

双-(1-杂环- $\beta$ -咔啉)-3-烷氨基衍生物的合成与抗肿瘤活性郭亮<sup>a,b</sup> 谢建伟<sup>a,b</sup> 范文玺<sup>c</sup> 陈伟<sup>c</sup>  
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**摘要** 为寻找高效、低毒的抗肿瘤候选化合物,以1-杂环取代- $\beta$ -咔啉-3-羧酸乙酯为原料,合成了一系列的双-(1-杂环- $\beta$ -咔啉)-3-烷氨基衍生物。所有目标化合物经  $^1\text{H}$  NMR、 $^{13}\text{C}$  NMR 和 HRMS 进行结构确证。以顺铂为阳性对照药,采用四甲基偶氮唑盐(MTT)法考察了目标化合物体外抗肿瘤(22RV1, SK-OV-3, MCF-7, BGC-823, A375 和 769-P 等 10 株细胞)活性。结果显示化合物 **5a**~**5h** 与阳性对照药和单取代  $\beta$ -咔啉衍生物相比具有很好的抗肿瘤活性,其  $\text{IC}_{50}$  值均小于  $10\ \mu\text{mol}\cdot\text{L}^{-1}$ ,特别是化合物 **5d** 对 769-P 的抑制活性达到  $0.8\ \mu\text{mol}\cdot\text{L}^{-1}$ ,化合物 **5h** 对 22RV1 的抑制活性达到  $0.6\ \mu\text{mol}\cdot\text{L}^{-1}$ 。

**关键词** 双  $\beta$ -咔啉; 合成; 抗肿瘤活性; 构效关系

Synthesis and Antitumor Activities of Novel Bivalent 1-Heterocyclic- $\beta$ -carbolines Linked by Alkylamino SpacerGuo, Liang<sup>a,b</sup> Xie, Jianwei<sup>a,b</sup> Fan, Wenxi<sup>c</sup> Chen, Wei<sup>c</sup>  
Dai, Bin<sup>\*,a,b</sup> Ma, Qin<sup>c</sup><sup>(a)</sup> School of Chemistry and Chemical Engineering, Shihezi University, Shihezi 832003)<sup>(b)</sup> Key Laboratory for Green Processing of Chemical Engineering of Xinjiang Bingtuan, Shihezi 832003)<sup>(c)</sup> Xinjiang Huashidan Pharmaceutical Research Co., Ltd., Urumqi 830011)

**Abstract** In order to find novel antitumor candidate compounds with high efficiency and low toxicity, a series of 1-heterocyclic substituted bivalent  $\beta$ -carbolines with a spacer of four or five methylene units between the two 3-methylamino group were synthesized, and the chemical structures were characterized by  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, and HRMS. The cytotoxic activities of all bivalent  $\beta$ -carbolines were evaluated *in vitro* against a panel of human tumor cell lines (22RV1, SK-OV-3, MCF-7, LLC, Eca-109, BGC-823, HT-29, HepG-2, A375, and 769-P) and compared with the positive control cisplatin and monovalent  $\beta$ -carbolines. The results demonstrated that compounds **5a**~**5h** exhibited potent cytotoxic activities with  $\text{IC}_{50}$  values lower than  $10\ \mu\text{mol}\cdot\text{L}^{-1}$ . In particular, compounds **5d** and **5h**, both of which had a spacer of five methylene units, exhibited significant inhibitory activity against 769-P and 22RV1 with  $\text{IC}_{50}$  values of  $0.8\ \mu\text{mol}\cdot\text{L}^{-1}$  and  $0.6\ \mu\text{mol}\cdot\text{L}^{-1}$ , respectively.

**Keywords** bivalent  $\beta$ -carboline; synthesis; antitumor activity; structure-activity relationship

$\beta$ -Carboline alkaloids are a class of natural and synthetic products that have a broad spectrum of biochemical effects and pharmacological properties.<sup>[1-6]</sup> The reported biological applications of  $\beta$ -carboline alkaloids include sedative and anxiolytic,<sup>[1]</sup> antitumor,<sup>[2,3]</sup> antimalarial,<sup>[3]</sup> antiparasitic,<sup>[4]</sup> anti-HIV<sup>[5]</sup> agents, and other pharmacological activities.

Recently, the structures of modified  $\beta$ -carboline alkaloids as a new class of antitumor agents have attracted the attention of chemists. It has been reported that  $\beta$ -carboline alkaloids can exhibit antitumor activities through multiple mechanisms, such as DNA binding,<sup>[6]</sup> inhibition topoisomerases I and II,<sup>[7,8]</sup> cyclin-dependent kinase (CDK),<sup>[9]</sup> polo-like ki-

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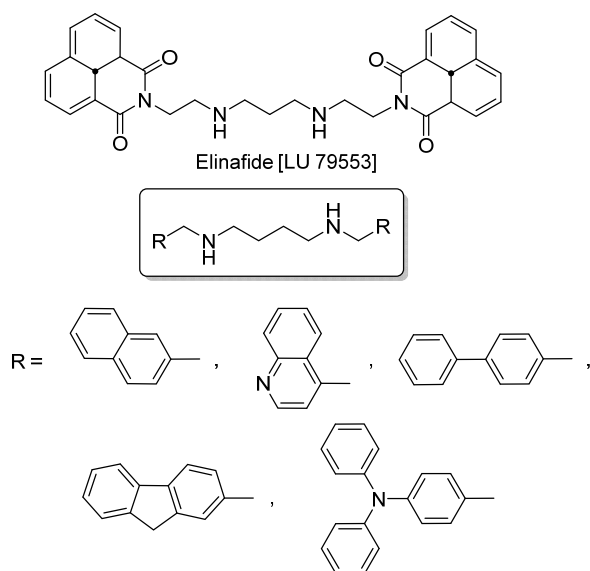
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nase (PLK1),<sup>[10]</sup> kinesin-like protein Eg5,<sup>[11]</sup> and I $\kappa$ B kinases.<sup>[12]</sup>

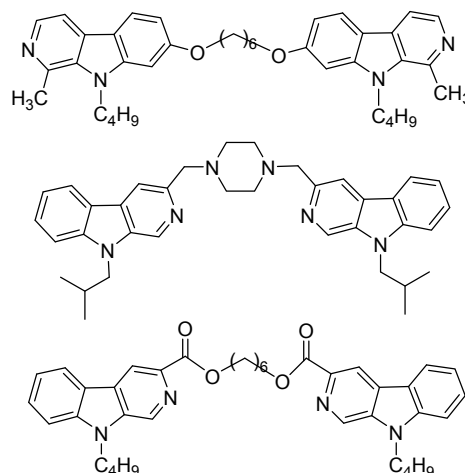
Recent reports suggest that bivalent  $\beta$ -carboline alkaloids have much better cytotoxic efficacies than monovalent ones.<sup>[13–15]</sup> This is most likely because of the dimerization of various intercalating agents by an appropriate spacer, which can increase DNA binding affinity. Thus, there has been tremendous interest in the design of novel polyvalent molecules that possess significant properties different from their monovalent counterparts.<sup>[16]</sup> For example, Elnafide [LU 79553]<sup>[17]</sup> (Figure 1), a naphthalimide derivative, has antitumor activities as a DNA intercalator and topoisomerase II inhibitor. Burns *et al.*<sup>[18]</sup> synthesized a series of aromatic-substituted diamines (Figure 1) and evaluated their cytotoxic profiles against human breast and prostate tumor cell lines. The results showed that some aryl-substituted diamine compounds demonstrated a nearly 300-fold improvement over the monovalent compounds with respect to cytotoxic properties.



**Figure 1** Structure of Elnafide and symmetric-substituted diamines

For more than a decade, our group<sup>[19–23]</sup> has focused on incorporating substituents into positions-1, 2, 3, 7 and 9 of the  $\beta$ -carboline nucleus as an antitumor agent. Our group has also investigated several novel bivalent  $\beta$ -carbolines with an alkyl spacer in position-1, 3 and 7 of  $\beta$ -carboline nucleus<sup>[24–26]</sup> (Figure 2), and these have exhibited more potent antitumor efficacies than monomers. In the present work, we designed and synthesized a series of 1-heterocyclic substituted bivalent  $\beta$ -carbolines with a spacer of four or five methylene units between the two 3-methylamino groups and to find congeners more active as potential antitumor agents and to study in depth the influence of the substituent in positions-1 and 3 of the  $\beta$ -carboline nucleus. The compounds for this study were synthesized from the starting material ethyl 1-heterocyclic substituted- $\beta$ -carboline-3-carboxylate via the alkylation, oxidation, condensation, and reduction (Scheme 1). The

antitumor activities of these compounds were studied *in vitro*. The details of the synthesis and biological activities of these compounds are presented herein.



**Figure 2** Representative reported bivalent  $\beta$ -carbolines

## 1 Results and discussion

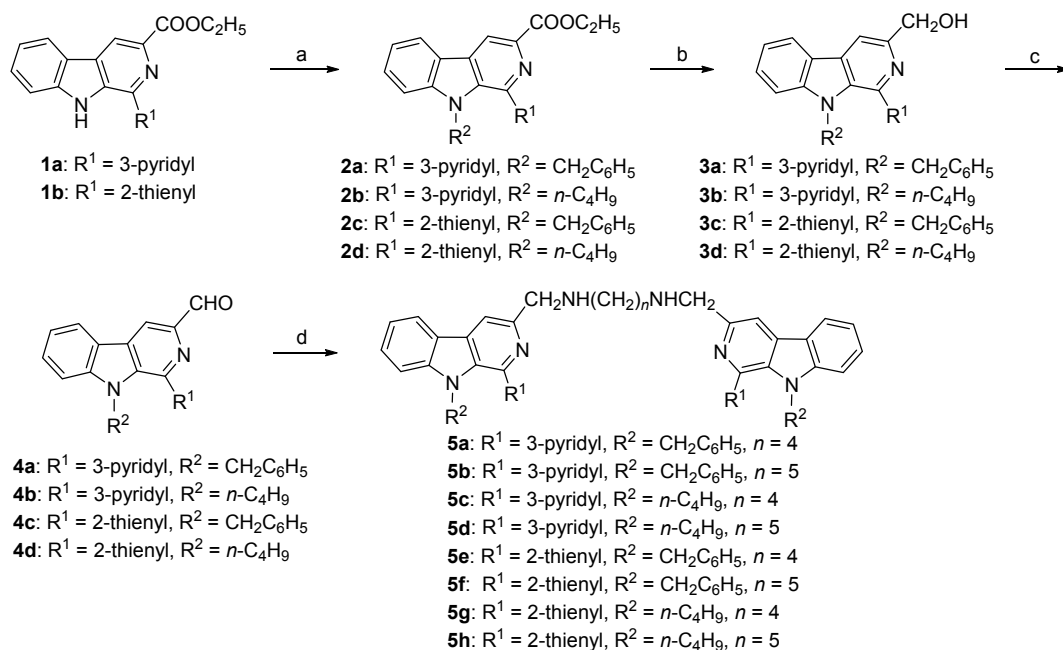
### 1.1 Synthesis

The synthetic route for the 1-heterocyclic substituted bivalent  $\beta$ -carbolines is outlined in Scheme 1. The starting material ethyl 1-heterocyclic substituted- $\beta$ -carboline-3-carboxylate (**1a**~**1b**). The  $N^9$  of **1** was alkylated or arylated by the action of sodium hydride in anhydrous DMF followed by the addition of 1-iodobutane or benzyl bromide to obtain intermediates **2**. The ester group in position-3 of **2** was reduced to its corresponding alcohols by  $\text{LiBH}_4$  in dry THF to provide compounds **3**, and further oxidized by  $\text{MnO}_2$  in  $\text{CH}_3\text{CN}$  to give 3-carboxaldehydes **4**. The reaction of compounds **4** with the corresponding diamines to form schiff bases took place readily at room temperature in good yield. The crude Schiff bases without further purification were directly reduced with  $\text{NaBH}_3\text{CN}$  in anhydrous methanol to give the target 1-heterocyclic substituted bivalent  $\beta$ -carbolines **5** in 40%~64% yield.

### 1.2 *In vitro* cell cytotoxicity assay

All the synthesized compounds were subjected to *in vitro* anticancer evaluation using methyl thiazolyl tetrazolium (MTT)<sup>[26]</sup> assay in ten human cancer cell lines and compared with the reference drug cisplatin (DDP). In order to enhance the solubility in aqueous solution, all compounds were prepared in the form of hydrochloride salt before use.  $\text{IC}_{50}$  ( $\mu\text{mol}\cdot\text{L}^{-1}$ ) are presented in Table 1.

As expected, all the 1-heterocyclic substituted bivalent  $\beta$ -carbolines exhibited excellent cytotoxic activities ( $\text{IC}_{50}$  values lower than  $10 \mu\text{mol}\cdot\text{L}^{-1}$ ) against all human tumor cell lines tested. In particular, these compounds were more potent than the positive control drug cisplatin. Meanwhile, the monovalent intermediates **3a**~**3d** and **4a**~**4d** showed weaker cytotoxic activities, all bivalent  $\beta$ -carbolines had significant cytotoxic efficacies in comparable to the monovalent  $\beta$ -carbolines. The results indicate that  $C^3$ -sub-



Reagent and conditions: (a) DMF, NaH, C<sub>4</sub>H<sub>9</sub>I or Benzyl bromide; (b) THF, LiBH<sub>4</sub>; (c) CH<sub>3</sub>CN, MnO<sub>2</sub>, reflux; (d) CH<sub>3</sub>OH, CH<sub>2</sub>Cl<sub>2</sub>, NH<sub>2</sub>(CH<sub>2</sub>)<sub>n</sub>NH<sub>2</sub>, r.t., NaBH<sub>3</sub>CN

**Scheme 1** Synthetic route of target compounds **5a~5h**

stituted dimers markedly improve cytotoxic activities.

We also examined the influence of the spacer length of 1-heterocyclic substituted bivalent  $\beta$ -carbolines and the effect of the substituent in position-9 of the  $\beta$ -carboline ring on the cytotoxic activities. The data show that compounds **5b**, **5d**, **5f** and **5h** (each with a spacer of five methylene units) had the highest cytotoxicity. Moreover, the 22RV1 and 769-P cell lines were more sensitive to these compounds than other cell lines. Compounds **5d** and **5h**, both of which had *n*-butyl in position-9 of the  $\beta$ -carboline ring, had the most potent inhibitory activity against 769-P and 22RV1. The IC<sub>50</sub> values for compounds **5d** and **5h** were 0.8 and 0.6  $\mu\text{mol}\cdot\text{L}^{-1}$ , respectively.

## 2 Conclusions

A new series of 1-heterocyclic substituted bivalent  $\beta$ -carbolines with a spacer of four or five methylene units between the 3-methylamino group have been designed and synthesized. Their cytotoxic potential against human tumor cell lines in culture were investigated. The results demonstrated that all bivalent  $\beta$ -carbolines had significant cytotoxic efficacies in comparable to the reference drug cisplatin and monovalent  $\beta$ -carbolines, and compound **5d** showed significant inhibitory activity against 769-P with IC<sub>50</sub> values of 0.8  $\mu\text{mol}\cdot\text{L}^{-1}$  and compound **5h** showed significant inhibitory activity against 22RV1 with IC<sub>50</sub> values of 0.6  $\mu\text{mol}\cdot\text{L}^{-1}$ . Preliminary SARs analysis indicated that 2-thienyl into position-1 of the  $\beta$ -carboline nucleus and the spacer of five methylene units between the 3-methylamino group provided compounds with greatly enhanced cytotoxic potencies. Further investigations to confirm the antitumor efficacy in animal models and elu-

cidate the pharmacological mechanisms of this class of compounds are underway in our laboratory.

## 3 Experimental

### 3.1 Materials and characterization

MS spectra were obtained from a Micromass ZQ4000 spectrometer; HRMS were obtained from a Bruker ultrafleXtreme MALDI-TOF/TOF spectrometer. <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra were recorded on a Varian inova-400 spectrometer at 400 MHz and 100 MHz, respectively, using TMS as internal standard and CDCl<sub>3</sub> or DMSO-*d*<sub>6</sub> as solvent. Melting points were determined in capillary tubes on an electrothermal WRS-3 apparatus and without correction. Elemental analyses (C, H and N) were carried out on an Elementar Vario ELIII CHNS Elemental Analyzer.

All solvents and reagents were obtained from commercial sources without further purification. Silica GF<sub>254</sub> used in the analytical thin-layer chromatography (TLC) and silica gel (200-300 mesh) for column chromatography were produced by the Qingdao Haiyang Chemical Co., Ltd.

### 3.2 Chemistry

#### 3.2.1 General procedure for the preparation of **2a~2d**

A mixture of **1a** (3.17 g, 10 mmol) and anhydrous DMF (60 mL) was stirred at room temperature for 0.5 h, and then 95% NaH (0.37 g, 15 mmol) and benzyl bromide (1.3 mL, 12 mmol) were added. The mixture was stirred at room temperature for 0.5~2 h. After completion of the reaction as indicated by TLC, the solution was poured into H<sub>2</sub>O (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

Table 1 Cytotoxicity of compounds **3a**~**3d**, **4a**~**4d** and **5a**~**5h** against human tumour cells

Comp.	IC <sub>50</sub> /(μmol·L <sup>-1</sup> )									
	22RV1	SK-OV-3	MCF-7	LLC	Eca-109	BGC-823	HT-29	HepG2	769-P	A375
<b>3a</b>	>100	>100	72.4	70.7	>100	>100	67.5	53.6	41.6	>100
<b>3b</b>	90.5	>100	>100	42.4	>100	>100	>100	>100	66.1	>100
<b>3c</b>	>100	>100	76.7	56.8	>100	76.7	74.9	54.3	32.7	90.1
<b>3d</b>	>100	>100	97.8	>100	>100	>100	89.2	98.5	79.5	93.2
<b>4a</b>	>100	90.1	>100	>100	>100	>100	>100	48.9	18.2	58.8
<b>4b</b>	>100	89.2	>100	>100	>100	>100	>100	19.9	66.6	>100
<b>4c</b>	>100	69.1	77.5	91.1	>100	83.1	69.1	55.9	27.5	83.2
<b>4d</b>	72.2	65.8	54.8	67.4	36.2	58.7	17.7	56.2	19.9	51.1
<b>5a</b>	3.7	2.8	5.7	5.7	6.0	6.0	6.2	7.6	4.1	6.2
<b>5b</b>	1.9	1.4	7.5	1.3	5.5	5.2	1.6	9.9	1.0	5.5
<b>5c</b>	2.3	1.6	6.9	1.7	7.2	4.0	7.4	7.9	1.8	6.6
<b>5d</b>	1.6	5.5	3.8	1.3	2.1	1.6	2.0	4.0	0.8	1.6
<b>5e</b>	3.9	2.7	2.7	1.4	5.3	7.5	5.8	7.0	2.6	4.7
<b>5f</b>	1.3	2.6	6.3	1.4	5.4	7.4	4.7	5.9	1.6	3.4
<b>5g</b>	3.4	2.2	4.6	1.9	1.8	1.5	5.2	4.4	2.1	2.3
<b>5h</b>	0.6	2.2	2.1	1.9	1.8	1.9	5.4	2.6	1.5	1.8
<b>DDP</b>	5.2	5.6	12.4	7.6	8.9	11.6	26.8	14.8	19.2	9.4

oil was crystallized from ethyl ether or ethyl ether-petroleum ether.

Ethyl 9-benzyl-1-(3-pyridyl)-β-carboline-3-carboxylate (**2a**): White solid, yield 89%. m.p. 148.4~149.7 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ: 8.98 (s, 1H), 8.70 (d, *J*=2.4 Hz, 1H), 8.66 (dd, *J*=4.8, 1.6 Hz, 1H), 8.32 (d, *J*=7.2 Hz, 1H), 7.61~7.68 (m, 2H), 7.42~7.46 (m, 2H), 7.21~7.24 (m, 1H), 7.15~7.18 (m, 1H), 7.08~7.12 (m, 2H), 6.45 (d, *J*=7.6 Hz, 2H), 5.29 (s, 2H), 4.53 (q, *J*=7.2 Hz, 2H), 1.47 (t, *J*=7.2 Hz, 3H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ: 165.87, 149.88, 149.36, 143.06, 138.10, 137.06, 136.05, 135.80, 131.04, 129.47, 129.44, 128.70, 128.61, 127.56, 125.29, 122.91, 121.93, 121.56, 121.44, 117.11, 110.78, 61.72, 48.35, 14.50; ESI-MS *m/z*: 409 [M+H]<sup>+</sup>. Anal. calcd for C<sub>26</sub>H<sub>21</sub>N<sub>3</sub>O<sub>2</sub>: C 76.64, H 5.19, N 10.31; found C 76.39, H 5.37, N 10.38.

Ethyl 9-*n*-butyl-1-(3-pyridyl)-β-carboline-3-carboxylate (**2b**): White solid, yield 91%. m.p. 156.1~156.9 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ: 8.93 (s, 1H), 8.92 (s, 1H), 8.78 (d, *J*=5.6 Hz, 1H), 8.27 (d, *J*=8.0 Hz, 1H), 8.06 (d, *J*=7.2 Hz, 1H), 7.64~7.68 (m, 1H), 7.49~7.54 (m, 2H), 7.38~7.42 (m, 1H), 4.53 (q, *J*=7.2 Hz, 2H), 4.01 (t, *J*=8.0 Hz, 2H), 1.48 (t, *J*=7.2 Hz, 3H), 1.33~1.40 (m, 2H), 0.84~0.96 (m, 4H), 0.66 (t, *J*=7.2 Hz, 3H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ: 165.91, 149.91, 149.33, 142.49, 140.14, 137.67, 137.43, 135.87, 130.87, 129.12, 123.33, 121.89, 121.04, 117.13, 110.61, 61.67, 44.67, 30.92, 19.77, 14.50, 13.45; ESI-MS *m/z*: 374 [M+H]<sup>+</sup>. Anal. calcd for C<sub>23</sub>H<sub>23</sub>N<sub>3</sub>O<sub>2</sub>: C 73.97, H 6.21, N 11.25; found C 73.85, H 6.07, N 11.36.

Ethyl 9-benzyl-1-(2-thienyl)-β-carboline-3-carboxylate (**2c**): White solid, yield 94%. m.p. 171.2~172.6 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ: 8.91 (s, 1H), 8.28 (d, *J*=8.0 Hz, 1H), 7.55~7.59 (m, 1H), 7.36~7.43 (m, 3H), 7.12~7.19 (m, 3H), 7.03 (dd, *J*=3.6, 1.2 Hz, 1H), 6.95 (dd, *J*=5.2, 3.6 Hz, 1H), 6.68~6.70 (m, 2H), 5.40 (s, 2H), 4.52 (q,

*J*=7.2 Hz, 2H), 1.47 (t, *J*=7.2 Hz, 3H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ: 165.94, 143.05, 139.98, 137.82, 137.39, 136.64, 136.62, 131.02, 129.23, 128.71, 128.59, 127.59, 127.33, 126.68, 125.65, 121.81, 121.71, 121.30, 116.96, 111.14, 61.66, 48.10, 14.48; ESI-MS *m/z*: 414 [M+H]<sup>+</sup>. Anal. calcd for C<sub>25</sub>H<sub>20</sub>N<sub>2</sub>O<sub>2</sub>S: C 72.79, H 4.89, N 6.79, S 7.77; found C 72.52, H 4.63, N 6.52, S 7.49.

Ethyl 9-*n*-butyl-1-(2-thienyl)-β-carboline-3-carboxylate (**2d**): White solid, yield 86%. m.p. 100.3~100.9 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ: 8.88 (s, 1H), 8.25 (d, *J*=7.6 Hz, 1H), 7.62~7.66 (m, 1H), 7.49~7.53 (m, 2H), 7.36~7.40 (m, 1H), 7.30 (dd, *J*=3.6, 1.2 Hz, 1H), 7.17 (dd, *J*=5.2, 3.6 Hz, 1H), 4.52 (q, *J*=7.2 Hz, 2H), 4.13 (t, *J*=8.0 Hz, 2H), 1.43~1.52 (m, 5H), 0.93~1.04 (m, 2H), 0.73 (t, *J*=7.2 Hz, 3H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ: 165.94, 142.58, 140.12, 137.25, 136.97, 136.41, 130.78, 128.96, 128.89, 127.39, 126.73, 121.85, 121.63, 120.89, 117.02, 110.68, 61.64, 44.49, 31.36, 19.96, 14.50, 13.56; ESI-MS *m/z*: 379 [M+H]<sup>+</sup>. Anal. calcd for C<sub>22</sub>H<sub>22</sub>N<sub>2</sub>O<sub>2</sub>S: C 69.81, H 5.86, N 7.40, S 8.47; found C 69.47, H 6.05, N 7.12, S 8.24.

### 3.2.2 General procedure for the preparation of **3a**~**3d**

To a solution of compound **2a** (4.07g, 10 mmol) in THF (200 mL) was added LiBH<sub>4</sub> (30 mmol). The mixture was then stirred at room temperature until the reaction is completed. Then the reaction was quenched with cool water (200 mL), and adjusted the pH of the aqueous phase to 3~4 by the addition of aqueous HCl, and stirred for 4 h. The reaction mixture was neutralized with aqueous NaOH solution and extracted with ethyl acetate. The organic layer was washed with water and saturated brine, dried over anhydrous sodium sulfate, filtered and concentrated. The residue obtained was purified by silica column chromatography with ethyl acetate as the eluent. Upon recrystallization, compound **3a** was obtained. Products **3b**~**3d** were prepared according to the same method of **3a**.



9-Benzyl-1-(3-pyridyl)-3-hydroxymethyl- $\beta$ -carboline (**3a**): White solid, yield 72.3%. m.p. 162.3~163.8 °C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$ : 8.60~8.71 (m, 2H), 8.23 (d,  $J=8.0$  Hz, 1H), 8.06 (s, 1H), 7.53~7.63 (m, 2H), 7.32~7.40 (m, 2H), 7.05~7.21 (m, 4H), 6.46 (d,  $J=7.2$  Hz, 2H), 5.24 (s, 2H), 4.97 (s, 2H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$ : 149.90, 149.36, 148.57, 143.37, 139.62, 136.60, 136.21, 135.20, 134.04, 132.17, 129.24, 128.61, 127.39, 125.36, 122.75, 121.81, 121.15, 120.56, 111.02, 110.43, 64.71, 48.21; ESI-MS  $m/z$ : 366  $[\text{M}+\text{H}]^+$ . Anal. calcd for  $\text{C}_{24}\text{H}_{19}\text{N}_3\text{O}$ : C 78.88, H 5.24, N 11.50; found C 78.69, H 5.36, N 11.73.

9-*n*-Butyl-1-(3-pyridyl)-3-hydroxymethyl- $\beta$ -carboline (**3b**): Light yellow solid, yield 78.2%. m.p. >270 °C;  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ , 400 MHz)  $\delta$ : 9.18 (d,  $J=1.6$  Hz, 1H), 9.02 (dd,  $J=5.2$ , 1.2 Hz, 1H), 8.87 (s, 1H), 8.62 (d,  $J=8.0$  Hz, 1H), 8.53~8.56 (m, 1H), 7.89~7.93 (m, 2H), 7.82~7.86 (m, 1H), 7.47~7.51 (m, 1H), 4.98 (s, 2H), 3.99 (t,  $J=7.2$  Hz, 2H), 1.26~1.34 (m, 2H), 0.80~0.89 (m, 2H), 0.60 (t,  $J=7.2$  Hz, 3H);  $^{13}\text{C}$  NMR ( $\text{DMSO}-d_6$ , 100 MHz)  $\delta$ : 147.87, 146.92, 146.00, 145.17, 143.46, 135.67, 132.81, 132.55, 129.17, 125.64, 124.02, 122.25, 119.68, 115.20, 111.87, 60.11, 44.59, 30.83, 19.60, 13.71; ESI-MS  $m/z$ : 332  $[\text{M}+\text{H}]^+$ . Anal. calcd for  $\text{C}_{21}\text{H}_{21}\text{N}_3\text{O}$ : C 76.11, H 6.39, N 12.68; found C 76.27, H 6.15, N 12.56.

9-Benzyl-1-(2-thienyl)-3-hydroxymethyl- $\beta$ -carboline (**3c**): Light yellow solid, yield 73.5%. m.p. 111.4~112.8 °C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$ : 8.17~8.20 (m, 1H), 7.98 (s, 1H), 7.52~7.56 (m, 1H), 7.42 (dd,  $J=5.2$ , 1.2 Hz, 1H), 7.30~7.34 (m, 2H), 7.12~7.18 (m, 3H), 7.02 (dd,  $J=3.6$ , 1.2 Hz, 1H), 6.96 (dd,  $J=5.2$ , 3.6 Hz, 1H), 6.70~6.72 (m, 2H), 5.37 (s, 2H), 4.95 (s, 2H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$ : 148.39, 143.41, 140.45, 137.02, 136.10, 134.55, 132.21, 129.06, 128.55, 128.23, 127.37, 127.20, 126.71, 125.73, 121.71, 121.32, 120.47, 110.98, 110.81, 64.68, 47.98; ESI-MS  $m/z$ : 371  $[\text{M}+\text{H}]^+$ . Anal. calcd for  $\text{C}_{23}\text{H}_{18}\text{N}_2\text{OS}$ : C 74.57, H 4.90, N 7.56, S 8.65; found C 74.84, H 4.99, N 7.41, S 8.78.

9-*n*-Butyl-1-(2-thienyl)-3-hydroxymethyl- $\beta$ -carboline (**3d**): Light yellow solid, yield 66.4%. m.p. 101.8~103.1 °C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$ : 8.16 (d,  $J=8.0$  Hz, 1H), 7.95 (s, 1H), 7.58~7.62 (m, 1H), 7.52 (dd,  $J=5.2$ , 0.8 Hz, 1H), 7.46 (d,  $J=8.0$  Hz, 1H), 7.28~7.32 (m, 2H), 7.19 (dd,  $J=5.2$ , 3.6 Hz, 1H), 4.96 (s, 2H), 4.12 (t,  $J=8.0$  Hz, 2H), 1.38~1.46 (m, 2H), 0.93~1.03 (m, 2H), 0.72 (t,  $J=7.2$  Hz, 3H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$ : 147.95, 142.91, 140.75, 135.75, 134.35, 132.02, 128.73, 128.38, 127.15, 126.72, 121.73, 121.22, 119.97, 111.01, 110.36, 64.72, 44.27, 31.14, 19.97, 13.59; ESI-MS  $m/z$ : 337  $[\text{M}+\text{H}]^+$ . Anal. calcd for  $\text{C}_{20}\text{H}_{20}\text{N}_2\text{OS}$ : C 71.40, H 5.99, N 8.33, S 9.53; found C 71.03, H 6.10, N 8.20, S 9.74.

### 3.2.3 General procedure for the preparation of **4a**~**4d**

The mixture of compound **3a** (2.20 g, 6 mmol), activated  $\text{MnO}_2$  (30 mmol) in  $\text{CH}_3\text{CN}$  (150 mL), was stirred under reflux for 2 h. After the completion of the reaction (monitored by TLC), then cooled to room temperature and filtered through Celite. The filtrate was passed through silica gel

and washed with dichloromethane, and the solvent was removed under reduced pressure. The residue was crystallized from acetone or acetone-petroleum ether to give the corresponding compound **4a**.

9-Benzyl-1-(3-pyridyl)- $\beta$ -carboline-3-carbaldehyde (**4a**): White solid, yield 86.7%. m.p. 127.6~128.9 °C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$ : 10.23 (s, 1H), 8.84 (s, 1H), 8.68~8.71 (m, 2H), 8.31~8.34 (m, 1H), 7.61~7.66 (m, 2H), 7.43~7.48 (m, 2H), 7.22~7.26 (m, 2H), 7.16~7.20 (m, 1H), 7.10~7.15 (m, 2H), 6.47~6.49 (m, 2H), 5.32 (s, 2H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$ : 193.36, 148.95, 143.45, 142.74, 140.12, 137.88, 137.76, 136.42, 131.13, 128.57, 129.47, 128.33, 127.33, 126.91, 125.41, 122.11, 121.85, 121.62, 120.87, 113.40, 111.24, 49.23; ESI-MS  $m/z$ : 364  $[\text{M}+\text{H}]^+$ . Anal. calcd for  $\text{C}_{24}\text{H}_{17}\text{N}_3\text{O}$ : C 79.32, H 4.72, N 11.56; found C 79.29, H 4.83, N 11.33.

9-*n*-Butyl-1-(3-pyridyl)- $\beta$ -carboline-3-carbaldehyde (**4b**): Yellow solid, yield 94.2%. m.p. 146.1~148.7 °C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$ : 10.23 (s, 1H), 8.93 (dd,  $J=2.0$ , 0.8 Hz, 1H), 8.82 (dd,  $J=5.2$ , 1.6 Hz, 1H), 8.79 (s, 1H), 8.25~8.28 (m, 1H), 8.02~8.05 (m, 1H), 7.65~7.69 (m, 1H), 7.53~7.56 (m, 1H), 7.51 (d,  $J=8.4$  Hz, 1H), 7.39~7.43 (m, 1H), 4.02 (t,  $J=8.0$  Hz, 2H), 1.36~1.44 (m, 2H), 0.86~0.94 (m, 2H), 0.68 (t,  $J=7.2$  Hz, 3H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$ : 193.00, 149.96, 149.94, 143.49, 142.52, 140.49, 136.78, 136.61, 135.38, 130.88, 129.42, 123.30, 121.99, 121.79, 121.38, 113.79, 110.71, 44.72, 30.95, 19.78, 13.44; ESI-MS  $m/z$ : 330  $[\text{M}+\text{H}]^+$ . Anal. calcd for  $\text{C}_{21}\text{H}_{19}\text{N}_3\text{O}$ : C 76.57, H 5.81, N 12.76; found C 76.92, H 5.85, N 12.66.

9-Benzyl-1-(2-thienyl)- $\beta$ -carboline-3-carbaldehyde (**4c**): White solid, yield 87.3%. m.p. 117.7~119.5 °C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$ : 10.27 (s, 1H), 8.79 (s, 1H), 8.29 (d,  $J=8.0$  Hz, 1H), 7.58~7.62 (m, 1H), 7.47 (dd,  $J=5.2$ , 1.2 Hz, 1H), 7.37~7.44 (m, 2H), 7.16~7.20 (m, 3H), 7.04 (dd,  $J=3.6$ , 1.2 Hz, 1H), 6.99 (dd,  $J=5.2$ , 3.6 Hz, 1H), 6.70~6.73 (m, 2H), 5.41 (s, 2H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$ : 193.21, 143.64, 143.11, 139.47, 137.49, 137.46, 136.36, 131.07, 129.59, 128.68, 128.65, 127.78, 127.46, 126.93, 125.59, 121.94, 121.84, 121.67, 113.49, 111.26, 48.18; ESI-MS  $m/z$ : 369  $[\text{M}+\text{H}]^+$ . Anal. calcd for  $\text{C}_{23}\text{H}_{16}\text{N}_2\text{OS}$ : C 74.98, H 4.38, N 7.60, S 8.70; found C 74.69, H 4.50, N 7.48, S 8.80.

9-*n*-Butyl-1-(2-thienyl)- $\beta$ -carboline-3-carbaldehyde (**4d**): White solid, yield 86%. m.p. 105.2~107.2 °C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$ : 10.25 (s, 1H), 8.75 (s, 1H), 8.25 (d,  $J=8.0$  Hz, 1H), 7.64~7.68 (m, 1H), 7.58 (dd,  $J=5.2$ , 1.2 Hz, 1H), 7.51 (d,  $J=8.4$  Hz, 1H), 7.40 (t,  $J=7.6$  Hz, 1H), 7.34 (dd,  $J=3.6$ , 1.2 Hz, 1H), 7.22 (dd,  $J=5.2$ , 3.6 Hz, 1H), 4.13 (t,  $J=8.0$  Hz, 2H), 1.46~1.54 (m, 2H), 0.98~1.07 (m, 2H), 0.75 (t,  $J=7.2$  Hz, 4H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$ : 193.21, 143.21, 142.57, 139.80, 137.22, 137.03, 130.78, 129.29, 128.76, 127.56, 126.98, 121.94, 121.79, 121.26, 113.56, 110.76, 44.57, 31.39, 20.00, 13.57; ESI-MS  $m/z$ : 335  $[\text{M}+\text{H}]^+$ . Anal. calcd for  $\text{C}_{20}\text{H}_{18}\text{N}_2\text{OS}$ : C 71.83, H 5.43, N 8.38, S 9.59; found C 71.50, H 5.53, N 8.26, S 9.58.

### 3.2.4 General procedure for the preparation of the target compounds **5a**~**5h**

To a solution of compound **4a** (0.73g, 2 mmol) in anhydrous methanol (30 mL) and anhydrous  $\text{CH}_2\text{Cl}_2$  (10 mL) was stirred at room temperature for 10 min, and the corresponding diamine (1.0 mmol) was added. The mixture was refluxed for 2~5 h. The solvent was evaporated under vacuum to give the crude schiff base, which was used directly in the next step without further purification. Then the crude Schiff base in anhydrous  $\text{CH}_3\text{OH}$  (30 mL) at 0 °C,  $\text{NaBH}_3\text{CN}$  (5 mmol) was added. The mixture was stirred at room temperature for 4~6 h. After the completion of the reaction (monitored by TLC), the pH of the aqueous phase was adjusted to 2~3 by the addition of aqueous HCl, and stirred for 1 h. The reaction mixture was neutralized with aqueous NaOH and the reaction mixture was concentrated under vacuum. The residue was dissolved in  $\text{CH}_2\text{Cl}_2$  (150 mL) and washed with aqueous  $\text{Na}_2\text{CO}_3$  (pH 10, 50 mL). The organic layer was separated, dried over anhydrous  $\text{Na}_2\text{SO}_4$ , filtered, and concentrated under vacuum. The residue was purified by flash chromatography on silica gel [ $V(\text{CH}_2\text{Cl}_2) : V(\text{CH}_3\text{OH}) : V(\text{NH}_4\text{OH}) = 100 : 1 : 0.8$ ] to provide target products.

*N,N*-Bis[[(9-benzyl-1-(3-pyridyl)- $\beta$ -carboline)-3-yl)methyl]butane-1,4-diamine (**5a**): Yellow solid, yield 47.4%. m.p. 159.0~161.2 °C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$ : 8.59 (m, 4H), 8.21 (d,  $J=7.6$  Hz, 2H), 8.08 (s, 2H), 7.50~7.57 (m, 4H), 7.30~7.35 (m, 4H), 7.05~7.14 (m, 8H), 6.45 (d,  $J=7.6$  Hz, 4H), 5.19 (s, 4H), 4.08 (s, 4H), 2.79 (t,  $J=6.4$  Hz, 4H), 1.67~1.70 (m, 4H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$ : 149.89, 149.23, 143.06, 140.17, 136.52, 136.29, 135.48, 133.73, 131.71, 128.94, 128.53, 127.28, 125.33, 122.69, 121.82, 121.24, 120.41, 112.87, 110.27, 54.89, 49.40, 48.10, 27.99. HRMS calcd for  $\text{C}_{52}\text{H}_{47}\text{N}_8$   $[\text{M} + \text{H}]^+$  783.3918, found 783.3918.

*N,N*-Bis[[(9-benzyl-1-(3-pyridyl)- $\beta$ -carboline)-3-yl)methyl]pentane-1,5-diamine (**5b**): Yellow solid, yield 40.1%. m.p. 97.8~99.9 °C;  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ , 400 MHz)  $\delta$ : 8.82~8.85 (m, 4H), 8.40 (d,  $J=7.6$  Hz, 2H), 8.27 (d,  $J=8.0$  Hz, 2H), 7.82 (d,  $J=8.4$  Hz, 2H), 7.67~7.76 (m, 4H), 7.41~7.45 (m, 4H), 7.04~7.13 (m, 6H), 6.35 (d,  $J=7.2$  Hz, 4H), 5.44 (s, 4H), 4.45 (t,  $J=5.2$  Hz, 4H), 2.91 (t,  $J=8.0$  Hz, 4H), 1.68~1.75 (m, 4H), 1.33~1.40 (m, 2H);  $^{13}\text{C}$  NMR ( $\text{DMSO}-d_6$ , 100 MHz)  $\delta$ : 143.78, 143.38, 143.10, 142.81, 140.61, 136.93, 136.28, 133.33, 131.68, 129.74, 128.45, 127.09, 125.26, 124.96, 121.89, 120.93, 120.08, 115.52, 111.11, 50.16, 47.65, 46.34, 24.60, 23.03. HRMS calcd for  $\text{C}_{53}\text{H}_{49}\text{N}_8$   $[\text{M} + \text{H}]^+$  797.4080, found 797.4077.

*N,N*-Bis[[(9-*n*-butyl-1-(3-pyridyl)- $\beta$ -carboline)-3-yl)methyl]butane-1,4-diamine (**5c**): Yellow solid, yield 57.7%. m.p. 225.7~227.2 °C;  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ , 400 MHz)  $\delta$ : 9.32 (s, 2H), 9.00 (dd,  $J=5.2$ , 1.2 Hz, 2H), 8.71 (d,  $J=8.0$  Hz, 2H), 8.62 (s, 2H), 8.35 (d,  $J=8.0$  Hz, 2H), 8.03~8.06 (m, 2H), 7.84 (d,  $J=8.4$  Hz, 2H), 7.70~7.74 (m, 2H), 7.41 (t,  $J=8.0$  Hz, 2H), 4.50 (s, 4H), 4.08 (t,  $J=8.0$  Hz, 4H), 3.03~3.10 (m, 4H), 1.80~1.89 (m, 4H), 1.21~1.29 (m, 4H), 0.77~0.87 (m, 4H), 0.57 (t,  $J=7.2$  Hz, 6H);  $^{13}\text{C}$  NMR

( $\text{DMSO}-d_6$ , 100 MHz)  $\delta$ : 145.19, 144.96, 142.56, 139.82, 136.90, 135.97, 133.25, 131.50, 129.59, 125.18, 121.81, 120.66, 120.02, 115.52, 111.17, 50.02, 45.90, 43.91, 30.10, 22.50, 18.96, 13.12. HRMS calcd for  $\text{C}_{46}\text{H}_{51}\text{N}_8$   $[\text{M} + \text{H}]^+$  715.4231, found 715.4231.

*N,N*-Bis[[(9-*n*-butyl-1-(3-pyridyl)- $\beta$ -carboline)-3-yl)methyl]pentane-1,5-diamine (**5d**): Yellow solid, yield 64.5%. m.p. 100.4~102.8 °C;  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ , 400 MHz)  $\delta$ : 9.40 (s, 2H), 9.05 (d,  $J=4.4$  Hz, 2H), 8.82 (d,  $J=6.0$  Hz, 2H), 8.67 (s, 2H), 8.35 (d,  $J=8.0$  Hz, 2H), 8.11~8.14 (m, 2H), 7.85 (d,  $J=8.4$  Hz, 2H), 7.73 (t,  $J=7.6$  Hz, 2H), 7.41 (t,  $J=8.0$  Hz, 2H), 4.50 (s, 4H), 4.08 (t,  $J=8.0$  Hz, 4H), 3.01~3.04 (m, 4H), 1.73~1.81 (m, 4H), 1.39~1.45 (m, 2H), 1.11~1.29 (m, 4H), 0.77~0.87 (m, 4H), 0.57 (t,  $J=7.2$  Hz, 6H);  $^{13}\text{C}$  NMR ( $\text{DMSO}-d_6$ , 100 MHz)  $\delta$ : 145.01, 144.76, 143.25, 140.25, 136.87, 136.62, 133.81, 132.28, 130.31, 126.16, 122.45, 121.33, 120.57, 116.40, 111.80, 50.41, 46.83, 44.56, 31.18, 30.65, 25.24, 23.61, 19.54, 13.70. HRMS calcd for  $\text{C}_{47}\text{H}_{53}\text{N}_8$   $[\text{M} + \text{H}]^+$  729.4388, found 729.4348.

*N,N*-Bis[[(9-benzyl-1-(2-thienyl)- $\beta$ -carboline)-3-yl)methyl]butane-1,4-diamine (**5e**): Yellow solid, yield 45.6%. m.p. 161.5~163.7 °C;  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ , 400 MHz)  $\delta$ : 8.52 (s, 2H), 8.34 (d,  $J=8.0$  Hz, 2H), 7.63~7.75 (m, 6H), 7.39 (t,  $J=7.6$  Hz, 2H), 7.30 (d,  $J=2.4$  Hz, 2H), 7.08~7.17 (m, 8H), 6.54~6.56 (m, 4H), 5.55 (s, 4H), 4.43 (s, 4H), 3.08 (t,  $J=7.6$  Hz, 4H), 1.76~1.84 (m, 4H);  $^{13}\text{C}$  NMR ( $\text{DMSO}-d_6$ , 100 MHz)  $\delta$ : 142.97, 140.15, 138.81, 137.01, 136.01, 133.64, 131.56, 129.58, 129.27, 128.57, 128.31, 127.00, 126.81, 125.51, 121.79, 120.88, 120.29, 114.92, 111.51, 50.16, 47.02, 46.12, 22.53. HRMS calcd for  $\text{C}_{50}\text{H}_{45}\text{N}_6\text{S}_2$   $[\text{M} + \text{H}]^+$  793.3142, found 793.3142.

*N,N*-Bis[[(9-benzyl-1-(2-thienyl)- $\beta$ -carboline)-3-yl)methyl]pentane-1,5-diamine (**5f**): Yellow solid, yield 49.4%. m.p. 242.6~244.4 °C;  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ , 400 MHz)  $\delta$ : 8.47 (s, 2H), 8.33 (d,  $J=8.0$  Hz, 2H), 7.71~7.75 (m, 4H), 7.62~7.66 (m, 2H), 7.39 (t,  $J=7.6$  Hz, 2H), 7.30 (dd,  $J=3.6$ , 1.2 Hz, 2H), 7.24 (s, 2H), 7.08~7.16 (m, 10H), 5.55 (s, 4H), 4.42 (s, 4H), 3.03 (t,  $J=7.6$  Hz, 4H), 1.68~1.76 (m, 4H), 1.35~1.43 (m, 2H);  $^{13}\text{C}$  NMR ( $\text{DMSO}-d_6$ , 100 MHz)  $\delta$ : 142.80, 140.66, 139.85, 137.11, 136.48, 133.63, 131.31, 129.31, 128.87, 128.26, 126.97, 126.77, 125.51, 121.64, 120.72, 120.36, 114.33, 111.47, 50.50, 46.95, 46.51, 24.74, 23.02. HRMS calcd for  $\text{C}_{51}\text{H}_{47}\text{N}_6\text{S}_2$   $[\text{M} + \text{H}]^+$  807.3298, found 807.3304.

*N,N*-Bis[[(9-*n*-butyl-1-(2-thienyl)- $\beta$ -carboline)-3-yl)methyl]butane-1,4-diamine (**5g**): Yellow solid, yield 43.1%. m.p. 195.9~197.7 °C;  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ , 400 MHz)  $\delta$ : 8.69 (s, 2H), 8.33 (d,  $J=8.0$  Hz, 2H), 7.91 (dd,  $J=5.2$ , 0.8 Hz, 2H), 7.83 (d,  $J=8.4$  Hz, 2H), 7.68~7.75 (m, 2H), 7.54 (dd,  $J=3.6$ , 1.2 Hz, 2H), 7.41 (t,  $J=7.6$  Hz, 2H), 7.30 (dd,  $J=5.2$ , 3.6 Hz, 2H), 4.50 (t,  $J=5.2$  Hz, 4H), 4.21 (t,  $J=8.0$  Hz, 4H), 3.06~3.13 (m, 4H), 1.80~1.91 (m, 4H), 1.28~1.36 (m, 4H), 0.86~0.96 (m, 4H), 0.65 (t,  $J=7.6$  Hz, 6H);  $^{13}\text{C}$  NMR ( $\text{DMSO}-d_6$ , 100 MHz)  $\delta$ : 142.84, 138.81, 135.10, 133.46, 131.65, 130.08, 129.81, 128.82, 127.02, 121.90, 120.77, 119.92, 115.65, 111.25, 49.53, 46.03, 43.68, 30.72,

22.54, 19.16, 13.23. HRMS calcd for  $C_{44}H_{49}N_6S_2$   $[M+H]^+$  725.3455, found 725.3447.

*N,N*-Bis[[(9-*n*-butyl-1-(2-thienyl)- $\beta$ -carboline)-3-yl]methyl]pentane-1,5-diamine (**5h**): Yellow solid, yield 50%. m.p. 262.7~264.6 °C;  $^1H$  NMR (DMSO- $d_6$ , 400 MHz)  $\delta$ : 8.63 (s, 2H), 8.32 (d,  $J=8.0$  Hz, 2H), 7.89 (dd,  $J=5.2, 0.8$  Hz, 2H), 7.82 (d,  $J=8.4$  Hz, 2H), 7.69~7.74 (m, 2H), 7.52 (dd,  $J=3.6, 1.2$  Hz, 2H), 7.40 (t,  $J=7.6$  Hz, 2H), 7.28~7.30 (m, 2H), 4.48 (t,  $J=5.2$  Hz, 4H), 4.21 (t,  $J=8.0$  Hz, 4H), 3.00~3.09 (m, 4H), 1.73~1.80 (m, 4H), 1.41~1.46 (m, 2H), 1.27~1.35 (m, 4H), 0.85~0.95 (m, 4H), 0.64 (t,  $J=7.6$  Hz, 6H);  $^{13}C$  NMR (DMSO- $d_6$ , 100 MHz)  $\delta$ : 142.75, 139.07, 135.29, 133.46, 131.52, 129.90, 129.68, 128.67, 127.00, 121.84, 120.69, 119.95, 115.42, 111.23, 49.71, 46.40, 43.64, 30.71, 24.72, 23.04, 19.16, 13.23; HRMS calcd for  $C_{45}H_{51}N_6S_2$   $[M+H]^+$  739.3611, found 739.3611.

### 3.2.5 Biology cell viability assay

Cytotoxicity assays *in vitro* were carried out using 96-well plate cultures and MTT staining according to the procedures described by Chen *et al.*<sup>[27]</sup> Briefly, the cancer cells were grown in RPMI-1640 medium, supplemented with 10% (*V/V*) fetal calf serum, 100  $\mu g \cdot mL^{-1}$  penicillin and 100  $\mu g \cdot mL^{-1}$  streptomycin. Cultured cells were propagated at 37 °C in a humidified atmosphere containing 5%  $CO_2$ . In the experiments, cells were allowed to acclimate for 24 h before any treatments. Cell lines were obtained from Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Science. DMSO was used as the solution for drugs. The human tumor cell lines were inoculated into fresh media containing fivefold diluted target compounds. Final concentration of DMSO in the growth medium was 2% (*V/V*) or lower, concentration without effect on cell replication. After incubation for 48 h, MTT assay was carried out in triplication and automatic microplate reader was used to determine absorbance. Cisplatin was the positive control. The optical density (OD) was read at 490 nm. In all of these experiments, three replicate wells were used to determine each point.  $IC_{50}$  values were calculated by the Logit method.

**Supporting Information**  $^1H$  NMR,  $^{13}C$  NMR, HRMS data of all the target compounds. The Supporting Information is available free of charge via the Internet at <http://sioc-journal.cn/>.

## References

- [1] Michael, C.; Robert, W. W.; Fil, G.; James, M. C.; Steven, A. B.; Kenner, C. R.; Jacqueline, N. C.; Steven, M. P.; Phil, S. *J. Med. Chem.* **1982**, 25, 1081.
- [2] Rashid, M. A.; Gustafson, K. R.; Boyd, M. R. *J. Nat. Prod.* **2001**, 64, 1454.
- [3] Kuo, P.-C.; Shi, L.-S.; Damu, A. G.; Su, C.-R.; Huang, C.-H.; Ke, C.-H.; Wu, J.-B.; Lin, A.-J.; Bastow, K. F.; Lee, K.-H.; Wu, T.-S. *J. Nat. Prod.* **2003**, 66, 1324.
- [4] Srivastava, S. K.; Agarwal, A.; Chauhan, P. M. S.; Agarwal, S. K.; Bhaduri, A. P.; Singh, S. N.; Fatima, N.; Chatterjee, R. K. *Bioorg. Med. Chem.* **1999**, 7, 1223.
- [5] Wang, Y.-H.; Tang, J.-G.; Wang, R.-R.; Yang, L.-M.; Dong, Z.-J.; Du, L.; Shen, X.; Liu, J.-K.; Zheng, Y.-T. *Biochem. Biophys. Res. Commun.* **2007**, 355, 1091.
- [6] Shankaraiah, N.; Siraj, K. P.; Nekkanti, S.; Srinivasulu, V.; Sharma, P.; Senwar, K. R.; Sathish, M.; Vishnuvardhan, M. V. P. S.; Ramakrishna, S.; Jadala, C.; Nagesh, N.; Kamal, A. *Bioorg. Chem.* **2015**, 59, 130.
- [7] Kamal, A.; Sathish, M.; Nayak, V. L.; Srinivasulu, V.; Kavitha, B.; Tangella, Y.; Thummuri, D.; Bagul, C.; Shankaraiah, N.; Nagesh, N. *Bioorg. Med. Chem.* **2015**, 23, 5511.
- [8] Figueiredo, P. O.; Perdomo, R. T.; Garcez, F. R.; Matos, M. F. C.; Carvalho, J. E.; Garcez, W. S. *Bioorg. Med. Chem. Lett.* **2014**, 24, 1358.
- [9] Li, Y.; Liang, F.-S.; Jiang, W.; Yu, F.-S.; Cao, R.-H.; Ma, Q.-H.; Dai, X.-Y.; Jiang, J.-D.; Wang, Y.-C.; Si, S.-Y. *Cancer Biol. Ther.* **2007**, 6, 1193.
- [10] Zhang, J.; Li, Y.; Guo, L.; Cao, R.-H.; Zhao, P.; Jiang, W.; Ma, Q.; Yi, H.; Li, Z.-R.; Jiang, J.-D.; Wu, J.-L.; Wang, Y.-C.; Si, S.-Y. *Cancer Biol. Ther.* **2009**, 8, 2374.
- [11] Barsanti, P. A.; Wang, W.; Ni, Z.; Duhl, D.; Brammeier, N.; Martin, E.; Bussiere, D.; Walter, A. O. *Bioorg. Med. Chem. Lett.* **2010**, 20, 157.
- [12] Castro, A. C.; Dang, L. C.; Soucy, F.; Grenier, L.; Mazdiyasni, H.; Hottelet, M.; Parent, L.; Pien, C.; Palombella, V.; Adams, J. *Bioorg. Med. Chem. Lett.* **2003**, 13, 2419.
- [13] Gauguier, B.; Barbet, J.; Capelle, N.; Roques, B. P.; Le Pecq, J. B.; Le Bret, M. *Biochemistry* **1978**, 17, 5078.
- [14] Capelle, N.; Barbet, J.; Dessen, P.; Blanquet, S.; Roques, B. P.; Le Pecq, J. B. *Biochemistry* **1979**, 18, 3354.
- [15] Wang, K.-B.; Di, Y.-T.; Bao, Y.; Yuan, C.-M.; Chen, G.; D. Li, H.; Bai, J.; He, H.-P.; Hao, X.-J.; Pei, Y.-H.; Jing, Y.-K.; Li, Z.-L.; Hua, H.-M. *Org. Lett.* **2014**, 16, 4028.
- [16] Joshi, A.; Vance, D.; Rai, P.; Thiagarajan, A.; Kane, R. S. *Chem. Eur. J.* **2008**, 14, 7738.
- [17] Brana, M. F.; Castellano, J. M.; Moran, M.; Perez de Vega, M. J.; Perron, D.; Conlon, D.; Bousquet, P. F.; Romerdahl, C. A.; Robinson, S. P. *Anticancer Drug Des.* **1996**, 11, 297.
- [18] Burns, M. R.; Turner, S. L.; Ziemer, J.; Veau, M. M.; Devens, B.; Carlson, C. L.; Graminski, G. F.; Vanderwerf, S. M.; Weeks, R. S.; Carreon, J. *Bioorg. Med. Chem. Lett.* **2002**, 12, 1263.
- [19] Guo, L.; Sun, J.; Fan, W.-X.; Ma, Q. *Chin. J. Modern Appl. Pharm.* **2012**, 29, 385 (in Chinese). (郭亮, 孙洁, 范文玺, 马芹, 中国现代应用药学, **2012**, 29, 385.)
- [20] Guo, L.; Cao, R.-H.; Fan, W.-X.; Ma, Q. *Chem. J. Chin. Univ.* **2014**, 35, 518 (in Chinese). (郭亮, 曹日晖, 范文玺, 马芹, 高等学校化学学报, **2014**, 35, 518.)
- [21] Guo, L.; Fan, W.-X.; Chen, X.-M.; Ma, Q.; Cao, R.-H. *Chin. J. Org. Chem.* **2013**, 33, 332 (in Chinese). (郭亮, 范文玺, 陈雪梅, 马芹, 曹日晖, 有机化学, **2013**, 33, 332.)
- [22] Guo, L.; Fan, W.-X.; Chen, W.; Ma, Q.; Cao, R.-H. *J. Chin. Pharm. Sci.* **2015**, 24, 801.
- [23] Zhang, G.-X.; Cao, R.-H.; Guo, L.; Ma, Q.; Fan, W.-X.; Chen, X.-M.; Li, J.-R.; Shao, G.; Qiu, L.-Q.; Ren, Z.-H. *Eur. J. Med. Chem.* **2013**, 65, 21.
- [24] Shi, B.-X.; Cao, R.-H.; Fan, W.-X.; Guo, L.; Ma, Q.; Chen, X.-M.; Zhang, G.-X.; Qiu, L.-Q.; Song, H.-C. *Eur. J. Med. Chem.* **2013**, 60, 10.
- [25] Wu, Q.-F.; Bai, Z.-S.; Ma, Q.; Fan, W.-X.; Guo, L.; Zhang, G.-X.; Qiu, L.-Q.; Yu, H.; Shao, G.; Cao, R.-H. *Med. Chem. Commun.* **2014**, 5, 953.
- [26] Guo, L.; Cao, R.-H.; Fan, W.-X.; Gan, Z.-Y.; Ma, Q. *Chem. J. Chin. Univ.* **2016**, 37, 1093 (in Chinese). (郭亮, 曹日晖, 范文玺, 甘紫云, 马芹, 有机化学, **2016**, 37, 1093.)
- [27] Chen, J.; Zhang, Y.-K.; Zhan, X.-P.; Liu, Z.-L.; Mao, Z.-M. *Chin. J. Org. Chem.* **2016**, 36, 572 (in Chinese). (陈简, 张袁魁, 詹晓平, 刘增路, 毛振民, 有机化学, **2016**, 36, 572.)

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