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Original article

Design, synthesis and in vitro and in vivo antitumor activities of novel bivalent β-carbolines

Buxi Shi [a], Rihui Cao [a,*], Wenxi Fan [b], Liang Guo [b], Qin Ma [b], Xuemei Chen [b], Guoxian Zhang [a], Liqin Qiu [a], Huacan Song [a,*]

[a] School of Chemistry and Chemical Engineering, Sun Yat-sen University, 135 Xin Gang Xi Road, Guangzhou 510275, PR China
[b] Xinjiang Huashidan Pharmaceutical Co. Ltd., 175 He Nan East Road, Urumqi 830011, PR China

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ABSTRACT

A series of bivalent β-carbolines with a spacer of three to ten methylene units between the indole nitrogen was synthesized and evaluated as antitumor agents. The results demonstrated that compounds **18c**, **21b**, **25a** and **31b** exhibited strong cytotoxic activities with IC_{50} value of lower than 20 μM against four tumor cell lines. Acute toxicities and antitumor efficacies of the selected compounds in mice were also evaluated, compounds **18b**, **21b**, **26a** and **31b** exhibited potent antitumor activities with tumor inhibition rate of over 40% in animal models. Preliminary structure–activity relationships analysis indicated that (**1**) the spacer length affected antitumor potencies, and four to six methylene units were more favorable; (**2**) the introduction of appropriate substituent into position-1 of β-carboline facilitated antitumor potencies.

1. Introduction

The β-carboline alkaloids are a large group of natural and synthetic indole alkaloids with a broad spectrum of biochemical effects and pharmaceutical functions [1]. Many previous reports focused on the effects of β-carbolines on the central nervous system (CNS) [1], however, there have been intense research efforts in recent years in the design and development of β-carbolines as a new class of antitumor agents [2–21].

β-Carbolines are initially discovered to exert their antitumor effects by intercalating into DNA [22,23]. Subsequently, Top I and II (topoisomerase I and II) [24–26], CDK (cyclin-dependent kinase) [4,27,28], MK-2 (mitogen activated protein kinase-activated protein kinase 2) [29], PLK1 (polo-like kinase) [30], kinesin-like protein Eg5 [31] and IKK (I-Kappa-B kinase) [32] were also found to be the pharmacological targets of this class of compounds.

Previous studies indicated that dimerization of various intercalating agents by an appropriate linker could lead to a dramatic increase in the DNA binding affinity [33,34]. Such compounds can bind to DNA by bis-intercalation mode, which produces binding sites that are at least twice as potent as monointercalation sites and causes much more pronounced structural changes of DNA [35]. Consequently, bivalent β-carbolines were expected to exhibit more potent antitumor efficacies than monomers and exert tumor cell killing effects through similar mechanisms of action.

Bivalent β-carbolines have been synthesized before [36–38]. In these cases, bivalent β-carbolines were linked at postion-6 or 9 of β-carboline ring (Fig. 1). Such compounds were found to be potential anti-Alzheimer agents. In addition, the synthesis and evaluation of bivalent β-carbolines with a spacer of four to ten methylene units at position-1 of β-carboline nucleus as antitumor agents were also reported [35] (Fig. 1). However, there was no information of systematic and detailed studies of structure–activity relationships on antitumor efficacies in vitro and in vivo of this class of compounds. In the present investigation, we reported the synthesis, in vitro evaluation, in vivo efficacies and preliminary structure–activity relationships for the new bivalent β-carbolines with a spacer of three to ten methylene units between the indole nitrogen. To the best of our knowledge, all bivalent β-carbolines except **18b** are novel compounds, and this is the first time to report the acute toxicities and antitumor efficacies of this class of compounds in mice.

2. Chemistry

The overall synthetic routes of monovalent and bivalent β-carbolines are outlined in Schemes 1–4. Monovalent β-carbolines **2**, **3**,

* Corresponding authors. Tel.: +86 20 84110918; fax: +86 20 84112245.
E-mail addresses: caorihui@mail.sysu.edu.cn (R. Cao), yjhchx@mail.sysu.edu.cn (H. Song).

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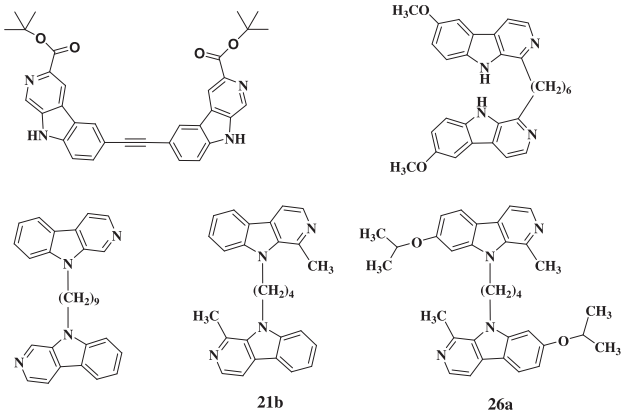


Fig. 1. The chemical structure of the representative reported and newly synthesized bivalent β-carbolines.

6 and **9** were synthesized according to already published methods [2,39]. The preparation of monovalent β-carbolines **1**, **4**–**5**, **7**–**8** and **16**–**17** have been already described as antitumor agents in our previous reports [5–7,12].

7-Methoxy-1-methyl-β-carboline **7** was converted to 1-methyl-β-carboline-7-ol **8a** by using acetic acid and hydrobromic acid as demethylation solvent, and then reacted with isopropyl bromide by the action of anhydrous K_2CO_3 in acetone to give monovalent β-carboline **8** (Scheme 1). The 1-substituted 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acids **10**–**14a** were prepared by the condensation of L-tryptophan with appropriate aldehydes via well-known Pictet–Spengler condensation in acid media, and were subsequently oxidated and decarboxylated in a single step through the reaction of potassium dichromate according to the method previously described by Snyder et al. [40] to afford monovalent β-carbolines **10**–**14** (Scheme 2).

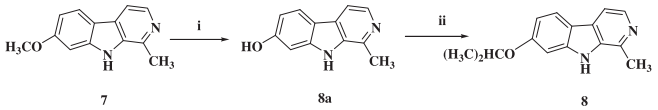
Esterification of the intermediate **14a** with ethanol in the presence of $SOCl_2$ by heating yielded the corresponding ethyl 1,2,3,4-tetrahydro-β-carboline-3-carboxylate **14b**, and followed by dehydrogenation with sulfur in refluxing xylene to afford monovalent β-carboline **15** (Scheme 3).

The symmetrical bivalent β-carbolines **18**–**34** were prepared by reaction of appropriate dibromoalkane with monovalent β-carbolines **1**–**17** in anhydrous DMF. The chemical structures of all the newly synthesized compounds were characterized by EI-MS, ^1H NMR and ^{13}C NMR.

3. Results and discussion

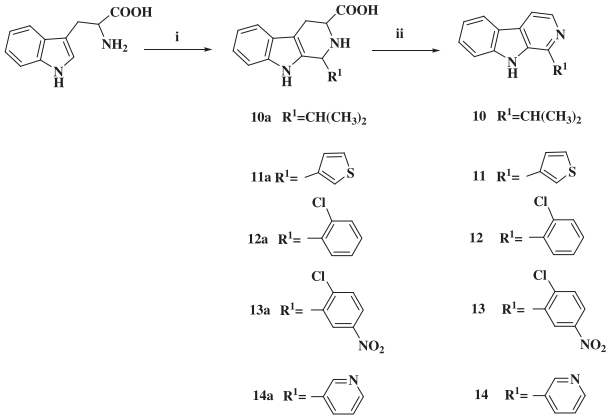
3.1. Cytotoxicity in vitro

The cytotoxic potencies of all the newly synthesized bivalent β-carbolines against a panel of human tumor cell lines were investigated and compared with the monovalent β-carbolines **4**



Reagents and conditions: (i) acetic acid, 40% HBr, reflux, 8 h; (ii) DMF, K_2CO_3, isopropyl bromide

Scheme 1. Synthesis of the monovalent β-carboline **8**.



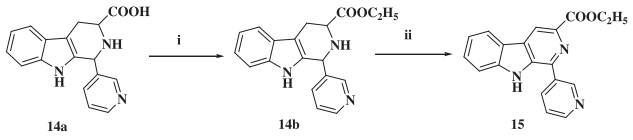
Reagents and conditions: (i) acetic acid, appropriate aldehyde, reflux, 3 h; (ii) acetic acid, K_2CrO_7, $NaHSO_3$, NaOH, stirred at 100℃, 20 min.

Scheme 2. Synthesis of the monovalent β-carbolines **10**–**14**.

and **7** and the reference drugs cisplatin. The human tumor cell line panel consisted of gastric carcinoma (BGC-823), malignant melanoma (A375), renal carcinoma (769-P and OS-RC-2), epidermoid carcinoma of the nasopharynx (KB). In order to enhance the solubility in aqueous solution, compounds **18**–**34** were converted into their water-soluble hydrochloride salt by the usual methods before use. The results were summarized in Table 1.

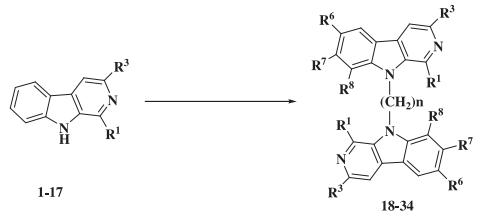
As shown in Table 1, compounds **18c**, **21b**, **25a** and **31b** displayed a broad spectrum of cytotoxic activities with IC_{50} value of lower than 20 μM against four tumor cell lines, while compounds **24a**, **24b**, **24e**, **26a**, **32a** and **33b** only exhibited strong cytotoxic effects with IC_{50} value of lower than 20 μM against three tumor cell lines. Interestingly, compounds **27b**, **30a** and **33a** were selectively active against A375 and 769-P cell lines with IC_{50} value of lower than 20 μM but fail to show cytotoxic effects in other cell lines at the concentration of 200 μM. Similarly, compounds **19** and **21h** displayed selective activities against 769-P and SK-OV-3 cell lines but were inactive against BGC, A375 and KB cell lines. Moreover, compounds **21c**, **21e** and **24c** were found to exhibit selective activity against SK-OV-3 cell lines. Unfortunately, compounds **20**, **21g**, **23b**, **25b**, **28a**, **32b**, **34a** and **34b** were almost inactive against all tumor cell lines tested.

We examined the influence of the spacer length of bivalent β-carbolines on cytotoxic activities. The data collected in Table 1 showed that compounds **18a**–**c** with a spacer of four to six methylene units exhibited moderate to strong cytotoxic activities, and compound **18c** with a spacer of six methylene units was found to be the highest cytotoxic agent with all IC_{50} value of lower than 40 μM



Reagents and conditions: (i) ethanol/$SOCl_2$, reflux, 4 h; (ii) xylene/sulphur, reflux, 8 h.

Scheme 3. Synthesis of the monovalent β-carboline **15**.



Reagents and conditions: (i) DMF, NaH, dibromoalkane, stirred at room temperature.

Scheme 4. Synthesis of the bivalent β-carbolines **18**–**34**.

against all tumor cell lines tested. While compounds **18d**,**e** with a spacer of seven to eight methylene units only had marginal or no cytotoxic effect in any cell lines. Similarly, compounds **21a**–**h** with a spacer of three to ten methylene units showed low to high cytotoxic effects against tumor cell lines, compound **21b** with a spacer of four methylene units displayed the highest cytotoxic effects with IC_{50} value of lower than 30 μM against all tumor cell lines tested, and compounds **21a** and **21d** with a spacer of three and six methylene units, respectively, showed moderate cytotoxic activities, while other compounds only exhibited selective activities against one or two tumor cell lines. These results suggested that the length of the spacer affected cytotoxic activities and four to six methylene units were more favorable.

In comparison with compounds **21a**–**h**, compounds **24a**–**e** had an additional methoxy group appended to position-7 of β-carboline ring. It was interesting to note that the spacer length in these bivalent β-carbolines has little influence on their cytotoxic activities. These results suggested that the electron-donating group of β-carboline ring might be facilitated their cytotoxic potencies. Moreover, the A375 and SK-OV-3 cell lines were more sensitive to these compounds than other cell lines.

Next, we evaluated the effect of various substituents in β-carboline ring on cytotoxic activities in these bivalent compounds. Table 1 showed that compound **18c** with a spacer of six methylene units showed strong cytotoxic activities with all IC_{50} value of lower than 40 μM against all tumor cell lines. Introduction of a bromo group in position-6 of compound **18c** led to compound **19**, which only exhibited selective cytotoxic activities against 769-P and SK-OV-3 cell lines. Replacement of the hydrogen atom in position-8 of compound **18c** by a bromo group to yield compound **20** failed to show cytotoxic effects in other cell lines at the concentration of 200 μM. Similarly, compound **21d** displayed moderate cytotoxic activities, while compound **23b** bearing a bromo group in position-6 of β-carboline ring was almost inactive. These results suggested that the bromo group in position-6 or 8 of β-carboline ring was detrimental to their cytotoxic activities.

Compound **21d** having a methyl group in position-1 displayed moderate cytotoxic activities. The cytotoxic potency of compound **27a** bearing an isopropyl group in position-1 was comparable to compound **21d**. Replacement of the methyl group in position-1 of compound **21d** by a 2-chloro-5-nitrophenyl group (compound **30a**) and a 3,4,5-trimethoxyphenyl substituent (compound **33a**) resulted in some enhancement in the activity against A375 and 769-P cell lines but loss of activity against BGC, KB and SK-OV-3 cell lines. Interestingly, replacement of the methyl group in position-1 of compound **21d** by a pyridin-3-yl group to yield compound **31b** showed significant enhance the activity with IC_{50} value of 14.1, 5.3, 13.3 and 14.9 μM against BGC, A375, KB and SK-OV-3 cell lines, respectively. Unfortunately, replacement of the methyl group in position-1 of compound **21d** by a thiophen-3-yl group (compound **28a**) and a 2-chlorophenyl group (compound **29a**) resulted in significant loss of cytotoxic activities. These results suggested that introducing an appropriate substituent into position-1 of β-carboline ring facilitated cytotoxic potency.

3.2. Preliminary assessment of acute toxicity

The LD_{50} values and scores for neurotoxicity of the selected bivalent β-carbolines in mice after administration by i.p. route were summarized in Table 2. All the tested bivalent β-carbolines resulted in acute toxic manifestation but caused no obvious neurotoxic effects including tremor, twitch, jumping and supination just like monovalent β-carboline **7** (Harmine). Animals showed a decrease in locomotor activity after the administration of various bivalent β-carbolines. Death occurred mostly in the high dosage group with 30 min after injection. All surviving animals returned to normal in the next day. Autopsy of the animals that died in the course of experiment and the necropsy findings in surviving animals at the end of experimental period (14 days) revealed no obvious changes in any organs.

Of all investigated bivalent β-carbolines, compound **24a** having a methoxy group in position-7 of β-carboline ring displayed remarkable acute toxicity with LD_{50} value of 50 mg kg^{-1}. Replacement of the methoxy group of compound **24a** in postion-7 with an isopropoxy substituent gave compound **26a** which exhibited lower acute toxicity with LD_{50} value of 220 mg kg^{-1}. In addition, compounds **18a**–**c**, **21b**, **21d**, **31b** and **33a** having no substituent in position-7 of β-carboline ring also demonstrated weaker acute toxicities with LD_{50} value of over 150 mg kg^{-1}. These results suggested that (1) the methoxy group in position-7 of β-carboline ring might play an important role in determining acute toxicity; (2) the acute toxicity decreased remarkably by replacing the methoxy substituent with a bulky alkoxy group or removing the methoxy substituent.

3.3. Evaluation of antitumor activity

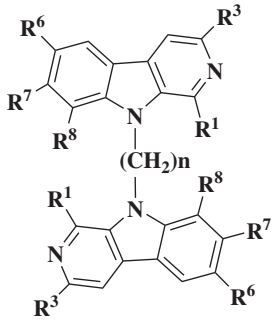
Nine bivalent β-carbolines were selected for evaluation in vivo against mice bearing CT-26 colon cancer, Lewis lung cancer, Sarcoma 180, H22 liver cancer and B16 melanoma and compared with monovalent β-carbolines **4** and **7** (harmine) and the reference drugs Cyclophosphamide (CTX). The tumor inhibition rates of all investigated bivalent β-carbolines were summarized in Table 2.

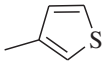
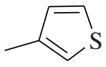
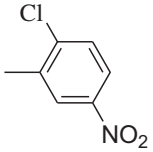
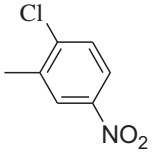
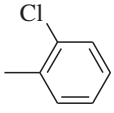
As shown in Table 2, all the tested bivalent β-carbolines displayed moderate to strong antitumor activities in animal model. Interestingly, compounds **18b** and **21b** exhibited remarkable antitumor activities with the tumor inhibition rate of over 40% against mice bearing CT-26 colon cancer, Lewis lung cancer, Sarcoma 180 and H22 liver cancer at dose 30 and 40 mg kg^{-1}, respectively. Similarly, compounds **26a** and **31b** also exhibited excellent antitumor activities with the tumor inhibition rate of over 40% against CT-26 colon cancer, Lewis lung cancer and Sarcoma 180 at dose 40 and 50 mg kg^{-1}, respectively.

Particularly, compound **21b** was found to be the most potent antitumor agent with the tumor inhibition rate of 50.8 and 56.2% against mice bearing CT-26 colon cancer and Sarcoma 180, respectively. Moreover, compounds **26a** and **31b** exhibited good antitumor activities with the tumor inhibition rate of 51.2 and 50.6% against mice bearing CT-26 colon cancer. It is interesting to note that CT-26 colon cancer was more susceptible to all tested compounds than Lewis lung cancer, Sarcoma 180, H22 liver cancer and B16 melanoma.

Compounds **18a** and **18c** with a spacer of 4 and 6 methylene units, respectively, showed moderate antitumor activities in all animal models with the tumor inhibition rate of lower than 40%, whereas compound **18b** with a spacer of 5 methylene units exhibited strong antitumor activities against mice bearing CT-26

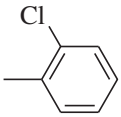
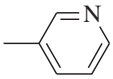
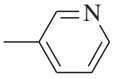
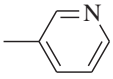
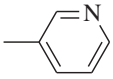
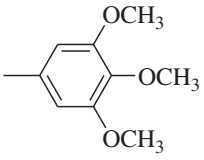
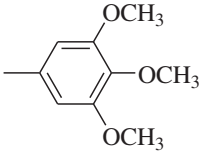
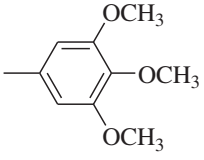
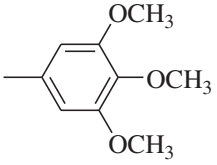
Table 1
Cytotoxic activities of bivalent β-carbolines in vitro.



Compounds	R^1	R^3	R^6	R^7	R^8	*n*	IC_{50} (μM[a])				
							BGC[b]	A375	769-P	KB	SK-OV-3
18a	H	H	H	H	H	4	59.7[c]	>200	199	>200	>200
18b	H	H	H	H	H	5	23.5	10.2	15.3	>200	>200
18c	H	H	H	H	H	6	13.8	10.8	12.8	13.9	38.9
18d	H	H	H	H	H	7	>200	>200	53.0	>200	>200
18e	H	H	H	H	H	8	>200	>200	>200	>200	33.5
19	H	H	Br	H	H	4	>200	>200	3.1	>200	8.9
20	H	H	H	H	Br	6	>200	>200	>200	>200	>200
21a	CH_3	H	H	H	H	3	>200	69.5	145	160	26.4
21b	CH_3	H	H	H	H	4	22.3	13.1	6.5	6.4	4.8
21c	CH_3	H	H	H	H	5	>200	>200	>200	>200	18.8
21d	CH_3	H	H	H	H	6	134	38.0	149	87.9	16.9
21e	CH_3	H	H	H	H	7	>200	>200	51.7	>200	96.8
21f	CH_3	H	H	H	H	8	>200	>200	>200	>200	>200
21g	CH_3	H	H	H	H	9	>200	67.2	>200	>200	61.9
21h	CH_3	H	H	H	H	10	>200	>200	16.2	>200	8.5
22	CH_3	$COOC_2H_5$	H	H	H	6	>200	>200	>200	>200	>200
23a	CH_3	H	Br	H	H	5	>200	>200	4.3	>200	>200
23b	CH_3	H	Br	H	H	6	>200	>200	>200	>200	63.4
24a	CH_3	H	H	OCH_3	H	4	75.7	6.2	65.6	2.8	7.1
24b	CH_3	H	H	OCH_3	H	5	58.6	<1.4	81.0	5.0	1.7
24c	CH_3	H	H	OCH_3	H	6	>200	35.3	>200	155	11.7
24d	CH_3	H	H	OCH_3	H	7	104	113	114	38.6	10.2
24e	CH_3	H	H	OCH_3	H	8	75.7	6.2	65.6	2.8	7.1
25a	CH_3	H	H	OC_4H_9	H	4	8.5	3.8	>200	6.8	5.5
25b	CH_3	H	H	OC_4H_9	H	6	>200	>200	56.0	>200	>200
26a	CH_3	H	H	$OCH(CH_3)_2$	H	6	194	18.5	95.4	19.8	17.1
26b	CH_3	H	H	$OCH(CH_3)_2$	H	9	>200	43.3	40.0	112	101
27a	$CH(CH_3)_2$	H	H	H	H	6	>200	53.5	18.5	>200	31.8
27b	$CH(CH_3)_2$	H	H	H	H	10	>200	19.6	4.6	>200	>200
28a		H	H	H	H	6	>200	>200	>200	>200	>200
28b		H	H	H	H	8	>200	32.1	>200	>200	>200
29a		H	H	H	H	6	>200	7.0	3.8	>200	>200
29b		H	H	H	H	9	>200	>300	1.8	>200	>200
30a		H	H	H	H	6	>200	23.2	>200	>200	>200

(*continued on next page*)

Table 1 (*continued*)

Compounds	R^1	R^3	R^6	R^7	R^8	*n*	IC_{50} (μM[a])				
							BGC[b]	A375	769-P	KB	SK-OV-3
30b		H	H	H	H	7	>200	10.4	82.1	>200	>200
31a		H	H	H	H	4	>200	85.3	>200	>200	36.8
31b		H	H	H	H	6	14.1	5.3	>200	13.3	14.9
32a		$COOC_2H_5$	H	H	H	5	24.1	13.0	132	15.7	>200
32b		$COOC_2H_5$	H	H	H	6	>200	>200	>200	>200	68.3
33a		H	H	H	H	6	>200	5.9	16.8	>200	>200
33b		H	H	H	H	7	>200	4.0	3.3	7.6	>200
34a		$COOC_2H_5$	H	H	H	6	>200	>200	>200	>200	>200
34b		$COOC_2H_5$	H	H	H	9	>200	>200	>200	>200	>200
4							>200	>200	175	>200	92.6
7							63.2	72.5	48.9	57.8	74.6
Cisplatin							17.3	39.2	11.6	13.1	4.2

[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-823), malignant melanoma (A375), renal carcinoma (769-P and OS-RC-2), epidermoid carcinoma of the nasopharynx (KB).
[c] Data represent the mean values of three independent determinations.

colon cancer, Lewis lung cancer, Sarcoma 180, H22 liver cancer with the tumor inhibition rate of 48.6, 42.5, 43.8 and 40.4%. These results indicated that the length of spacer played a vital role in determining antitumor activities in animal models.

In comparison with compound **21b**, methoxy substituent in position-7 (**24a**) and replacement of methyl group in position-1 with hydrogen atom (**18a**) reduced the antitumor activities of the compounds. Of all bivalent β-carbolines with a spacer of 6 methylene units, compound **18c** having no substituents in β-carboline ring showed moderate antitumor activities, while introducing a methyl (**21d**), a pyridin-3-yl (**31b**) and a 3,4,5-trimethoxyphenyl (**33a**) substituent into position-1 of β-carboline nucleus, respectively, increased the antitumor activities of these compounds. These results implied that introducing an appropriate substituent into position-1 of β-carboline nucleus enhanced their antitumor activities.

4. Conclusions

In this study, a series of bivalent β-carbolines with a spacer of three to ten methylene units between the indole nitrogen was designed, synthesized and evaluated as potential antitumor agents. The results demonstrated that compounds **18c**, **21b**, **25a** and **31b**

Table 2
Acute toxic effects of bivalent β-carbolines in mice and antitumor activities of these compounds against mice bearing CT-26 colon cancer, Lewis lung cancer, Sarcoma 180, H22 liver cancer and B16 melanoma.

Compounds	Acute toxicity		Dosage (mg kg^{-1})	% Maximum weight loss	Tumor inhibition rate (%)				
	LD_{50} (mg kg^{-1})	Neurotoxic effect			CT-26 colon cancer	Lewis lung cancer	Sarcoma 180	H22 liver cancer	B16 melanoma
18a	200	–[a]	40	+14.5	39.7	34.2	ND[b]	ND	ND
18b	150	–	30	+10.0	48.6	42.5	43.8	40.4	26.1
18c	200	–	40	+15.0	32.4	37.7	ND	31.2	24.8
21b	200	–	40	+21.5	50.8	40.4	56.2	42.3	29.9
21d	200	–	40	+5.1	39.6	42.1	ND	ND	ND
24a	50	–	10	+7.7	45.5	38.1	ND	ND	ND
26a	220	–	40	+10.2	51.2	49.6	40.6	39.7	20.5
31b	250	–	50	+11.9	50.6	43.2	40.5	35.0	24.3
33a	200	–	40	+7.2	45.8	43.3	ND	ND	ND
4[c]	32.6	–	10	+11.8	ND	ND	35.2	ND	ND
7 (Harmine)[d]	59.0	++	7.5	+8.5	ND	34.1	15.3	ND	ND
CTX			50	+7.7	97.2	97.8	85.6	83.8	86.5

[a] Acute neurotoxic manifestation were denoted by "+" and "–". A "+" represents toxic responses including tremble, twitch, jumping and supination, while "–" means no such reaction.
[b] ND = not determined.
[c] See Ref. [7].
[d] See Ref. [22].

exhibited strong cytotoxic activities with IC_{50} value of lower than 20 μM against four tumor cell lines. Acute toxic assays of the selected bivalent β-carbolines in mice indicated that all compounds caused no obvious neurotoxic effects including tremor, twitch, jumping and supination just like harmine. In addition, nine bivalent β-carbolines were selected for evaluation in vivo against mice bearing CT-26 colon cancer, Lewis lung cancer, Sarcoma 180, H22 liver cancer and B16 melanoma, compounds **18b**, **21b**, **26a** and **31b** exhibited remarkable antitumor efficacies with tumor inhibition rate of over 40% in three animal models, and the CT-26 colon cancer model was more susceptible to all tested compounds than other animal models. Preliminary structure–activity analysis indicated that (1) the spacer length affected antitumor activities, and four to six methylene units were more favorable; (2) the introduction of appropriate substituent into position-1 of β-carboline ring facilitated antitumor potencies.

Although our current investigation described a preliminary structure–activity analysis between bivalent β-carbolines and antitumor activities, while the antitumor efficacies of the studied compounds presented here remained relatively moderate. Moreover, the low water solubility of bivalent β-carbolines in this study limited their in vitro and in vivo efficacies. Therefore, further design and synthesize more potent antitumor agents together with improved water solubility is needed.

Our previous studies indicated that intercalation into DNA is a major cellular event of monovalent β-carbolines [26] and these compounds can pass through cell membrane and penetrate into nucleus quickly resulting in intercalating into DNA in cells [41]. Moreover, previous reports showed that dimerization of various intercalating agents could lead to a very large increase in the DNA binding affinity by bis-intercalation [33,34]. Consequently, such bivalent β-carbolines were expected to exert their tumor cell killing effects through similar mechanisms of action. Further investigation to elucidate the pharmacological mechanisms of this class of compounds are underway in our laboratory, and the data will be published elsewhere.

5. Experimental section

5.1. Reagents and general methods

All reagents were purchased from commercial suppliers and were dried and purified when necessary. Melting points were determined in capillary tubes on an electrothermal PIF YRT-3 apparatus and without correction. ESI-MS spectra were obtained from VG ZAB-HS spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Mercury-Plus 300 spectrometer at 300 MHz and 75 MHz and a Varian INOVA 500NB spectrometer at 500 MHz and 125 MHz, respectively, using TMS as internal standard and $CDCl_3$ or DMSO-d_6 as solvent and chemical shifts (δ) were expressed in ppm. Silica gel F254 were used in analytical thin-layer chromatography (TLC) and silica gel were used in column chromatography respectively.

The preparation of the following monovalent β-carbolines was described earlier: β-carboline **1** [5], 6-bromo-β-carboline **2** [39], 8-bromo-β-carboline **3** [39], 1-methyl-β-carboline **4** [7], ethyl 1-methyl-β-carboline-3-carboxylate **5** [6], 6-bromo-1-methyl-β-carboline **6** [39], 7-methoxy-1-methyl-β-carboline **7** [5], 7-butoxy-1-methyl-β-carboline **9** [2], 1-(3,4,5-trimethoxyphenyl)-β-carboline **16** [12], ethyl 1-(3,4,5-trimethoxyphenyl)-β-carboline-3-carboxylate **17** [12].

5.2. Synthesis of 7-isopropoxy-1-methyl-β-carboline (*8*)

A mixture of 7-methoxy-1-methyl-β-carboline **7** (2.12 g, 10 mmol), acetic acid (100 ml) and 40% hydrobromic acid (50 ml) was refluxed for 12 h. After completion of the reaction as indicated by TLC, the mixture was cooled and poured onto ice. The aqueous mixture, made basic with sodium hydroxide. The precipitate formed was collected by filtration, washed well with water and dried in vacuum to give the intermediate 1-methyl-β-carboline-7-ol **8a**. The intermediate **8a** prepared in these ways could be used directly in the next step without further purification.

A mixture of 1-methyl-β-carboline 7-ol **8a** (1.98 g, 10 mmol) and acetone (60 ml) was stirred for 10 min, and then anhydrous K_2CO_3 (2.76 g, 20 mmol) and isopropyl bromide (20 mmol) were added. The mixture was stirred and refluxed. 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 made acidic with concentrated hydrochloric acid. Upon removal of solvent, the residue was crystallized from acetone to afford yellow solid. The solid was dissolved in water and made basic with sodium bicarbonate, and the aqueous mixture 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 to give white solids

(2.1 g, 87%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.58 (1H, s), 8.29 (1H, d, J = 5.4 Hz), 7.94 (1H, d, J = 8.7 Hz), 7.71 (1H, d, J = 5.4 Hz), 6.95 (1H, d, J = 2.1 Hz), 6.87 (1H, dd, J = 2.1 Hz, 8.7 Hz), 4.58–4.70 (1H, m), 2.81 (3H, s), 1.41 (3H, s), 1.39 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ 158.8, 142.5, 141.8, 138.2, 135.1, 128.0, 123.3, 115.4, 112.6, 110.9, 97.4, 70.3, 22.6, 21.0; ESI-MS m/z: 241 $[M + H]^+$.

5.3. General procedure for the preparation of monovalent β-carbolines (**10**–**14**)

A mixture of L-tryptophan (20.4 g, 100 mmol), acetic acid (300 ml) and the appropriate aldehydes (100 mmol) was refluxed for 3 h, then cooled and adjusted pH to 5 with concentrated ammonium hydroxide, the precipitated product was collected by filtration and washed well with water and dried to provide the intermediates 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid **10a**–**14a**. Further purification was not necessary and used directly for the next steps.

1,2,3,4-Tetrahydro-β-carboline-3-carboxylic acid **10a**–**14a** (10 mmol) was diluted to 100 ml with water, and then heated to boiling. To the hot solution was added potassium dichromate (15 g) and acetic acid (20 ml). The brown suspension was heated for 20 min and then cooled under the tap. After treating the cold solution with sodium sulfite to remove the excess oxidizing agent, the mixture was made definitely alkaline with sodium hydroxide. The solution was extracted exhaustively with ethyl acetate (1000 ml), the extracts dried over anhydrous sodium sulfate, and the solvent removed. The residue was crystallized from anhydrous ethanol, and compounds **10**–**14** were obtained.

5.3.1. 1-Isopropyl-β-carboline (**10**)

Afforded white crystals (1.41 g, 67%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.50 (1H, s), 8.43 (1H, d, J = 5.4 Hz), 8.12 (1H, d, J = 7.8 Hz), 7.82 (1H, d, J = 5.4 Hz), 7.52–7.54 (2H, m), 7.27–7.30 (1H, m), 3.45–3.59 (1H, m), 1.52 (3H, s), 1.49 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ 150.8, 140.7, 138.5, 133.8, 129.1, 128.4, 122.2, 121.9, 120.1, 113.2, 111.9, 32.4, 22.0; ESI-MS m/z: 211 $[M + H]^+$.

5.3.2. 1-(Thiophen-3-yl)-β-carboline (**11**)

Afforded yellow solids (2.1 g, 83%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.77 (1H, s), 8.47 (1H, d, J = 5.1 Hz), 8.11 (1H, d, J = 8.1 Hz), 7.87 (2H, d, J = 5.1 Hz), 7.74–7.75 (1H, m), 7.53–7.55 (2H, m), 7.47–7.49 (1H, m), 7.28–7.33 (1H, m), 7.19–7.22 (1H, m); ^{13}C NMR (75 MHz, DMSO): δ 144.2, 141.7, 138.7, 137.1, 131.4, 130.4, 129.1 (2C), 128.6, 126.4, 122.1, 121.4, 120.6, 114.5, 113.2; ESI-MS m/z: 251$[M + H]^+$.

5.3.3. 1-(2-Chlorophenyl)-β-carboline (**12**)

Afforded white solids (1.38 g, 50%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.55 (1H, d, J = 5.1 Hz), 8.29 (1H, s), 8.14–8.19 (1H, m), 8.00 (1H, dd, J = 0.6 Hz, 5.4 Hz), 7.51–7.62 (3H, m), 7.39–7.46 (3H, m), 7.27–7.33 (1H, m); ^{13}C NMR (75 MHz, DMSO): δ 141.9, 141.4, 138.4, 137.8, 134.4, 133.1, 132.5, 130.9, 130.4, 128.9 (2C), 128.0, 122.4, 121.3, 120.1, 115.1, 112.8; ESI-MS m/z: 279 $[M + H]^+$.

5.3.4. 1-(2-Chloro-5-nitrophenyl)-β-carboline (**13**)

Afforded yellow solids (1.62 g, 50%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.57 (1H, d, J = 5.1 Hz), 8.51 (1H, d, J = 2.7 Hz), 8.25 (1H, dd, J = 2.7 Hz, 8.7 Hz), 8.18 (1H, d, J = 7.8 Hz), 8.08 (1H, d, J = 5.1 Hz), 7.72 (1H, d, J = 8.7 Hz), 7.55–7.61 (1H, m), 7.50 (1H, d, J = 7.8 Hz), 7.32–7.37 (1H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ 147.0, 141.4, 140.3, 139.5, 138.8, 138.6, 134.4, 132.0, 129.5, 129.3, 127.2, 125.5, 122.5, 121.2, 120.3, 115.8, 112.6; ESI-MS m/z: 324 $[M + H]^+$.

5.3.5. 1-(Pyridin-3-yl)-β-carboline (**14**)

Afforded yellow solids (1.97 g, 80%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.11 (1H, s), 9.39 (1H, d, J = 1.8 Hz), 8.65 (1H, dd, J = 1.8 Hz, 4.5 Hz), 8.61 (1H, d, J = 5.4 Hz), 8.38–8.42 (1H, m), 8.18 (1H, d, J = 7.8 Hz), 8.04 (1H, d, J = 5.4 Hz), 7.52–7.61 (3H, m), 7.30–7.36 (1H, m); ^{13}C NMR (75 MHz, DMSO): δ 149.9, 149.5, 141.7, 139.8, 139.2, 136.5, 134.6, 133.8, 130.1, 129.1, 124.5, 122.3, 121.3, 120.4, 115.1, 113.0; ESI-MS m/z: 246 $[M + H]^+$.

5.4. Synthesis of ethyl 1-(pyridin-3-yl)-β-carboline-3-carboxylate (**15**)

A mixture of the **14a** (30 mmol), anhydrous ethanol (500 ml) and $SOCl_2$ (20 ml) was heated at reflux for 4 h, and then evaporated in reduced pressure. The resulting mixture was poured into H_2O (300 ml) and neutralized with sodium hydrogen carbonate. The solution was extracted with ethyl acetate (3 × 300 ml). The organic phase was washed with water and brine, then dried over anhydrous sodium sulfate, filtered and evaporated. The residue was crystallized from ethyl acetate to afford white solid **14b**. Further purification was not necessary and used directly for the next steps.

A suspension of compound **14b** (30 mmol) and sulfur (4.8 g, 150 mmol) in xylene (250 ml) was heated at reflux for 8 h. The solution was cooled and stored at 4 °C for 3 h, and then filtered and washed generously with petroleum ether, the solid was dried and recrystallized from ethyl acetate to afford white crystals (8.2 g, 86%), ^{1}H NMR (300 MHz, $CDCl_3$): δ 11.6 (1H, s), 9.48 (1H, s), 8.91 (1H, s), 8.52–8.54 (1H, m), 8.42–8.45 (1H, m), 8.23 (1H, d, J = 7.8 Hz), 7.58 (2H, d, J = 3.9 Hz), 7.43–7.48 (1H, m), 7.35–7.40 (1H, m), 4.55 (2H, q, J = 7.2 Hz), 1.52 (3H, t, J = 7.2 Hz); ^{13}C NMR (75 MHz, DMSO): δ 165.9, 150.3, 149.6, 142.0, 139.8, 137.7, 136.9, 135.2, 133.9, 130.0, 129.5, 124.6, 122.6, 121.6, 121.2, 117.7, 113.3, 61.5, 15.2; ESI-MS m/z: 318 $[M + H]^+$.

5.5. General procedure for the preparation of bivalent β-carbolines **18**–**34**

To a stirred solution of monovalent β-carbolines **1**–**17** (2.0 mmol) in anhydrous DMF (30 ml) was added 60% NaH (0.4 g, 10 mmol). After stirring for 20 min at room temperature, the appropriate dibromoalkane (1.0 mmol) was added. And then the reaction mixture was stirred at room temperature for 8–20 h. After completion of the reaction as indicated by TLC, the solution was poured into ice-water and extracted with CH_2Cl_2. The combined organic layer was washed with water and brine, dried over anhydrous Na_2SO_4, filtered and evaporated in vacuum. The residue was purified by column chromatography with CH_2Cl_2/MeOH (50:1) to successfully afford the desirable target products.

5.5.1. 9-[4-(β-Carboline-9-yl)butyl]-β-carboline (**18a**)

Starting from β-carboline **1** (2.0 mmol) and 1,4-dibromobutane (1.0 mmol), compound **18a** was obtained as white solid (0.18 g, 46%), mp >270 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.03 (2H, s), 8.36 (2H, d, J = 5.0 Hz), 8.23 (2H, d, J = 8.0 Hz), 8.09 (2H, d, J = 5.0 Hz), 7.65 (2H, d, J = 8.5 Hz), 7.56 (2H, t, J = 7.5 Hz), 7.25 (2H, t, J = 7.5 Hz), 4.49 (4H, t, J = 7.5 Hz), 1.87–1.92 (4H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 141.0, 139.0, 136.4, 131.8, 128.5, 122.0, 121.1, 119.8, 114.7, 109.3, 43.0, 27.0. ESI-MS m/z: 391.3 $[M + H]^+$.

5.5.2. 9-[5-(β-Carboline-9-yl)pentyl]-β-carboline (**18b**)

Starting from β-carboline **1** (2.0 mmol) and 1,5-dibromopentane (1.0 mmol), compound **18b** was obtained as white solid (0.21 g, 54%), mp 191–192 °C (lit. [37], mp 174 °C). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.82 (2H, s), 8.47 (2H, d, J = 5.0 Hz), 8.13 (2H, d, J = 7.5 Hz), 7.95 (2H, d, J = 5.0 Hz), 7.56 (2H, t, J = 7.5 Hz), 7.35 (2H, d, J = 8.0 Hz), 7.28 (2H, t, J = 7.5 Hz), 4.30 (4H, t, J = 7.0 Hz), 1.88–1.94 (4H, m), 1.42–1.47 (2H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 141.0, 138.9, 136.3, 131.8, 128.3, 128.2, 121.8, 121.0, 119.6, 114.5, 109.2, 43.0, 28.9, 25.1; ESI-MS m/z: 404.9 $[M + H]^+$.

*5.5.3. 9-[6-(β-Carboline-9-yl)hexyl]-β-carboline (**18c**)*

Starting from β-carboline **1** (2.0 mmol) and 1,6-dibromohexane (1.0 mmol), compound **18c** was obtained as white solid (0.22 g, 52%), mp 114–115 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.83 (2H, s), 8.45 (2H, d, J = 5.0 Hz), 8.12 (2H, d, J = 8.0 Hz), 7.94 (2H, d, J = 5.0 Hz), 7.53–7.56 (2H, m), 7.36 (2H, d, J = 8.0 Hz), 7.26–7.28 (2H, m), 4.31 (4H, t, J = 7.5 Hz), 1.82–1.87 (4H, m), 1.35–1.38 (4H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ 140.9, 138.8, 136.3, 131.9, 128.4, 128.2, 121.8, 120.9, 119.4, 114.4, 109.2, 43.0, 28.9, 26.9; ESI-MS m/z: 418.9 $[M + H]^+$.

*5.5.4. 9-[7-(β-Carboline-9-yl)heptyl]-β-carboline (**18d**)*

Starting from β-carboline **1** (2.0 mmol) and 1,7-dibromoheptane (1.0 mmol), compound **18d** was obtained as white solid (0.22 g, 51%), mp 151–152 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.85 (2H, s), 8.46 (2H, d, J = 5.0 Hz), 8.13 (2H, d, J = 7.5 Hz), 7.95 (2H, d, J = 5.0 Hz), 7.55–7.57 (2H, m), 7.41 (2H, d, J = 8.0 Hz), 7.27–7.28 (2H, m), 4.32 (4H, t, J = 7.5 Hz), 1.81–1.88 (4H, m), 1.28–1.37 (6H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 141.0, 138.7, 136.3, 131.9, 128.2, 121.8, 120.9, 119.4, 114.4, 109.3, 43.1, 29.0, 28.9, 26.9; ESI-MS m/z: 433.3 $[M + H]^+$.

*5.5.5. 9-[8-(β-Carboline-9-yl)octyl]-β-carboline (**18e**)*

Starting from β-carboline **1** (2.0 mmol) and 1,8-dibromooctane (1.0 mmol), compound **18e** was obtained as white solid (0.20 g, 44%), mp 180–181 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.87 (2H, s), 8.45 (2H, d, J = 5.0 Hz), 8.13 (2H, d, J = 8.0 Hz), 7.94 (2H, d, J = 5.0 Hz), 7.55–7.59 (2H, m), 7.43 (2H, d, J = 8.5 Hz), 7.26–7.28 (2H, m), 4.32 (4H, t, J = 7.0 Hz), 1.81–1.87 (4H, m), 1.24–1.32 (8H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 141.0, 138.7, 136.4, 131.9, 128.1 (4C), 121.7, 120.9, 119.4, 114.4, 109.3, 43.1, 29.0, 28.9, 26.9; ESI-MS m/z: 447.1 $[M + H]^+$.

*5.5.6. 6-Bromo-9-[4-(6-bromo-β-carboline-9-yl)butyl]-β-carboline (**19**)*

Starting from 6-bromo-β-carboline **2** (2.0 mmol) and 1,4-dibromobutane (1.0 mmol), compound **19** was obtained as white solid (0.28 g, 51%), mp 316–317 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.78 (2H, s), 8.46 (2H, d, J = 5.1 Hz), 8.20 (2H, d, J = 1.8 Hz), 7.87 (2H, dd, J = 5.1 Hz, 0.9 Hz), 7.55 (2H, dd, J = 8.7 Hz, 1.8 Hz), 7.07 (2H, d, J = 8.7 Hz), 4.28–4.32 (4H, m), 1.92–1.98 (4H, m); ESI-MS m/z: 549.1 $[M + H]^+$.

*5.5.7. 8-Bromo-9-[6-(8-bromo-β-carboline-9-yl)hexyl]-β-carboline (**20**)*

Starting from 8-bromo-β-carboline **3** (2.0 mmol) and 1,6-dibromohexane (1.0 mmol), compound **20** was obtained as yellow oil (0.21 g, 37%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.89 (2H, s), 8.49 (2H, d, J = 5.5 Hz), 8.09 (2H, dd, J = 1.0 Hz, 7.5 Hz), 7.91 (2H, d, J = 5.5 Hz), 7.72 (2H, dd, J = 1.0 Hz, 7.5 Hz), 7.11 (2H, t, J = 7.5 Hz), 4.79 (4H, t, J = 7.5 Hz), 1.89–1.93 (4H, m), 1.46–1.49 (4H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 139.5, 137.3, 133.7, 132.6, 127.8, 124.4, 120.9, 120.7, 114.2, 103.4, 44.3, 30.7, 26.5; ESI-MS m/z: 577.8 $[M + H]^+$.

*5.5.8. 1-Methyl-9-[3-(1-methyl-β-carboline-9-yl)propyl]-β-carboline (**21a**)*

Starting from 1-methyl-β-carboline **4** (2.0 mmol) and 1,3-dibromopropane (1.0 mmol), compound **21a** was obtained as white solid (0.18 g, 46%), mp 239–240 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.31 (2H, d, J = 5.0 Hz), 8.09 (2H, d, J = 8.0 Hz), 7.81 (2H, d, J = 5.0 Hz), 7.49–7.51 (2H, m), 7.24–7.27 (2H, m), 7.20 (2H, d, J = 8.0 Hz), 4.54 (4H, t, J = 7.5 Hz), 2.74 (6H, s), 2.31–2.37 (2H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 141.0, 140.8, 138.4, 134.8, 129.1, 128.3, 121.6121.4, 119.9, 112.9, 109.1, 41.9, 31.3, 23.1; ESI-MS m/z: 404.9 $[M + H]^+$.

*5.5.9. 1-Methyl-9-[4-(1-methyl-β-carboline-9-yl)butyl]-β-carboline (**21b**)*

Starting from 1-methyl-β-carboline **4** (2.0 mmol) and 1,4-dibromobutane (1.0 mmol), compound **21b** was obtained as white solid (0.26 g, 62%), mp 232–233 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.35 (2H, d, J = 3.9 Hz), 8.13 (2H, d, J = 5.7 Hz), 7.85 (2H, d, J = 3.9 Hz), 7.55–7.57 (2H, m), 7.28–7.35 (4H, m), 4.53 (4H, t, J = 6.9 Hz), 2.97 (6H, s), 1.94–1.97 (4H, m). ^{13}C NMR (75 MHz, $CDCl_3$): δ 141.4, 141.0, 138.3, 135.0, 129.3, 128.3, 121.6, 121.4, 119.8, 113.0, 109.5, 44.4, 28.1, 23.5. ESI-MS m/z: 419.3 $[M + H]^+$.

*5.5.10. 1-Methyl-9-[5-(1-methyl-β-carboline-9-yl)pentyl]-β-carboline (**21c**)*

Starting from 1-methyl-β-carboline **4** (2.0 mmol) and 1,5-dibromopentane (1.0 mmol), compound **21c** was obtained as white solid (0.17 g, 40%), mp 213–214 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.33 (2H, d, J = 5.0 Hz), 8.11 (2H, d, J = 8.0 Hz), 7.83 (2H, d, J = 5.0 Hz), 7.53–7.55 (2H, m), 7.36 (2H, d, J = 8.0 Hz), 7.27 (2H, d, J = 8.0 Hz), 4.48 (4H, t, J = 7.5 Hz), 2.99 (6H, s), 1.83–1.89 (4H, m), 1.44–1.49 (2H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 141.3, 141.0, 138.1, 134.9, 129.0, 128.1, 121.4, 121.2, 119.6, 112.8, 109.5, 44.4, 30.6, 24.3, 23.5; ESI-MS m/z: 433.3 $[M + H]^+$.

*5.5.11. 1-Methyl-9-[6-(1-methyl-β-carboline-9-yl)hexyl]-β-carboline (**21d**)*

Starting from 1-methyl-β-carboline **4** (2.0 mmol) and 1,6-dibromohexane (1.0 mmol), compound **21d** was obtained as white solid (0.26 g, 58%), mp 220–221 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.19 (4H, d, J = 4.5 Hz), 7.95 (2H, d, J = 5.0 Hz), 7.64 (2H, d, J = 8.5 Hz), 7.54–7.57 (2H, m), 7.22–7.25 (2H, m), 4.54 (4H, t, J = 8.0 Hz), 2.93 (6H, s), 1.69–1.74 (4H, m), 1.36–1.43 (4H, m). ^{13}C NMR (125 MHz, $CDCl_3$): δ 141.6, 141.0, 137.8, 135.0, 129.2, 128.2, 121.6, 121.3, 119.7, 113.0, 109.6, 44.6, 30.8, 26.8, 23.4. ESI-MS m/z: 447.4 $[M + H]^+$.

*5.5.12. 1-Methyl-9-[7-(1-methyl-β-carboline-9-yl)heptyl]-β-carboline (**21e**)*

Starting from 1-methyl-β-carboline **4** (2.0 mmol) and 1,7-dibromoheptane (1.0 mmol), compound **21e** was obtained as white solid (0.12 g, 26%), mp 189–190 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.31 (2H, d, J = 5.5 Hz), 8.10 (2H, d, J = 7.5 Hz), 7.83 (2H, d, J = 5.5 Hz), 7.53–7.55 (2H, m), 7.41 (2H, d, J = 8.0 Hz), 7.27 (2H, t, J = 8.0 Hz), 4.48 (4H, t, J = 8.0 Hz), 3.02 (6H, s), 1.77–1.84 (4H, m), 1.36–1.42 (6H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ 141.4, 141.1, 138.0, 135.0, 129.0, 128.0, 121.4, 121.3, 119.5, 112.9, 109.5, 44.7, 30.6, 29.1, 26.7, 23.5; ESI-MS m/z: 460.9 $[M + H]^+$.

*5.5.13. 1-Methyl-9-[8-(1-methyl-β-carboline-9-yl)octyl]-β-carboline (**21f**)*

Starting from 1-methyl-β-carboline **4** (2.0 mmol) and 1,8-dibromooctane (1.0 mmol), compound **21f** was obtained as white solid (0.19 g, 41%), mp 141–142 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.19 (2H, d, J = 5.0 Hz), 8.17 (2H, d, J = 8.0 Hz), 7.93–7.94 (2H, d, J = 5.0 Hz), 7.63 (2H, d, J = 8.0 Hz), 7.54–7.57 (2H, m), 7.21–7.24 (2H, m), 4.52 (4H, t, J = 7.5 Hz), 2.94 (6H, s), 1.65–1.69 (4H, m), 1.22–1.31 (8H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 141.3, 141.0, 137.7, 134.9, 128.8, 127.9, 121.3, 121.1, 119.4, 112.7, 109.5, 44.6, 30.5, 29.0, 26.6, 23.3. ESI-MS m/z: 475.4 $[M + H]^+$.

*5.5.14. 1-Methyl-9-[9-(1-methyl-β-carboline-9-yl)nonyl]-β-carboline (**21g**)*

Starting from 1-methyl-β-carboline **4** (2.0 mmol) and 1,9-dibromononane (1.0 mmol), compound **21g** was obtained as white solid (0.26 g, 53%), mp 155–156 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.32 (2H, d, J = 5.0 Hz), 8.11 (2H, d, J = 7.5 Hz), 7.83 (2H, d,

J = 5.0 Hz), 7.54–7.57 (2H, m), 7.44 (2H, d, J = 8.5 Hz), 7.26–7.28 (2H, m), 4.51 (4H, t, J = 7.5 Hz), 3.04 (6H, s), 1.78–1.85 (4H, m), 1.25–1.40 (10H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 141.4, 141.1, 137.9, 135.0, 128.9, 127.9, 121.4, 121.2, 119.5, 112.8, 109.6, 44.8, 30.7, 29.1, 26.8, 23.5; ESI-MS m/z: 488.9 $[M + H]^+$.

*5.5.15. 1-Methyl-9-[10-(1-methyl-β-carboline-9-yl)decyl]-β-carboline (**21h**)*

Starting from 1-methyl-β-carboline **4** (2.0 mmol) and 1,10-dibromodecane (1.0 mmol), compound **21h** was obtained as white solid (0.28 g, 56%), mp 152–154 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.18–8.20 (4H, m), 7.94 (2H, d, J = 4.5 Hz), 7.66 (2H, d, J = 8.5 Hz), 7.55–7.58 (2H, m), 7.21–7.24 (2H, m), 4.56 (4H, t, J = 7.5 Hz), 2.96 (6H, s), 1.68–1.73 (4H, m), 1.15–1.32 (12H, m), ^{13}C NMR (125 MHz, $CDCl_3$): δ 141.5, 141.2, 137.9, 135.1, 129.0, 128.0, 121.4, 121.3, 119.5, 112.9, 109.7, 44.8, 30.8, 29.3, 29.2, 26.8, 23.5. ESI-MS m/z: 503.4 $[M + H]^+$.

*5.5.16. 3-Ethoxycarbonyl-1-methyl-9-[6-(3-ethoxycarbonyl-1-methyl-β-carboline-9-yl)hexyl]-β-carboline (**22**)*

Starting from ethyl 1-methyl-β-carboline-3-carboxylate **5** (2.0 mmol) and 1,6-dibromohexane (1.0 mmol), compound **22** was obtained as white solid (0.32 g, 54%), mp 268–269 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.73 (2H, s), 8.15 (2H, d, J = 7.8 Hz), 7.55–7.60 (2H, m), 7.29–7.42 (4H, m), 4.48–4.56 (8H, m), 2.99 (6H, s), 1.77–1.87 (4H, m), 1.49 (6H, t, J = 7.2 Hz), 1.38–1.44 (4H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ 166.2, 141.9, 141.4, 137.1, 136.7, 129.1, 128.7, 121.9, 121.8, 120.8, 116.5, 110.2, 61.7, 45.1, 31.2, 27.1, 24.5, 14.9; ESI-MS m/z: 590.1 $[M]^+$.

*5.5.17. 6-Bromo-1-methyl-9-[5-(6-bromo-1-methyl-β-carboline-9-yl)pentyl]-β-carboline (**23a**)*

Starting from 6-bromo-1-methyl-β-carboline **6** (2.0 mmol) and 1,5-dibromopentane (1.0 mmol), compound **23a** was obtained as white solid (0.21 g, 35%), mp 208–209 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.33 (2H, d, J = 5.4 Hz), 8.20 (2H, d, J = 1.8 Hz), 7.76 (2H, d, J = 5.4 Hz), 7.58 (2H, dd, J = 1.8 Hz, 8.7 Hz), 7.18 (2H, d, J = 8.7 Hz), 4.45 (4H, t, J = 7.5 Hz), 2.98 (6H, s), 1.78–1.88 (4H, m), 1.32–1.42 (2H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ 141.5, 140.0, 138.6, 135.3, 131.0, 128.1, 124.4, 123.0, 113.1, 112.6, 111.3, 44.9, 31.1, 24.8, 24.0; ESI-MS m/z: 605.5 $[M]^+$.

*5.5.18. 6-Bromo-1-methyl-9-[6-(6-bromo-1-methyl-β-carboline-9-yl)hexyl]-β-carboline (**23b**)*

Starting from 6-bromo-1-methyl-β-carboline **6** (2.0 mmol) and 1,6-dibromohexane (1.0 mmol), compound **23b** was obtained as white solid (0.41 g, 67%), mp >270 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.33 (2H, d, J = 5.0 Hz), 8.22 (2H, d, J = 2.0 Hz), 7.77 (2H, d, J = 5.0 Hz), 7.62 (2H, dd, J = 8.5, 2.0 Hz), 7.24 (2H, d, J = 8.5 Hz), 4.47 (4H, t, J = 7.5 Hz), 2.99 (6H, s), 1.76–1.83 (4H, m), 1.35–1.41 (4H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 141.5, 140.0, 138.5, 135.3, 130.8, 128.0, 124.2, 123.0, 113.0, 112.4, 111.1, 44.8, 30.7, 26.8, 23.5; ESI-MS m/z: 604.0 $[M]^+$.

*5.5.19. 7-Methoxy-1-methyl-9-[4-(7-methoxy-1-methyl-β-carboline-9-yl)butyl]-β-carboline (**24a**)*

Starting from 7-methoxy-1-methyl-β-carboline **7** (2.0 mmol) and 1,4-dibromobutane (1.0 mmol), compound **24a** was obtained as white solid (0.23 g, 49%), mp >270 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.27 (2H, d, J = 5.1 Hz), 7.93 (2H, d, J = 8.4 Hz), 7.70 (2H, d, J = 5.1 Hz), 6.86 (2H, dd, J = 8.4, 2.1 Hz), 6.70 (2H, d, J = 2.1 Hz), 4.48 (4H, t, J = 7.2 Hz), 3.87 (6H, s), 2.93 (6H, s), 1.90–1.92 (4H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.9, 142.9, 140.3, 138.6, 135.2, 129.6, 122.5, 115.3, 112.3, 108.7, 93.4, 55.7, 44.3, 27.7, 23.4. ESI-MS m/z: 479.6 $[M + H]^+$.

*5.5.20. 7-Methoxy-1-methyl-9-[5-(7-methoxy-1-methyl-β-carboline-9-yl)pentyl]-β-carboline (**24b**)*

Starting from 7-methoxy-1-methyl-β-carboline **7** (2.0 mmol) and 1,5-dibromopentane (1.0 mmol), compound **24b** was obtained as white solid (0.26 g, 52%), mp 180–181 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.29 (2H, d, J = 5.5 Hz), 7.97 (2H, d, J = 8.5 Hz), 7.73 (2H, d, J = 5.5 Hz), 6.88 (2H, dd, J = 8.5, 2.0 Hz), 6.77 (2H, d, J = 2.0 Hz), 4.43 (4H, t, J = 7.5 Hz), 3.88 (6H, s), 2.96 (6H, s), 1.83–1.89 (4H, m), 1.43–1.49 (4H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 160.8, 142.9, 140.3, 138.3, 135.1, 129.4, 122.3, 115.1, 112.2, 108.5, 93.4, 55.6, 44.4, 30.5, 24.3, 23.4; ESI-MS m/z: 492.9 $[M + H]^+$.

*5.5.21. 7-Methoxy-1-methyl-9-[6-(7-methoxy-1-methyl-β-carboline-9-yl)hexyl]-β-carboline (**24c**)*

Starting from 7-methoxy-1-methyl-β-carboline **7** (2.0 mmol) and 1,6-dibromohexane (1.0 mmol), compound **24c** was obtained as yellow oil (0.23 g, 45%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.28 (2H, d, J = 5.0 Hz), 7.96 (2H, d, J = 8.5 Hz), 7.72 (2H, d, J = 5.5 Hz), 6.88 (2H, dd, J = 8.5, 2.0 Hz), 6.81 (2H, d, J = 2.0 Hz), 4.43 (4H, t, J = 7.5 Hz), 3.92 (6H, s), 2.98 (6H, s), 1.79–1.82 (4H, m), 1.41–1.44 (4H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 160.8, 143.0, 140.4, 138.2, 135.2, 129.4, 122.4, 115.2, 112.2, 108.4, 93.5, 55.6, 44.6, 30.6, 26.8, 23.3; ESI-MS m/z: 507.1 $[M + H]^+$.

*5.5.22. 7-Methoxy-1-methyl-9-[7-(7-methoxy-1-methyl-β-carboline-9-yl)heptyl]-β-carboline (**24d**)*

Starting from 7-methoxy-1-methyl-β-carboline **7** (2.0 mmol) and 1,7-dibromoheptane (1.0 mmol), compound **24d** was obtained as white solid (0.27 g, 53%), mp 199–200 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.28 (2H, d, J = 5.0 Hz), 7.96 (2H, d, J = 8.5 Hz), 7.72 (2H, d, J = 5.5 Hz), 6.87 (2H, dd, J = 8.5, 2.0 Hz), 6.81 (2H, d, J = 2.0 Hz), 4.41 (4H, t, J = 7.5 Hz), 3.91 (6H, s), 2.98 (6H, s), 1.78–1.81 (4H, m), 1.36–1.44 (6H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 160.7, 142.9, 140.4, 138.3, 135.2, 129.3, 122.3, 115.2, 112.2, 108.4, 93.4, 55.6, 44.7, 30.5, 29.2, 26.8, 23.4; ESI-MS m/z: 520.9 $[M + H]^+$.

*5.5.23. 7-Methoxy-1-methyl-9-[8-(7-methoxy-1-methyl-β-carboline-9-yl)octyl]-β-carboline (**24e**)*

Starting from 7-methoxy-1-methyl-β-carboline **7** (2.0 mmol) and 1,8-dibromooctane (1.0 mmol), compound **24e** was obtained as white solid (0.38 g, 71%), mp 181–182 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.27 (2H, d, J = 5.0 Hz), 7.97 (2H, d, J = 8.5 Hz), 7.76 (2H, d, J = 5.0 Hz), 6.89 (2H, dd, J = 8.5, 2.0 Hz), 6.83 (2H, d, J = 2.0 Hz), 4.44 (4H, t, J = 7.5 Hz), 3.93 (6H, s), 3.04 (6H, s), 1.76–1.82 (4H, m), 1.31–1.39 (8H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 160.8, 143.1, 140.3, 137.9, 135.2, 129.4, 122.3, 115.2, 112.2, 108.5, 93.5, 55.6, 44.8, 30.4, 29.2, 26.8, 23.2; ESI-MS m/z: 535.0 $[M + H]^+$.

*5.5.24. 7-Butoxy-1-methyl-9-[4-(7-butoxy-1-methyl-β-carboline-9-yl)butyl]-β-carboline (**25a**)*

Starting from 7-butoxy-1-methyl-β-carboline **9** (2.0 mmol) and 1,4-dibromobutane (1.0 mmol), compound **25a** was obtained as white solid (0.22 g, 62%), mp 193–194 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.26 (2H, d, J = 5.1 Hz), 7.92 (2H, d, J = 8.4 Hz), 7.68 (2H, d, J = = 5.1 Hz), 6.85 (2H, dd, J = 1.8 Hz, 8.4 Hz), 6.71 (2H, d, J = 1.8 Hz), 4.41 (4H, t, J = 6.9 Hz), 4.01 (4H, t, J = 6.6 Hz), 2.91 (6H, s), 1.79–1.89 (8H, m), 1.48–1.61 (4H, m), 1.02 (6H, t, J = 7.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.6, 143.1, 140.3, 138.6, 135.3, 129.8, 122.6, 115.3, 112.5, 109.4, 94.3, 68.5, 44.6, 31.8, 28.2, 23.7, 19.7, 14.3; ESI-MS m/z: 563.4 $[M + H]^+$.

*5.5.25. 7-Butoxy-1-methyl-9-[6-(7-butoxy-1-methyl-β-carboline-9-yl)hexyl]-β-carboline (**25b**)*

Starting from 7-butoxy-1-methyl-β-carboline **9** (2.0 mmol) and 1,6-dibromohexane (1.0 mmol), compound **25b** was obtained as

white solid (0.23 g, 65%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.27 (2H, d, J = = 5.0 Hz), 7.95 (2H, d, J = 8.5 Hz), 7.71 (2H, d, J = 5.0 Hz), 6.87 (2H, dd, J = 8.5, 2.0 Hz), 6.81 (2H, d, J = 2.0 Hz), 4.42 (4H, t, J = 7.5 Hz), 4.06 (4H, t, J = 7.0 Hz), 2.97 (6H, s), 1.79–1.83 (8H, m), 1.49–1.57 (4H, m), 1.41–1.44 (4H, m), 0.99 (6H, t, J = 6.0 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 160.3, 143.0, 140.3, 138.2, 135.2, 129.4, 122.2, 115.1, 121.1, 108.8, 94.2, 68.1, 44.6, 31.3, 30.6, 26.8, 23.3, 19.2, 13.8; ESI-MS m/z: 590.4 $[M + H]^+$.

*5.5.26. 7-Isoproxy-1-methyl-9-[6-(7-isoproxy-1-methyl-β-carboline-9-yl)hexyl]-β-carboline (**26a**)*

Starting from 7-isopropoxy-1-methyl-β-carboline **8** (2.0 mmol) and 1,6-dibromohexane (1.0 mmol), compound **26a** was obtained as white solid (0.25 g, 71%), mp 155–156 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.25 (2H, d, J = 5.4 Hz), 7.95 (2H, d, J = 8.7 Hz), 7.71 (2H, d, J = 5.4 Hz), 6.86 (2H, dd, J = 1.8 Hz, 8.7 Hz), 6.82 (2H, d, J = 1.8 Hz), 4.62–4.74 (2H, m), 4.41 (4H, t, J = 7.5 Hz), 2.98 (6H, s), 1.75–1.85 (4H, m), 1.39–1.41 (16H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.2, 143.3, 140.5, 138.3, 135.4, 129.7, 122.6, 115.4, 112.5, 110.1, 96.4, 70.9, 45.0, 31.0, 27.3, 23.7, 22.6; ESI-MS m/z: 562.5 $[M + H]^+$.

*5.5.27. 7-Isoproxy-1-methyl-9-[9-(7-isoproxy-1-methyl-β-carboline-9-yl)nonyl]-β-carboline (**26b**)*

Starting from 7-isopropoxy-1-methyl-β-carboline **8** (2.0 mmol) and 9-dibromononane (1.0 mmol), compound **26b** was obtained as white solid (0.4 g, 67%), mp 161–162 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.25 (2H, d, J = 5.1 Hz), 7.94 (2H, d, J = 9.3 Hz), 7.70 (2H, d, J = 5.1 Hz), 6.84–6.87 (4H, m), 4.63–4.75 (2H, m), 4.41 (4H, t, J = 7.8 Hz), 3.00 (6H, s), 1.75–1.85 (4H, m), 1.26–1.45 (22H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.1, 143.2, 140.6, 138.4, 135.5, 129.5, 122.5, 115.4, 112.4, 110.0, 96.3, 70.8, 45.1, 31.0, 29.8, 29.6, 27.3, 23.8, 22.5; ESI-MS m/z: 605.5 $[M + H]^+$.

*5.5.28. 1-Isopropyl-9-[6-(1-isopropyl-β-carboline-9-yl)hexyl]-β-carboline (**27a**)*

Starting from 1-isopropyl-β-carboline **10** (2.0 mmol) and 1,6-dibromohexane (1.0 mmol), compound **27a** was obtained as white solid (0.14 g, 28%), mp 241–242 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.41 (2H, d, J = 4.5 Hz), 8.09 (2H, d, J = = 7.5 Hz), 7.82 (2H, d, J = 4.5 Hz), 7.51–7.56 (2H, m), 7.37 (2H, d, J = 8.4 Hz), 7.22–7.27 (2H, m), 4.46 (4H, t, J = 7.5 Hz), 3.62–3.73 (2H, m), 1.76–1.86 (4H, m), 1.48 (6H, s), 1.46 (6H, s), 1.22–1.34 (4H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ 150.6, 142.0, 138.5, 133.5, 129.9, 128.3, 121.7, 121.6, 119.8, 112.7, 109.8, 45.5, 31.7, 30.7, 27.2, 23.1, 23.0; ESI-MS m/z: 503.4 $[M + H]^+$.

*5.5.29. 1-Isopropyl-9-[10-(1-isopropyl-β-carboline-9-yl)decyl]-β-carboline (**27b**)*

Starting from 1-isopropyl-β-carboline **10** (2.0 mmol) and 1,10-dibromodecane (1.0 mmol), compound **27b** was obtained as yellow oil (0.18 g, 36%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.42 (2H, d, J = 5.1 Hz), 8.09 (2H, d, J = 7.8 Hz), 7.81 (2H, 5.1 Hz), 7.53–7.58 (2H, m), 7.43 (2H, d, J = 8.4 Hz), 7.22–7.27 (2H, m), 4.48 (4H, t, J = 7.8 Hz), 3.68–3.81 (2H, m), 1.79–1.89 (4H, m), 1.52 (6H, s), 1.49 (6H, s), 1.28–1.42 (12H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ 150.7, 142.0, 138.4, 133.6, 129.9, 128.2, 121.7, 121.5, 119.7, 112.7, 109.9, 45.8, 31.7, 30.6, 29.7, 29.6, 27.3, 23.1; ESI-MS m/z: 559.5 $[M + H]^+$.

*5.5.30. 1-(Thiophen-3-yl)-9-[6-[1-(thiophen-3-yl) -β-carboline-9-yl]hexyl]-β-carboline (**28a**)*

Starting from 1-(thiophen-3-yl)-β-carboline **11** (2.0 mmol) and 1,6-dibromohexane (1.0 mmol), compound **28a** was obtained as white solid (0.37 g, 64%), mp 198–199 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.50 (2H, d, J = 5.1 Hz), 8.15 (2H, d, J = 7.8 Hz), 7.96 (2H, d, J = 5.1 Hz), 7.55–7.60 (2H, m), 7.27–7.43 (6H, m), 7.19–7.21 (2H, m), 7.08–7.10 (2H, m), 4.03 (4H, t, J = 7.8 Hz), 1.21–1.30 (4H, m), 0.69–0.74 (4H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ 142.3, 141.3, 138.7, 137.3, 134.9, 131.0, 128.8, 128.5, 127.2, 126.9, 121.9, 121.5, 120.3, 114.4, 110.5, 44.5, 29.2, 26.5; ESI-MS m/z: 583.4 $[M + H]^+$.

*5.5.31. 1-(Thiophen-3-yl)-9-[8-[1-(thiophen-3-yl) -β-carboline-9-yl]octyl]-β-carboline (**28b**)*

Starting from 1-(thiophen-3-yl)-β-carboline **11** (2.0 mmol) and 1,8-dibromooctane (1.0 mmol), compound **28b** was obtained as yellow solid (0.43 g, 70%), mp 161–162 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.50 (2H, d, J = 5.1 Hz), 8.15 (2H, d, J = 7.2 Hz), 7.96 (2H, d, J = 5.1 Hz), 7.56–7.61 (2H, m), 7.41–7.44 (4H, m), 7.22–7.32 (4H, m), 7.08–7.11 (2H, m), 4.09 (4H, t, J = 7.5 Hz), 1.32–1.42 (4H, m), 0.80–0.88 (8H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ 142.4, 141.3, 138.6, 137.3, 135.0, 131.0, 128.8, 128.4, 127.2, 126.8, 121.9, 121.5, 120.2, 114.4, 110.5, 44.7, 29.3, 29.2, 26.9; ESI-MS m/z: 611.4 $[M + H]^+$.

*5.5.32. 1-(2-Chlorophenyl)-9-[6-[1-(2-chlorohenyl)-β-carboline-9-yl]hexyl]-β-carboline (**29a**)*

Starting from 1-(2-chlorophenyl)-β-carboline **12** (2.0 mmol) and 1,6-dibromohexane (1.0 mmol), compound **29a** was obtained as white solid (0.57 g, 90%), mp 210–211 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.52 (2H, d, J = 5.1 Hz), 8.18 (2H, 7.8 Hz), 8.02 (2H, d, J = 5.1 Hz), 7.54–7.59 (2H, m), 7.43–7.49 (4H, m), 7.27–7.39 (8H, m), 3.67–3.89 (4H, m), 1.17–1.39 (4H, m), 0.56–0.69 (4H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ 141.8, 141.1, 139.0, 138.5, 134.4, 134.3, 131.7, 130.2, 130.1, 129.6, 128.7, 126.9, 121.9, 121.4, 120.1, 114.5, 110.0, 44.3, 29.4, 26.6; ESI-MS m/z: 639.3 $[M]^+$.

*5.5.33. 1-(2-Chlorophenyl)-9-[7-[1-(2-chlorohenyl)-β-carboline-9-yl]heptyl]-β-carboline (**29b**)*

Starting from 1-(2-chlorophenyl)-β-carboline **12** (2.0 mmol) and 1,7-dibromoheptane (1.0 mmol), compound **29b** was obtained as yellow oil (0.49 g, 75%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.53 (2H, d, J = 5.1 Hz), 8.18 (2H, d, J = 7.8 Hz), 8.02 (2H, d, J = 5.1 Hz), 7.55–7.60 (2H, m), 7.46–7.52 (4H, m), 7.27–7.39 (8H, m), 3.70–3.93 (4H, m), 1.32–1.42 (4H, m), 0.56–0.85 (6H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ 141.9, 141.1, 139.0, 138.3, 134.4, 134.3, 131.7, 130.2, 130.1, 129.6, 128.7, 126.9, 121.9, 121.4, 120.1, 114.6, 110.1, 44.4, 29.4, 28.9, 26.9; ESI-MS m/z: 653.3 $[M]^+$.

*5.5.34. 1-(2-Chloro-5-nitrophenyl)-9-[6-[1-(2-chloro-5-nitrophenyl)-β-carboline-9-yl]-hexyl]-β-carboline (**30a**)*

Starting from 1-(2-chloro-5-nitrophenyl)-β-carboline **13** (2.0 mmol) and 1,6-dibromohexane (1.0 mmol), compound **30a** was obtained as yellow oil (0.40 g, 55%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.52–8.54 (2H, m), 8.38–8.40 (2H, m), 8.16–8.27 (4H, m), 8.05–8.08 (2H, m), 7.57–7.67 (4H, m), 7.29–7.37 (4H, m), 3.78–3.91 (2H, m), 3.52–3.67 (2H, m), 1.09–1.27 (4H, m), 0.44–0.65 (4H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ 146.3, 141.9, 141.5, 140.4, 138.6, 138.3, 134.1, 131.0, 130.7, 129.2, 126.5, 124.7, 122.0, 121.3, 120.5, 115.4, 110.2, 44.3, 29.5, 26.8; ESI-MS m/z: 729.3 $[M]^+$.

*5.5.35. 1-(2-Chloro-5-nitrophenyl)-9-[9-[1-(2-chloro-5-nitrophenyl)-β-carboline-9-yl]-nonyl]-β-carboline (**30b**)*

Starting from 1-(2-chloro-5-nitrophenyl)-β-carboline **13** (2.0 mmol) and 1,9-dibromononane (1.0 mmol), compound **30b** was obtained as yellow oil (0.32 g, 41%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.52–8.54 (2H, m), 8.46 (2H, d, J = 2.7 Hz), 8.26–8.32 (2H, m), 8.19 (2H, d, J = 7.8 Hz), 8.07 (2H, dd, J = 1.8 Hz, 5.1 Hz), 7.68–7.73 (2H, m), 7.58–7.64 (2H, m), 7.41–7.45 (2H, m), 7.29–7.35 (2H, m), 3.91–4.02 (2H, m), 3.60–3.75 (2H, m), 1.24–1.34 (4H, m), 0.68–0.90 (10H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ 146.4, 142.0, 141.5, 140.5, 138.5, 138.4, 134.2, 130.8, 130.7, 129.1, 126.6, 124.8, 121.9, 121.2, 120.4, 115.3, 110.2, 44.6, 29.6, 29.3, 27.1; ESI-MS m/z: 771.3 $[M]^+$.

*5.5.36. 1-(Pyridin-3-yl)-9-[4-[1-(pyridine-3-yl)-β-carboline-9-yl] butyl]-β-carboline (**31a**)*

Starting from 1-(pyridin-3-yl)-β-carboline **14** (2.0 mmol) and 1,4-dibromobutane (1.0 mmol), compound **31a** was obtained as yellow solid (0.26 g, 46%), mp 224–225 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.60–8.62 (4H, m), 8.50 (2H, d, J = 5.1 Hz), 8.17 (2H, d, J = 7.8 Hz), 7.95 (2H, d, J = 5.1 Hz), 7.52–7.62 (4H, m), 7.32–7.37 (2H, m), 7.18–7.24 (4H, m), 3.67 (4H, t, J = 7.2 Hz), 0.76–0.84 (4H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ 150.1, 149.6, 142.2, 140.3, 139.1, 136.6, 135.9, 133.9, 130.9, 129.0, 123.2, 122.0, 121.3, 120.5, 114.4, 110.2, 43.9, 26.1; ESI-MS m/z: 545.5 $[M + H]^+$.

*5.5.37. 1-(Pyridin-3-yl)-9-[6-[1-(pyridine-3-yl)-β-carboline-9-yl] hexyl]-β-carboline (**31b**)*

Starting from 1-(pyridin-3-yl)-β-carboline **14** (2.0 mmol) and 1,6-dibromohexane (1.0 mmol), compound **31b** was obtained as yellow solid (0.32 g, 56%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.81–8.82 (2H, m), 8.67 (2H, d, J = 3.9 Hz), 8.52 (2H, d, J = 5.1 Hz), 8.16 (2H, d, J = 7.8 Hz), 7.99 (2H, d, J = 5.1 Hz), 7.87–7.91 (2H, m), 7.55–7.60 (2H, m), 7.28–7.39 (6H, m), 3.84 (4H, t, J = 7.8 Hz), 1.08–1.18 (4H, m), 0.52–0.57 (4H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ 150.1, 149.6, 142.2, 140.5, 138.9, 136.8, 136.1, 134.4, 131.0, 128.9, 123.3, 121.9, 121.4, 120.4, 114.5, 110.4, 44.6, 28.8, 26.3; ESI-MS m/z: 573.5 $[M + H]^+$.

*5.5.38. 3-Ethoxycarbonyl-1-(pyridin-3-yl)-9-[5-[3-ethoxycarbonyl-1-(pyridine-3-yl)-β-carboline-9-yl]pentyl]-β-carboline (**32a**)*

Starting from ethyl 1-(pyridin-3-yl)-β-carboline-3-carboxylate **15** (2.0 mmol) and 1,5-dibromopentane (1.0 mmol), compound **32a** was obtained as yellow solid (0.3 g, 43%), mp 172–174 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.90 (2H, s), 8.76 (2H, d, J = 2.1 Hz), 8.46–8.48 (2H, dd, J = 1.5, 5.1 Hz), 8.26 (2H, d, J = 8.1 Hz), 7.83–7.87 (2H, m), 7.61–7.66 (2H, m), 7.33–7.43 (4H, m), 7.21–7.26 (2H, m), 4.53 (4H, q, J = 6.9 Hz), 3.81 (4H, t, J = 7.8 Hz), 1.49 (6H, t, J = 6.9 Hz), 1.01–1.11 (4H, m), 0.31–0.39 (2H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ 165.9, 150.3, 149.9, 142.4, 140.3, 137.9, 137.1, 135.8, 135.5, 131.0, 129.4, 123.2, 122.1, 121.7, 121.4, 117.3, 110.6, 62.0, 44.5, 28.6, 23.5, 14.8; ESI-MS m/z: 703.5 $[M + H]^+$.

*5.5.39. 3-Ethoxycarbonyl-1-(pyridin-3-yl)-9-[6-[3-ethoxycarbonyl-1-(pyridine-3-yl)-β-carboline-9-yl]hexyl]-β-carboline (**32b**)*

Starting from ethyl 1-(pyridin-3-yl)-β-carboline-3-carboxylate **15** (2.0 mmol) and 1,6-dibromohexane (1.0 mmol), compound **32b** was obtained as yellow solid (0.35 g, 49%), mp 221–222 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.88 (2H, s), 8.83 (2H, d, J = 1.5 Hz), 8.67 (2H, dd, J = 1.5 Hz, 5.1 Hz), 8.23 (2H, d, J = 7.8 Hz), 7.91–7.95 (2H, m), 7.59–7.65 (2H, m), 7.35–7.41 (6H, m), 4.51 (4H, q, J = 7.2 Hz), 3.87 (4H, t, J = 7.8 Hz), 1.48 (6H, t, J = 7.2 Hz), 1.11–1.21 (4H, m), 0.51–0.60 (4H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ 165.9, 150.4, 149.9, 142.5, 140.4, 137.8, 137.2, 135.8, 135.6, 131.1, 129.3, 123.3, 122.1, 121.7, 121.3, 117.2, 110.7, 61.9, 44.8, 29.0, 26.2, 14.8; ESI-MS m/z: 717.5 $[M + H]^+$.

*5.5.40. 1-(3,4,5-Trimethoxyphenyl)-9-[6-[1-(3,4,5-trimethoxyphenyl)]-β-carboline-9-yl]-hexyl]-β-carboline (**33a**)*

Starting from 1-(3,4,5-trimethoxyphenyl)-β-carboline **16** (2.0 mmol) and 1,6-dibromohexane (1.0 mmol), compound **33a** was obtained as white solid (0.57 g, 76%), mp 199–200 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.47 (2H, d, J = 5.5 Hz), 8.16 (2H, d, J = 8.0 Hz), 7.96 (2H, d, J = 5.5 Hz), 7.55–7.59 (2H, m), 7.36 (2H, d, J = 8.5 Hz), 7.28–7.31 (2H, m), 6.73 (4H, s), 3.88 (4H, t, J = 7.5 Hz), 3.84 (6H, s), 3.74 (12H, s), 1.22–1.32 (4H, m), 0.67–0.70 (4H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 152.9, 143.8, 141.8, 138.1, 138.0, 135.4, 133.7, 130.2, 128.4, 121.5, 121.2, 119.8, 113.6, 110.0, 106.4, 60.8, 56.0, 44.2, 28.9, 26.1; ESI-MS m/z: 750.6 $[M]^+$.

*5.5.41. 1-(3,4,5-Trimethoxyphenyl)-9-[7-[1-(3,4,5-trimethoxyphenyl)]-β-carboline-9-yl]-heptyl]-β-carboline (**33b**)*

Starting from 1-(3,4,5-trimethoxyphenyl)-β-carboline **16** (2.0 mmol) and 1,7-dibromoheptane (1.0 mmol), compound **33b** was obtained as yellow oil (0.56 g, 62%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.46 (2H, d, J = 5.1 Hz), 8.14 (2H, d, J = 7.8 Hz), 7.95 (2H, d, J = 5.1 Hz), 7.54–7.59 (2H, m), 7.39 (2H, d, J = 7.8 Hz), 7.25–7.29 (2H, m), 6.75 (4H, s), 3.91 (4H, t, J = 7.8 Hz), 3.83 (6H, s), 3.80 (12H, s), 1.25–1.38 (4H, m), 0.72–0.86 (6H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 153.1, 144.0, 142.1, 138.3, 135.7, 134.0, 130.4, 128.6, 121.8, 121.5, 120.1, 114.0, 110.4, 106.7, 61.2, 56.4, 44.7, 29.5, 29.1, 26.8; ESI-MS m/z: 765.4 $[M + H]^+$.

*5.5.42. 3-Ethoxycarbonyl-1-(3,4,5-trimethoxyphenyl)-9-[6-[3-ethoxycarbonyl-1-(3,4,5-trimethoxyphenyl)-β-carboline-9-yl]hexyl]-β-carboline (**34a**)*

Starting from ethyl 1-(3,4,5-trimethoxyphenyl)-β-carboline-3-carboxylate **17** (2.0 mmol) and 1,6-dibromohexane (1.0 mmol), compound **34b** was obtained as white solid (0.6 g, 67%), mp 264–265 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.83 (2H, s), 8.21 (2H, d, J = 7.8 Hz), 7.58–7.64 (2H, m), 7.33–7.40 (4H, m), 6.73 (4H, s), 4.50 (4H, q, J = 7.2 Hz), 3.83–3.89 (10H, m), 3.73 (12H, s), 1.23–1.28 (4H, m), 1.46 (6H, t, J = 7.2 Hz), 0.65–0.73 (4H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ 166.1, 153.2, 143.8, 142.4, 138.6, 137.1, 135.5, 134.9, 130.3, 129.1, 122.0, 121.7, 121.0, 116.9, 110.7, 107.2, 61.8, 61.1, 56.4, 44.7, 29.6, 26.6, 14.9; ESI-MS m/z: 895.5 $[M + H]^+$.

*5.5.43. 3-Ethoxycarbonyl-1-(3,4,5-trimethoxyphenyl)-9-[9-[3-ethoxycarbonyl-1-(3,4,5-trimethoxyphenyl)-β-carboline-9-yl]nonyl]-β-carboline (**34b**)*

Starting from ethyl 1-(3,4,5-trimethoxyphenyl)-β-carboline-3-carboxylate **17** (2.0 mmol) and 1,9-dibromononane (1.0 mmol), compound **34b** was obtained as yellow oil (0.25 g, 53%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.85 (2H, s), 8.23 (2H, d, J = 7.8 Hz), 7.58–7.63 (2H, m), 7.32–7.47 (4H, m), 6.82 (2H, s), 6.79 (2H, s), 4.51 (4H, q, J = 7.2 Hz), 3.84–4.00 (22H, m), 1.37–1.58 (10H, m), 1.24–1.28 (4H, m), 0.84–0.97 (6H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ 166.1, 153.2, 143.8, 142.4, 138.6, 136.9, 135.6, 135.0, 130.3, 129.0, 121.9, 121.8, 120.9, 116.9, 110.7, 107.0, 61.7, 61.2, 56.5, 45.0, 29.8, 29.6, 29.4, 27.0, 14.9; ESI-MS m/z: 937.5 $[M + H]^+$.

5.6. Cytotoxicity assays in vitro

Cytotoxicity assays in vitro were carried out using 96 microtitre plate cultures and MTT staining according to the procedures described by Cao et al. [5]. Briefly, cells were grown in RPMI-1640 medium containing 10% (v/v) fetal calf serum and 100 μg ml^{-1} penicillin and 100 μg ml^{-1} streptomycin. Cultures were propagated at 37 °C in a humidified atmosphere containing 5% CO_2. Cell lines were obtained from Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Science. DMSO was used as the solution for drugs. Final concentration of DMSO in the growth medium was 2% (v/v) or lower, concentration without effect on cell replication. In all of these experiments, three replicate wells were used to determine each point.

5.7. Assay of acute toxicities

Acute toxicity assay was performed according to the method described by Cao et al. [5]. Briefly, healthy C57BL/6 mice (9–12 weeks) weighing 18–22 g were housed in rooms where the temperature was approximately 24 ± 2 °C, with a relative humidity 60–70%, and in 12 h light–dark cycle. The sterile food and water were provided according to institutional guidelines. All animals were provided by Shanghai Laboratory Animal Center of Chinese Academy

of Science. All animal procedures were approved by the Animal Ethical Committee of the Sun Yat-sen University. Prior to each experiment, mice were fastened overnight and allowed free access to water. Various doses of the bivalent β-carbolines ranging from 1.0 to 500 mg kg^{-1} dissolved in 0.5% carboxymethyl cellulose sodium (CMC-Na) salt solution were given via intraperitoneal (i.p.) to different groups of healthy C57BL/6 mice, and each group contained 10 mice (5 males and 5 females). After the administration of the compounds, 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 animals were sacrificed at the 14th day after drug administration and checked macroscopically for possible damage to the heart, liver and kidneys. Mice of immediate death following drug administration were also examined for any possible organ damage. LD_{50} values were calculated graphically as described [42].

5.8. Assay of antitumor activity

Antitumor activity against mice bearing CT-26 colon cancer, Lewis lung cancer, Sarcoma 180, H22 liver cancer and B16 melanoma was performed as described by Cao et al. [5] with a slightly modification. Briefly, CT-26 colon cancer, Lewis lung cancer, Sarcoma 180, H22 liver cancer and B16 melanoma cell lines were provided by Shanghai Institute of Pharmaceutical Industry. Tumor cells of CT-26 colon cancer, Lewis lung cancer, Sarcoma 180, H22 liver cancer and B16 melanoma were inoculated to mice. After 7 days, tumors were taken out and cells harvested. Viable tumor cells (2×10^6 cells/mouse) were inoculated to the armpit of mice by subcutaneous injection. Each compound was injected by intraperitoneal (i.p.) to different group mice (each group containing 10 female mice) 24 h after the inoculation at a dosage about one fifth of LD_{50} value once a day for consecutive 7 days. Cyclophosphamide (CTX) at 30 mg kg^{-1} was used as a positive control and vehicle as negative control. The weights of animals were recorded every 3 days. All animals were sacrificed at the 21st day after tumor inoculation and the tumors were excised and weighed. The inhibition rate was calculated as follows:

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

T: average tumor weight of treated group; *C*: average tumor weight of negative control group.

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