European Journal of Medicinal Chemistry 147 (2018) 253–265

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

Synthesis and structure-activity relationships of asymmetric dimeric β-carboline derivatives as potential antitumor agents

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ARTICLE INFO

Article history:
Received 8 November 2017
Received in revised form
2 February 2018
Accepted 2 February 2018
Available online 7 February 2018

Keywords:
Harmine
Asymmetric dimeric β-carboline
Cytotoxic
Antitumor
Structure-activity relationship

ABSTRACT

A series of newly asymmetric dimeric β-carbolines with a spacer of 4–6 methylene units between the indole nitrogen and the harmine oxygen were synthesized. Structures of all the novel synthesized compounds were confirmed by their spectral and analytical studies. All of the synthesized compounds were screened for their *in vitro* cytotoxic activity against nine cancer cell lines. The results revealed that compounds **7c**, **7o** and **7s** exhibited the highest cytotoxic activities with IC_{50} values of less than 20 μM against the tumor cell lines tested. Acute toxicities and antitumor efficacies of the selected compounds in mice were also evaluated, and compound **7o** exhibited potent antitumor activities with the tumor inhibition rate of over 40%. The wound healing assay displayed a specific impairment in the motility of the HT-29 cells, which suggested the anti-metastatic potential of compound **7o**. Moreover, compound **7o** had obvious angiogenesis inhibitory effects in the chicken chorioallantoic membrane (CAM) assay. Preliminary structure-activity relationship (SAR) analysis indicated that: (1) 3-phenylpropyl substituent at the N^9-position of the indole ring was the most suitable group giving rise to potent cytotoxic agents; (2) the spacer length affected the antitumor potencies, and four methylene units were more favorable.

1. Introduction

Peganum harmala L. is a perennial, glabrous plant that is distributed in the Xinjiang Uyghur and Inner Mongolia Autonomous Regions of China. The extracts of *Peganum harmala* seeds have been traditionally used for hundreds of years in these areas. *Peganum harmala* L. has been regarded as a traditional herb to that possesses a wide spectrum of pharmacological actions with different applications, including nervous system [1–3], antimicrobial [4–6], and antineoplasm treatments [2,7–10], and it is effective in the treatment of dermatoses [11].

Harmine, originally isolated from *Peganum harmala* seeds in 1847, is the most representative naturally occurring β-carboline alkaloid, having a core indole structure and a pyridine ring. In the last several decades, harmine has been confirmed as an important active ingredient to treatalimentary tract cancers [12,13]. Recent reports [12–15] have demonstrated that harmine and its derivatives have remarkable antitumor activities, together with potential neurotoxicity. Moreover, it has been reported that harmine and its derivatives can exert antitumor activities through multiple mechanisms, such as DNA binding [16–18], inhibition topoisomerases I and II [19,20], CDK (cyclin-dependent kinase) [21,22], PLK1 (polo-like kinase) [23], kinesin-like protein Eg5 [24] and IκB kinases [25].

For more than a decade, our group [15,26–31] has focused on incorporating substituents into positions-1, 2, 3, 7 and 9 of the β-carboline nucleus as antitumor agents. Structure-activity relationship (Fig. 1) analysis has demonstrated that: (1) the methoxy group substituent at position-7 of harmine might play a crucial role in determining their remarkable neurotoxic effects; (2) prolonged or enlarged alkoxy substituents at position-7 led to enhanced cytotoxic activities and eliminated completely neurotoxic effects; and (3) the substituents in position-9 of the β-carboline nucleus played a vital role in the modulation of their antitumor activities.

Previous literature [32–35] has shown that some dimer antitumor agents via an appropriate linker could lead to significantly improved antitumor activities (100- to 500-fold improvement over

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https://doi.org/10.1016/j.ejmech.2018.02.003
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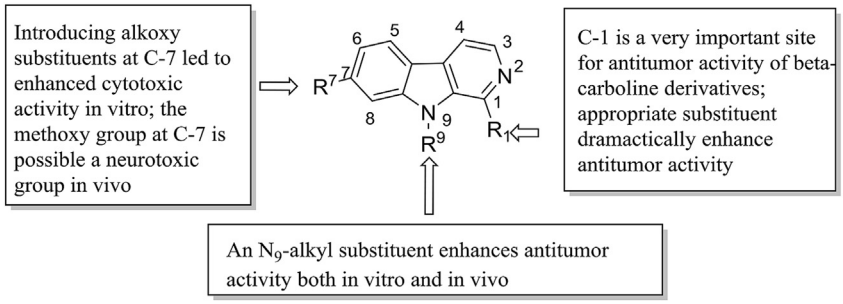


Fig. 1. The reported structure-activity relationships of β-carbolines against tumor cells.

the corresponding monomers). Therefore, our group reported the synthesis, *in vitro* evaluation, *in vivo* efficacies and structure-activity relationships for the novel symmetric bivalent β-carbolines with an alkyl spacer or alkylamino spacer in position-1, 3, 7 and 9 of the β-carboline nucleus, respectively (Fig. 2) [36–40,44]. Some of bivalent β-carbolines have exhibited more potent antitumor efficacies than monomers, and the others had limited utility for cancer therapy because of their poor water solubility. The conclusion of structure-activity relationship information revealed that: (1) the length of the spacer affected the cytotoxic activities *in vitro* and 4–6 methylene units were more favorable; and (2) the introduction of substituents into position-1 of the β-carboline ring might be detrimental to antitumor potency *in vivo* models.

We have continued our search for novel antitumor agents endowed with better antitumor activities and less neurotoxicities, and we provide detailed studies of structure-activity relationships (SARs) on the antitumor efficacies *in vitro* and *in vivo* of this class of compounds. Here, we designed and synthesized a series of methylene units linked with asymmetric dimeric β-carboline derivatives as potent antitumor agents. These compounds were expected to exhibit significantly improved cytotoxic activities. We report herein the preparation of the novel asymmetric dimeric β-carbolines and their biological evaluation as antitumor agents.

2. Chemistry

The overall synthetic routes that were used to design the asymmetric dimeric β-carbolines are shown in Schemes 1–3. The starting material L-tryptophan was reacted with the corresponding aldehyde via the Pictet-Spengler condensation followed by oxidation and decarboxylation to afford the intermediate 1-substituted-β-carbolines **3a-i** [36,41], and the result of yield in Table 1. Then, **3a-b** further reacted with the appropriate dibromoalkane by N^9-alkylation to obtain the intermediates **4a-f**. Harmine, which we extracted from *Peganum harmala* L., was N^9-alkylated by treatment with sodium hydride (NaH) and 1,4-dibrombutane or an alkyl halide in dimethylformamide (DMF) at room temperature, to yield the harmine derivatives **4g**, and **5a-c** [13]. The preparation of compounds **6a-c** followed a common synthetic scheme, which was characterized by the demethylation of compounds **5a-c** using acetic acid and hydrobromic acid as the reaction solvent [14]. The reaction of compounds **6a-c** with the corresponding intermediates **4a-f**, and **4g** readily took place at room temperature to provide the target asymmetric dimeric β-carbolines **7a-m**, and **7u** in a 51–87% yield. After considering the drawbacks of the previous synthesis, we sought to explore an alternative synthetic strategy for these compounds (Scheme 3). The reaction of compound **6c** with 1,4-

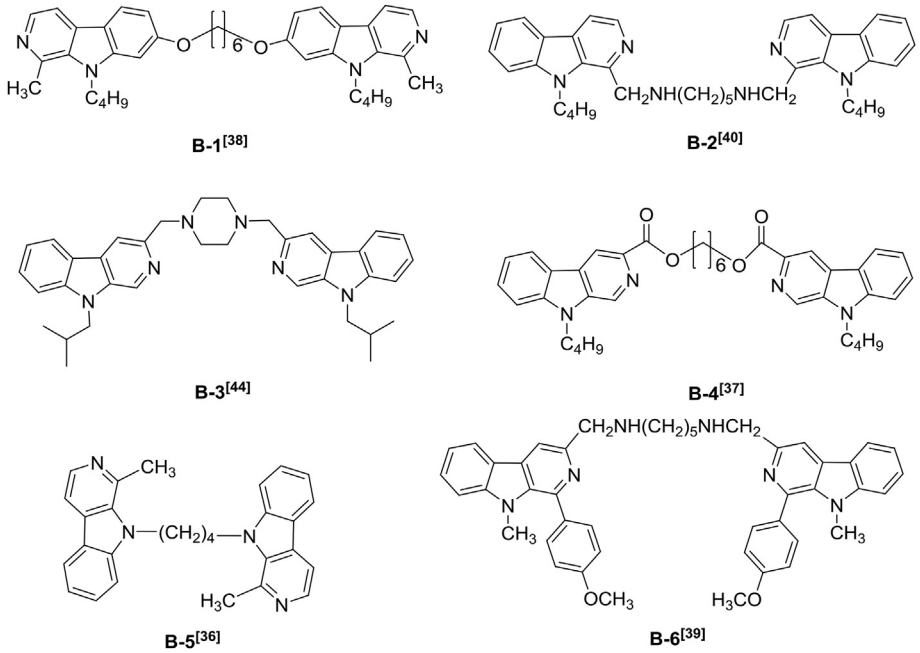
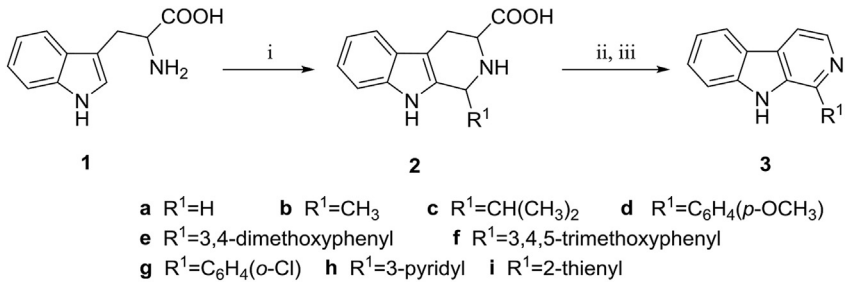
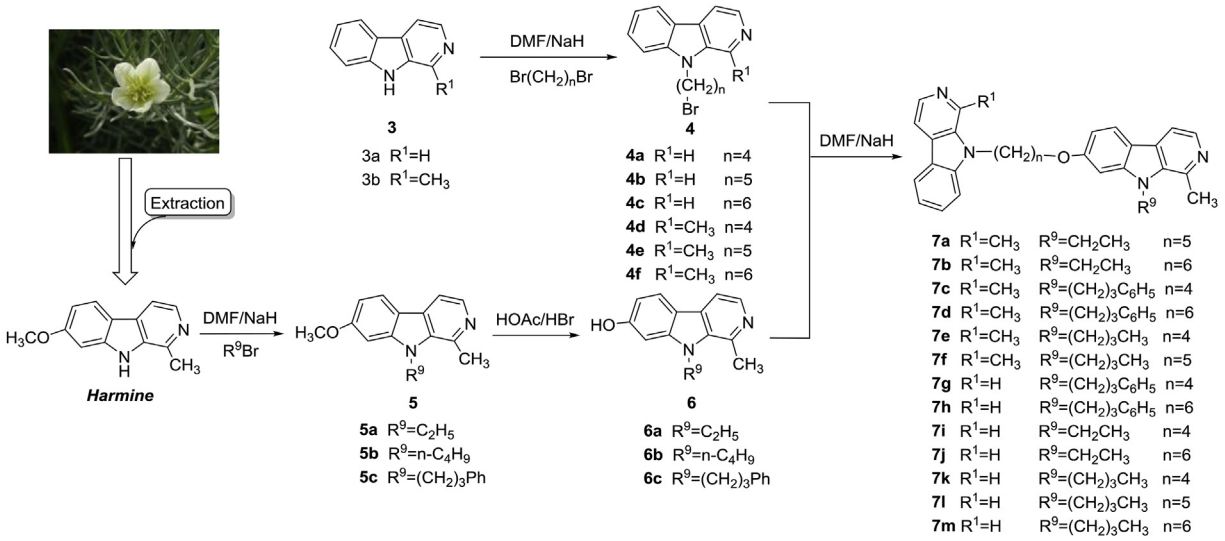


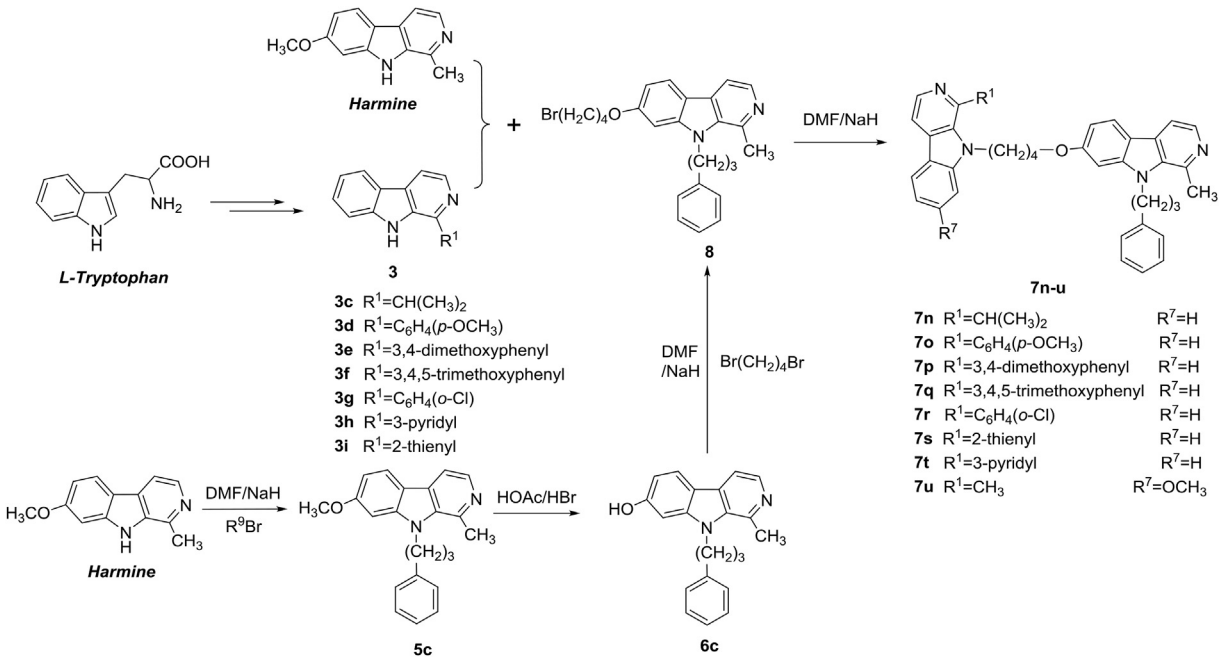
Fig. 2. The chemical structure of the representative reported symmetric bivalent β-carbolines.



Scheme 1. Synthesis of the 1-substituted β-carboline derivatives **3a-3i**.
Reagents and conditions: (i) a: NaOH, H_2O, formaldehyde, reflux, 3 h; b: H_2SO_4, H_2O, acetaldehyde, room temperature, 3 h; c-i: HOAc, appropriate aldehyde, reflux, 3 h; (ii) H_2O, K_2CrO_7, HOAc, stirred at 100 °C, 20 min (iii) $NaHSO_3$, NaOH.



Scheme 2. Synthesis of the asymmetric dimeric β-carboline derivatives **7a-m**.



Scheme 3. Synthesis of the asymmetric dimeric β-carboline derivatives **7n-u**.

Table 1
Substrate scope of primary 1-substituted β-carboline derivatives.

Entry	R_1	Product	Yield(%)
1	H	**3a**	75
2	CH_3	**3b**	81
3	Isopropyl	**3c**	73
4	C_6H_4(*p*-OCH_3)	**3d**	69
5	3,4-dimethoxyphenyl	**3e**	82
6	3,4,5-trimethoxyphenyl	**3f**	84
7	2-chlorophenyl	**3g**	65
8	3-pyridyl	**3h**	67
9	2-thienyl	**3i**	71

dibrombutane by the action of NaH in anhydrous DMF furnished intermediate **8**, and then the dimeric β-carbolines **7n-t** were prepared by reaction of compounds **3c-i** with intermediate **8** in anhydrous DMF. The chemical structures of all the novel asymmetric dimeric β-carbolines were confirmed by ^{1}H NMR, ^{13}C NMR and HRMS. By contrasting the ^{1}H NMR spectrum data of compounds **7n-t**, we found an interesting phenomenon that compounds **7n-q**, **7s-t** show a triplet peak at δ 4.3 and a group of multiplets at δ 1.5–1.8, respectively. But compound **7r** in the region have two sets of multiple peaks.

3. Results and discussion

3.1. In vitro cell cytotoxicity assay

All of the synthesized newly asymmetric dimeric β-carbolines were evaluated to *in vitro* cytotoxic potencies using the MTT assay in a panel of human tumor cell lines, and they were compared with the reference drug cisplatin and the reported symmetric bivalent β-carbolines (**B-1**, **B-3**, **B-4**, **B-5**). The human tumor cell line panel consisted of gastric carcinoma (BGC-823), liver carcinoma (HepG2), breast carcinoma (MCF-7), malignant melanoma (A375), colon carcinoma (HT-29), renal carcinoma (769-P), ovarian carcinoma (SK-OV-3), esophageal carcinoma (Eca-109), and Lewis lung carcinoma (LLC). In order to enhance the solubility in aqueous solution, all of the compounds were prepared in the form of hydrochloride salts before use. The activity was expressed as the concentration (IC_{50}) that causes 50% inhibition of cancer cell growth and is summarized in Table 2.

As shown in Table 2, compounds **7c**, **7d**, **7g**, **7h** and **7o-u** displayed significant and selective cytotoxicities with IC_{50} values lower than 20 μM against at least six tumor cell lines. Of these compounds, **7o** exhibited good activity against 769-P, SK-OV-3, Eca-109 and LLC with IC_{50} values of 4.9, 4.2, 4.5 and 3.4 μM, respectively. On the other hand, compounds **7a**, **7b**, **7i**, **7j**, **7k**, **7l** and **7m** exhibited weak to inactive cytotoxic activities.

When asymmetric dimeric β-carbolines had the same linker, we examined the influence of the substituents in position-9 of the β-carboline core on the cytotoxic activities. In the series of compounds having an ethyl, *n*-butyl, or 3-phenylpropyl substituent at the N^9-position of the indole ring. Compounds **7c**, **7d**, **7g** and **7h** with a 3-phenylpropyl displayed higher cytotoxic activities against human tumor cell lines tested (**7c** > **7e**, **7d** > **7b**, **7g** > **7i** and **7k**, **7h** > **7j** and **7m**). The results implied that the 3-phenylpropyl group represented the most optimal structure for these compounds class to exhibit remarkable cytotoxicity. Table 2 also showed that the different spacer lengths of the asymmetric dimeric β-carbolines had a great effect on the cytotoxic activities. Of all asymmetric dimeric β-carbolines **7a-m**, compounds **7c-d**, **7g-h** bearing a 3-phenylpropyl substituent in position-9 of β-carboline nucleus and with a spacer of four or six methylene units, dimeric β-carbolines with four methylene units as linkers had stronger cytotoxic activities than compounds with a spacer of six methylene units (**7c** > **7d**, **7g** > 7**h**). Similarly, dimers **7e-f**, **7k-m**, employing *n*-butyl in position-9 of β-carboline nucleus, showed the same tendency. It was interesting to note that the spacer length in ethyl substituent dimeric β-carbolines has little influence on their cytotoxic activities (**7a** vs. **7b**, **7i** vs. **7j**). These results suggested that the length of the spacers affected the cytotoxic activities and four methylene units were more favorable.

Next, we examined the influence of the substituents in position-1 of the β-carboline ring on cytotoxic potencies. In comparison with compound **7g**, **7c**, and **7n-t**, bearing an additional methyl, isopropyl, 4-methoxyphenyl, 3,4-dimethoxyphenyl, 3,4,5-trimethoxyphenyl, 2-chlorophenyl, 2-thienyl, and 3-pyridyl group in position-1 of the β-carboline core, respectively. Of all these dimeric β-carbolines, the compound **7o** having a 4-methoxyphenyl in position-1 of the β-carboline ring displayed strong cytotoxic activities against 769-P, SK-OV-3, Eca-109 and LLC with IC_{50} values of 4.9, 4.2, 4.5, and 3.4 μM, respectively. Meanwhile, **7c**, **7g**, **7p-t** only showed significant cytotoxic activities against one or two cell lines with IC_{50} values lower than 5 μM, and compound **7p** demonstrated the broader spectrum of cytotoxic activities against the tested tumor cell lines with IC_{50} values lower than 10 μM (except for HT-29 cell line). Compound **7n** with an isopropyl in position-1 showed moderate cytotoxic activities with IC_{50} values ranging from 10.8 to 46.2 μM. In addition, in the LLC cell line assay, most compounds exhibited selective activities against it with IC_{50} values lower than 10 μM (except **7n** and **7r**). On the other hand, most compounds displayed moderate cytotoxic activities against the HT-29 cell line with IC_{50} values ranging from 11.2 to 22.7 μM (except **7q**).

3.2. Assessment of acute toxicity

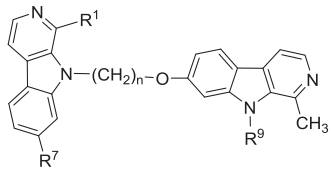
The LD_{50} values of the selected asymmetric dimeric β-carbolines in mice after intraperitoneal (i.p.) administration are shown in Table 3. All of the tested asymmetric dimeric β-carbolines resulted in acute toxic manifestation but they did not cause any obvious neurotoxic effects, including tremors, twitch, jumping, and supination. The animals showed a decrease in locomotive activity after the administration of various bivalent β-carbolines. Death occurred mostly in the high dosage group within 4–8 h after injection. All of the surviving animals returned to normal within the next day. Autopsies of the animals that died during the course of experiment and the necropsy findings in the surviving animals at the end of the experimental period (14 days) revealed no obvious changes in any of the organs.

Of all of the investigated asymmetric dimeric β-carbolines, compound **7u,** with a methoxy group in position-7 of the β-carboline ring, displayed remarkable acute toxicity with the LD_{50} value of 8 mg kg^{-1}, while compound **7c**, which had no substituent in position-7 of the β-carboline ring, demonstrated weaker acute toxicities with the LD_{50} value of 150 mg kg^{-1}. Replacement of the 3-phenylpropyl group in position-9 of compound **7c** with an *n*-butyl substituent gave compound **7e**, which also exhibited higher acute toxicity with the LD_{50} value of 50 mg kg^{-1}. In addition, compound **7g**, with no substituent in position-1 of the β-carboline ring, exhibited same acute toxicity with the LD_{50} value of 50 mg kg^{-1}. Compounds **7o** and **7s**, which have the 3-phenylpropyl group in position-9 and a different group in position-1 of the β-carboline ring, displayed lower acute toxicity with the LD_{50} values of 41.5 mg kg^{-1} and 37.5 mg kg^{-1}, respectively. These results suggested that the introduction of the substituent into position-1 and the methoxy group in position-7 of the β-carboline ring might play an important role in determining acute toxicity.

3.3. Evaluation of antitumor activity of asymmetric dimeric β-carbolines in vivo

Based on the *in vitro* assay results, we further tested the

Table 2
Cytotoxic activities of derivatives *in vitro*[c] (IC_{50}, μM[a]).



Comp.	R^1	R^7	R^9	n	IC_{50}								
					BGC[b]	HepG2	MCF7	A375	HT-29	769-P	SK-OV-3	Eca-109	LLC
7a	CH_3	H	ethyl	5	35.4	111.9	56.3	31.5	14.9	70.6	30.8	46.6	>100
7b	CH_3	H	ethyl	6	44.7	74.6	75.2	74.6	36.2	75.2	11.2	61.4	51.1
7c	CH_3	H	phenylpropyl	4	7.4	9.1	4.0	8.4	14.1	9.9	13.1	9.9	4.4
7d	CH_3	H	phenylpropyl	6	12.5	14.1	11.7	15.8	28.1	16.1	19.4	15.1	14.1
7e	CH_3	H	*n*-butyl	4	33.6	15.7	11.2	14.1	16.4	15.3	21.7	12.2	13.3
7f	CH_3	H	*n*-butyl	5	>100	>100	>100	50.8	10.1	11.6	14.3	>100	12.4
7g	H	H	phenylpropyl	4	8.5	9.7	4.5	33.4	15.9	10.7	13.8	9.5	9.6
7h	H	H	phenylpropyl	6	11.2	12.1	8.5	37.1	16.2	13.1	76.4	12.6	28.2
7i	H	H	ethyl	4	41.5	>100	19.4	30.7	37.0	27.4	19.8	37.0	16.5
7j	H	H	ethyl	6	40.7	>100	19.0	34.7	60.3	>100	>100	17.4	21.8
7k	H	H	*n*-butyl	4	41.5	29.5	28.8	72.4	21.8	22.9	17.3	21.8	38.0
7l	H	H	*n*-butyl	5	>100	65.1	16.7	>100	32.6	31.1	65.1	>100	>100
7m	H	H	*n*-butyl	6	>100	51.7	>100	78.3	>100	>100	>100	>100	46.1
7n	isopropyl	H	phenylpropyl	4	37.8	46.2	14.4	23.8	12.1	18.5	28.1	16.2	10.8
7o	4-methoxyphenyl	H	phenylpropyl	4	12.1	14.1	8.5	13.6	13.3	4.9	4.2	4.5	3.4
7p	3,4-dimethoxyphenyl	H	phenylpropyl	4	9.7	2.4	7.2	6.7	22.7	8.2	8.8	9.3	6.2
7q	3,4,5-trimethoxyphenyl	H	phenylpropyl	4	18.2	24.9	11.7	7.1	3.9	14.1	10.3	9.7	8.7
7r	2-chlorophenyl	H	phenylpropyl	4	12.4	14.4	5.9	3.9	20.2	21.9	22.9	18.4	10.1
7s	2-thienyl	H	phenylpropyl	4	11.4	8.1	12	14.6	18.1	11.9	11.2	4.9	4.2
7t	3-pyridyl	H	phenylpropyl	4	4.2	10.7	29.8	18.9	11.2	7.2	22.9	12.9	9.4
7u	CH_3	CH_3O	phenylpropyl	4	4.7	5.6	23.4	15.3	21.6	8.5	30	18.4	14.4
B-1 [38]					NR[d]	13.0	>100	NR	6.1	NR	NR	NR	NR
B-3 [44]					10.8	9.66	7.16	13.0	11.3	12.7	16.0	14.3	7.68
B-4 [37]					43.2	NR	NR	18.2	NR	>100	>100	NR	NR
B-5 [36]					22.3	NR	NR	13.1	NR	6.5	4.8	NR	NR
Cisplatin					11.6	14.8	12.4	9.4	26.8	19.2	5.6	8.9	7.6

[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 cells using the MTT assay.
[b] Cell lines include gastric carcinoma (BGC), liver carcinoma (HepG2), breast carcinoma (MCF-7), malignant melanoma (A375), colon carcinoma (HT-29), renal carcinoma (769-P), ovarian carcinoma (SK-OV-3), esophageal carcinoma (Eca-109), Lewis lung carcinoma (LLC).
[c] Data represent the mean values of three independent determinations.
[d] NR = not reported.

Table 3
Acute toxic effects of asymmetric dimeric β-carbolines in mice and antitumor activities of these compounds against mice bearing Sarcoma 180 and Lewis lung cancer.

Comp.	Acute toxicity		Dosage (mg kg^{-1})	Tumor inhibition rate (%)	
	LD_{50}(mg kg^{-1})	Neurotoxic effect		Sarcoma 180	Lewis lung cancer
7c	150	–[a]	30	36.7	ND[b]
7e	50	–	10	53.3	27.2
7g	50	–	10	33.5	ND
7o	41.5	–	8.3	54.6	48.1
7s	37.5	–	7.5	47.3	31.6
7u	8	+	1.6	49.9	38.2
CTX			30	82.5	80.7

[a] Acute neurotoxic manifestation were denoted by "+" and "-". "+" represents toxic responses including tremble, twitch, jumping and supination, while "-" means no such reaction.
[b] ND = not determined.

antitumor activity of six asymmetric dimeric β-carbolines *in vivo* against mice bearing Sarcoma 180 and Lewis lung cancer, respectively, and the positive control Cyclophosphamide (CTX). Our previous investigation demonstrated that mice bearing Lewis lung cancer were more susceptible to β-carbolines than other animal models; therefore, these animal models were selected and evaluated in the present investigation [15]. The tumor inhibition rates of all of the investigated asymmetric dimeric β-carbolines are illustrated in Table 3.

As shown in Table 3, all of the tested asymmetric dimeric β-carbolines displayed moderate-to-strong antitumor activities in the animal model. Compounds **7e**, **7o**, **7s**, and **7u** showed remarkable antitumor activity with the tumor inhibition rate of over 40% against Sarcoma 180-bearing mice at doses of 10, 8.3, 7.5, and 1.6 mg kg^{-1}, respectively. However, the four compounds showed moderate antitumor activity against mice with Lewis lung cancer with the tumor inhibition rate ranging from 27.2% to 48.1% at the same dose. In particular, compound **7o**, which has a 3-phenylpropyl

group in position-9 of the β-carboline ring, exhibited the most potent antitumor agent with the tumor inhibition rate of 54.6% and 48.1% against Sarcoma 180-bearing mice and Lewis lung cancer-bearing mice, respectively. Compounds **7c** and **7g** displayed moderate antitumor activity with the tumor inhibition rate of 36.7% and 33.5% against Sarcoma 180-bearing mice at doses of 30 and 10 mg kg^{-1}, respectively. Interestingly, the Sarcoma 180 mice were more susceptible to all of the tested compounds than the Lewis lung cancer-bearing mice. These results suggested that introducing the 3-phenylpropyl group in position-9 of the β-carboline nucleus significantly improved the antitumor activity.

3.4. Inhibitory effect of **7o** on tumor cell migration

Cell migration plays an important role in tumor formation and cancer metastasis. It is relevant for angiogenesis to ensure tumor nutrition as well as for the formation of metastases, in which tumor cells leave the primary tumor site and spread to other tissues. In the present study, the effect of **7o** on the migration ability of HT-29 and LLC cells was examined by the wound healing technique. Fig. 3 shows that the two cancer cell lines were treated with different concentrations of positive control Combretastatin A4 phosphate (CA4P) and **7o** after 24 h. Surprisingly, **7o** showed an inhibitory effect on cell migration on the LLC cells, especially at 50 μM.

3.5. Anti-angiogenic activity in vivo of compound **7o**

The most potent compound, **7o**, was selected to evaluate anti-angiogenic activity by chicken chorioallantoic membrane (CAM) assay. The inhibitory effects of compound **7o** on the angiogenesis of CAM are shown in Fig. 4. The anti-angiogenetic activity of compound **7o** was semi-quantitatively analyzed using Graph Pad Prism 5.0 (shown in Fig. 5). The results showed that compound **7o** ($p < 0.05$) could inhibit the angiogenesis of CAM. The anti-angiogenetic activity of compound **7o** was comparable to the CA4P *in vivo* CAM assay at the same dose (50 μM).

4. Conclusions

We designed and synthesized a novel series of asymmetric dimeric β-carboline derivatives with a spacer of 4–6 methylene units between the indole nitrogen and the harmine oxygen atom. All of the compounds were screened for their *in vitro* cytotoxic activity against BGC-823, HepG2, MCF-7, A375, HT-29, 769-P, SK-OV-3, Eca-109 and LLC cancer cell lines. The results demonstrated that compounds **7c**, **7o** and **7s** exhibited prominent cytotoxic activity with IC_{50} values of lower than 20 μM against the test cell lines. Antitumor evaluation of the selected asymmetric dimeric β-carbolines in animal models, revealed that compound **7o**, which has a 3-phenylpropyl group in position-9 of the β-carboline ring, exhibited the most potent antitumor agent with the tumor inhibition rate of 54.6% and 48.1% against mice both bearing Sarcoma 180 and Lewis lung cancer, respectively. Moreover, the pharmacological mechanisms showed that compound **7o** has a certain impairment in the motility of LLC cells, which suggests the anti-metastatic potential. The *in vivo* study indicated that compound **7o** could retard in the CAM assay, and anti-angiogenetic potency was more potent than the reference drug CA4P. Preliminary SARs analysis indicated that: (1) 3-phenylpropyl substituent at the N^9-position of the indole ring was the most suitable group giving rise to potent cytotoxic agents; (2) the spacer length affected antitumor potencies, and four methylene units were more favorable.

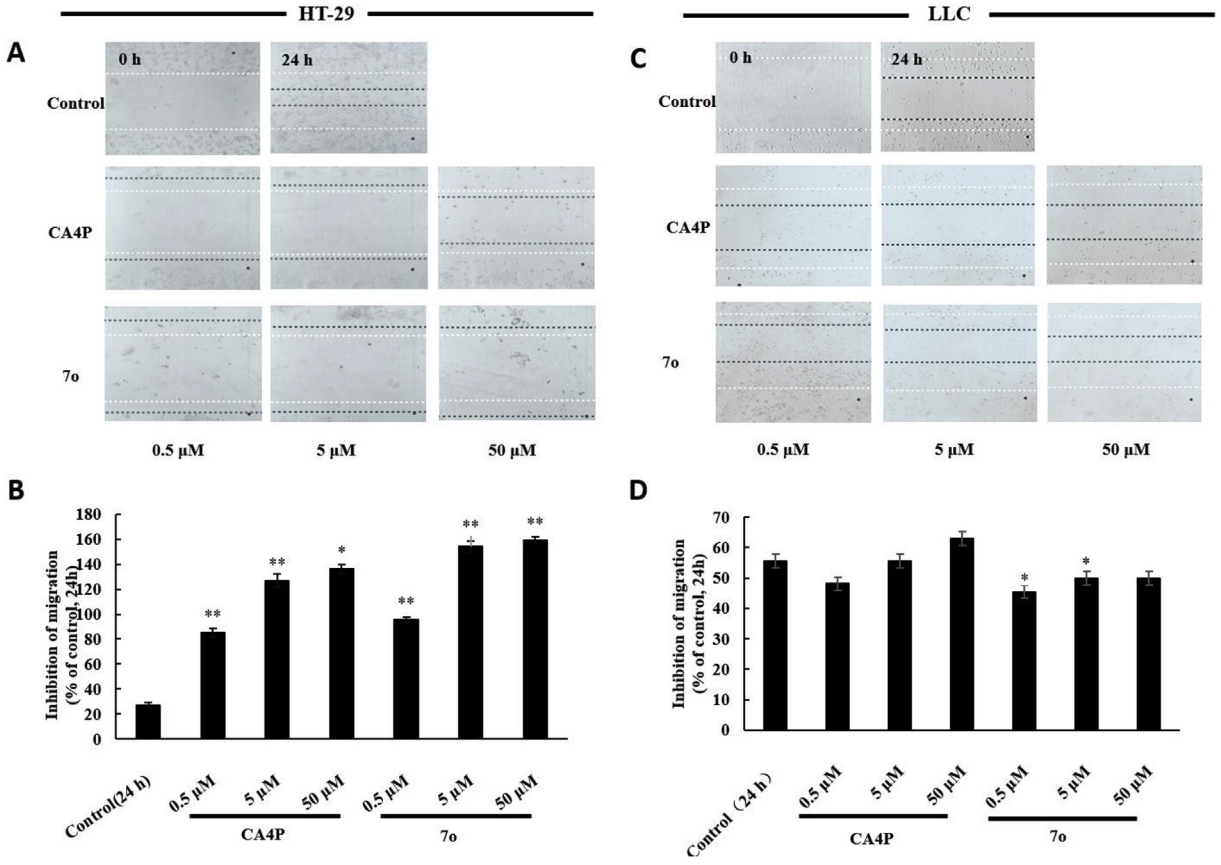


Fig. 3. Wound healing migration assay of HT29 (A and B) and LLC (C and D) cells after 24 h of treatment with **7o**.

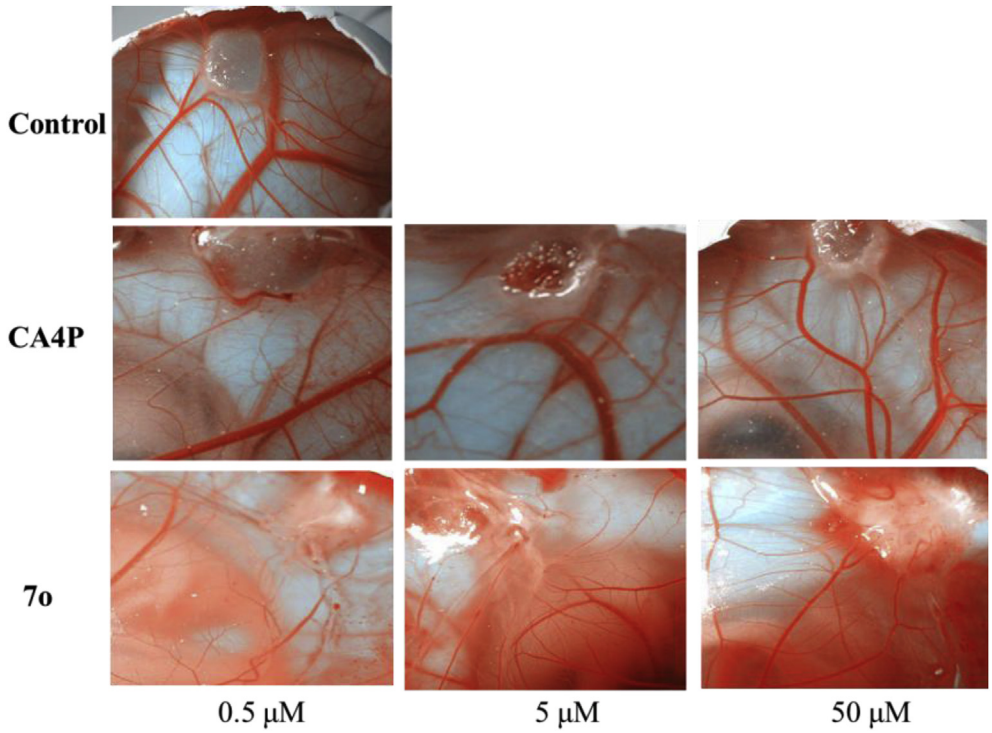


Fig. 4. Inhibitory effects of compound **7o** on the angiogenesis of CAM.

5. Experimental section

5.1. Reagents and general methods

Melting points were determined in capillary tubes on an electrothermal WRS-3 apparatus and without correction. NMR spectra were recorded at room temperature on a Bruker Avance III HD 400 instrument at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR. HRMS were measured on Bruker ultrafleXtreme MALDI-TOF/TOF-MS and HCCA (alpha-cyano-4-hydroxycinnamic acid) is used as matrix. Column chromatography was performed with silica gel (200–300 mesh) and analytical TLC on silica gel 60-F_{254}.

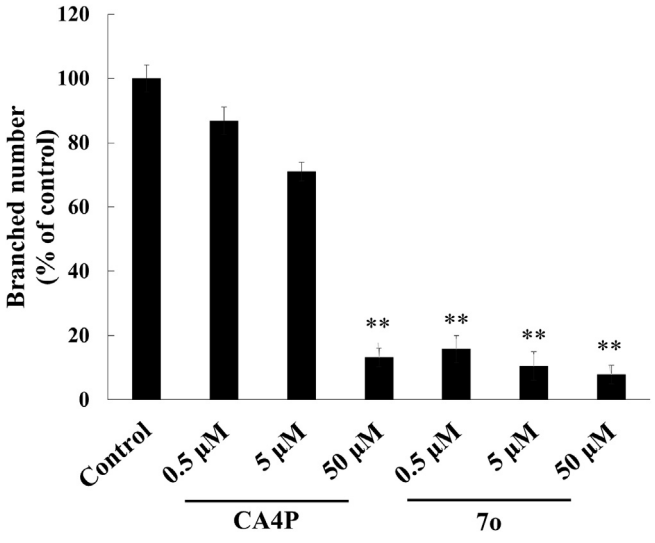


Fig. 5. Anti-angiogenic activity of compound **7o**.

All reagents were purchased from commercial suppliers and were dried and purified when necessary. The following intermediates, β-carboline **3a** [41], 1-methyl-β-carboline **3b** [41], 1-isopropyl-β-carboline **3c** [36], 1-(4-methoxyphenyl)-β-carboline **3d** [13], 1-(3,4-dimethoxyphenyl)-β-carboline **3e** [13], 1-(3,4,5-trimethoxyphenyl)-β-carboline **3f** [42], 1-(2-chlorophenyl)-β-carboline **3g** [36], 1-(pyridyl-3-yl)-β- carboline **3h** [36], 1-(thiophen-2-yl)-β-carboline **3i** [36],. 7-methoxy-9-ethyl-1-methyl-β-carboline **5a** [13], 7-methoxy-9-*n*-butyl-1-methyl- β-carboline **5b** [13], 7-methoxy-9-(3-phenylpropyl)-1-methyl-β-carboline **5c** [13], 9-ethyl-1-methyl-β-carboline7-ol **6a** [14], 9-*n*-butyl-1-methyl-β-carboline-7-ol **6b** [14], and 1-methyl-9-(3-phenyl-propyl)- β-carboline-7-ol **6c** [14], were synthesized according to published procedures.

5.2. General procedure for the preparation of compounds ***4a-f***

A mixture of **3a-b** (10 mmol) and anhydrous DMF (50 mL) was stirred at room temperature for 0.5 h, then NaH (0.50 g, 20 mmol) and the appropriate dibromoalkane (20 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 (150 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 crystallized from ethyl ether or ethyl ether-petroleum ether, giving white crystals.

5.2.1. 9-(4-bromobutoxyl)-β-carboline (***4a***)

White crystals (2.65 g, 87%) were obtained. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.10 (s, 1H), 8.40 (d, J = 5.2 Hz, 1H), 8.28 (d, J = 8.0 Hz, 1H), 8.14 (dd, J = 5.2, 0.8 Hz, 1H), 7.75 (d, J = 8.4 Hz, 1H), 7.60–7.64 (m, 1H), 7.27–7.31 (m, 1H), 4.56 (t, J = 6.8 Hz, 2H), 3.56 (t, J = 6.4 Hz, 2H), 1.90–1.97 (m, 2H), 1.81–1.88 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 141.13, 138.88, 136.47, 133.13, 128.77, 127.66, 122.44,

120.80, 119.92, 115.04, 110.62, 42.15, 35.09, 30.23, 27.87.

*5.2.2. 9-(5-bromopentyl)-β-carboline (**4b**)*

White crystals (2.72 g, 86%) were obtained. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.45 (d, J = 5.2 Hz, 1H), 8.53 (t, J = 5.2 Hz, 1H), 8.44 (d, J = 6.0 Hz, 1H), 8.32 (dt, J = 8.0, 1.0 Hz, 1H), 7.84–7.88 (m, 1H), 7.67 (d, J = 8.4 Hz, 1H), 7.49–7.54 (m, 1H), 4.63 (t, J = 7.2 Hz, 2H), 3.45 (dt, J = 54.0, 6.4 Hz, 2H), 1.96–2.04 (m, 2H), 1.80–1.95 (m, 2H), 1.56–1.64 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 144.19, 135.44, 133.92, 132.44, 128.57, 124.18, 123.42, 122.24, 119.70, 116.69, 110.66, 44.45, 32.03, 28.57, 25.63, 24.40.

*5.2.3. 9-(6-bromohexyl)-β-carboline (**4c**)*

Yellow oil (2.31 g, 70%) were obtained. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.83 (s, 1H), 8.43 (d, J = 5.6 Hz, 1H), 8.05 (d, J = 7.6 Hz, 1H), 7.86 (d, J = 5.2 Hz, 1H), 7.52 (t, J = 7.6 Hz, 1H), 7.36 (d, J = 8.4 Hz, 1H), 7.22 (t, J = 7.6 Hz, 1H), 4.22 (t, J = 7.2 Hz, 2H), 3.27 (t, J = 6.8 Hz, 2H), 1.84–1.76 (m, 2H), 1.74–1.67 (m, 2H), 1.41–1.26 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 141.02, 138.71, 136.35, 131.91, 128.30, 128.17, 121.83, 120.93, 119.54, 114.51, 109.40, 43.06, 33.71, 32.44, 28.92, 27.78, 26.33.

*5.2.4. 9-(4-bromobutoxyl)-1-methyl-β-carboline (**4d**)*

White crystals (2.81 g, 88%) were obtained. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.71 (dd, J = 6.2, 1.2 Hz, 1H), 8.54 (dd, J = 8.0, 1.2 Hz, 1H), 8.49 (dd, J = 6.2, 2.0 Hz, 1H), 8.00 (d, J = 8.4 Hz, 1H), 7.83–7.87 (m, 1H), 7.46–7.50 (m, 1H), 4.75 (t, J = 7.6 Hz, 2H), 3.70 (t, J = 6.4 Hz, 2H), 3.27 (s, 3H), 2.04–1.77 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 144.33, 139.00, 133.74, 133.26, 132.07, 129.29, 123.86, 122.14, 119.67, 116.15, 111.78, 45.35, 44.31, 29.63, 28.29, 18.31.

*5.2.5. 9-(5-bromopentyl)-1-methyl-β-carboline (**4e**)*

White crystals (2.97 g, 90%) were obtained. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.42–8.33 (m, 2H), 8.29 (dt, J = 8.0, 1.2 Hz, 1H), 7.82–7.86 (m, 1H), 7.66 (dt, J = 8.4, 0.8 Hz, 1H), 7.48–7.51 (m, 1H), 4.69 (t, J = 7.6 Hz, 2H), 3.52 (s, 3H), 3.48 (dt, J = 54.0, 6.4 Hz, 1H), 1.82–1.98 (m, 4H), 1.60–1.68 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 144.30, 137.12, 133.87, 133.76, 132.22, 128.57, 123.11, 122.38, 119.70, 115.13, 110.68, 45.35, 44.43, 31.86, 30.29, 24.04, 18.07.

*5.2.6. 9-(6-bromohexyl)-1-methyl-β-carboline (**4f**)*

White crystals (2.37 g, 69%) were obtained. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.33 (d, J = 5.2 Hz, 1H), 8.12 (d, J = 7.6 Hz, 1H), 7.84 (d, J = 5.2 Hz, 1H), 7.56–7.60 (m, 1H), 7.45 (d, J = 8.4 Hz, 1H), 7.25–7.29 (m, 1H), 4.54 (t, J = 7.6 Hz, 2H), 3.38 (t, J = 6.4 Hz, 2H), 3.05 (s, 3H), 1.81–1.89 (m, 4H), 1.40–1.55 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 141.51, 141.13, 137.94, 135.09, 129.14, 128.17, 121.55, 121.33, 119.66, 112.99, 109.67, 44.76, 33.56, 30.70, 27.90, 26.13, 23.55.

*5.3. General procedure for the preparation of compounds **7a-m***

A solution of compound **6a-c** (4 mmol) in anhydrous DMF (10 mL) was added slowly with stirring to a solution of **4a-f** (6 mmol), NaH (0.25 g, 10 mmol), potassium iodide (1.68 g, 10 mmol) in anhydrous DMF (25 mL). The mixture was stirred at room temperature until the reaction is completed. Then the mixture was poured into ice-cold water. The reaction mixture was extracted with ethyl acetate (3 × 70 mL), washed with water and brine, dried over anhydrous Na_2SO_4 and evaporated to give the dimeric β-carbolines **7a-m** as crude product, which was purified using silica gel (200–300 mesh) and using CH_2Cl_2:CH_3OH (100:1) as the eluent. Then the compounds were dissolved in hydrochloric acid alcohol, removed by evaporation and the residue was the **7a-m** hydrochloride salts.

*5.3.1. 9-Ethyl-1-methyl-7-((5-(1-methyl-β-carboline-9-yl)pentyl) oxy)-β-carboline (**7a**)*

White crystals (1.42 g, 74%) were obtained, m.p. 155.4–156.8 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.33 (d, J = 5.2 Hz, 1H), 8.28 (d, J = 5.2 Hz, 1H), 8.12 (d, J = 7.6 Hz, 1H), 7.96 (d, J = 8.8 Hz, 1H), 7.85 (d, J = 5.2 Hz, 1H), 7.73 (d, J = 5.2 Hz, 1H), 7.55–7.59 (m, 1H), 7.48 (d, J = 8.4 Hz, 1H), 7.25–7.28 (m, 1H), 6.82–6.87 (m, 2H), 4.59 (t, J = 7.6 Hz, 2H), 4.53 (q, J = 7.2 Hz, 2H), 4.08 (t, J = 6.0 Hz, 2H), 3.07 (s, 3H), 3.03 (s, 3H), 1.88–2.01 (m, 4H), 1.63–1.71 (m, 2H), 1.43 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.24, 142.68, 141.45, 141.14, 140.41, 138.01, 135.10, 135.04, 129.43, 129.08, 128.11, 122.44, 121.52, 121.33, 119.62, 115.31, 112.97, 112.26, 109.65, 109.05, 93.78, 67.97, 44.79, 39.46, 30.66, 29.15, 23.63, 23.60, 23.15, 15.53. HRMS (ESI) calcd for $C_{31}H_{33}N_4O$ 477.2649 $[M+H]^+$, found 477.2647.

*5.3.2. 9-Ethyl-1-methyl-7-((6-(1-methyl-β-carboline-9-yl)hexyl) oxy)-β-carboline (**7b**)*

White crystals (1.38 g, 70%) were obtained, m.p. 127.1–128.3 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.34 (d, J = 5.2 Hz, 1H), 8.28 (d, J = 5.2 Hz, 1H), 8.15 (d, J = 8.0 Hz, 1H), 7.95 (d, J = 8.8 Hz, 1H), 7.84 (d, J = 5.2 Hz, 1H), 7.73 (d, J = 5.2 Hz, 1H), 7.55–7.59 (m, 1H), 7.50 (d, J = 8.4 Hz, 1H), 7.27–7.31 (m, 1H), 6.85 (dd, J = 8.8, 2.0 Hz, 1H), 6.81 (d, J = 2.0 Hz, 1H), 4.68 (t, J = 8.0 Hz, 2H), 4.42 (t, J = 8.0 Hz, 2H), 4.12 (t, J = 6.0 Hz, 2H), 3.08 (s, 3H), 3.02 (s, 3H), 2.08–2.16 (m, 2H), 1.94–2.00 (m, 2H), 1.75–1.82 (m, 2H), 1.38–1.47(m, 2H), 0.96 (t, J = 7.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.91, 143.05, 141.45, 141.17, 140.49, 138.10, 138.01, 135.28, 135.08, 129.35, 129.13, 128.12, 122.42, 121.54, 121.38, 119.67, 115.32, 112.97, 112.24, 109.68, 108.78, 94.10, 67.73, 44.67, 44.59, 32.72, 27.80, 26.69, 23.59, 23.30, 20.17, 13.90. HRMS (ESI) calcd for $C_{32}H_{35}N_4O$ 491.2805 $[M+H]^+$, found 491.2813.

*5.3.3. 1-Methyl-7-(4-(1-methyl-β-carboline-9-yl)butoxy)-9-phenylpropyl-β-carboline (**7c**)*

White crystals (1.57 g, 71%) were obtained, m.p. 147.7–148.7 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.35 (d, J = 5.2 Hz, 1H), 8.27 (d, J = 5.2 Hz, 1H), 8.12–8.14 (m, 1H), 7.94 (d, J = 8.4 Hz, 1H), 7.86 (d, J = 5.2 Hz, 1H), 7.73 (d, J = 5.2 Hz, 1H), 7.56–7.60 (m, 1H), 7.51 (d, J = 8.4 Hz, 1H), 7.24–7.30 (m, 3H), 7.14–7.19 (m, 3H), 6.84 (dd, J = 8.4, 2.0 Hz, 1H), 6.60 (d, J = 2.0 Hz, 1H), 4.69 (t, J = 7.6 Hz, 2H), 4.43 (t, J = 7.6 Hz, 2H), 4.01 (t, J = 6.0 Hz, 2H), 3.09 (s, 3H), 2.90 (s, 3H), 2.74 (t, J = 7.2 Hz, 2H), 2.08–2.17 (m, 4H), 1.93–1.98(m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.07, 143.10, 141.51, 141.14, 140.58, 140.27, 138.02, 135.14, 135.09, 129.63, 129.22, 128.58, 128.41, 128.20, 126.31, 122.53, 121.59, 121.39, 119.74, 115.21, 113.02, 112.32, 109.70, 109.34, 93.74, 67.67, 44.62, 44.06, 32.89, 31.70, 27.82, 26.61, 23.53, 22.76. HRMS (ESI) calcd for $C_{37}H_{37}N_4O$ 553.2962 $[M+H]^+$, found 553.2953.

*5.3.4. 1-Methyl-7-((6-(1-methyl-β-carboline-9-yl)hexyl)oxy)-9-phenylpropyl-β-carboline (**7d**)*

White crystals (1.79 g, 77%) were obtained, m.p. 126.1–127.4 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.33 (d, J = 5.2 Hz, 1H), 8.26 (d, J = 5.2 Hz, 1H), 8.15–8.09 (m, 1H), 7.93 (d, J = 8.4 Hz, 1H), 7.85 (d, J = 5.2 Hz, 1H), 7.71 (d, J = 5.2 Hz, 1H), 7.55–7.59 (m, 1H), 7.47 (d, J = 8.4 Hz, 1H), 7.25–7.31 (m, 3H), 7.18–7.22 (m, 3H), 6.83 (dd, J = 8.4, 2.0 Hz, 1H), 6.61 (d, J = 2.0 Hz, 1H), 4.57 (t, J = 7.6 Hz, 2H), 4.43 (t, J = 7.6 Hz, 2H), 3.95 (t, J = 6.0 Hz, 2H), 3.07 (s, 3H), 2.89 (s, 3H), 2.75 (t, J = 7.2 Hz, 2H), 2.10–2.17 (m, 2H), 1.87–1.98 (m, 2H), 1.80–1.85 (m, 2H), 1.52–1.61 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.29, 143.03, 141.49, 141.17, 140.65, 140.41, 138.00, 135.19, 135.12, 129.49, 129.08, 128.58, 128.41, 128.09, 126.31, 122.37, 121.53, 121.34, 119.61, 115.08, 112.97, 112.24, 109.66, 109.36, 93.69, 68.03, 44.82, 44.06, 32.93, 31.71, 30.83, 29.24, 26.80, 26.04, 23.61, 23.02. HRMS (ESI) calcd for $C_{39}H_{41}N_4O$ 581.3275 $[M+H]^+$, found 581.3273.

*5.3.5. 9-Butyl-1-methyl-7-(4-(1-methyl-β-carboline-9-yl)butoxy)-β-carboline (**7e**)*

White crystals (1.42 g, 72%) were obtained, m.p. 130.1–131.5 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.33 (d, J = 5.2 Hz, 1H), 8.28 (d, J = 5.2 Hz, 1H), 8.10–8.12 (m, 1H), 7.95 (d, J = 8.8 Hz, 1H), 7.83 (d, J = 5.2 Hz, 1H), 7.71 (d, J = 5.2 Hz, 1H), 7.54–7.59 (m, 1H), 7.49 (d, J = 8.4 Hz, 1H), 7.26–7.30 (m, 1H), 7.25 (d, J = 0.8 Hz, 1H), 6.84 (dd, J = 8.4, 2.0 Hz, 1H), 6.80 (d, J = 2.0 Hz, 1H), 4.66 (t, J = 7.6 Hz, 2H), 4.41 (t, J = 8.0 Hz, 2H), 4.11 (t, J = 6.0 Hz, 2H), 3.07 (s, 3H), 3.01 (s, 3H), 2.07–2.15 (m, 2H), 1.93–1.99 (m, 2H), 1.74–1.82 (m, 2H), 1.37–1.46 (m, 2H), 0.96 (t, J = 7.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.24, 142.68, 141.45, 141.14, 140.41, 138.01, 135.10, 135.04, 129.43, 129.08, 128.11, 122.44, 121.52, 121.33, 119.62, 115.31, 112.97, 112.26, 109.65, 109.05, 93.78, 67.97, 44.79, 39.46, 30.66, 29.68, 29.15, 23.63, 23.60, 23.15, 15.53. HRMS (ESI) calcd for $C_{32}H_{35}N_4O$ 491.2805 $[M+H]^+$, found 491.2807.

*5.3.6. 9-Butyl-1-methyl-7-((5-(1-methyl-β-carboline-9-yl)pentyl)oxy)-β-carboline (**7f**)*

White crystals (1.21 g, 61%) were obtained, m.p. 118.9–120.2 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.33 (d, J = 5.2 Hz, 1H), 8.28 (d, J = 5.2 Hz, 1H), 8.09–8.12 (m, 1H), 7.95 (d, J = 8.4 Hz, 1H), 7.83 (d, J = 5.2 Hz, 1H), 7.72 (d, J = 5.2 Hz, 1H), 7.54–7.58 (m, 1H), 7.46 (d, J = 8.4 Hz, 1H), 7.24–7.28 (m, 1H), 6.80–6.85 (m, 2H), 4.57 (t, J = 7.6 Hz, 2H), 4.41 (t, J = 7.6 Hz, 2H), 4.07 (t, J = 6.0 Hz, 2H), 3.06 (s, 3H), 3.01 (s, 3H), 1.87–1.99 (m, 4H), 1.74–1.82 (m, 2H), 1.62–1.70 (m, 2H), 1.38–1.47 (m, 2H), 0.97 (t, J = 7.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.13, 143.06, 141.43, 141.15, 140.50, 138.05, 135.29, 135.09, 129.34, 129.05, 128.09, 122.34, 121.51, 121.34, 119.61, 115.22, 112.95, 112.22, 109.64, 108.86, 94.12, 67.96, 44.77, 44.65, 32.72, 30.67, 29.17, 23.63, 23.35, 20.17, 13.91. HRMS (ESI) calcd for $C_{33}H_{37}N_4O$ 505.2962 $[M+H]^+$, found 505.2957.

*5.3.7. 1-Methyl-7-(4-(β-carboline-9-yl)butoxy)-9-phenylpropyl-β-carboline (**7g**)*

White crystals (1.56 g, 73%) were obtained, m.p. 154.5–155.2 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.96 (s, 1H), 8.49 (d, J = 5.2 Hz, 1H), 8.26 (d, J = 5.2 Hz, 1H), 8.15–8.17 (m, 1H), 7.97 (d, J = 5.2 Hz, 1H), 7.92 (d, J = 8.4 Hz, 1H), 7.71 (d, J = 5.2 Hz, 1H), 7.58–7.63 (m, 1H), 7.52 (d, J = 8.4 Hz, 1H), 7.24–7.32 (m, 3H), 7.13–7.18 (m, 3H), 6.81 (dd, J = 8.4, 2.0 Hz, 1H), 6.57 (d, J = 2.0 Hz, 1H), 4.53 (t, J = 7.2 Hz, 2H), 4.41 (t, J = 8.0 Hz, 2H), 3.97 (t, J = 6.4 Hz, 2H), 2.88 (s, 3H), 2.73 (t, J = 7.2 Hz, 2H), 2.17–2.23 (m, 2H), 2.08–2.16 (m, 2H), 1.87–1.94 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.99, 142.96, 141.11, 140.62, 140.40, 139.02, 137.95, 136.48, 135.17, 132.07, 129.44, 128.56, 128.41, 128.36, 126.28, 122.44, 121.98, 121.14, 119.66, 115.19, 114.62, 112.27, 109.44, 109.21, 93.71, 67.69, 44.03, 43.14, 32.89, 31.70, 26.95, 26.18, 22.98. HRMS (ESI) calcd for $C_{36}H_{35}N_4O$ 539.2805 $[M+H]^+$, found 539.2807.

*5.3.8. 7-((6-(β-carboline-9-yl)hexyl)oxy)-1-methyl-9-phenylpropyl-β-carboline (**7h**)*

White crystals (1.26 g, 56%) were obtained, m.p. 122.2–123.3 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.92 (s, 1H), 8.47 (d, J = 5.2 Hz, 1H), 8.26 (d, J = 5.2 Hz, 1H), 8.14–8.16 (m, 1H), 7.97 (d, J = 5.2 Hz, 1H), 7.92 (d, J = 8.4 Hz, 1H), 7.71 (d, J = 5.2 Hz, 1H), 7.56–7.61 (m, 1H), 7.48 (d, J = 8.4 Hz, 1H), 7.26–7.30 (m, 3H), 7.17–7.21 (m, 3H), 6.81 (dd, J = 8.4, 2.0 Hz, 1H), 6.60 (d, J = 2.0 Hz, 1H), 4.40–4.44 (m, 4H), 3.92 (t, J = 6.4 Hz, 2H), 2.89 (s, 3H), 2.74 (t, J = 7.2 Hz, 2H), 2.11–2.17 (m, 2H), 1.95–2.02 (m, 2H), 1.76–1.81 (m, 2H), 1.53–1.59 (m, 2H), 1.46–1.52 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.32, 143.06, 141.14, 140.63, 140.31, 138.86, 137.83, 136.51, 135.13, 132.04, 129.55, 128.57, 128.40, 128.30, 126.30, 122.36, 121.93, 121.08, 119.55, 114.98, 114.59, 112.24, 109.43, 93.63, 68.01, 44.03, 43.30, 32.91, 31.71, 29.69, 29.15, 27.10, 25.99, 22.90. HRMS (ESI) calcd for $C_{38}H_{39}N_4O$ 567.3118 $[M+H]^+$, found 567.3112.

*5.3.9. 1-Methyl-7-(4-(β-carboline-9-yl)butoxy)-9-ethyl-β-carboline (**7i**)*

White crystals (1.46 g, 81%) were obtained, m.p. 186.2–186.7 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.96 (s, 1H), 8.48 (d, J = 5.2 Hz, 1H), 8.28 (d, J = 5.2 Hz, 1H), 8.18–8.13 (m, 1H), 8.00–7.92 (m, 2H), 7.74 (d, J = 5.2 Hz, 1H), 7.58–7.62 (m, 1H), 7.52 (d, J = 8.4 Hz, 1H), 7.28–7.32 (m, 1H), 6.84 (dd, J = 8.4, 2.0 Hz, 1H), 6.81 (d, J = 2.0 Hz, 1H), 4.52 (dt, J = 8.0, 7.2 Hz, 4H), 4.10 (t, J = 6.4 Hz, 2H), 3.04 (s, 3H), 2.17–2.24 (m, 2H), 1.90–1.97 (m, 2H), 1.42 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.13, 142.73, 141.12, 140.28, 138.97, 137.71, 136.47, 134.99, 132.03, 129.55, 128.36, 122.52, 121.96, 121.13, 119.65, 115.33, 114.60, 112.30, 109.43, 109.06, 93.79, 67.79, 43.13, 39.48, 27.01, 26.14, 22.97, 15.51. HRMS (ESI) calcd for $C_{29}H_{29}N_4O$ 449.2336$[M+H]^+$, found 449.2332.

*5.3.10. 9-Ethyl-1-methyl-7-((6-(β-carboline-9-yl)hexyl)oxy)-β-carboline (**7j**)*

White crystals (1.66 g, 87%) were obtained, m.p. 150.0–151.1 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.92 (s, 1H), 8.47 (d, J = 5.2 Hz, 1H), 8.28 (d, J = 5.2 Hz, 1H), 8.14–8.17 (m, 1H), 7.94–7.98 (m, 2H), 7.75 (d, J = 5.2 Hz, 1H), 7.57–7.61 (m, 1H), 7.49 (d, J = 8.4 Hz, 1H), 7.27–7.31 (m, 1H), 6.83–6.86 (m, 2H), 4.54 (q, J = 7.2 Hz, 2H), 4.43 (t, J = 7.2 Hz, 2H), 4.05 (t, J = 6.4 Hz, 2H), 3.06 (s, 3H), 1.95–2.02 (m, 2H), 1.79–1.86 (m, 2H), 1.55–1.62 (m, 2H), 1.47–1.53 (m, 2H), 1.44 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.48, 142.88, 141.14, 140.13, 138.82, 137.48, 136.51, 134.94, 132.01, 129.71, 128.30, 122.48, 121.93, 121.07, 119.55, 115.10, 114.58, 112.31, 109.42, 109.32, 93.70, 68.11, 43.29, 39.49, 29.15, 27.05, 25.96, 22.83, 15.53. HRMS (ESI) calcd for $C_{31}H_{33}N_4O$ 477.2649 $[M+H]^+$, found 477.2646.

*5.3.11. 9-Butyl-1-methyl-7-(4-(β-carboline-9-yl)butoxy)-β-carboline (**7k**)*

White crystals (1.62 g, 85%) were obtained, m.p. 124.4–124.7 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.96 (s, 1H), 8.48 (d, J = 5.2 Hz, 1H), 8.28 (d, J = 5.2 Hz, 1H), 8.16 (d, J = 7.6 Hz, 1H), 7.97 (dd, J = 5.2, 1.2 Hz, 1H), 7.94 (d, J = 8.4 Hz, 1H), 7.73 (d, J = 5.2 Hz, 1H), 7.58–7.62 (m, 1H), 7.50 (d, J = 8.4 Hz, 1H), 7.28–7.32 (m, 1H), 6.84 (dd, J = 8.4, 2.0 Hz, 1H), 6.80 (d, J = 2.0 Hz, 1H), 4.53 (t, J = 7.2 Hz, 2H), 4.43 (t, J = 7.6 Hz, 2H), 4.10 (t, J = 6.4 Hz, 2H), 3.02 (s, 3H), 2.17–2.24 (m, 2H), 1.90–1.97 (m, 2H), 1.75–1.82 (m, 2H), 1.38–1.47 (m, 2H), 0.96 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.00, 143.09, 141.11, 140.40, 139.00, 137.85, 136.47, 135.25, 132.06, 129.42, 128.35, 122.42, 121.97, 121.13, 119.64, 115.25, 114.61, 112.27, 109.44, 108.85, 94.10, 67.78, 44.67, 43.13, 32.72, 27.04, 26.18, 23.21, 20.16, 13.90. HRMS (ESI) calcd for $C_{31}H_{33}N_4O$ 477.2649 $[M+H]^+$, found 477.2660.

*5.3.12. 9-Butyl-1-methyl-7-((5-(β-carboline-9-yl)pentyl)oxy)-β-carboline (**7l**)*

White crystals (1.67 g, 85%) were obtained, m.p. 127.2–127.9 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.93 (s, 1H), 8.47 (d, J = 5.2 Hz, 1H), 8.27 (d, J = 5.2 Hz, 1H), 8.15 (d, J = 7.6 Hz, 1H), 7.96 (d, J = 5.2 Hz, 1H), 7.93 (d, J = 8.4 Hz, 1H), 7.71 (d, J = 5.2 Hz, 1H), 7.57–7.61 (m, 1H), 7.48 (d, J = 8.4 Hz, 1H), 7.26–7.30 (m, 1H), 6.78–6.82 (m, 2H), 4.39–4.44 (m, 4H), 4.03 (t, J = 6.0 Hz, 2H), 3.01 (s, 3H), 1.99–2.07 (m, 2H), 1.85–1.92 (m, 2H), 1.74–1.81 (m, 2H), 1.58–1.65 (m, 2H), 1.37–1.46 (m, 2H), 0.98 (t, J = 7.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.11, 143.06, 141.10, 140.46, 138.92, 137.99, 136.50, 135.26, 132.05, 129.36, 128.31, 128.28, 122.32, 121.93, 121.09, 119.58, 115.14, 114.58, 112.23, 109.43, 108.88, 94.07, 67.94, 44.64, 43.27, 32.72, 29.16, 29.06, 23.96, 23.33, 20.17, 13.92. HRMS (ESI) calcd for $C_{32}H_{35}N_4O$ 491.2805 $[M+H]^+$, found 491.2805.

*5.3.13. 9-Butyl-1-methyl-7-((6-(β-carboline-9-yl)hexyl)oxy)-β-carboline (**7m**)*

White crystals (1.52 g, 75%) were obtained, m.p. 154.4–155.1 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.91 (s, 1H), 8.47 (d, *J* = 5.2 Hz, 1H), 8.27 (d, *J* = 5.2 Hz, 1H), 8.15 (d, *J* = 7.6 Hz, 1H), 7.92–7.96 (m, 2H), 7.71 (d, *J* = 5.2 Hz, 1H), 7.56–7.60 (m, 1H), 7.47 (d, *J* = 8.4 Hz, 1H), 7.26–7.30 (m, 1H), 6.81–6.84 (m, 2H), 4.38–4.44 (m, 4H), 4.04 (t, *J* = 6.4 Hz, 2H), 2.99 (s, 3H), 1.94–2.01 (m, 2H), 1.75–1.85 (m, 4H), 1.54–1.61 (m, 2H), 1.38–1.52 (m, 4H), 0.96 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.20, 143.09, 141.11, 140.45, 138.87, 138.00, 136.50, 135.26, 132.05, 129.37, 128.28, 128.26, 122.30, 121.92, 121.06, 119.53, 115.09, 114.57, 112.22, 109.42, 108.89, 94.07, 68.08, 44.65, 43.28, 32.72, 29.19, 29.14, 27.08, 25.97, 23.33, 20.17, 13.91. HRMS (ESI) calcd for $C_{33}H_{37}N_4O$ 505.2962 $[M+H]^+$, found 505.2962.

*5.4. Synthesis of 7-(4-bromobutoxyl)-1-methyl-9-(3-phenylpropyl)-β-carboline (**8**)*

Compound **6c** (3.16 g, 10 mmol) and NaH (0.50 g, 20 mmol) were dissolved in anhydrous DMF (50 mL). 1,4-dibromobutane (20 mmol) was added and then stirred at room temperature until the reaction is completed. Then the reaction solution was poured into cool water (20 mL), the organic compounds were extracted with ethyl acetate and washed sequentially with water and brine, then dried over anhydrous sodium sulfate, filtered and evaporated. The residue was crystallized from ethyl ether-petroleum ether to afford white crystals (0.36 g, 80%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.26 (d, *J* = 5.2 Hz, 1H), 7.95 (d, *J* = 8.4 Hz, 1H), 7.73 (d, *J* = 5.2 Hz, 1H), 7.21–7.35 (m, 5H), 6.85 (dd, *J* = 8.4, 2.0 Hz, 1H), 6.61 (d, *J* = 2.0 Hz, 1H), 4.45 (t, *J* = 6.4 Hz, 2H), 4.01 (t, *J* = 6.0 Hz, 2H), 3.53 (t, *J* = 6.4 Hz, 2H), 2.91 (s, 3H), 2.77 (t, *J* = 7.2 Hz, 3H), 2.10–2.19 (m, 4H), 1.98–2.05 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.32, 143.20, 140.62, 140.19, 139.78, 135.13, 129.73, 128.65, 128.47, 126.41, 122.51, 115.08, 112.34, 109.61, 93.66, 67.24, 44.06, 33.46, 32.92, 31.73, 29.49, 27.87, 22.70.

*5.5. General procedure for the preparation of compounds **7n-u***

A mixture of compound **8** (6 mmol) and potassium iodide (1.68 g, 10 mmol) in anhydrous DMF (25 mL), followed by stirring for 2 h at room temperature. Then NaH (0.25 g, 10 mmol) was added at 0 °C, and harmine or compound **3c-i** (4 mmol) in anhydrous DMF (10 mL) was added slowly. The reaction mixture was then allowed to reach room temperature and stirred. The progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was poured into ice-cold water and extracted with ethyl acetate (3 × 70 mL). The organic layer was washed with water and brine, dried over Na_2SO_4 and filtered. The filtrate was evaporated at reduced pressure. The residue obtained was purified by flash column chromatography using CH_2Cl_2:CH_3OH (100:1) as the eluent. Then the compounds were dissolved in hydrochloric acid alcohol, removed by evaporation and the residue was the **7n-u** hydrochloride salts.

*5.5.1. 1-Methyl-9-phenylpropyl-7-(4-(1-isopropyl-β-carboline-9-yl)butoxy)-β-carboline (**7n**)*

White crystals (1.58 g, 68%) were obtained, m.p. 100.2–100.9 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.44 (d, *J* = 5.2 Hz, 1H), 8.27 (d, *J* = 5.2 Hz, 1H), 8.11 (d, *J* = 8.0 Hz, 1H), 7.93 (d, *J* = 8.4 Hz, 1H), 7.83 (d, *J* = 5.2 Hz, 1H), 7.71 (d, *J* = 5.2 Hz, 1H), 7.55–7.59 (m, 1H), 7.50 (d, *J* = 8.4 Hz, 1H), 7.24–7.29 (m, 3H), 7.13–7.18 (m, 3H), 6.83 (dd, *J* = 8.4, 2.0 Hz, 1H), 6.59 (d, *J* = 2.0 Hz, 1H), 4.65 (t, *J* = 8.0 Hz, 2H), 4.41 (t, *J* = 8.0 Hz, 2H), 4.00 (t, *J* = 6.0 Hz, 2H), 3.73–3.83 (m, 1H), 2.88 (s, 3H), 2.73 (t, *J* = 7.2 Hz, 2H), 2.08–2.16 (m, 4H), 1.92–1.98 (m, 2H), 1.48 (d, *J* = 6.4 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.96, 150.47, 142.93, 141.78, 140.61, 140.48, 138.36, 138.09, 135.20, 133.36, 129.75, 129.40, 128.56, 128.41, 128.10, 126.28, 122.44, 121.54, 121.36, 119.62, 115.25, 112.50, 112.26, 109.63, 109.18, 93.65, 67.62, 45.12, 44.03, 32.90, 31.70, 31.37, 27.37, 26.63, 23.03, 22.67. HRMS (ESI) calcd for $C_{39}H_{41}N_4O$ 581.3275 $[M+H]^+$, found 581.3275.

*5.5.2. 7-(4-(1-(4-methoxyphenyl)-β-carboline-9-yl)butoxy)-1-methyl-9-phenylpropyl -β-carboline (**7o**)*

White crystals (1.79 g, 69%) were obtained, m.p. 117.2–117.9 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.52 (d, *J* = 5.2 Hz, 1H), 8.26 (d, *J* = 5.2 Hz, 1H), 8.17 (d, *J* = 7.6 Hz, 1H), 7.95 (d, *J* = 5.2 Hz, 1H), 7.90 (d, *J* = 8.4 Hz, 1H), 7.69 (d, *J* = 5.2 Hz, 1H), 7.56–7.60 (m, 3H), 7.50 (d, *J* = 8.4 Hz, 1H), 7.29–7.32 (m, 1H), 7.22–7.26 (m, 2H), 7.13–7.17 (m, 3H), 6.99–7.02 (m, 2H), 6.73 (dd, *J* = 8.4, 2.0 Hz, 1H), 6.48 (d, *J* = 2.0 Hz, 1H), 4.39 (t, *J* = 8.0 Hz, 2H), 4.14 (t, *J* = 8.0 Hz, 2H), 3.79 (s, 3H), 3.68 (t, *J* = 6.4 Hz, 2H), 2.88 (s, 3H), 2.72 (t, *J* = 7.2 Hz, 2H), 2.07–2.14 (m, 2H), 1.58–1.66 (m, 2H), 1.34–1.41 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.93, 159.76, 144.12, 142.90, 142.09, 140.62, 140.46, 138.54, 138.12, 135.17, 134.23, 132.55, 130.55, 130.45, 129.37, 128.56, 128.41, 128.33, 126.28, 122.33, 121.65, 121.59, 119.88, 115.11, 113.67, 113.40, 112.22, 110.21, 109.13, 93.66, 67.43, 55.35, 44.14, 43.99, 32.89, 31.68, 26.32, 25.84, 23.08. HRMS (ESI) calcd for $C_{43}H_{41}N_4O_2$ 645.3224 $[M+H]^+$, found 645.3234.

*5.5.3. 1-Methyl-9-phenylpropyl-7-(4-(1-(3,4-dimethoxyphenyl)-β-carboline-9-yl)-butoxy)-β-carboline (**7p**)*

White crystals (1.89 g, 70%) were obtained, m.p. 76.5–77.4 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.53 (d, *J* = 5.2 Hz, 1H), 8.26 (d, *J* = 5.2 Hz, 1H), 8.20 (d, *J* = 7.6 Hz, 1H), 7.98 (d, *J* = 5.2 Hz, 1H), 7.91 (d, *J* = 8.8 Hz, 1H), 7.71 (d, *J* = 5.2 Hz, 1H), 7.57–7.61 (m, 1H), 7.49 (d, *J* = 8.4 Hz, 1H), 7.30–7.33 (m, 1H), 7.12–7.26 (m, 7H), 6.96 (d, *J* = 8.0 Hz, 1H), 6.73 (dd, *J* = 8.4, 2.0 Hz, 1H), 6.51 (d, *J* = 2.0 Hz, 1H), 4.42 (t, *J* = 8.0 Hz, 2H), 4.15 (t, *J* = 8.0 Hz, 2H), 3.91 (s, 3H), 3.89 (s, 3H), 3.72 (t, *J* = 6.0 Hz, 2H), 2.88 (s, 3H), 2.73 (t, *J* = 7.2 Hz, 2H), 2.08–2.15 (m, 2H), 1.64–1.71 (m, 2H), 1.35–1.43 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.94, 149.26, 148.76, 144.06, 142.94, 142.07, 140.60, 140.44, 138.41, 138.05, 135.17, 134.19, 132.72, 130.49, 129.41, 128.55, 128.38, 126.27, 122.37, 121.89, 121.67, 121.56, 119.92, 115.14, 113.54, 112.48, 112.22, 110.82, 110.24, 109.10, 93.70, 67.48, 65.84, 56.01, 44.17, 44.02, 32.89, 31.68, 26.36, 26.00, 22.99, 15.26. HRMS (ESI) calcd for $C_{44}H_{43}N_4O_3$ 675.3330 $[M+H]^+$, found 675.3330.

*5.5.4. 1-Methyl-9-phenylpropyl-7-(4-(1-(3,4,5-trimethoxyphenyl)-β-carboline-9-yl)-butoxy)-β-carboline (**7q**)*

White crystals (1.97 g, 70%) were obtained, m.p. 91.3–91.8 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.52 (d, *J* = 5.2 Hz, 1H), 8.25 (d, *J* = 5.2 Hz, 1H), 8.18–8.21 (m, 1H), 8.00 (d, *J* = 5.2 Hz, 1H), 7.89 (d, *J* = 8.8 Hz, 1H), 7.69 (d, *J* = 8.8 Hz, 1H), 7.57–7.61 (m, 1H), 7.49 (d, *J* = 8.4 Hz, 1H), 7.29–7.33 (m, 1H), 7.22–7.27 (m, 2H), 7.12–7.17 (m, 3H), 6.87 (s, 2H), 6.73 (dd, *J* = 8.4, 2.0 Hz, 1H), 6.53 (d, *J* = 2.0 Hz, 1H), 4.41 (t, *J* = 8.0 Hz, 2H), 4.13 (t, *J* = 8.0 Hz, 2H), 3.94 (s, 3H), 3.87 (s, 6H), 3.76 (t, *J* = 6.0 Hz, 2H), 2.87 (s, 3H), 2.72 (t, *J* = 7.2 Hz, 2H), 2.07–2.14 (m, 2H), 1.71–1.78 (m, 2H), 1.41–1.48 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.91, 153.13, 143.94, 142.89, 141.98, 140.61, 140.48, 138.24, 138.13, 135.64, 135.18, 133.98, 130.41, 129.33, 128.54, 128.49, 128.37, 126.27, 122.34, 121.68, 121.45, 119.99, 115.17, 113.83, 112.20, 110.20, 109.10, 106.52, 93.68, 67.53, 65.83, 61.01, 56.21, 44.27, 32.89, 31.70, 26.44, 26.27, 23.06. HRMS (ESI) calcd for $C_{45}H_{45}N_4O_4$ 705.3435 $[M+H]^+$, found 705.3424.

*5.5.5. 1-Methyl-9-phenylpropyl-7-(4-(1-(2-chlorophenyl)-β-carboline-9-yl)butoxy)-β-carboline (**7r**)*

White crystals (1.83 g, 71%) were obtained, m.p. 172.8–173.4 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.55 (d, *J* = 5.2 Hz, 1H), 8.27 (d, *J* =

5.2 Hz, 1H), 8.19–8.21 (m, 1H), 8.03 (d, *J* = 5.2 Hz, 1H), 7.93 (d, *J* = 8.8 Hz, 1H), 7.71 (d, *J* = 5.2 Hz, 1H), 7.57–7.61 (m, 2H), 7.45–7.50 (m, 2H), 7.35–7.40 (m, 2H), 7.29–7.33 (m, 1H), 7.23–7.27 (m, 2H), 7.13–7.18 (m, 3H), 6.77 (dd, *J* = 8.4, 2.0 Hz, 1H), 6.53 (d, *J* = 2.0 Hz, 1H), 4.41 (t, *J* = 8.0 Hz, 2H), 3.91–4.14 (m, 2H), 3.72–3.77 (m, 2H), 2.89 (s, 3H), 2.73 (t, *J* = 7.2 Hz, 2H), 2.08–2.16 (m, 2H), 1.58–1.80 (m, 2H), 1.35–1.49 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.96, 142.94, 141.67, 140.98, 140.60, 140.45, 138.78, 138.29, 138.07, 135.19, 134.18, 134.10, 131.45, 130.09, 130.01, 129.47, 129.42, 128.57, 128.52, 128.39, 126.80, 126.30, 122.36, 121.71, 121.29, 119.90, 115.16, 114.31, 112.25, 109.87, 109.15, 93.73, 67.47, 44.04, 43.91, 32.90, 31.71, 26.53, 26.27, 23.03. HRMS (ESI) calcd for $C_{42}H_{38}ClN_4O$ 649.2729 $[M+H]^+$, found 649.2729.

5.5.6. 1-Methyl-9-phenylpropyl-7-(4-(1-(2-thienyl)-β-carboline-9-yl)butoxy)-β-carboline (**7s**)

White crystals (1.89 g, 76%) were obtained, m.p. 132.7–134.4 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.53 (d, *J* = 5.2 Hz, 1H), 8.26 (d, *J* = 5.2 Hz, 1H), 8.17–8.19 (m, 1H), 7.98 (d, *J* = 5.2 Hz, 1H), 7.92 (d, *J* = 8.8 Hz, 1H), 7.71 (d, *J* = 5.2 Hz, 1H), 7.58–7.62 (m, 1H), 7.51 (d, *J* = 8.4 Hz, 1H), 7.45 (dd, *J* = 5.2, 1.2 Hz, 1H), 7.29–7.34 (m, 2H), 7.23–7.27 (m, 2H), 7.11–7.18 (m, 4H), 6.76 (dd, *J* = 8.4, 2.0 Hz, 1H), 6.51 (d, *J* = 2.0 Hz, 1H), 4.40 (t, *J* = 8.0 Hz, 2H), 4.27 (t, *J* = 8.0 Hz, 2H), 3.75 (t, *J* = 6.4 Hz, 2H), 2.88 (s, 3H), 2.73 (t, *J* = 7.2 Hz, 2H), 2.08–2.16 (m, 2H), 1.67–1.75 (m, 2H), 1.44–1.51 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.95, 142.91, 142.19, 141.10, 140.61, 140.43, 138.60, 138.05, 137.10, 135.17, 134.82, 130.88, 129.41, 128.63, 128.57, 128.41, 128.28, 127.03, 126.73, 126.30, 122.35, 121.71, 121.39, 120.12, 115.10, 114.21, 112.25, 110.27, 109.21, 93.65, 67.43, 44.16, 44.01, 32.90, 31.71, 26.43, 26.08, 23.06. HRMS (ESI) calcd for $C_{40}H_{37}N_4OS$ 621.2683 $[M+H]^+$, found 621.2689.

5.5.7. 1-Methyl-9-phenylpropyl-7-(4-(1-(3-pyridyl)-β-carboline-9-yl)butoxy)-β-carboline (**7t**)

White crystals (1.97 g, 80%) were obtained, m.p. 124.6–125.5 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.94 (dd, *J* = 2.0, 0.8 Hz, 1H), 8.71 (dd, *J* = 4.8, 1.6 Hz, 1H), 8.57 (d, *J* = 5.2 Hz, 1H), 8.27 (d, *J* = 5.2 Hz, 1H), 8.19–8.22 (m, 1H), 8.03 (d, *J* = 5.2 Hz, 1H), 7.99–8.02 (m, 1H), 7.92 (d, *J* = 8.8 Hz, 1H), 7.71 (d, *J* = 5.2 Hz, 1H), 7.59–7.64 (m, 1H), 7.50 (d, *J* = 8.4 Hz, 1H), 7.38–7.41 (m, 1H), 7.32–7.36 (m, 1H), 7.24–7.27 (m, 2H), 7.13–7.19 (m, 3H), 6.74 (dd, *J* = 8.4, 2.0 Hz, 1H), 6.52 (d, *J* = 2.0 Hz, 1H), 4.43 (t, *J* = 8.0 Hz, 2H), 4.12 (t, *J* = 8.0 Hz, 2H), 3.71 (t, *J* = 6.0 Hz, 2H), 2.88 (s, 3H), 2.74 (t, *J* = 7.2 Hz, 2H), 2.09–2.16 (m, 2H), 1.60–1.67 (m, 2H), 1.36–1.42 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.82, 150.09, 149.57, 142.88, 142.10, 140.59, 140.46, 138.79, 138.08, 136.59, 136.00, 135.16, 134.36, 130.86, 129.34, 128.74, 128.51, 128.37, 126.23, 123.11, 122.32, 121.72, 121.35, 120.21, 115.13, 114.23, 112.20, 110.17, 109.14, 93.61, 67.29, 44.35, 44.01, 32.86, 31.68, 26.23, 25.75, 23.02. HRMS (ESI) calcd for $C_{41}H_{38}N_5O$ 616.3071 $[M+H]^+$, found 616.3074.

5.5.8. 1-Methyl-9-phenylpropyl-7-(4-(1-methyl-7-methoxy-β-carboline-9-yl)butoxy)-β-carboline (**7u**)

White crystals (1.80 g, 77%) were obtained, m.p. 127.4–128.1 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.30 (d, *J* = 5.2 Hz, 1H), 8.27 (d, *J* = 5.2 Hz, 1H), 7.98 (d, *J* = 8.8 Hz, 1H), 7.93 (d, *J* = 8.8 Hz, 1H), 7.75 (d, *J* = 5.2 Hz, 1H), 7.71 (d, *J* = 5.2 Hz, 1H), 7.21–7.27 (m, 2H), 7.10–7.18 (m, 3H), 6.88–6.90 (m, 2H), 6.83 (dd, *J* = 8.4, 2.0 Hz, 1H), 6.59 (d, *J* = 2.0 Hz, 1H), 4.61 (t, *J* = 8.0 Hz, 2H), 4.41 (t, *J* = 8.0 Hz, 2H), 4.01 (t, *J* = 6.0 Hz, 2H), 3.89 (s, 3H), 3.05 (s, 3H), 2.88 (s, 3H), 2.73 (t, *J* = 7.2 Hz, 2H), 2.04–2.17 (m, 4H), 1.91–1.98 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.94, 159.92, 143.12, 142.94, 140.60, 140.47, 140.41, 138.17, 138.03, 135.25, 135.20, 129.57, 129.42, 128.56, 128.50, 128.40, 128.25, 126.28, 122.47, 122.45, 115.25, 112.35, 112.29, 109.16, 108.70, 93.71, 93.52, 67.70, 55.65, 44.60, 44.02, 32.88, 31.69, 27.66, 26.55, 23.27, 22.94. HRMS (ESI) calcd for $C_{38}H_{39}N_4O_2$ 583.3068 $[M+H]^+$, found 583.3076.

5.6. Cytotoxicity assays in vitro

All of the synthesized compounds were evaluated against nine different cancer cell lines using MTT assay *in vitro*. The target tumor cell lines were cultured to log phase in RPMI-1640 medium supplemented with 10% (v/v) fetal bovine serum (FBS), 100 μg·mL^{-1} penicillin, and 100 μg·mL^{-1} streptomycin. After diluting to approximate 10^5 cells per mL with complete medium, 100 mL of the obtained cell suspension was plated onto each well of a 96-well culture plate and incubated in 5% CO_2 at 37 °C for 24 h. Cell lines were obtained from Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Science. DMSO was used as the solution for the drugs. The final concentration of DMSO in the growth medium was 2% (v/v) or lower without an effect on cell replication. Cisplatin was the positive control. The optical density (OD) was read at 490 nm. Three replicate wells were used for each drug concentration in all of the experiments. Each assay was carried out at least three times. The results that were expressed as IC_{50} (inhibitory concentration 50%) values were calculated using the Logit method.

5.7. Assay of acute toxicities

Specific pathogen-free KM mice (6–8 weeks old) weighing 19–22 g were housed in a mouse room at 24 ± 2 °C and 60–70% humidity with 12 h light/dark cycles. The mice were provided rodent laboratory chow pellets and tap water for a week to adapt to the environment of the mouse room. The experimental protocol was approved by the Institutional Animal Ethical Committee, and all of the animals were provided by Laboratory Animal Center of Xinjiang Uygur Autonomous Region. Prior to each experiment, mice were fastened overnight and allowed free access to water. Various doses of the asymmetric dimeric β-carboline derivatives, ranging from 5.0 to 500 mg kg^{-1} dissolved in 0.5% carboxymethyl cellulose sodium (CMC-Na) salt solution, were given intraperitoneally to different groups of healthy KM mice, and each group contained 10 mice (5 males and 5 females). After the administration of the compounds, the mice were observed continuously for the first 2 h for any gross behavioral changes and deaths, then intermittently for the next 24 h, and occasionally thereafter for 14 days, and for the onset of any delayed effects. All of the animals were killed on the 14th day after drug administration, and they were checked macroscopically for possible damage to the heart, liver, and kidneys. Mice that experienced immediate death following drug administration were also examined for any possible organ damage. LD_{50} values were calculated graphically as described [43].

5.8. In vivo antitumor activity

Sarcoma180 and Lewis lung cancer cell lines were provided by Shanghai Institute of Pharmaceutical Industry. Mice were inoculated with Sarcoma180 and Lewis lung cancer tumor cells. After 7 days, the tumors were removed and the cells were harvested. Mice received subcutaneous injections of viable tumor cells (2×10^6 cells/mouse) in the armpit. Each compound was administered via i.p. injection to different groups of mice (each group contained 10 female mice) 24 h after the inoculation at a dosage about one-fifth of the LD_{50} value once a day for seven consecutive days. This dose was the maximum tolerated dose for most of the compounds based on our preliminary studies. CTX at 30 mg kg^{-1} was used as the positive control and the vehicle as the negative control. The weight of the animals was recorded every three days.

All of the animals were killed on the 21st day after tumor inoculation, and the tumors were excised and weighed. The inhibition rate was calculated as follows:

$(C - T)/C \times 100$,

where T is the average tumor weight of the treated group and C is the average tumor weight of the negative control group.

5.9. Wound healing assay in vitro

HT-29 and LLC cells (about 1×10^6/mL) were seeded in a 24-well plate at a density that after 24 h of growth, and they were allowed to reach 90% confluence in complete medium. A single scratch wound was created on the confluent monolayers using a micropipette tip across the center of the well and a straight line was scratched in one direction. Then, wounded monolayers were washed with phosphate buffer saline (PBS) to remove the detached cells, and each assay was carried out three times. After washing, fresh media with FBS was added, various concentrations of **7o** were added to their respective wells, and then they were incubated for 24 h. The medium in each well was discarded and washed several times with PBS. Cells migrated to the wound surface and the average distance of migrating cells was determined under an inverted microscope at designated time points. Pictures of three different regions of each wound were taken. The experiment was performed three times.

5.10. CAM assay in vivo

Antiangiogenic activity of the selected compound **7o** was investigated *in vivo* using a CAM assay. Five-day-old fertilized eggs were obtained from a local hatchery. We injected 5 mL of albumin, and the eggs were incubated horizontally to allow the CAM to detach from the shell to produce a sham chamber. Compound **7o** was prepared in gelatin sponge discs ($5 \times 5 \times 5$ mm^3) at the concentration of 0.5, 5.0, and 50 μM/disc, respectively. CA4P was used as the positive control drug. Discs containing the vehicle only (DMSO) were used as the negative control. A small window opening was made in the shell, and the discs were directly applied onto the CAM. The square opening was covered with sterilized surgical tape and the embryos were incubated for 48 h at 38.5 °C. The CAMs were photographed under a dissecting microscope and blood vessels in each CAM were counted. The results are presented as a mean percentage of inhibition to the control ± SD, ($n = 3$).

Acknowledgments

This work was supported by the scientific research innovation project in Xinjiang (No. XJGRI2016052), the Program for Outstanding Youth Science and Technology Innovation Talents Training in Xinjiang Uygur Region, China (No. qn2015jc067) and Xinjiang Huashidan Pharmaceutical Research Co. Ltd.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ejmech.2018.02.003.

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