6-H), 7.33 (m, 1H, 7-H), 4.92 (t, J = 5.5Hz, 1H, OH), 4.72(m, 2H, 9- C**H**$_2$$CH_3$), 3.56 (m, 2H, $NHCH_2C$**H**$_2$OH), 3.44 (m, 2H, NHC**H**$_2$$CH_2OH$), 1.39 (t, J = 6Hz, 3H, 9- CH_2C**H**$_3$); IR (KBr): 3326, 2928, 2851, 1627, 1575, 1534, 1466, 1438, 1312, 1244, 750 cm^{-1}; UV:λ_{max} 356, 341, 277, 248, 222, 202 nm; FAB-MS (M + 1): $[M + 1]^+$ calcd for $C_{16}H_{17}N_3O_2$, 284; found 284; Analysis (calcd, found for $C_{16}H_{17}N_3O_2$): C (67.8, 67.7), H (6.0, 5.8), N (14.8, 14.7).

4.1.27. N-(2-hydroxylethyl)-9-n-butyl-β-carboline-3-carboxamide (46)

Prepared by the same procedure as compound **14** from **29** (0.89 g, 3 mmol) and ethanolamide (8 ml, 133 mmol). White crystals of **46** were obtained (0.63 g, 68%). m.p.: 96–97°C; ^1H NMR (500 MHz, DMSO-d_6): δ 9.05 (s, 1H, 1-H), 8.84 (s, 1H, 4-H), 8.62 (t, J = 6Hz, 1H, CONH), 8.41 (d, J = 8Hz, 1H, 8-H), 7.76 (d, J = 8.5Hz, 1H, 5-H), 7.65 (m, 1H, 6-H), 7.33 (m, 1H, 7-H), 4.80 (t, J = 5.5Hz, 1H, OH), 4.57 (t, J = 8Hz, 2H, 9-C**H**$_2$$CH_2CH_2CH_3$), 3.57 (m, 2H, $NHCH_2C$**H**$_2$OH), 3.45 (m, 2H, NHC**H**$_2$$CH_2OH$), 1.82 (m, 2H, 9- CH_2C**H**$_2$$CH_2CH_3$), 1.30 (m, 2H, 9- CH_2CH_2C**H**$_2$$CH_3$), 0.88 (t, J = 7.5Hz, 3H, 9-$CH_2CH_2CH_2C$**H**$_3$); IR (KBr): 3377, 3056, 2957, 2871, 1651, 1533, 1497, 1464, 1359, 1334, 1264, 750 cm^{-1}; UV:λ_{max} 359, 345, 292, 267, 255, 250, 225, 214 nm; FAB-MS (M + 1): $[M + 1]^+$ calcd for $C_{18}H_{21}N_3O_2$, 312; found 312; Analysis (calcd, found for $C_{18}H_{21}N_3O_2$): C (69.5, 69.3), H (6.8, 7.1), N (13.5, 13.3).

4.1.28. N-(2-hydroxylethyl)-9-benzyl-β-carboline-3-carboxamide (47)

Prepared by the same procedure as compound **14** from **30** (0.99 g, 3 mmol) and ethanolamide (8 ml, 133 mmol). White crystals of **47** were obtained (0.73 g, 71%). m.p.: 210–211°C; ^1H NMR (500 MHz, DMSO-d_6): δ 9.02 (s, 1H, 1-H), 8.77 (s, 1H, 4-H), 8.56(t, J = 6Hz, 1H, CONH), 8.38 (d, J = 7.5Hz, 1H, 8-H), 7.70 (d, J = 8.5Hz, 1H, 5-H), 7.56 (m, 1H, 6-H), 7.33 (m, 1H, 7-H), 7.20–7.38 (m, 5H, 9-CH_2C_6**H**$_5$), 5.89 (s, 2H, 9-C**H**$_2$$C_6H_5$), 3.56 (m, 2H, $NHCH_2C$**H**$_2$OH), 3.45 (m, 2H, NHC**H**$_2$$CH_2OH$); IR (KBr): 3332, 2932, 2853, 1631, 1551, 1456, 1366, 1222, 749 cm^{-1}; UV:λ_{max} 359, 345, 291, 249, 223, 212 nm; FAB-MS (M + 1): $[M + 1]^+$ calcd for $C_{21}H_{19}N_3O_2$, 346; found 346; Analysis (calcd, found for $C_{21}H_{19}N_3O_2$): C (73.0, 73.1), H (5.5, 5.8), N (12.2, 12.0).

4.1.29. N-(2-hydroxyl)-9-(2′,3′,4′,5′,6′-pentafluoro)benzyl -β-carboline-3-carboxamide (48)

Prepared by the same procedure as compound **14** from **31** (1.26 g, 3 mmol) and ethanolamide (8 ml, 133 mmol). White crystals of **48** were obtained (1.02 g, 78%). m.p.: 216–218°C; ^1H NMR (500 MHz, DMSO-d_6): δ 9.07 (s, 1H, 1-H), 8.86 (s, 1H, 4-H), 8.68(t, J = 6Hz, 1H, CONH), 8.43 (d, J = 7.5Hz, 1H, 8-H), 7.73 (m, 1H, 5-H), 7.67 (m, 1H, 6-H), 7.35 (m, 1H, 7-H), 5.83 (s, 2H, 9-C**H**2C6F5), 3.57 (m, 2H, $NHCH_2C$**H**$_2$OH), 3.41 (m, 2H, NHC**H**$_2$$CH_2OH$); IR (KBr): 3340, 2930, 2875, 1658, 1538, 1500, 1464, 1334, 1269, 1064, 1014, 746 cm^{-1}; UV:λ_{max} 352, 338, 268, 256, 246, 225, 212 nm; FAB-MS (M + 1): $[M + 1]^+$ calcd for $C_{21}H_{14}N_3O_2F_5$, 436; found 436; Analysis (calcd, found for $C_{21}H_{14}N_3O_2F_5$): C (57.9, 58.0), H (3.2, 3.4), N (9.7, 9.5).

4.1.30. N-(2-aminohexyl)-9-benzyl-1-methyl-β-carboline-3-carboxamide (49)

Prepared by the same procedure as compound **15** from **25** (1.03 g, 3 mmol) and 1,6-hexanediamide (3.48 g, 30 mmol). Light yellow crystals of **49** were obtained (0.22 g, 18%). m.p.: 96–97°C; ^1H NMR (500 MHz, DMSO-d_6): δ 8.73 (s, 1H, 4-H), 8.53 (t, J = 6Hz, 1H, CONH), 8.42 (d, J = 7.5Hz, 1H, 8-H), 7.69 (d, J = 8.5Hz, 1H, 5-H), 7.60 (m, 1H, 6-H), 7.34 (m, 1H, 7-H), 7.21–7.33 (m, 5H, 9-CH_2C_6**H**$_5$), 5.97 (s, 2H, 9-C**H**$_2$$C_6H_5$), 3.35 (m, 2H, NHC**H**$_2$$(CH_2)_5NH_2$), 3.00 (m, 2H, $NH(CH_2)_5C$**H**$_2$$NH_2$), 2.98 (s, 3H, 1- CH_3), 1.55 (m, 2H, $NHCH_2C$**H**$_2$$(CH_2)_3NH_2$), 1.33–1.38 (m, 6H, CH_2×3); IR (KBr): 3296, 3061, 2928, 2854, 1649, 1620, 1559, 1528, 1453, 1370, 1342, 1206, 729 cm^{-1}; UV:λ_{max} 352, 339, 309, 297, 286, 275, 265, 253, 211 nm; FAB-MS (M + 1): $[M + 1]^+$ calcd for $C_{26}H_{30}N_4O$, 436; found 436; Analysis (calcd, found for $C_{26}H_{30}N_4O$): C (75.4, 75.4), H (7.3, 7.0), N (13.5, 13.4).

4.1.31. N-(2-aminohexyl)-9-methyl-β-carboline-3-carboxamide (50)

Prepared by the same procedure as compound **15** from **27** (1.02 g, 4 mmol) and 1,6-hexanediamide (3.48 g, 30 mmol). Light yellow crystals of **50** were obtained (0.27 g, 21%). m.p.: 167–168°C; ^1H NMR (500 MHz, DMSO-d_6): δ 8.73 (s, 1H, 4-H), 8.53 (t, J = 6Hz, 1H, CONH), 8.42 (d, J = 7.5Hz, 1H, 8-H), 7.69 (d, J = 8.5Hz, 1H, 5-H), 7.60 (m, 1H, 6-H), 7.34 (m, 1H, 7-H), 7.21–7.33 (m, 5H, 9-CH_2C_6**H**$_5$), 5.97 (s, 2H, 9-C**H**$_2$$C_6H_5$), 3.35 (m, 2H, NHC**H**$_2$$(CH_2)_5NH_2$), 3.00 (m, 2H, NH$(CH_2)_5C$**H**$_2$$NH_2$), 2.98 (s, 3H, 1- CH_3), 1.55 (m, 2H, $NHCH_2C$**H**$_2$$(CH_2)_3NH_2$), 1.33–1.38 (m, 6H, CH_2 × 3); IR (KBr): 3397, 2932, 1612, 1564, 1508, 1480, 1397, 1336, 1137, 734 cm^{-1}; UV:λ_{max} 360, 341, 309, 286, 274, 246, 204 nm; FAB-MS (M + 1): $[M + 1]^+$ calcd for $C_{19}H_{24}N_4O$, 325; found 325; Analysis (calcd, found for $C_{19}H_{24}N_4O$): C (70.4, 70.2), H (7.4, 7.7), N (17.3, 17.1).

4.1.32. N-(2-aminohexyl)-9-ethyl-β-carboline-3-carboxamide (51)

Prepared by the same procedure as compound **15** from **28** (0.80 g, 3 mmol) and 1,6-hexanediamide (3.48 g, 30 mmol). Light yellow crystals of **51** were obtained (0.20 g, 20%). m.p.: 146–148°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.05 (s, 1H, 1-H), 8.77 (s, 1H, 4-H), 8.52 (t, J = 6Hz, 1H, CONH), 8.41 (d, J = 7.5Hz, 1H, 8-H), 7.69 (d, J = 8.5Hz, 1H, 5-H), 7.61 (m, 1H, 6-H), 7.32 (m, 1H, 7-H), 4.66 (s, 3H, 9- C**H**$_2$CH$_3$), 3.35 (m, 2H, NHC**H**$_2$(CH$_2$) $_5$NH$_2$), 3.03 (m, 2H, NH(CH$_2$)$_5$C**H**$_2$NH$_2$), 1.52 (m, 2H, NHCH$_2$C**H**$_2$(CH$_2$) $_3$CH$_2$NH$_2$), 1.41 (t, J = 6Hz, 3H, 9- CH$_2$C**H**$_3$). 1.32–1.36 (m, 6H, CH$_2$×3); IR (KBr): 3342, 2937, 2854, 1645, 1454, 1395, 1371, 1319, 1286, 710 cm^{-1}; UV:λ_{max} 360, 341, 305, 289, 274, 245, 204 nm; FAB-MS (M + 1): [M + 1]$^+$ calcd for $C_{20}H_{26}N_4O$, 339; found 339; Analysis (calcd, found for $C_{20}H_{26}N_4O$): C (71.0, 70.9), H (7.7, 7.4), N (16.6, 16.3).

4.1.33. N-(2-aminohexyl)-9-benzyl-β-carboline-3-carboxamide (52)

Prepared by the same procedure as compound **15** from **30** (0.99 g, 3 mmol) and 1,6-hexanediamide (3.48 g, 30 mmol). White crystals of **52** were obtained (0.23 g, 19%). m.p.: 162–164°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.03 (s, 1H, 1-H), 8.74 (s, 1H, 4-H), 8.53 (t, J = 6Hz, 1H, CONH), 8.41 (d, J = 7.5Hz, 1H, 8-H), 7.69 (d, J = 8.5Hz, 1H, 5-H), 7.58 (m, 1H, 6-H), 7.34 (m, 1H, 7-H), 7.21–7.36 (m, 5H, 9-CH$_2$C$_6$**H**$_5$), 5.89 (s, 2H, 9-C**H**2C6H5), 3.35 (m, 2H, NHC**H**$_2$(CH$_2$)$_5$NH$_2$), 3.03 (m, 2H, NH(CH$_2$)$_5$C**H**$_2$NH$_2$), 1.53 (m, 2H, NHCH$_2$C**H**$_2$(CH$_2$) $_3$NH$_2$), 1.32–1.37 (m, 6H, CH$_2$×3); IR (KBr): 3295, 3054, 2932, 1646, 1559, 1534, 1453, 1382, 1342, 1206, 730 cm^{-1}; UV:λ_{max} 360, 344, 290, 250, 202 nm; FAB-MS (M + 1): [M + 1]$^+$ calcd for $C_{25}H_{28}N_4O$, 401; found 401; Analysis (calcd, found for $C_{25}H_{28}N_4O$): C (75.0, 75.1), H (7.0, 6.6), N (14.0, 13.8).

4.1.34. (2-(N-9-benzyl-β-carboline-3-carboxyl)amino)ethyl-glycine amide (53)

Compound **38** (1.03 g, 3 mmol) was diluted and boiled in ethanol (50 ml), and then glycine (0.23 g, 3 mmol) diluted in ethanol (10 ml) was added drop-wise in 1 hour. After heated for another 2 hour at 90°C, the mixture was poured into water (200 ml) and extracted with ethyl acetate (5 × 100 ml). The organic layer was dried with anhydrous sodium sulfate, removed by evaporation and the residue was purified by silica column chromatography with methanol as the eluent to remove the side-product of dimmer and excess ethylenediamide. After recrystallization with methanol twice, white crystals of **53** were obtained (0.14 g, 12%). m.p.: 171–172°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.07 (s, 1H, 1-H), 8.87 (s, 1H, 4-H), 8.76 (t, J = 6Hz, 1H, CONH), 8.44 (d, J = 8.5Hz, 1H, 8-H), 7.78 (d, J = 8.5Hz, 1H, 5-H), 7.63 (m, 1H, 6-H), 7.46 (m, 1H, 7-H), 7.20–7.30 (m, 5H, 9-CH$_2$C$_6$**H**$_5$), 5.85 (s, 2H, 9-C**H**$_2$C$_6$H$_5$), 4.29 (t, J = 4.29Hz, 2H, NHCH$_2$CH$_2$NHOCC**H**$_2$NH$_2$), 3.44 (m, 2H, NHC**H**$_2$CH$_2$NHOCCH$_2$NH$_2$), 3.31 (m, 2H, NHCH$_2$C**H**$_2$NHOCCH$_2$NH$_2$); IR (KBr): 3468, 3237, 3054, 2946, 2868, 1672, 1644, 1544, 1495, 1464, 1394, 1353, 1264, 740 cm^{-1}; UV:λ_{max} 357, 342, 273, 245, 205 nm; FAB-MS (M + 1): [M + 1]$^+$ calcd for $C_{23}H_{23}N_5O_2$, 402; found 402; Analysis (calcd, found for $C_{23}H_{23}N_5O_2$): C (68.8, 68.9), H (5.7, 5.6), N (17.5, 17.7).

4.1.35. (2-(N-9-benzyl-β-carboline-3-carboxyl)amino)ethyl-tryptophan amide (54)

Prepared by the same procedure as compound **53** from **38** (1.03 g, 3 mmol) and tryptophan (0.61 g, 3 mmol). White crystals of m were obtained (0.25 g, 16%). m.p.: 178–179°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.06 (s, 1H, 1-H), 8.86 (s, 1H, 4-H), 8.75 (t, J = 6Hz, 1H, N**H**CH$_2$CH$_2$NHOCCH), 8.43 (d, J = 8.5Hz, 1H, 8-H), 8.08 (t, J = 5Hz, 1H, NHCH$_2$CH$_2$N**H** OCCH**),** 7.90–8.03 (m, 5H, 2′,3′,4′,5′,6′-H), 7.77 (d, J = 8.5Hz, 1H, 5-H), 7.62 (m, 1H, 6-H), 7.34 (m, 1H, 7-H), 7.21–7.30 (m, 5H, 9-CH$_2$C$_6$**H**$_5$), 5.84 (s, 2H, 9-C**H**$_2$C$_6$H$_5$), 4.03 (m, 1H, NHCH$_2$CH$_2$NHOCC**H**), 3.44 (m, 2H, NHC**H**$_2$CH$_2$NHOCCH), 3.32 (m, 2H, NHCH$_2$C**H**$_2$NHOCCH), 2.89 (d, J = 8Hz, 2H, NHCH$_2$CH$_2$NHOCCHC**H**$_2$); IR (KBr): 3469, 3315, 3055, 2941, 2893, 1670, 1642, 1528, 1496, 1463, 1390, 1333, 1264, 738 cm^{-1}; UV:λ_{max} 356, 342, 304, 279, 265, 259, 252, 231, 226, 213 nm; FAB-MS (M + 1): [M + 1]$^+$ calcd for $C_{32}H_{30}N_6O_2$, 531; found 531; Analysis (calcd, found for $C_{32}H_{30}N_6O_2$): C (72.5, 72.5), H (5.7, 5.5), N (15.8, 15.8).

4.1.36. N,N′-bis (9-ethyl-1-methyl-β-carboline-3-carboxyl)-1, 6-diaminohexane (55)

1,6-Hexanediamide (1.39 g, 12 mmol) was diluted in DMF (30 ml) and boiled, and then compound **22** (0.81 g, 3 mmol) was added drop-wise in 1 h. After heated for 48 h, the mixture was poured into water (200 ml) and extracted with ethyl acetate (5 × 100 ml). The organic layer was dried with anhydrous sodium sulfate, removed by evaporation and the residue was purified by silica column chromatography with methanol as the eluent. After recrystallization with methanol, Light yellow crystals of **55** were obtained (0.21 g, 24%). m.p.: 143–145°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.67 (s, 2H, 4-H), 8.55 (t, J = 6Hz, 2H, CONH), 8.36 (d, J = 8Hz, 2H, 8-H), 7.75 (d, J = 8.5Hz, 2H, 5-H), 7.64 (m, 2H, 6-H), 7.31 (m, 2H, 7-H), 4.69 (s, 4H, 9- C**H**$_2$CH$_3$), 3.36 (m, 4H, CONHC**H**$_2$CH$_2$CH$_2$), 3.06 (s, 6H, 1- CH$_3$), 1.57 (m, 4H, CONHCH$_2$C**H**$_2$CH$_2$), 1.43 (m, 4H, CONHCH$_2$CH$_2$C**H**$_2$), 1.38 (t, J = 7Hz, 6H, 9-CH$_2$C**H**$_3$); IR (KBr): 3290, 3049, 2932, 2857, 1682, 1651, 1527, 1449, 1377, 1334, 1235, 751 cm^{-1}; UV:λ_{max} 354, 340, 308, 294, 288, 276, 250, 224, 213 nm; FAB-MS (M + 1): [M + 1]$^+$ calcd for $C_{36}H_{40}N_6O_2$, 589; found 589; Analysis (calcd, found for $C_{36}H_{40}N_6O_2$): C (73.5, 73.4), H (6.8, 7.1), N (14.3, 14.4).

4.1.37. N,N′-bis (9-benzyl-1-methyl-β-carboline-3-carboxyl)-1, 2-diaminoethane (56)

Prepared by the similar procedure as compound **55** from **25** (1.03 g, 3 mmol) and ethylenediamide (7.1 ml, 8 mmol) except that DMF was replaced by ethanol. Light yellow crystals of **56** were obtained (0.22 g, 22%). m.p.: 210–211°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.75 (s, 2H, 4-H), 8.71 (t, J = 5.5Hz, 2H, CONH), 8.43 (d, J = 7.5Hz, 2H, 8-H), 7.70 (d, J = 8.0Hz, 2H, 5-H), 7.60 (m, 2H, 6-H), 7.34 (m, 2H, 7-H), 7.21–7.35 (m, 10H, 9-CH$_2$C$_6$**H**$_5$), 5.98 (s, 4H, 9-C

$\mathbf{H}_2C_6H_5$), 3.45 (m, 4H, NHC$\mathbf{H}_2$CH$_2$NH), 2.88 (s, 6H, 1- CH_3); IR (KBr): 3286, 3060, 2939, 2840, 1695, 1651, 1449, 1379, 1342, 1270, 1204, 727 cm^{-1}; UV:λ_{max} 352, 339, 302, 287, 275, 264, 256, 245, 222, 213 nm; FAB-MS (M + 1): $[M + 1]^+$ calcd for $C_{42}H_{36}N_6O_2$, 657; found 657; Analysis (calcd, found for $C_{42}H_{36}N_6O_2$): C (76.8, 76.6), H (5.5, 5.8), N (12.8, 13.0).

4.2. Cell culture

Cell lines were cultured in plastic culture flasks (Life Technologies) with RPMI 1640 medium (Life Technology) supplemented with 10% fetal bovine serum (HyClone Co.), 100 U ml^{-1} of penicillin, and 100 μl ml^{-1} of streptomycin in a humidified atmosphere of 5% CO_2/95% air at and given fresh medium every 3 or 4 days.

4.3. In vitro cellular proliferation inhibition assay

MTT assay was carried out as described before [13,23,24]. Briefly, 20 ml cell suspension (1×10^5cells ml^{-1}) was seeded into a 96-well microtiter plate (Falcon Co.) and compounds at various concentrations were added. After incubation for 48 hours at 37°C, 20 μl MTT solution (5 mg ml^{-1} in PBS) was added to each well. And after another 4-hour incubation, the medium was carefully removed from each well and 100 μl DMSO was added to dissolve the formazan crystals. Plates were measured at 490 nm with an absorbance reader (Bio-Rad). Each experiment was performed in seven replicate wells for each drug concentration and carried out independently for three times. The IC_{50} value was defined as the concentration needed for a 50% reduction in the absorbance calculated based on the survival curves. Unless stated, compounds that used in the drug screening were in the hydrochloride forms.

4.4. Determination of ΔT_m

Experiments on the determination of ΔT_m were performed as described before [7]. A quartz cuvette (1 cm path length) was heated by circulating water in a thermostatically controlled cell hold at an uprising temperature (0.5 °C min^{-1}) from 20 to 97 °C. The melting temperature (T_m) was taken as the midpoint of the hyperchromic transition in the melting curve measured at 260 nm by a Shimadzu UV 2501PC Spectrometer. Experiments were performed in buffer A (0.5 mM Tris–HCl, 0.1 mM EDTA, pH 8.0), presenting a T_m of 74.0 °C of CT-DNA. In all cases, the ratio of compound to CT-DNA was 0.5. All experiments were carried out independently for four times and the data were analyzed by Student's *t*-test.

4.5. Cell cycle analysis by flow cytometry

Seven ml Hela cell suspension (1×10^5cells ml^{-1}) in culture medium was seeded into a 100 mm × 20 mm plate (Falcon Co.) and incubated for 24 hours, then compounds at different concentrations were added. After treatment, cells were trypsinized, washed with PBS for two times and fixed with 70% ethanol for 12 hours. After ethanol was removed, cells were stained with 50 μg ml^{-1} propidium iodide (containing 10 mg ml^{-1} RNase A) and analyzed by flow cytometry with 10,000 cells counted.

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