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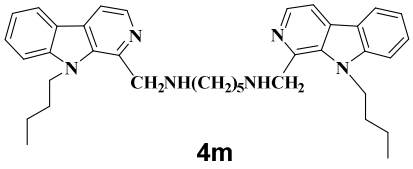
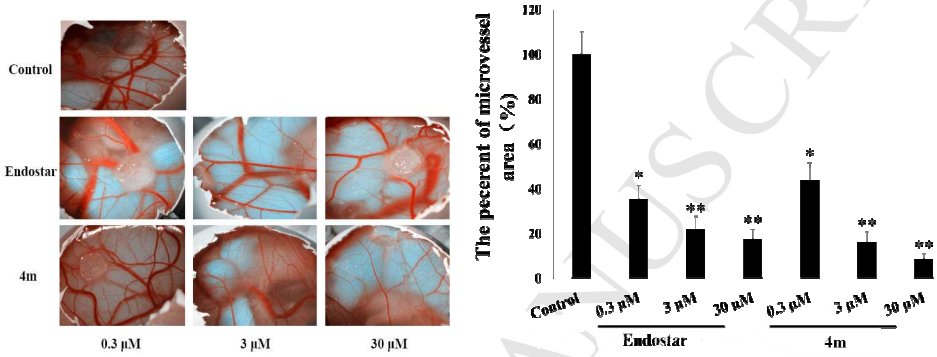
Graphic abstract





Synthesis and biological evaluation of novel alkyl diamine linked bivalent β-carbolines as angiogenesis inhibitors

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Abstract

We have synthesized and evaluated a series of novel alkyl diamine linked bivalent β-carbolines as potent angiogenesis inhibitors. The results demonstrated that most bivalent β-carbolines exhibited significant antiproliferative effects against human umbilical vein cell lines EA.HY926. Compound **4m** was found to be the most potent antiproliferative agent with IC_{50} value of 2.16 μM against EA.HY926 cell lines. Mechanism investigations revealed that compound **4m** could significantly inhibit EA.HY926 cells migration and tube formation in a dose-dependent manner. Moreover, compound **4m** also showed obvious angiogenesis inhibitory effects in CAM assay, and the antiangiogenetic potency was more potent than the reference drug Endostar. The bivalent β-carbolines might be served as candidates for the development of vascular targeting antitumor drugs.

Keywords: Synthesis; Bivalent β-carbolines; Antitumor; Angiogenesis inhibitors; Structure-activity relationships.

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1. Introduction

Angiogenesis, the sprouting of new blood capillaries from pre-existing vessels, is a physiological process involving endothelial cells activation, invasion, migration, proliferation, tube formation, and finally capillary network formation[1]. Angiogenesis is also vital for the sustained growth, proliferation, invasion and metastasis of solid tumors [2]. Without the development and progression of new blood vessels, tumors cannot deteriorate beyond a critical size or metastasize to other organs [3]. Endothelial cells play an important role in the complicated process of tumor angiogenesis. Therefore, inhibition of the proliferation, migration, and tube formation of endothelial cells might be an effective therapy for suppressing tumor progression and metastasis. Accordingly, the search for inhibitors of this process has become a hot topic in the treatment of tumor, and several tumor angiogenesis drugs have been marketed. Nowadays, there are still more than 80 angiogenesis inhibitors currently in clinical trials [4].

The β-carbolines represent a large group of naturally occurring and synthetic alkaloids associated with a broad spectrum of biochemical and pharmaceutical effects [5]. In the last two decades, the β-carbolines have been characterized as a new class of potential antitumor agents, and a large number of synthetic β-carbolines acting as antitumor agents were reported [6-13, 51]. Previous investigations indicated that this class of compounds exerted their antitumor effects through multiple mechanisms of action including intercalating into DNA [14-15], inhibiting Topo I and II (topoisomerase I and II) [16-17], CDK (cyclin-dependent kinase) [18-19], MK-2

(mitogen activated protein kinase-activated protein kinase 2) [20], kinesin-like protein Eg5 [21] and IKK (I-Kappa-B kinase) [22].

In our previous reports, we described the preparation and antitumor activities *in vitro* [23-36] and *in vivo* [23, 25, 35-36] of numerous β-carboline derivatives bearing various substituents in position-1, 2, 3, 7 and 9 of β-carboline nucleus. The SARs analysis indicated that (1) the common β-carboline moiety was very important for their potential antitumor activities; (2) the introduction of appropriate substituents into position-1, 3 and 9 of β-carboline nucleus faciliated their antitumor potencies. Our previous studies on the mechanism of action demonstrated that the ability of β-carbolines to act as DNA intercalating agents and Top I inhibitors was related to their potent antitumor activities [37], and these molecules could pass through cell membrane and penetrate into cell nucleus quickly resulting in intercalating into DNA in cells [26]. In addition, some β-carbolines were observed to induce apoptosis in HepG2 cells and down-regulate the expression of *Bcl-2* gene and upregulate the expression of death receptor *Fas* without altering the level of *Bax* and p53 [38]. Our recent investigations revealed that N^2-benzyl substituted quaternary β-carbolines was new and potent PLK inhibitors with potential for cancer treatment [39-40].

Previous reports [41-45] demonstrated that dimerization of various antitumor agents by an appropriate linker could lead to significantly improved antitumor activities (100 to 500-fold better than the corresponding monomers). Consequently, dimers of β-carboline were also expected to show more significant *in vitro* antitumor activity and more potent *in vivo* efficacy than monomers. Firstly, our group reported

the synthesis, *in vitro* evaluation, *in vivo* efficacies and structure-activity relationships for the new bivalent β-carbolines linked at the N-9 and C-3 position with a spacer of three to ten methylene units, respectively (**Figure 1**) [46-47], however, these bivalent β-carbolines had limited utility for cancer therapy because of their poor water solubility. To circumvent the water solubility problem and find congeners more active as potential antitumor agents, recently, we described the synthesis and cytotoxic potencies of novel bivalent β-carbolines linked with piperazine group (**Figure 1**), and these compounds exhibited significantly improved antitumor activities and water solubility [48]. The bivalent β-carbolines was originally expected to exerted their antitumor effects by DNA intercalating or Top I inhibiting effect. Unfortunately, our group found that these compounds had no effect on DNA and Top I, but exhibitd significant inhibitory effect on the vessel growth in chicken chorioallantoic membrane (CAM) assay [48]. These molecules might be served as angiogenesis inhibitors for the development of vascular targeting antitumor drugs.

In a continuing effort to develop novel bivalent β-carbolines endowed with better antiangiogenic activity, in the present investigation, we designed and synthesized a series of novel alkyl diamine linked bivalent β-carbolines as potent antivascular agents. These compounds were expected to exhibit significantly improved antivascular activity due to the improved water solubility. We report herein the preparation of novel bivalent β-carbolines and their biological evaluation as angiogenesis inhibitors.

2. Chemistry

The synthetic route for the preparation of monovalent and bivalent β-carbolines is shown in **Scheme 1**. The monovalent β-carboline **1** was obtained by the condensation of L-tryptophan with acetaldehyde in acid solution and followed by aromatization, oxidation and decarboxylation in a single step through the action of potassium dichromate [49]. The N^9 of monovalent β-carboline **1** was alkylated or arylated by the action of sodium hydride in anhydrous DMF followed by the addition of the appropriate alkylating and arylating agents to afford intermediates **2a**–**l** [23] in 65-82% yield. The methyl group in position-1 of **2a**–**l** was further oxidized to carboxaldehyde by SeO_2 in anhydrous dioxane to provide β-carboline-1-carboxaldehydes **3a–l** in 31-61% yield [34].

The reaction of compounds **3a-l** with the corresponding sym-diamines to form schiff bases took place readily at room temperature in good yield. The crude schiff bases without further purification were directly reduced with $NaBH_3CN$ in anhydrous methanol to give the target bivalent β-carbolines **4a-ad** in 48-89% yield. The chemical structures of all new bivalent β-carbolines were characterized by ESI-MS, ^{1}H NMR, ^{13}C NMR and HRMS.

3. Results and Discussion

3.1 Inhibitory effect on EA.HY926s proliferation

The inhibitory potencies of novel bivalent β-carbolines against human umbilical vein cell lines EA.HY926 were investigated and compared with the reference drugs

CA_4P and Endostar. In order to enhance the solubility in aqueous solution, all bivalent β-carbolines were prepared in the form of hydrochloride salt before use. As predicted, the hydrochloride salt of novel bivalent β-carbolines linked with a spacer of alkyl diamine in position-3 showed good water-solubility (more than 10 mg/ml). The results were summarized in **Table 1**.

As shown in **Table 1**, most bivalent β-carbolines exhibited good antiproliferative effects with IC_{50} value of lower than 10.0μM against human umbilical vein cell lines EA.HY926. Compounds **4l**, **4m**, **4n**, **4v**, **4x**, **4z**, **4ab**, **4ac** and **4ad** displayed significant antiproliferative potencies with IC_{50} value of lower than 5.0μM against EA.HY926 cell lines, and compound **4m** was found to be the most potent antiproliferative agent with IC_{50} value of 2.16μM against EA.HY926 cell lines, and the antiproliferative potency of compound **4m** was comparable with positive control drug Endostar (1.76 μM).

We examined the influence of the substituents in position-9 of β-carboline ring on antiproliferative effects. Compounds **4a** and **4b** having no substituent in position-9 of β-carboline nucleus showed weak antiproliferative effects with IC_{50} value of higher than 10μM against EA.HY926 cell lines. Similarly, compounds **4c** and **4d** bearing a methyl group in position-9 of β-carboline nucleus also displayed weak antiproliferative potencies against EA.HY926 cell lines. While compounds **4e-ad** bearing an ethyl (**4e-f**), isorpropyl (**4g-h**), isobutyl (**4i-j**), n-butyl (**4k-n**), n-hexyl (**4o-r**), n-octyl (**4s-t**), benzyl (**4u-v**), 4-fluorobenzyl (**4w-x**), 3-chlorobenzyl (**4y-z**) and 3-phenylpropyl (**4aa-ad**) group in position-9 of β-carboline ring, respectively,

exhibited good to potent antiproliferative potencies with IC_{50} value of lower than 10.0μM against EA.HY926 cell lines.

Of all bivalent β-carbolines with a linker of five methylene units, compound **4b** with no subsitiuent in position-9 of β-carboline nucleus showed weak antiproliferative effect against EA.HY926 cell lines. Interestingly, compounds bearing an n-butyl (**4m**), n-hexyl (**4q**), n-octyl (**4t**), 3-chlorobenzyl (**4z**) and 3-phenylpropyl (**4ac**) group in position-9 of β-carboline nucleus, respectively, exhibited good to strong antiproliferative effects with the tendency of n-butyl > 3-phenylpropyl > 3-chlorobenzyl > n-hexyl > n-octyl. Similarly, the antiproliferative potencies of compounds **4d**, **4h**, **4j**, **4n**, **4r**, **4v** and **4ad** with a linker of six methylene units followed the sequence of **4n** (n-butyl) > **4ad** (3-phenylpropyl) > **4v** (benzyl) > **4f** (ethyl) > **4j** (isobutyl) > **4r** (n-hexyl) > **4h** (isopropyl) > **4d** (methyl), and the antiproliferative potencies of compounds **4a**, **4c**, **4f**, **4i**, **4l**, **4p**, **4s**, **4x** and **4ab** with a linker of four methylene units followed the sequence of **4l** (n-butyl) > **4ab** (3-phenylpropyl) > **4x** (4-fluorobenzyl) > **4f** (ethyl) > **4p** (n-hexyl) > **4i** (isobutyl) > **4s** (n-octyl) > **4c** (methyl) > **4a** (H). Exceptionally, compounds **4e**, **4g**, **4k**, **4o**, **4u**, **4w**, **4y** and **4aa** with a linker of three methylene units showed no distinct difference and the IC_{50} values of this class of compounds ranged from 7.77 to 9.39μM. These data suggested that the introduction substituent into position-9 of β-carboline ring might facilitated their antiproliferative potencies, and the four to six methylene linear alkyl and arylated alkyl subsitituents were the optimal group giving rise to potent antiproliferative agents.

We also examined the influence of the linker length of bivalent β-carbolines on antiproliferative activities. Compounds **4b**, **4t** and **4z** with a linker of five methylene units exhibited more potent antiproliferative effects than the analogues **4a (**n=4**)**, **4s (**n=4**)** and **4y (**n=3**)**. Of all bivalent β-carbolines having an n-butyl group in position-9 of β-carboline nucleus, compounds **4m** with a linker of five methylene units exhibited more potent antiproliferative effects than the analogues **4k**, **4l** and **4n** and followed the tendency of **4m (**n=5**)** > **4n (**n=6**)** > **4l (**n=4**)** > **4k (**n=3**)**. A similar tendency also applied to compounds **4o-r** and **4aa-ad** bearing an n-hexyl and 3-phenylpropyl group in position-9 of of β-carboline nucleus, respectively. These results showed clearly that the length of the linker had a major effect on the bivalent β-carbolines to inhibit tumor cell growth and five to six methylene units might be more favorable.

3.2 Inhibitory effect on EA.HY926s migration

Cell migration is an essential feature for vascular endothelial cells in angiogenesis. Logically, we investigated the inhibitory effect on the EA.HY926s chemotactic motility of the most potent compound **4m** by wound-healing assay. As shown in **Figure 2**, compound **4m** could inhibit VEGF-induced EA.HY926s migration in a dose-dependent manner ranging from 0.3μM to 30μM. Treatment with compound **4m** significantly inhibited EA.HY926s migration at concentration of 30μM and the inhibitory potency of compound **4m** was more potent than positive drug Endostar.

3.3 Inhibitory effect on EA.HY926s tube formation

We also evaluated the ability of the selected compound **4m** in a tube formation assay. EA.HY926s plated on a Matrigel coated plate could formed capillary-like

tubules with multicentric junctions in cell cultured in the absence of compound (control). After 24 h treatment in different concentrantions (0.3-30μM) of compound **4m**, the capillary-like tubes were interrupted in different levels (**Figure 3A**). Quantitative image analysis showed that compound **4m** significantly decreased the capillary-like tubules in a concentration-dependent manner (**Figure 3B**).

3.4 Anti-angiogenic activity *in vivo*

The most potent compound **4m** was selected to evaluate the angiogenetic activity by CAM assay. The inhibitory effects of compound **4m** on angiogenesis of CAM are shown in **Figure 4A**. The anti-angiogenetic activities of compounds **7g** was semiquantitatively analyzed using Graph Pad Prism 5.0 (shown in **Figure 4B**). The result showed that compound **4m** ($p < 0.05$) could inhibit the angiogenesis of CAM. The anti-angiogenetic activity of compound **4m** was more potent than Endostar *in vivo* CAM assay at the same dose (30μM).

4. Conclusion

A series of novel alkyl diamine linked bivalent β-carbolines with linkers of various lengths and incorporating different substituents into position-9 was synthesized and evaluated as angiogenesis inhibitors. Most bivalent β-carbolines exhibited significant antiproliferative activities against human umbilical vein cell lines EA.HY926. Preliminary structure-activity relationships information revealed that (1) the introduction substituent into position-9 of β-carboline ring might facilitate their antiproliferative potencies, and the four to six methylene linear alkyl and arylated

alkyl subsitituents were the optimal group giving rise to potent antiproliferative agents. (2) the length of the linker had a major effect on the bivalent β-carbolines to inhibit tumor cell growth and five to six methylene units might be more favorable. The most potent compound **4m** was found to significantly inhibit EA.HY926s migration and tube formation in a dose-dependent manner. Moreover, compound **4m** showed obvious angiogenesis inhibitory effects in CAM assay, and the anti-angiogenetic potency was more potent than the reference drug Endostar. Further investigations to confirm antitumor efficacy in animal models and elucidate the pharmacological mechanisms of this class of compounds are underway in our laboratory, and the data will be published elsewhere.

5. Experimental protocols

5. Experimental Section

5.1 Reagents and general methods

All reagents were purchased from commercial suppliers and were dried and purified when necessary, and the preparation of monovalent β-carbolines **2a-c, 2f, 2i-l**, **3a-c, 3f** and **3i-l** has been already described in our previous reports [25, 34].

Melting points were determined in capillary tubes on an electrothermal PIF YRT-3 apparatus and without correction. 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, respectively, using TMS as internal standard and $CDCl_3$ as solvent and chemical shifts (δ) were expressed in ppm.

HRMS were obtained from ESI-Q-TOF maxis 4G spectrometer. Silica gel F254 were used in analytical thin-layer chromatography (TLC) and silica gel were used in column chromatography respectively.

5.2 General procedure for the preparation of 9-alkyl substituted β-carbolines 2d-e and 2g-h.

A mixture of 1-methyl-β-carboline (3.64 g, 20 mmol) and anhydrous DMF (75 ml) was stirred at room temperature until clear, and then 60% NaH (1.2 g, 30 mmol) and halogenated alkane (40 mmol) were added. The mixture was stirred at room temperature. After completion of the reaction as indicated by TLC, the solution was poured into H_2O (100 mL) and extracted with ethyl acetate. The organic phase was washed with water and brine, then dried over anhydrous sodium sulfate, filtered and evaporated. The resulting oil was purified by silica column chromatography with ethyl acetate as the eluent to successfully afford the desirable products.

5.2.1 9-Isopropyl-1-methyl-β-carboline (2d): White solid was obtained (2.91g, 65%). Mp 135.3-138.1℃. ESI-MS m/z: 224.9 $[M+H]^+$. ^{1}H NMR (300MHz, $CDCl_3$): δ 8.31 (d, J = 5.1 Hz, 1H, ArH); 8.12 (d, J = 7.8 Hz, 1H, ArH); 7.82 (d, J = 5.1 Hz, 1H, ArH); 7.70 (d, J = 8.4 Hz, 1H, ArH); 7.51 (t, J = 8.4 Hz, 1H, ArH); 7.29-7.22 (m, 1H, ArH); 5.62-5.50 (m, 1H, NC***H***$[CH_3]_2$); 3.06 (s, 3H, C***H***$_3$); 1.76 (d, J = 7.2 Hz, 6H, NCH[C***H***$_3]_2$). ^{13}C NMR (75MHz, $CDCl_3$): δ 141.2, 140.0, 137.8, 135.9, 129.1, 127.6, 122.8, 121.8, 119.5, 113.4, 113.0, 48.4, 25.2, 21.7.

5.2.2 9-Isobutyl-1-methyl-β-carboline (2e): White solid was obtained (3.76g, 79%). Mp 71.8-72.6℃. ESI-MS m/z: 238.8 $[M+H]^+$. ^{1}H NMR (300MHz, $CDCl_3$): δ 8.33 (d,

J = 5.1 Hz, 1H, ArH); 8.11 (d, *J* = 7.8 Hz, 1H, ArH); 7.84 (d, *J* = 5.1 Hz, 1H, ArH); 7.55 (t, *J* = 8.4 Hz, 1H, ArH); 7.46 (d, *J* = 8.4 Hz, 1H, ArH); 7.28-7.23 (m, 1H, ArH); 4.36 (d, *J* = 7.5 Hz, 2H, NC***H***$_2$CH[CH_3]$_2$); 3.04 (s, 3H, C***H***$_3$); 2.35-2.20 (m, 1H, NCH_2C***H***[CH_3]$_2$); 0.94 (d, *J* = 6.6 Hz, 6H, NCH_2CH[C***H***$_3$]$_2$). ^{13}C NMR (75MHz, $CDCl_3$): δ 142.1, 141.5, 138.2, 135.5, 129.1, 128.0, 121.5, 119.6, 113.0, 110.6, 52.0, 31.0, 24.2, 20.5.

5.2.3 9-n-Hexyl-1-methyl-β-carboline (2g): White solid was obtained (4.36g, 82%). Mp 49.2-52.3℃. ESI-MS m/z: 266.9 $[M+H]^+$. ^{1}H NMR (300MHz, $CDCl_3$): δ 8.31 (d, *J* = 5.1 Hz, 1H, ArH); 8.11 (d, *J* = 7.8 Hz, 1H, ArH); 7.83 (d, *J* = 5.1 Hz, 1H, ArH); 7.57 (t, *J* = 8.4 Hz, 1H, ArH); 7.45 (d, *J* = 8.4 Hz, 1H, ArH); 7.29-7.25 (m, 1H, ArH); 4.52 (t, *J* = 8.1 Hz, 2H, NC***H***$_2$[CH_2]$_4$$CH_3$); 3.06 (s, 3H, C***H***$_3$); 1.91-1.77 (m, 2H, NCH_2C***H***$_2$[CH_2]$_3$$CH_3$); 1.50-1.23 (m, 6H, NCH_2CH_2[C***H***$_2$]$_3$$CH_3$); 0.90 (t, *J* = 6.6 Hz, 3H, NCH_2[CH_2]$_4$C***H***$_3$). ^{13}C NMR (75MHz, $CDCl_3$): δ 141.4, 141.2, 137.9, 135.1, 129.0, 128.1, 121.5, 121.3, 119.6, 113.0, 109.7, 45.0, 31.8, 31.0, 26.9, 23.8, 22.9, 14.3.

5.2.4 9-n-Octyl-1-methyl-β-carboline (2h): Yellow oil was obtained (4.76g, 81%). ESI-MS m/z: 294.8 $[M+H]^+$. ^{1}H NMR (300MHz, $CDCl_3$): δ 8.31 (d, *J* = 5.1 Hz, 1H, ArH); 8.10 (d, *J* = 7.8 Hz, 1H, ArH); 7.82 (d, *J* = 5.1 Hz, 1H, ArH); 7.57 (t, *J* = 8.4 Hz, 1H, ArH); 7.44 (d, *J* = 8.4 Hz, 1H, ArH); 7.29-7.22 (m, 1H, ArH); 4.50 (t, *J* = 7.8 Hz, 2H, NC***H$_2$***[CH_2]$_6$$CH_3$); 3.05 (s, 3H, C***H***$_3$); 1.90-1.77 (m, 2H, NCH_2C***H***$_2$[CH_2]$_5$$CH_3$); 1.48-1.23 (m, 10H, NCH_2CH_2[C***H***$_2$]$_5$$CH_3$); 0.89 (t, *J* = 6.6 Hz, 3H, NCH_2[CH_2]$_6$C***H***$_3$). ^{13}C NMR (75MHz, $CDCl_3$): δ 141.6, 141.4, 138.1, 135.3, 129.2, 128.2, 121.6, 121.5,

119.7, 113.1, 109.9, 45.2, 32.1, 31.1, 29.6, 29.5, 27.3, 23.9, 22.9, 14.4.

5.3. General procedures for the synthesis of 9-alkyl substituted β-carboline-1-carboxaldehydes 3a-d and 3g-h.

To a solution of **2a-d** and **2g-h** (15 mmol) in dioxane (100 mL) was added SeO_2 (40 mmol). The suspension was refluxed for 2 h. After completion of the reaction as indicated by TLC, the mixture was cooled and filtered through Celite. The filtrate was evaporated under reduced pressure. The residue was crystallized from acetone or acetone-petroleum ether to afford the desirable products.

5.3.1 β-Carboline-1-carboxaldehyde (3a): Yellow solid was obtained (1.09g, 31%). Mp 197.5-199.3℃. ESI-MS m/z: 294.8 $[M+H]^+$. ^{1}H NMR (300MHz, $CDCl_3$): δ 10.35 (s, 1H, C***H***O); 10.06 (s, 1H, N***H***); 8.64 (d, J = 5.1 Hz, 1H, ArH); 8.20-8.14 (m, 2H, ArH); 7.67-7.57 (m, 2H, ArH); 7.37 (t, J = 6.3 Hz, 1H, ArH). ^{13}C NMR (75MHz, $CDCl_3$): δ 195.7, 141.4, 139.7, 136.1, 135.3, 131.7, 129.7, 122.0, 121.3, 120.6, 119.4, 112.2.

5.3.2 9-Methyl-β-carboline-1-carboxaldehyde (3b): Yellow solid was obtained (1.63g, 52%). Mp 130.4-132.4℃. ESI-MS m/z: 196.8 $[M+H]^+$. ^{1}H NMR (300MHz, $CDCl_3$): δ 10.31 (s, 1H, C***H***O); 8.62 (d, J = 4.8 Hz, 1H, ArH); 8.17-8.12 (m, 2H, ArH); 7.67 (t, J = 8.4 Hz, 1H, ArH); 7.52 (d, J = 8.4 Hz, 1H, ArH); 4.24 (s, 3H, NC$\boldsymbol{H}_3$). ^{13}C NMR (75MHz, $CDCl_3$): δ 193.6, 143.1, 138.3, 137.6, 135.9, 132.2, 129.4, 121.3, 120.8, 120.5, 118.5, 110.2, 34.6.

5.3.3 9-Ethyl-β-carboline-1-carboxaldehyde (3c): Yellow solid was obtained (1.71g, 51%). Mp 58.3-59.9℃. ESI-MS m/z: 224.9 $[M+H]^+$. ^{1}H NMR (300MHz, $CDCl_3$): δ

10.33 (s, 1H, C***H***O); 8.64 (d, *J* = 4.8 Hz, 1H, ArH); 8.20-8.14 (m, 2H, ArH); 7.67 (t, *J* = 7.2 Hz, 1H, ArH); 7.56 (d, *J* = 8.1 Hz, 1H, ArH); 7.36 (t, *J* = 7.2 Hz, 1H, ArH); 4.98-4.88 (m, 2H, NC***H***$_2$CH$_3$); 1.43 (t, *J* = 6.9 Hz, 1H, NCH$_2$C***H***$_3$). ^{13}C NMR (75MHz, $CDCl_3$): δ 193.9, 142.0, 138.2, 137.5, 134.8, 132.4, 129.4, 121.4, 120.8, 120.8, 118.6, 110.3, 41.9, 15.2.

5.3.4 9-Isopropyl-β-carboline-1-carboxaldehyde (3d): Yellow solid was obtained (1.89g, 53%). Mp 43.3-46.7℃. ESI-MS m/z: 238.8 $[M+H]^+$. ^{1}H NMR (300MHz, $CDCl_3$): δ 10.31 (s, 1H, C***H***O); 8.62 (d, *J* = 4.8 Hz, 1H, ArH); 8.18-8.12 (m, 2H, ArH); 7.79 (t, *J* = 8.4 Hz, 1H, ArH); 7.60 (d, *J* = 7.2 Hz, 1H, ArH); 7.33 (t, *J* = 7.2 Hz, 1H, ArH); 5.88-5.72 (m, 1H, NC***H***$[CH_3]_2$); 1.74 (d, *J* = 6.9 Hz, 6H, NCH$[C\boldsymbol{H}_3]_2$). ^{13}C NMR (75MHz, $CDCl_3$): δ 194.1, 140.9, 138.4, 137.8, 136.3, 132.3, 128.7, 122.6, 121.8, 120.6, 118.5, 114.1, 51.4, 21.4.

5.3.5 9-n-Hexyl-β-carboline-1-carboxaldehyde (3g):Yellow solid was obtained (2.56g, 61%). Mp 39.1-42.5℃. ESI-MS m/z: 280.9 $[M+H]^+$. ^{1}H NMR (300MHz, $CDCl_3$): δ 10.32 (s, 1H, C***H***O); 8.63 (d, *J* = 4.8 Hz, 1H, ArH); 8.20-8.13 (m, 2H, ArH); 7.66 (t, *J* = 8.4 Hz, 1H, ArH); 7.54 (d, *J* = 8.4 Hz, 1H, ArH); 7.35 (t, *J* = 7.5 Hz, 1H, ArH); 4.86 (t, *J* = 7.5 Hz, 2H, NC***H***$_2[CH_2]_4CH_3$); 1.83-1.71 (m, 2H, NCH$_2$C***H***$_2[CH_2]_3CH_3$); 1.43-1.21 (m, 6H, NCH$_2$CH$_2[C\boldsymbol{H}_2]_3CH_3$); 0.87 (t, *J* = 6.6 Hz, 3H, NCH$_2[CH_2]_4C\boldsymbol{H}_3$). ^{13}C NMR (75MHz, $CDCl_3$): δ 193.9, 142.5, 138.3, 137.8, 135.2, 132.6, 129.4, 121.5, 120.9, 120.8, 118.7, 110.7, 47.1, 31.8, 30.0, 26.7, 22.9, 14.3.

5.3.6 9-n-Octyl-β-carboline-1-carboxaldehyde (3h): Yellow solid was obtained

(2.72g, 59%). Mp 45.5-47.4℃. ESI-MS m/z: 308.8 $[M+H]^+$. ^{1}H NMR (300MHz, $CDCl_3$): δ 10.32 (s, 1H, C***H***O); 8.64 (d, *J* = 5.1 Hz, 1H, ArH); 8.20-8.14 (m, 2H, ArH); 7.66 (t, *J* = 8.4 Hz, 1H, ArH); 7.54 (d, *J* = 8.4 Hz, 1H, ArH); 7.36 (t, *J* = 7.2 Hz, 1H, ArH); 4.87 (t, *J* = 7.5 Hz, 2H, NC***H***$_2[CH_2]_6CH_3$); 1.83-1.71 (m, 2H, NCH_2C***H***$_2[CH_2]_5CH_3$); 1.43-1.21 (m, 10H, $NCH_2CH_2[C$***H***$_2]_5CH_3$); 0.87 (t, *J* = 6.6 Hz, 3H, $NCH_2[CH_2]_6C$***H***$_3$). ^{13}C NMR (75MHz, $CDCl_3$): δ 193.9, 142.5, 138.3, 137.8, 135.2, 132.6, 129.4, 121.5, 120.9, 120.8, 118.7, 110.7, 47.1, 32.1, 30.0, 29.7, 29.5, 27.0, 23.0, 14.5.

5.4. General procedure for the preparation of bivalent β-carbolines 4a-ad.

A mixture of β-carboline-1-carboxaldehydes (2.2 mmol), anhydrous methanol (30mL) and anhydrous CH_2Cl_2 (10 mL) was stirred at room temperature for 10 min, and the corresponding sym-diamine (1.0 mmol) was added. The mixture was refluxed for 2 h, and the solvent was evaporated under vacuum to give the crude Schiff base, which was used directly in the next step without further purification.

$NaBH_3CN$ (5 mmol) was added to a solution of the above-mentioned crude Schiff base in anhydrous CH_3OH (30 mL) at 0 ^{o}C. The mixture was stirred at room temperature for 4-6 h. After completion of the reaction as indicated by TLC, the reaction mixture was concentrated under vacuum. The residue was dissolved in CH_2Cl_2 (150 mL) and washed with aqueous Na_2CO_3 (pH 10, 50 mL). The organic layer was separated, dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue was purified by flash chromatography on silica gel (CH_2Cl_2/CH_3OH/NH_4OH, 100:1:0.8) to provide target products.

5.4.1 N,N-Bis[(β-carboline-1-yl)methyl]butane-1,4-diamine (4a). Yellow oil was obtained (0.33g, 75%). ESI-MS m/z: 449.1 $[M+H]^+$. ^{1}H NMR (300MHz, $CDCl_3$): δ 10.25 (s, 2H, 2N***H***), 8.31 (d, *J* = 5.4 Hz, 2H, ArH), 8.09 (d, *J* = 7.8 Hz, 2H, ArH), 7.84 (d, *J* = 5.4 Hz, 2H, ArH), 7.52-7.45 (m, 4H, ArH), 7.27-7.22 (m, 2H, ArH), 4.35 (s, 4H, 2C***H***$_2$), 2.77-2.59 (m, 4H, HNC***H***$_2$CH$_2$CH$_2$C***H***$_2$NH), 1.68-1.54 (m, 4H, HNCH$_2$C***H***$_2$C***H***$_2$CH$_2$NH). ^{13}C NMR (75MHz, $CDCl_3$): δ 143.5, 140.5, 138.0, 135.1, 129.1, 128.4, 121.9, 121.6, 119.8, 114.0, 112.0, 54.8, 49.9, 28.0. HRMS (ESI) calcd for $C_{28}H_{34}Cl_6N_6$ $[M+H]^+$ 449.2448, found 449.2446.

5.4.2 N,N-Bis[(β-carboline-1-yl)methyl]pentane-1,5-diamine (4b)： Yellow oil was obtained (0.36g, 78%). ESI-MS m/z: 462.9 $[M+H]^+$. ^{1}H NMR (300MHz, $CDCl_3$)：δ 10.37 (s, 2H, 2N***H***), 8.32 (d, *J* = 5.4 Hz, 2H, ArH), 8.07 (d, *J* = 7.8 Hz, 2H, ArH), 7.82 (d, *J* = 5.4 Hz, 2H, ArH), 7.52-7.41 (m, 4H, ArH), 7.26-7.18 (m, 2H, ArH), 4.33 (s, 4H, 2C***H***$_2$), 2.57 (t, *J* = 6.6 Hz, 4H, HNC***H***$_2$CH$_2$CH$_2$CH$_2$C***H***$_2$NH), 1.50-1.38 (m, 4H, HNCH$_2$C***H***$_2$CH$_2$C***H***$_2$CH$_2$NH), 1.33-1.20 (m, 2H, HNCH$_2$CH$_2$C***H***$_2$CH$_2$CH$_2$NH). ^{13}C NMR (75MHz, $CDCl_3$)：δ 143.6, 140.5, 138.0, 135.1, 129.1, 128.4, 121.9, 121.6, 119.8, 113.9, 111.9, 54.9, 50.0, 30.1, 25.3. HRMS (ESI) calcd for $C_{29}H_{36}Cl_6N_6$ $[M+H]^+$ 463.2605, found 463.2596.

5.4.3 N,N-Bis[(9-methyl-β-carboline-1-yl)methyl]butane-1,4-diamine (4c)： Yellow oil was obtained (0.30g, 65%). ESI-MS m/z: 476.9 $[M+H]^+$. ^{1}H NMR (300MHz, $CDCl_3$): δ 8.30 (d, *J* = 5.1 Hz, 2H, ArH), 8.08 (d, *J* = 7.8 Hz, 2H, ArH), 7.84 (d, *J* = 5.1 Hz, 2H, ArH), 7.58 (t, *J* = 7.2 Hz, 2H, ArH), 7.42 (d, *J* = 7.8 Hz, 2H, ArH), 7.29-7.22 (m, 2H, ArH), 4.38 (s, 6H, 2NC***H***$_3$), 4.18 (s, 4H, 2C***H***$_2$), 2.88-2.78 (m, 4H,

HNC***H***$_2$CH$_2$CH$_2$C***H***$_2$NH), 1.74-1.66 (m, 4H, HNCH$_2$C***H***$_2$C***H***$_2$CH$_2$NH). ^{13}C NMR (75MHz, $CDCl_3$): δ 142.9, 142.2, 137.7, 135.6, 129.4, 128.3, 121.4, 121.1, 119.6, 113.9, 109.6, 54.3, 50.3, 32.1, 28.4. HRMS (ESI) calcd for $C_{30}H_{38}Cl_6N_6$ $[M+H]^+$ 477.2761, found 477.2764.

5.4.4 N,N-Bis[(9-methyl-β-carboline-1-yl)methyl]hexane-1,6-diamine (4d)： Yellow oil was obtained (0.33g, 66%). ESI-MS m/z: 506.1 $[M+H]^+$. ^{1}H NMR (300MHz, $CDCl_3$): δ 8.31 (d, *J* = 5.1 Hz, 2H, ArH), 8.06 (d, *J* = 7.8 Hz, 2H, ArH), 7.83 (d, *J* = 5.1 Hz, 2H, ArH), 7.56 (t, *J* = 8.1 Hz, 2H, ArH), 7.40 (d, *J* = 8.1 Hz, 2H, ArH), 7.27-7.20 (m, 2H, ArH), 4.35 (s, 6H, 2NC***H***$_3$), 4.16 (s, 4H, 2C***H***$_2$), 2.78 (t, *J* = 6.9 Hz, 4H, HNC***H***$_2$CH$_2$CH$_2$CH$_2$CH$_2$C***H***$_2$NH), 1.64-1.52 (m, 4H, HNCH$_2$C***H***$_2$CH$_2$CH$_2$-C***H***$_2$CH$_2$NH)，1.43-1.35 (m, 4H, HNCH$_2$CH$_2$C***H***$_2$C***H***$_2$CH$_2$CH$_2$NH). ^{13}C NMR (75MHz, $CDCl_3$): δ 143.10, 142.2, 137.7, 135.7, 129.4, 128.3, 121.4, 121.1, 119.6, 113.9, 109.6, 54.5, 50.5, 32.1, 30.6, 27.7. HRMS (ESI) calcd for $C_{32}H_{42}Cl_6N_6$ $[M+H]^+$ 505.3074, found 505.3170.

5.4.5 N,N-Bis[(9-ethyl-β-carboline-1-yl)methyl]propyl-1,3-diamine (4e)： Yellow oil was obtained (0.28g, 56%). ESI-MS m/z: 491.1 $[M+H]^+$. ^{1}H NMR (300MHz, $CDCl_3$): δ 8.27 (d, *J* = 5.4 Hz, 2H, ArH), 8.05 (d, *J* = 7.5 Hz, 2H, ArH), 7.81 (d, *J* = 5.4 Hz, 2H, ArH), 7.53 (t, *J* = 7.5 Hz, 2H, ArH), 7.37 (d, *J* = 8.4 Hz, 2H, ArH), 7.26-7.18 (m, 2H, ArH), 4.65-4.52 (m, 4H, 2NC***H***$_2$CH$_3$), 4.37 (s, 4H, 2C***H***$_2$), 2.97 (t, *J* = 6.6 Hz, 4H, HNC***H***$_2$CH$_2$C***H***$_2$NH), 1.97-1.86 (m, 2H, HNCH$_2$C***H***$_2$CH$_2$NH), 1.41 (t, *J* = 7.2 Hz, 6H, 2NCH$_2$C***H***$_3$). ^{13}C NMR (75MHz, $CDCl_3$): δ 141.7, 141.4, 137.6, 134.6, 129.9, 128.5,

121.6, 121.5, 119.9, 114.1, 109.8, 53.8, 49.0, 40.0, 30.0, 16.1. HRMS (ESI) calcd for $C_{31}H_{40}Cl_6N_6$ $[M+H]^+$ 491.2918, found 491.2913.

5.4.6 N,N-Bis[(9-ethyl-β-carboline-1-yl)methyl]butane-1,4-diamine (4f) : Yellow oil was obtained (0.28g, 55%). ESI-MS m/z: 504.9 $[M+H]^+$. ^{1}H NMR (300MHz, $CDCl_3$): δ 8.29 (d, J = 5.4 Hz, 2H, ArH), 8.03 (d, J = 7.5 Hz, 2H, ArH), 7.78 (d, J = 5.4 Hz, 2H, ArH), 7.53 (t, J = 7.5 Hz, 2H, ArH), 7.39 (d, J = 8.1 Hz, 2H, ArH), 7.27-7.18 (m, 2H, ArH), 4.68-4.53 (m, 4H, 2NC***H***$_2$$CH_3$), 4.32 (s, 4H, 2C***H***$_2$), 2.88-2.80 (m, 4H, HNC***H***$_2$$CH_2CH_2$C***H***$_2$NH), 1.75-1.66 (m, 4H, $HNCH_2$C***H***$_2$C***H***$_2$$CH_2$NH), 1.41 (t, J = 6.9 Hz, 6H, $2NCH_2$C***H***$_3$); ^{13}C NMR (75MHz, $CDCl_3$) δ 142.1, 141.3, 137.7, 134.7, 129.8, 128.4, 121.6, 119.8, 114.0, 109.8, 54.2, 50.30, 40.0, 28.4, 16.1. HRMS (ESI) calcd for $C_{32}H_{42}Cl_6N_6$ $[M+H]^+$ 505.3074, found 505.3067.

5.4.7 N,N-Bis[(9-isopropyl-β-carboline-1-yl)methyl]propyl-1,3-diamine (4g) :Yellow oil was obtained (0.28g, 53%). ESI-MS m/z: 518.9 $[M+H]^+$. ^{1}H NMR (300MHz, $CDCl_3$): δ 8.26 (d, J = 5.1 Hz, 2H, ArH), 8.12 (d, J = 7.8 Hz, 2H, ArH), 7.87 (d, J = 5.1 Hz, 2H, ArH), 7.63 (d, J = 8.4 Hz, 2H, ArH), 7.48 (t, J = 8.4 Hz, 2H, ArH), 7.25-7.20 (m, 2H, ArH), 5.68-5.57 (m, 2H, 2NC***H***$[CH_3]_2$), 4.04 (s, 4H, 2C***H***$_2$), 2.75-2.66 (m, 4H, HNC***H***$_2$$CH_2$C***H***$_2$NH), 1.80-1.70 (m, 2H, $HNCH_2$C***H***$_2$$CH_2$NH), 1.52 (d, J = 7.2 Hz, 12H, 2NCH[C***H***$_3$]$_2$). ^{13}C NMR (75MHz, $CDCl_3$): δ 141.5, 139.9, 137.1, 136.4, 129.7, 127.6, 122.7, 121.6, 119.3, 114.1, 113.5, 61.7, 52.6, 48.4, 23.9, 21.6. HRMS (ESI) calcd for $C_{33}H_{44}Cl_6N_6$ $[M+H]^+$ 519.3231, found 519.3296.

5.4.8 N,N-Bis[(9-isopropyl-β-carboline-1-yl)methyl]hexane-1,6-diamine (4h) : Yellow oil was obtained (0.50g, 89%). ESI-MS m/z: 560.9 $[M+H]^+$. ^{1}H NMR

(300MHz, $CDCl_3$) δ 8.31(d, J = 5.4 Hz, 2H, ArH), 8.12 (d, J = 7.8 Hz, 2H, ArH), 7.86 (d, J = 5.4 Hz, 2H, ArH), 7.70 (d, J = 8.4 Hz, 2H, ArH), 7.51 (t, J = 8.4 Hz, 2H, ArH), 7.28-7.20 (m, 2H, ArH), 5.74-5.62 (m, 2H, 2NC***H***$[CH_3]_2$), 4.31 (s, 4H, 2C***H***$_2$), 2.78 (t, J = 6.9 Hz, 2H, HNC***H***$_2CH_2CH_2CH_2CH_2C$***H***$_2$NH), 1.73 (d, J = 6.9 Hz, 12H, 2NCH[C***H***$_3]_2$), 1.63-1.52 (m, 4H, $HNCH_2C$***H***$_2$***C***H_2CH_2C***H***$_2CH_2$NH), 1.45-1.38 (m, 4H, $HNCH_2CH_2C$***H***$_2C$***H***$_2CH_2CH_2$NH). ^{13}C NMR (75MHz, $CDCl_3$): δ 142.7, 140.1, 137.5, 135.8, 129.6, 127.6, 122.9, 121.7, 119.4, 113.8, 113.5, 55.9, 50.5, 48.8, 30.6, 27.8, 21.8. HRMS (ESI) calcd for $C_{36}H_{50}Cl_6N_6$ $[M+H]^+$ 561.3700, found 561.3703.

5.4.9 N,N-Bis[(9-isobutyl-β-carboline-1-yl)methyl]butane-1,4-diamine (4i) *:* Yellow oil was obtained (0.42g, 74%). ESI-MS m/z: 562.1 $[M+H]^+$. ^{1}H NMR (300MHz, $CDCl_3$): δ 8.32 (d, J = 5.1 Hz, 2H, ArH), 8.08 (d, J = 7.8 Hz, 2H, ArH), 7.86 (d, J =5.1 Hz, 2H, ArH), 7.53 (t, J = 7.8 Hz, 2H, ArH), 7.46 (d, J = 8.4 Hz, 2H, ArH), 7.28-7.20 (m, 2H, ArH), 4.39 (d, J = 7.5 Hz, 4H, 2NC***H***$_2$CH$[CH_3]_2$), 4.33 (s, 4H, 2C***H***$_2$), 2.86-2.79 (m, 4H, HNC***H***$_2CH_2CH_2C$***H***$_2$NH), 2.32-2.17 (m, 2H, $2NCH_2C$***H***$[CH_3]_2$), 1.30-1.24 (m, 4H, $HNCH_2C$***H***$_2C$***H***$_2CH_2$NH), 0.93 (d, J =6.6 Hz, 12H, $2NCH_2CH[C$***H***$_3]_2$). ^{13}C NMR (75MHz, $CDCl_3$): δ 142.4, 142.2, 137.7, 135.0, 129.8, 128.1, 121.4, 121.2, 119.6, 113.9, 110.7, 54.2, 52.1, 50.3, 31.0, 28.5, 20.7. HRMS (ESI) calcd for $C_{36}H_{50}Cl_6N_6$ $[M+H]^+$ 561.3700, found 561.3699.

5.4.10 N,N-Bis[(9-isobutyl-β-carboline-1-yl)methyl]hexane-1,6-diamine (4j) .Yellow oil was obtained (0.46g, 78%). ESI-MS m/z: 588.9 $[M+H]^+$. ^{1}H NMR (300MHz, $CDCl_3$): δ 8.36 (d, J = 5.4 Hz, 2H, ArH), 8.10 (d, J = 7.8 Hz, 2H, ArH), 7.89 (d, J =5.4 Hz, 2H, ArH), 7.55 (t, J = 7.8 Hz, 2H, ArH), 7.47 (d, J = 8.4 Hz, 2H, ArH),

7.28-7.22 (m, 2H, ArH), 4.42 (d, *J* = 7.5 Hz, 4H, 2NC***H***$_2$CH[CH$_3$]$_2$), 4.33 (s, 4H, 2C***H***$_2$), 2.78 (t, *J* = 6.9 Hz, 4H, HNC***H***$_2$CH$_2$CH$_2$CH$_2$CH$_2$C***H***$_2$NH), 2.34-2.22 (m, 2H, 2NCH$_2$C***H***[CH$_3$]$_2$), 1.66-1.53 (m, 4H, HNCH$_2$C***H***$_2$CH$_2$CH$_2$C***H***$_2$CH$_2$NH), 1.46-1.36 (m, 4H, HNCH$_2$CH$_2$C***H***$_2$C***H***$_2$CH$_2$CH$_2$NH), 0.95 (d, *J* =6.6 Hz, 12H, 2NCH$_2$CH[C***H***$_3$]$_2$). ^{13}C NMR (75MHz, CDCl$_3$): δ 142.2, 141.3, 137.6, 134.9, 129.9, 128.2, 121.4, 121.1, 119.7, 114.0, 110.7, 53.5, 52.1, 50.1, 30.9, 29.9, 27.4, 20.6. HRMS (ESI) calcd for $C_{38}H_{54}Cl_6N_6$ $[M+H]^+$ 589.4013, found 589.4007.

5.4.11 N,N-Bis (9-butyl-β-carboline-1-yl)methyl]propane-1,3-diamine (4k) *:* Yellow oil was obtained (0.26g, 48%). ESI-MS m/z: 546.9 $[M+H]^+$. ^{1}H NMR (300MHz, CDCl$_3$): δ 8.31 (d, *J* = 5.1 Hz, 2H, ArH), 8.10 (d, *J* = 7.8 Hz, 2H, ArH), 7.87 (d, *J* =5.1 Hz, 2H, ArH), 7.55 (t, *J* = 7.8 Hz, 2H, ArH), 7.47 (d, *J* = 8.4 Hz, 2H, ArH), 7.29-7.22 (m, 2H, ArH), 4.56 (t, *J* = 7.8 Hz, 2H, 2NC***H***$_2$[CH$_2$]$_2$CH$_3$), 4.33 (s, 4H, 2C***H***$_2$), 2.92 (t, *J* = 6.6 Hz, 4H, HNC***H***$_2$CH$_2$C***H***$_2$NH), 1.90-1.78 (m, 6H, 2NCH$_2$C***H***$_2$CH$_2$CH$_3$, HNCH$_2$C***H***$_2$CH$_2$NH), 1.51-1.37 (m, 4H, 2NCH$_2$CH$_2$C***H***$_2$CH$_3$), 0.97 (t, *J* = 7.2 Hz, 6H, 2NCH$_2$[CH$_2$]$_2$C***H***$_3$). ^{13}C NMR (75MHz, CDCl$_3$): δ 142.6, 141.7, 137.7, 134.9, 129.8, 128.3, 121.5, 121.4, 119.7, 114.1, 110.0, 54.7, 49.1, 45.9, 33.3, 31.0, 20.8, 14.4. HRMS (ESI) calcd for $C_{35}H_{48}Cl_6N_6$ $[M+H]^+$ 547.3544, found 547.3536.

5.4.12 N,N-Bis[(9-butyl-β-carboline-1-yl)methyl]butane-1,4-diamine (4l) *:* Yellow oil was obtained (0.33g, 59%). ESI-MS m/z: 560.9 $[M+H]^+$. ^{1}H NMR (300MHz, CDCl$_3$): δ 8.29 (d, *J* = 5.1 Hz, 2H, ArH), 8.07 (d, *J* = 7.8 Hz, 2H, ArH), 7.82 (d, *J* =5.1 Hz, 2H, ArH), 7.56 (t, *J* = 7.8 Hz, 2H, ArH), 7.44 (d, *J* = 8.4 Hz, 2H, ArH),

7.26-7.21 (m, 2H, ArH), 4.56 (t, *J* = 7.2 Hz, 2H, 2NC***H***$_2$[CH$_2$]$_2$CH$_3$), 4.34 (s, 4H, 2C***H***$_2$), 2.90-2.82 (m, 4H, HNC***H***$_2$CH$_2$CH$_2$C***H***$_2$NH), 1.87-1.78 (m, 4H, 2NCH$_2$C***H***$_2$CH$_2$CH$_3$), 1.77-1.67 (m, 4H, HNCH$_2$C***H***$_2$C***H***$_2$CH$_2$NH), 1.52-1.37 (m, 4H, 2NCH$_2$CH$_2$C***H***$_2$CH$_3$), 0.98 (t, *J* = 6.9 Hz, 6H, 2NCH$_2$[CH$_2$]$_2$C***H***$_3$). ^{13}C NMR (75MHz, CDCl$_3$): δ 142.0, 141.7, 137.6, 134.8, 129.7, 128.3, 121.5, 121.4, 119.7, 114.0, 109.9, 54.1, 50.3, 45.1, 33.2, 28.5, 20.7, 14.3. HRMS (ESI) calcd for $C_{36}H_{50}Cl_6N_6$ $[M+H]^+$ 561.3700, found 561.3708.

5.4.13 N,N-Bis[(9-butyl-β-carboline-1-yl)methyl]pentane-1,5-diamine (4m) **:** Yellow oil was obtained (0.37g, 64%). ESI-MS m/z: 575.1 $[M+H]^+$. ^{1}H NMR (300MHz, CDCl$_3$) : δ 8.34 (d, *J* = 5.1 Hz, 2H, ArH), 8.10 (d, *J* = 7.8 Hz, 2H, ArH), 7.88 (d, *J* =5.1 Hz, 2H, ArH), 7.57 (t, *J* = 7.8 Hz, 2H, ArH), 7.44 (d, *J* = 8.4 Hz, 2H, ArH), 7.29-7.22 (m, 2H, ArH), 4.60 (t, *J* = 8.1 Hz, 4H, 2NC***H***$_2$[CH$_2$]$_2$CH$_3$), 4.33 (s, 4H, 2C***H***$_2$), 2.81 (t, *J* = 6.9 Hz, 4H, HNC***H***$_2$CH$_2$CH$_2$CH$_2$C***H***$_2$NH), 1.91-1.79 (m, 4H, 2NCH$_2$C***H***$_2$CH$_2$CH$_3$), 1.70-1.56 (m, 4H, HNCH$_2$C***H***$_2$CH$_2$C***H***$_2$CH$_2$NH), 1.54-1.40 (m, 6H, 2NCH$_2$CH$_2$C***H***$_2$CH$_3$, HNCH$_2$CH$_2$C***H***$_2$CH$_2$CH$_2$NH), 1.00 (t, *J* = 7.2 Hz, 6H, 2NCH$_2$[CH$_2$]$_2$C***H***$_3$). ^{13}C NMR (75MHz, CDCl$_3$): δ 142.6, 141.8, 137.7, 135.0, 129.8, 128.3, 121.5, 119.7, 114.0, 110.0, 54.5, 50.5, 45.1, 33.3, 30.5, 25.7, 20.7, 14.3. HRMS (ESI) calcd for $C_{37}H_{52}Cl_6N_6$ $[M+H]^+$ 575.3857, found 575.3856.

5.4.14 N,N-Bis[(9-butyl-β-carboline-1-yl)metyl]hexane-1,6-diamine (4n) **:** Yellow oil was obtained (0.40g, 68%). ESI-MS m/z: 590.2 $[M+H]^+$. ^{1}H NMR (300MHz, CDCl$_3$) : δ 8.34 (d, *J* = 5.1 Hz, 2H, ArH), 8.11 (d, *J* = 7.8 Hz, 2H, ArH), 7.88 (d, *J* =5.1 Hz, 2H, ArH), 7.57 (t, *J* = 7.8 Hz, 2H, ArH), 7.46 (d, *J* = 8.4 Hz, 2H, ArH),

7.29-7.22 (m, 2H, ArH), 4.61 (t, *J* = 7.5 Hz, 4H, 2NC***H***$_2$[CH$_2$]$_2$CH$_3$), 4.32 (s, 4H, 2C***H***$_2$), 2.79 (t, *J* = 6.9 Hz, 4H, HNC***H***$_2$CH$_2$CH$_2$CH$_2$CH$_2$C***H***$_2$NH), 1.90-1.79 (m, 4H, 2NCH$_2$C***H***$_2$CH$_2$CH$_3$), 1.66-1.54 (m, 4H, HNCH$_2$C***H***$_2$CH$_2$CH$_2$C***H***$_2$CH$_2$NH), 1.54-1.37 (m, 8H, 2NCH$_2$CH$_2$C***H***$_2$CH$_3$, HNCH$_2$CH$_2$C***H***$_2$C***H***$_2$CH$_2$CH$_2$NH), 1.00 (t, *J* = 7.5 Hz, 6H, 2NCH$_2$[CH$_2$]$_2$C***H***$_3$). ^{13}C NMR (75MHz, CDCl$_3$): δ 142.8, 141.7, 137.7, 135.0, 129.8, 128.3, 121.5, 121.5, 119.7, 114.0, 110.0, 54.7, 50.6, 45.1, 33.3, 30.6, 27.8, 20.8, 14.4. HRMS (ESI) calcd for $C_{38}H_{54}Cl_6N_6$ $[M+H]^+$ 589.4013, found 589.4014.

5.4.15 N,N-Bis[(9-hexyl-β-carboline-1-yl)methyl]propyl-1,3-diamine (4o) *:* Yellow oil was obtained (0.43g, 72%). ESI-MS m/z: 603.0 $[M+H]^+$. ^{1}H NMR (300MHz, CDCl$_3$) δ 8.31 (d, *J* = 5.4 Hz, 2H, ArH), 8.10 (d, *J* = 7.8 Hz, 2H, ArH), 7.86 (d, *J* =5.4 Hz, 2H, ArH), 7.56 (t, *J* = 7.8 Hz, 2H, ArH), 7.40 (d, *J* = 8.4 Hz, 2H, ArH), 7.29-7.22 (m, 2H, ArH), 4.54 (t, *J* = 7.8 Hz, 4H, 2NC***H***$_2$[CH$_2$]$_4$CH$_3$), 4.32 (s, 4H, 2C***H***$_2$), 2.92 (t, *J* = 6.9 Hz, 4H, HNC***H***$_2$CH$_2$C***H***$_2$NH), 1.92-1.76 (m, 6H, 2NCH$_2$C***H***$_2$[CH$_2$]$_3$CH$_3$, HNCH$_2$C***H***$_2$CH$_2$NH), 1.48-1.21 (m, 12H, 2NCH$_2$CH$_2$[C***H***$_2$]$_3$CH$_3$), 0.87 (t, *J* = 6.6 Hz, 6H, 2NCH$_2$[CH$_2$]$_4$C***H***$_3$). ^{13}C NMR (75MHz, CDCl$_3$): δ 137.9, 137.0, 133.0, 130.2, 125.1, 123.6, 116.8, 115.0, 109.3, 105.2, 49.9, 44.3, 40.6, 27.2, 26.4, 26.3, 22.4, 18.2, 9.6. HRMS (ESI) calcd for $C_{39}H_{56}Cl_6N_6$ $[M+H]^+$ 603.4170, found 603.4172.

5.4.16 N,N-Bis[(9-hexyl-β-carboline-1-yl)methyl]butane-1,4-diamine (4p) *:* Yellow oil was obtained (0.46g, 75%). ESI-MS m/z: 616.9 $[M+H]^+$. ^{1}H NMR (300MHz, CDCl$_3$): δ 8.33 (d, *J* = 5.4 Hz, 2H, ArH), 8.10 (d, *J* = 7.8 Hz, 2H, ArH), 7.87 (d, *J* =5.4 Hz, 2H, ArH), 7.55 (t, *J* = 7.8 Hz, 2H, ArH), 7.43 (d, *J* = 8.4 Hz, 2H, ArH), 7.28-7.22 (m, 2H, ArH), 4.57 (t, *J* = 8.1 Hz, 4H, 2NC***H***$_2$[CH$_2$]$_4$CH$_3$), 4.32 (s, 4H,

2C***H***$_2$), 2.88-2.79 (m, 4H, HNC***H***$_2$CH$_2$CH$_2$C***H***$_2$NH), 1.91-1.78 (m, 4H, 2NCH$_2$C***H***$_2$[CH$_2$]$_3$CH$_3$), 1.73-1.63 (m, 4H, HNCH$_2$C***H***$_2$C***H***$_2$CH$_2$NH), 1.51-1.24 (m, 12H, 2NCH$_2$CH$_2$[C***H***$_2$]$_3$CH$_3$), 0.89 (t, *J* = 6.6 Hz, 6H, 2NCH$_2$[CH$_2$]$_4$C***H***$_3$). ^{13}C NMR (75MHz, CDCl$_3$): *δ* 142.7, 141.7, 137.7, 134.9, 129.8, 128.3, 121.5, 121.5, 119.7, 114.0, 110.0, 54.7, 50.5, 45.4, 32.0, 31.2, 28.6, 27.1, 23.0, 14.5. HRMS (ESI) calcd for $C_{40}H_{58}Cl_6N_6$ $[M+H]^+$ 617.4326, found 617.4330.

5.4.17 N,N-Bis[(9-hexyl-β-carboline-1-yl)metyl]pentane-1,5-diamine (4q) *:* Yellow oil was obtained (0.40g, 71%). ESI-MS m/z: 632.3 $[M+H]^+$. ^{1}H NMR (300MHz, CDCl$_3$): *δ* 8.34 (d, *J* = 5.4 Hz, 2H, ArH), 8.11 (d, *J* = 7.8 Hz, 2H, ArH), 7.88 (d, *J* =5.4 Hz, 2H, ArH), 7.57 (t, *J* = 7.8 Hz, 2H, ArH), 7.44 (d, *J* = 8.4 Hz, 2H, ArH), 7.28-7.24 (m, 2H, ArH), 4.59 (t, *J* = 7.8 Hz, 4H, 2NC***H***$_2$[CH$_2$]$_4$CH$_3$), 4.32 (s, 4H, 2C***H***$_2$), 2.81 (t, *J* = 6.6 Hz, 4H, HNC***H***$_2$CH$_2$CH$_2$CH$_2$C***H***$_2$NH), 1.92-1.79 (m, 4H, NCH$_2$C***H***$_2$[CH$_2$]$_3$CH$_3$), 1.68-1.55 (m, 4H, HNCH$_2$C***H***$_2$CH$_2$C***H***$_2$CH$_2$NH), 1.53-1.25 (m, 14H, 2NCH$_2$CH$_2$[C***H***$_2$]$_3$CH$_3$, HNCH$_2$CH$_2$C***H***$_2$CH$_2$CH$_2$NH), 0.90 (t, *J* = 6.6 Hz, 6H, 2NCH$_2$[CH$_2$]$_4$C***H***$_3$). ^{13}C NMR (75MHz, CDCl$_3$): *δ* 142.7, 141.7, 137.7, 134.9, 129.7, 128.3, 121.5, 121.4, 119.7, 114.0, 109.9, 54.7, 50.6, 45.3, 31.9, 31.1, 30.6, 27.1, 25.7, 23.0, 14.5. HRMS (ESI) calcd for $C_{41}H_{60}Cl_6N_6$ $[M+H]^+$ 631.4483, found 631.4485.

5.4.18 N,N-Bis[(9-hexyl-β-carboline-1-yl)methyl]hexane-1,6-diamine (4r) *:* Yellow oil was obtained (0.53g, 82%). ESI-MS m/z: 645.3 $[M+H]^+$. ^{1}H NMR (300MHz, CDCl$_3$): *δ* 8.34 (d, *J* = 5.4 Hz, 2H, ArH), 8.10 (d, *J* = 7.8 Hz, 2H, ArH), 7.87 (d, *J* =5.4 Hz, 2H, ArH), 7.57 (t, *J* = 7.8 Hz, 2H, ArH), 7.45 (d, *J* = 8.1 Hz, 2H, ArH), 7.2-7.22 (m, 2H, ArH), 4.60 (t, *J* = 8.4 Hz, 4H, 2NC***H***$_2$[CH$_2$]$_4$CH$_3$), 4.32 (s, 4H,

2C***H***$_2$), 2.80 (t, *J* = 6.9 Hz, 4H, HNC***H***$_2$CH$_2$CH$_2$CH$_2$CH$_2$C***H***$_2$NH), 1.92-1.80 (m, 4H, NCH$_2$C***H***$_2$[CH$_2$]$_3$CH$_3$), 1.66-1.54 (m, 4H, HNCH$_2$C***H***$_2$CH$_2$CH$_2$C***H***$_2$CH$_2$NH), 1.53-1.28 (m, 16H, 2NCH$_2$CH$_2$[C***H***$_2$]$_3$CH$_3$, HNCH$_2$CH$_2$C***H***$_2$C***H***$_2$CH$_2$CH$_2$NH), 0.90 (t, *J* = 6.9 Hz, 6H, 2NCH$_2$[CH$_2$]$_4$C***H***$_3$). ^{13}C NMR (75MHz, CDCl$_3$) : δ 141.7, 137.5, 134.7, 129.8, 128.3, 121.5, 121.3, 119.7, 114.0, 109.9, 53.9, 50.3, 45.3, 31.9, 31.1, 30.2, 27.6, 27.0, 22.9, 14.4. HRMS (ESI) calcd for $C_{42}H_{62}Cl_6N_6$ $[M+H]^+$ 645.4639, found 645.4644.

5.4.19 N,N-Bis[(9-octyl-β-carboline-1-yl)methyl]butane-1,4-diamine (4s) :Yellow oil was obtained (0.41g, 61%). ESI-MS m/z: 672.9 $[M+H]^+$. ^{1}H NMR (300MHz, CDCl$_3$): δ 8.33 (d, *J* = 5.1 Hz, 2H, ArH), 8.07 (d, *J* = 7.8 Hz, 2H, ArH), 7.84 (d, *J* =5.1 Hz, 2H, ArH), 7.55 (t, *J* = 7.8 Hz, 2H, ArH), 7.41 (d, *J* = 8.4 Hz, 2H, ArH), 7.26-7.20 (m, 2H, ArH), 4.55 (t, *J* = 7.8 Hz, 4H, 2NC***H***$_2$[CH$_2$]$_6$CH$_3$), 4.33 (s, 4H, 2C***H***$_2$), 2.89-2.81 (m, 4H, HNC***H***$_2$CH$_2$CH$_2$C***H***$_2$NH), 1.90-1.77 (m, 4H, 2NCH$_2$C***H***$_2$[CH$_2$]$_5$CH$_3$), 1.74-1.65 (m, 4H, HNCH$_2$C***H***$_2$C***H***$_2$CH$_2$NH), 1.48-1.20 (m, 20H, 2NCH$_2$CH$_2$[C***H***$_2$]$_5$CH$_3$), 0.88 (t, *J* = 5.4 Hz, 6H, 2NCH$_2$[CH$_2$]$_6$C***H***$_3$). ^{13}C NMR (75MHz, CDCl$_3$): δ 142.5, 141.7, 137.7, 134.9, 129.7, 128.3, 121.5, 119.7, 113.9, 109.1, 54.5, 50.5, 45.3, 32.1, 31.1, 29.7, 29.6, 28.6, 27.4, 23.0, 14.5. HRMS (ESI) calcd for $C_{44}H_{66}Cl_6N_6$ $[M+H]^+$ 673.4952, found 673.4954.

5.4.20 N,N-Bis[(9-octyl-β-carboline-1-yl)methyl]pentane-1,5-diamine (4t) *:* Yellow oil was obtained (0.46g, 66%). ESI-MS m/z: 686.9 $[M+H]^+$. ^{1}H NMR (300MHz, CDCl$_3$): δ 8.34 (d, *J* = 5.1 Hz, 2H, ArH), 8.10 (d, *J* = 7.8 Hz, 2H, ArH), 7.88 (d, *J* =5.1 Hz, 2H, ArH), 7.57 (t, *J* = 7.8 Hz, 2H, ArH), 7.44 (d, *J* = 8.4 Hz, 2H, ArH),

7.29-7.22 (m, 2H, ArH), 4.59 (t, *J* = 7.8 Hz, 4H, 2NC***H***$_2$[CH_2]$_6$$CH_3$), 4.33 (s, 4H, 2C***H***$_2$), 2.81 (t, *J* = 6.9 Hz, 4H, HNC***H***$_2$$CH_2$$CH_2$$CH_2$C***H***$_2$NH), 1.92-1.80 (m, 4H, 2NCH_2C***H***$_2$[CH_2]$_5$$CH_3$), 1.68-1.55 (m, 4H, HNC$H_2$C***H***$_2CH_2$C***H***$_2$$CH_2$NH), 1.54-1.20 (m, 22H, 2NC$H_2$$CH_2$[C***H***$_2$]$_5$$CH_3$, HNC$H_2$$CH_2$C***H***$_2$$CH_2$$CH_2$NH), 0.88 (t, *J* = 6.0 Hz, 6H, 2NCH_2[CH_2]$_6$C***H***$_3$). ^{13}C NMR (75MHz, $CDCl_3$): δ 142.7, 141.8, 137.7, 135.0, 129.8, 128.3, 121.5, 119.7, 114.0, 110.0, 54.6, 50.5, 45.4, 32.1, 31.2, 30.6, 29.8, 29.6, 27.5, 25.7, 23.0, 14.4. HRMS (ESI) calcd for $C_{45}H_{68}Cl_6N_6$ $[M+H]^+$ 687.5109, found 687.5114.

5.4.21 N,N-Bis[(9-benzyl-β-carboline-1-yl)methyl]propane-1,3-diamine (4u) : Yellow oil was obtained (0.30g, 49%). ESI-MS m/z: 614.9 $[M+H]^+$. ^{1}H NMR (300MHz, $CDCl_3$): δ 8.34 (d, *J* = 5.1 Hz, 2H, ArH), 8.16 (d, *J* = 7.8 Hz, 2H, ArH), 7.93 (d, *J* =5.1 Hz, 2H, ArH), 7.52 (t, *J* = 7.8 Hz, 2H, ArH), 7.36-7.14 (m, 10H, ArH), 6.94 (d, *J* = 6.0 Hz, 4H, ArH), 5.94 (s, 4H, 2NC***H***$_2$Ph), 4.07 (s, 4H, 2C***H***$_2$), 2.74 (t, *J* = 6.6 Hz, 4H, HNC***H***$_2$$CH_2$C***H***$_2$NH), 1.74-1.62 (m, 2H, HNCH_2C***H***$_2$$CH_2$NH). ^{13}C NMR (75MHz, $CDCl_3$): δ 143.0, 142.2, 138.8, 138.2, 135.8, 129.2, 128.7, 127.4, 125.4, 121.6, 120.2, 114.3, 110.2, 54.6, 48.8, 48.5, 30.8. HRMS (ESI) calcd for $C_{41}H_{44}Cl_6N_6$ $[M+H]^+$ 615.3231, found 615.3229.

5.2.22 N,N-Bis[(9-benzyl-β-carboline-1-yl)methyl]hexane-1,6-diamine (4v) : Yellow oil was obtained (0.49g, 75%). ESI-MS m/z: 656.8 $[M+H]^+$. ^{1}H NMR (300MHz, $CDCl_3$): δ 8.38 (d, *J* = 5.1 Hz, 2H, ArH), 8.16 (d, *J* = 7.8 Hz, 2H, ArH), 7.94 (d, *J* =5.1 Hz, 2H, ArH), 7.53 (t, *J* = 7.8 Hz, 2H, ArH), 7.38-7.19 (m, 10H, ArH), 6.98 (d, *J* = 6.6 Hz, 4H, ArH), 6.00 (s, 4H, 2NC***H***$_2$Ph), 4.08 (s, 4H, 2C***H***$_2$), 2.66 (t, *J* = 6.9 Hz,

4H, HNC***H***$_2$CH$_2$CH$_2$CH$_2$CH$_2$C***H***$_2$NH), 1.54-1.43 (m, 4H, HNCH$_2$C***H***$_2$CH$_2$CH$_2$C***H***$_2$CH$_2$NH), 1.37-1.30 (m, 4H, HNCH$_2$CH$_2$C***H***$_2$C***H***$_2$CH$_2$CH$_2$NH). ^{13}C NMR (75MHz, $CDCl_3$): δ 143.2, 142.3, 138.9, 138.2, 135.9, 130.0, 129.2, 128.7, 127.5, 125.4, 121.6, 120.2, 114.2, 110.2, 54.5, 50.4, 48.6, 30.5, 27.7. HRMS (ESI) calcd for $C_{44}H_{50}Cl_6N_6$ $[M+H]^+$ 657.3700, found 657.3715.

5.2.23 N,N-Bis[[9-(4-fluorobenzyl)-β-carboline-1-yl]methyl]propane-1,3- diamine (4w) : Yellow oil was obtained (0.35g, 53%). ESI-MS m/z: 650.9 $[M+H]^+$. ^{1}H NMR (300MHz, $CDCl_3$): δ 8.29 (d, J = 5.1 Hz, 2H, ArH), 8.14 (d, J = 7.8 Hz, 2H, ArH), 7.90 (d, J =5.1 Hz, 2H, ArH), 7.52 (t, J = 7.8 Hz, 2H, ArH), 7.31-7.25 (m, 4H, ArH), 6.97-6.87 (m, 8H, ArH), 5.87 (s, 4H, 2NC***H***$_2$Ph[4-F]), 4.11 (s, 4H, 2C***H***$_2$), 2.79 (t, J = 6.3 Hz, 4H, HNC***H***$_2$CH$_2$C***H***$_2$NH), 1.79-1.67 (m, 2H, HNCH$_2$C***H***$_2$CH$_2$NH). ^{13}C NMR (75MHz, $CDCl_3$): δ 163.7, 160.4, 142.9, 142.0, 138.3, 135.7, 134.5, 130.0, 128.7, 127.1, 127.0, 121.6, 120.3, 116.2, 115.9, 114.2, 110.0, 54.5, 48.1, 47.9, 40.7, 34.0. HRMS (ESI) calcd for $C_{41}H_{42}Cl_6F_2N_6$ $[M+H]^+$ 651.3042, found 651.3045.

5.2.24 N,N-Bis[[9-(4-fluorobenzyl)-β-carboline-1-yl]methyl]butane-1,4-diamine (4x) : Yellow oil was obtained (0.42g, 63%). ESI-MS m/z: 665.9 $[M+H]^+$. ^{1}H NMR (300MHz, $CDCl_3$): δ 8.34 (d, J = 5.1 Hz, 2H, ArH), 8.14 (d, J = 7.8 Hz, 2H, ArH), 7.91 (d, J =5.1 Hz, 2H, ArH), 7.53 (t, J = 7.8 Hz, 2H, ArH), 7.34-7.25 (m, 4H, ArH), 6.92 (d, J =7.2 Hz, 8H, ArH), 5.92 (s, 4H, 2NC***H***$_2$Ph[4-F]), 4.07 (s, 4H, 2C***H***$_2$), 2.72-2.66 (m, 4H, HNC***H***$_2$CH$_2$CH$_2$C***H***$_2$NH), 1.60-1.54 (m, 4H, HNCH$_2$C***H***$_2$C***H***$_2$CH$_2$NH). ^{13}C NMR (75MHz, $CDCl_3$): δ 163.7, 160.5, 142.6, 142.1,

138.3, 135.6, 134.4, 130.1, 128.8, 127.1, 127.0, 126.8, 121.7, 121.6, 120.4, 120.2, 116.2, 116.0, 114.3, 110.0, 54.3, 50.2, 48.0, 28.4. HRMS (ESI) calcd for $C_{42}H_{44}Cl_6F_2N_6$ $[M+H]^+$ 665.3199, found 665.3206.

5.2.25 N,N-Bis[[9-(3-chlorobenzyl)-β-carboline-1-yl]methyl]propane-1,3-diamine (4y) *:* Yellow oil was obtained (0.35g, 51%). ESI-MS m/z: 684.6 $[M+H]^+$. ^{1}H NMR (300MHz, $CDCl_3$): δ 8.34 (d, J = 5.1 Hz, 2H, ArH), 8.13 (d, J = 7.8 Hz, 2H, ArH), 7.91 (d, J =5.1 Hz, 2H, ArH), 7.51 (t, J = 7.8 Hz, 2H, ArH), 7.32-7.24 (m, 4H, ArH), 7.19-7.06 (m, 4H, ArH), 7.00 (s, 2H, ArH), 6.87 (d, J = 7.5 Hz, 2H, ArH), 5.92 (s, 4H, 2N***CH₂***Ph[3-Cl]), 4.07 (s, 4H, 2C***H***$_2$), 2.77 (t, J = 6.6 Hz, 4H, HNC***H***$_2$CH$_2$C***H***$_2$NH), 1.74-1.64 (m, 2H, HNCH$_2$C***H***$_2$CH$_2$NH). ^{13}C NMR (75MHz, $CDCl_3$): δ 142.8, 142.1, 141.2, 138.4, 135.8, 135.1, 130.5, 130.1, 128.9, 127.7, 125.7, 123.7, 121.7, 121.6, 120.5, 114.4, 110.0, 54.5, 48.8, 48.2, 30.5. HRMS (ESI) calcd for $C_{41}H_{40}Cl_8N_6$ $[M+H]^+$ 683.2451, found 683.2442.

5.4.26 N,N-Bis[[9-(3-chlorobenzyl)-β-carboline-1-yl]methyl]pentane-1,5-diamine (4z) *:* Yellow oil was obtained (0.37g, 53%). ESI-MS m/z: 710.5 $[M+H]^+$. ^{1}H NMR (300MHz, $CDCl_3$): δ 8.38 (d, J = 5.1 Hz, 2H, ArH), 8.16 (d, J = 7.8 Hz, 2H, ArH), 7.95 (d, J =5.1 Hz, 2H, ArH), 7.50 (t, J = 7.8 Hz, 2H, ArH), 7.34-7.24 (m, 4H, ArH), 7.20-7.13 (m, 4H, ArH), 7.03 (s, 2H, ArH), 6.84 (d, J = 6.3 Hz, 2H, ArH), 6.00 (s, 4H, 2N***CH₂***Ph[3-Cl]), 4.05 (s, 4H, 2C***H***$_2$), 2.67 (t, J = 6.6 Hz, 4H, HNC***H***$_2$CH$_2$CH$_2$CH$_2$C***H***$_2$NH), 1.55-1.25 (m, 6H, HNCH$_2$C***H***$_2$C***H***$_2$C***H***$_2$CH$_2$NH). ^{13}C NMR (75MHz, $CDCl_3$) δ 143.1, 142.1, 141.2, 138.4, 135.2, 130.5, 130.1, 128.8,

127.7, 125.7, 123.6, 121.7, 120.4, 114.3, 110.0, 54.8, 50.3, 48.3, 30.5, 25.6. HRMS (ESI) calcd for $C_{43}H_{46}Cl_8N_6$ $[M+H]^+$ 711.2764, found 711.2781.

5.4.27 N,N-Bis[[9-(3-phenylpropyl)-β-carboline-1-yl]methyl]propane-1,3-diamine (4aa)： Yellow oil was obtained (0.33g, 50%). ESI-MS m/z: 670.7 $[M+H]^+$. ^{1}H NMR (300MHz, $CDCl_3$): δ 8.30 (d, J = 5.1 Hz, 2H, ArH), 8.09 (d, J = 7.8 Hz, 2H, ArH), 7.85 (d, J =5.1 Hz, 2H, ArH), 7.52 (t, J = 7.8 Hz, 2H, ArH), 7.30-7.12 (m, 14H, ArH), 4.57 (t, J = 8.1 Hz, 4H, 2NC***H***$_2$CH$_2$CH$_2$Ph), 4.19 (s, 4H, 2C***H***$_2$), 2.78-2.67 (m, 8H, 2NCH$_2$CH$_2$C***H***$_2$Ph, HNC***H***$_2$CH$_2$C***H***$_2$NH), 2.20-2.08 (m, 4H, 2NCH$_2$C***H***$_2$CH$_2$Ph), 1.83-1.68 (m, 2H, HNCH$_2$C***H***$_2$CH$_2$NH). ^{13}C NMR (75MHz, $CDCl_3$): δ 142.8, 141.7, 141.0, 137.8, 135.0, 129.9, 128.8, 128.6, 128.4, 126.5, 121.6, 119.8, 114.1, 110.0, 54.8, 48.8, 44.7, 33.6, 32.3, 31.0. HRMS (ESI) calcd for $C_{45}H_{46}Cl_6N_6$ $[M+H]^+$ 671.3857, found 671.3866.

5.4.28 N,N-Bis[[9-(3-phenylpropyl)-β-carboline-1-yl]methyl]butane-1,4-diamine (4ab) ：Yellow oil was obtained (0.38g, 56%). ESI-MS m/z: 685.1$[M+H]^+$. ^{1}H NMR (300MHz, $CDCl_3$): δ 8.70 (s, 2H, ArH), 8.11 (d, J = 7.8 Hz, 2H, ArH), 7.95 (s, 2H, ArH), 7.52 (t, J = 6.9 Hz, 2H, ArH), 7.36-7.12 (m, 14H, ArH), 4.34 (t, J = 7.2 Hz, 4H, 2NC***H***$_2$CH$_2$CH$_2$Ph), 4.08 (s, 4H, 2C***H***$_2$), 2.79-2.67 (m, 8H, 2NCH$_2$CH$_2$C***H***$_2$Ph , HNC***H***$_2$CH$_2$CH$_2$C***H***$_2$NH), 2.29-2.17 (m, 4H, 2NCH$_2$C***H***$_2$CH$_2$Ph), 1.70-1.64 (m, 4H, HNCH$_2$C***H***$_2$C***H***$_2$CH$_2$NH). ^{13}C NMR (75MHz, $CDCl_3$): δ 149.0, 141.6, 140.8, 135.7, 131.5, 131.4, 129.3, 128.8, 128.4, 126.4, 122.2, 121.4, 119.7, 113.3, 109.6, 56.0, 50.0, 43.0, 33.5, 30.6, 28.5. HRMS (ESI) calcd for $C_{46}H_{54}Cl_6N_6$ $[M+H]^+$ 685.4013, found 685.4005.

5.4.29 N,N-Bis[[9-(3-phenylpropyl)-β-carboline-1-yl]methyl]pentane-1,5-diamine (4ac) : Yellow oil was obtained (0.40g, 57%). ESI-MS m/z: 698.7 $[M+H]^+$. ^{1}H NMR (300MHz, $CDCl_3$): δ 8.33 (d, J = 5.4 Hz, 2H, ArH), 8.09 (d, J = 7.8 Hz, 2H, ArH), 7.87 (d, J =5.4 Hz, 2H, ArH), 7.54 (t, J = 7.8 Hz, 2H, ArH), 7.34-7.17 (m, 14H, ArH), 4.62 (t, J =8.1 Hz, 4H, 2NC***H***$_2$CH$_2$CH$_2$Ph), 4.21 (s, 4H, 2C***H***$_2$), 2.78 (t, J =7.5 Hz, 4H, HNC***H***$_2$CH$_2$CH$_2$CH$_2$C***H***$_2$NH), 2.67 (t, J =6.9 Hz, 4H, 2NCH$_2$CH$_2$C***H***$_2$Ph), 2.25-2.12 (m, 4H, 2NCH$_2$C***H***$_2$CH$_2$Ph), 1.62-1.50 (m, 4H, HNCH$_2$C***H***$_2$CH$_2$C***H***$_2$CH$_2$NH), 1.47-1.38 (m, 2H, HNCH$_2$CH$_2$C***H***$_2$CH$_2$CH$_2$NH). ^{13}C NMR (75MHz, $CDCl_3$): δ 142.8, 141.8, 141.0, 137.8, 135.0, 129.9, 128.8, 128.6, 128.3, 126.5, 121.5, 119.8, 114.0, 109.9, 54.6, 50.3, 44.8, 33.6, 32.3, 30.5, 25.6. HRMS (ESI) calcd for $C_{47}H_{56}Cl_6N_6$ $[M+H]^+$ 699.4170, found 699.4180.

5.4.30 N,N-Bis[[9-(3-phenylpropyl)-β-carboline-1-yl]methyl]hexane-1,6-diamine (4ad) : Yellow oil was obtained (0.47g, 66%). ESI-MS m/z: 712.7 $[M+H]^+$. ^{1}H NMR (300MHz, $CDCl_3$): δ 8.33 (d, J = 5.1 Hz, 2H, ArH), 8.09 (d, J = 7.8 Hz, 2H, ArH), 7.86 (d, J =5.1 Hz, 2H, ArH), 7.54 (t, J = 7.8 Hz, 2H, ArH), 7.36-7.16 (m, 14H, ArH), 4.63 (t, J = 8.1 Hz, 4H, 2NC***H***$_2$CH$_2$CH$_2$Ph), 4.21 (s, 4H, 2C***H***$_2$), 2.79 (t, J = 7.2 Hz, 4H, HNC***H***$_2$CH$_2$CH$_2$CH$_2$CH$_2$C***H***$_2$NH), 2.67 (t, J = 6.9 Hz, 4H, 2NCH$_2$CH$_2$C***H***$_2$Ph), 2.26-2.13 (m, 4H, 2NCH$_2$C***H***$_2$CH$_2$Ph), 1.58-1.48 (m, 4H, HNCH$_2$C***H***$_2$CH$_2$CH$_2$C***H***$_2$CH$_2$NH), 1.42-1.33 (m, 4H, HNCH$_2$CH$_2$C***H***$_2$C***H***$_2$CH$_2$CH$_2$-NH). ^{13}C NMR (75MHz, $CDCl_3$): δ 142.7, 141.7, 141.0, 137.8, 135.0, 129.9, 128.8, 128.6, 128.4, 126.5, 121.6, 119.8, 114.0, 110.0, 54.6, 50.4, 44.8, 33.7, 32.3, 30.6, 27.8. HRMS (ESI) calcd for $C_{48}H_{58}Cl_6N_6$ $[M+H]^+$ 713.4326, found 713.4335.

5.3 Cell proliferation assay

Cell proliferative assay were carried out using 96 microtitre plate cultures and MTT staining. The human umbilical vein cell line EA.HY926 was obtained from Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Science. Cells were grown in RPMI-1640 medium containing 10% (v/v) fetal calf serum and 100 μM penicillin and 100 μM streptomycin. Cultures were propagated at 37℃ in a humified atmosphere containing 5% CO_2, Cells in logarithmic phase were diluted to a density of 50,000 cells/mL in culture medium and treated with various concentrations of bivalent β-carbolines **4a-4ad** in 96-well plates for 48 h in final volumes of 200 μL. Then 20 μL MTT (5 mg/mL) was added to each well, and the cells were incubated for additional 4 h. After carefully removing the medium, the precipitates were dissolved in 150 μL of DMSO, shaken mechanically for 2 min, and then absorbance values at a wavelength of 570 nm were taken on a spectrophotometer (Bio-Rad iMark microplate absorbance Reader, USA). IC_{50} values were calculated using percentage of growth versus untreated control. Combretastain A4 phosphate (CA4P) and Endostar were used as positive controls and 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.

5.4 Cell migration assay

Cell migration assay were carried out using 24-well plate and wound-healing method according to the procedures described by Queiroz [50]. Briefly, EA.HY926

cells were seeded in a 24-well plate pre-coated with 0.1% gelatin and were allowed to grow to 100% confluence. Cell culture were injured by a 10 μL tip, cells were washed twice with PBS, and then incubated with fresh medium containing VEGF with or without compound **4m** at different concentrations for 24 h. Endostar was used as positive control. Cell migration to the damaged area was then visualized and photographed on a phase contrast microscope. Inhibition percentage was expressed as percentage of the control (100%).

5.5 Tube formation assay

Tube formation assay were generally performed as described by Queiroz's group [50]. Matrigel$^{@}$ basement membrane matrix (growth factor reduced) (Corning, USA) was thawed at 4℃, pipetted into pre-chilled 24-well plates (200μL matrigel/well) and incubated at 37℃ for 30 min. Then EA.HY926 cells (5× 10^4 cells/mL) were added to matrigel coated plates, followed by addition of various concentrations of the selected compound **4m** with VEGF (60 ng/mL). Endostar was used as positive control. After 24 h of incubation with 5% CO_2 at 37℃, the number of capillary-like tube formation of each well was photographed using a phase-contrast microscope at 100× magnifications. Tube formation was quantitated by manual counting the number of branch points. All experiments were done in triplicate. Inhibition percentage was expressed as percentage of control (100%).

5.6 Chicken chorioallantoic membrane (CAM) assay

Antiangiogenic activity of the selected compound **4m** was investigated *in vivo* using chicken chorioallantoic membrane (CAM) assay [48]. Five day-old fertilized

eggs were obtained from local hatchery. 5 mL of albumin was aspirated and the eggs were incubated horizontally to allow the CAM to detach from the shell. Compound **4m** was prepared in 1.2% agarose discs at concentration of 0.3, 3.0 and 30 μM/disc, respectively. Endostar was used as positive control. Discs containing the vehicle only (DMSO) were used as negative control. A small window opening was made in the shell, and the discs were directly applied onto the CAM. The square opening was covered with sterilized surgical tape and the embryos were incubated for 48 h at 37°C. The CAMs were photographed under a dissecting microscope and blood vessels in each CAM were counted. The results are presented as a mean percentage of inhibition to the control ± SD, (n = 3).

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References

[1] M. E. Maragoudakis, Angiogenesis in health and disease, *General. pharmacol.* 35 (2000), 225-226.

[2] N. Ferrara, R. S. Kerbel, Angiogenesis as a therapeutic target, Nature 438 (2005), 967–74.

[3] P. Carmeliet, Angiogenesis in health and disease, Nature Med. 9 (2003), 653-660.

[4] L. Ma, J. Chen, X. Wang, X. Liang, Y. Luo, W. Zhu, T. Wang, M. Peng, S. Li, S. Jie, A. Peng, Y. Wei and L. Chen, Structural modification of Honokiol, a biphenyl occurring in magnolia officinalis: the evaluation of Honokiol analogues as inhibitors of angiogenesis and for their cytotoxicity and structure-activity relationship, J. Med. Chem. 54 (2011) 6469-6481.

[5] R. Cao, W. Peng, Z. Wang, A. Xu, β-Carboline alkaloids: biochemical and pharmacological functions, Curr. Med. Chem. 14 (2007) 479-500.

[6] J. Ishida, H. Wang, K. Bastow, C. Hu, K. Lee, Antitumor agents 201, Cytotoxicity of harmine and β-carboline analogs, Bioorg. Med. Chem. Lett. 9 (1999) 3319-3324.

[7] S. Xiao, W. Lin, C. Wang, M. Yang, Synthesis and biological evaluation of DNA targeting flexible side-chain substituted β-carboline derivatives, Bioorg. Med. Chem. Lett. 11 (2001) 437-441.

[8] Y. Song, J. Wang, S. F. Teng, D. Kesuma, Y. Deng, J. Duan, J. H. Wang, R. Z. Qi, M. M. Sim, β-Carboline as specific inhibitors of cyclin-dependent kinases, Bioorg. Med. Chem. Lett. 12 (2002) 1129-1132.

[9] M. Zhao, L. Bi, W. Wang, C. Wang, M. Baudy-Floch, J. Ju, S. Peng, Synthesis and cytotoxic activities of β-carboline amino acid ester conjugates, Bioorg. Med. Chem. 14 (2006) 6998-7010.

[10] A. S. N. Formagio, L. T. D. Tonin, M. A. Foglio, C. Madjarof, J. E. de Carvalho, W. F. da Costa, F. P. Cardoso, M. H. Sarragiotto, Synthesis and antitumoral activity of novel 3-(2-substituted-1,3,4-oxadiazol-5-yl) and 3-(5-substituted-

1,2,4-triazol-3-yl) β-carboline derivatives, Bioorg. Med. Chem. 16 (2008) 9660-9667.

[11] J. Wu, M. Zhao, K. Qian, K. –H. Lee, S. Morris-Natschke, S. Peng, Novel N-(3-carboxyl-9-benzyl-β-carboline-1-yl)ethylamino acids: Synthesis, anti-tumor evaluation, intercalating determination, 3D QSAR analysis and docking investigation, Eur. J. Med. Chem. 44 (2009) 4153-4161.

[12] R. Ikeda, M. Kurosawa, T. Okabayashi, A. Takei, M. Yoshiwara, T. Kumakura, N. Sakai, O. Funatsu, A. Morita, M. Ikekita, Y. Nakaike, T. Konakahara, 3-(3-Phenoxybenzyl)amino-β-carboline: A novel antitumor drug targeting α-tubulin, Bioorg. Med. Chem. Lett. 21 (2011) 4784-4787.

[13] M. Yang, P. Kuo, T. Hwang, W. Chiou, K. Qian, C. Lai, K. Lee, T. Wu, Synthesis, in vitro anti-inflammatory and cytotoxic evaluation, and mechanism of action studies of 1-benzoyl-β-carboline and 1-benzoyl-3-carboxy-β-carboline derivatives, Bioorg. Med. Chem. 19 (2011) 1674-1682.

[14] G. Duportail, Linear and circular dichroism of harmine and harmaline interacting with DNA, Int. J. Biol. Macromol. 3 (1981) 188-193.

[15] Z. Taira, S. Kanzawas, C. Dohara, S. Ishida, M. Matsumoto, Y. Sakiya, Intercalation of six beta-carboline derivatives into DNA, Jpn. J. Toxicol. Environ. Health 43 (1997) 83-91.

[16] Y. Funayama, K. Nishio, K. Wakabayashi, M. Nagao, K. Shimoi, T. Ohira, S. Hasegawa, M. Saijo, Effects of β and γ-carboline derivatives on DNA topoisomerase activities, Mutat. Res. 349 (1996**)** 183-191.

[17] A. M. Sobhani, S. A. Ebrahimi, M. Mahmoudian, An in vitro evaluation of human DNA topoisomerase I inhibition by *Peganum harmala L.* seeds extract and its β-carboline alkaloids, J. Pharm. Pharmaceut. Sci. 5 (2002) 19-23.

[18] Y. Song, J. Wang, S. F. Teng, D. Kesuma, Y. Deng, J. Duan, J. H. Wang, R. Z. Qi, M. M. Sim, β-Carboline as specific inhibitors of cyclin-dependent kinases, Bioorg. Med. Chem. Lett. 12 (2002) 1129-1132.

[19] Y. Song, D. Kesuma, J. Wang, Y. Deng, J. Duan, J. H. Wang, R. Z. Qi, Specific inhibition of cyclin-dependent kinases and cell proliferation by harmine, Biochem. Biophys. Res. Commun. 317 (2004) 128-132.

[20] J. I. Trujillo, M. J. Meyers, D. R. Anderson, S. Hegde, M. W. Mahoney, W. F. Vernier, I. P. Buchler, K. K. Wu, S. Yang, S. J. Hartmann, D. B. Reitz, Novel tetrahydro-β-carboline-1-carboxylic acids as inhibitors of mitogen activated protein kinase-activated protein kinase 2 (MK-2), Bioorg. Med. Chem. Lett. 17 (2007), 4657-4663.

[21] P. A. Barsanti, W. Wang, Z. Ni, D. Duhl, N. Brammeier, E. Martin, The discovery of tetrahydro-β-carbolines as inhibitors of the kinesin Eg5, Bioorg. Med. Chem. Lett. 20 (2010) 157-160.

[22] A. C. Castro, L. C. Dang, F. Soucy, L. Grenier, H. Mazdiyasni, M. Hottelet, L. Parent, C. Pien, V. Palombella, J. Adams, Novel IKK inhibitors: β-carbolines, Bioorg. Med. Chem. Lett. 13 (2003) 2419-2422.

[23] R. Cao, Q. Chen, X. Hou, H. Chen, H. Guan, Y. Ma, W. Peng, A.Xu, Synthesis, acute toxicities and antitumor effects of novel 9-substituted β-carboline

derivatives, Bioorg. Med. Chem. 12 (2004) 4613-4623.

[24] R. Cao, W. Peng, H. Chen, X. Hou, H. Guan, Q. Chen, Y. Ma, A. Xu, Synthesis and in vitro cytotoxic evaluation of 1,3-bisubstituted and 1,3,9-trisubstituted β-carboline derivatives, Eur. J. Med. Chem. 40 (2005) 249-257.

[25] R. Cao, H. Chen, W. Peng, Y. Ma, X. Hou, H. Guan, X. Liu, A. Xu, Design, synthesis and *in vitro* and *in vivo* antitumor activities of novel β-carboline derivatives, Eur. J. Med. Chem. 40 (2005) 991-1001.

[26] H. Guan, H. Chen, W. Peng, Y. Ma, R. Cao, X. Liu, A. Xu, Design of β-carboline derivatives as DNA-targeting antitumor agents, Eur. J. Med. Chem. 41 (2006) 1167-1179.

[27] R. Cao, W. Yi, Q. Wu, X. Guan, M. Feng, C. Ma, Z. Chen, H. Song, W. Peng, Synthesis and cytotoxic activities of 1-benzylidine substituted β-carboline derivatives, Bioorg. Med. Chem. Lett. 18 (2008) 6558-6561.

[28] Q. Wu, R. Cao, M. Feng, X. Guan, C. Ma, J. Liu, H. Song, W. Peng, Synthesis and *in vitro* cytotoxic evaluation of novel 3,4,5-trimethoxyphenyl substituted β-carboline derivatives, Eur. J. Med. Chem. 44 (2009) 533-540.

[29] C. Ma, R. Cao, B. Shi, S. Li, Z. Chen, Y. Wei, W. Peng, Z. Ren, H. Song, Synthesis and cytotoxic evaluation of *N*2-benzylated quaternary β-carboline amino acid ester conjugates, Eur. J. Med. Chem. 45 (2010) 1515-1523.

[30] R. Cao, X. Guan, B. Shi, Z. Chen, Z. Ren, W. Peng, H. Song, Design, synthesis and 3D-QSAR of β-carboline derivatives as potent antitumor agents, Eur. J. Med. Chem. 45 (2010) 2503-2515.

[31] Z. Chen, R. Cao, B. Shi, Y. Wei, L. Yu, H. Song, Z. Ren, W. Peng, Synthesis of novel β-carbolines with efficient DNA-binding capacity and potent cytotoxicity, Bioorg. Med. Chem. Lett. 20 (2010) 3876-3879.

[32] Z. Chen, R. Cao, L. Yu, B. Shi, J. Sun, L. Guo, Q. Ma, W. Yi, X. Song, H. Song, Synthesis, cytotoxic activities and DNA binding properties of β-carboline derivatives, Eur. J. Med. Chem. 45 (2010) 4740-4745.

[33] C. Ma, R. Cao, B. Shi, X. Zhou, Q. Ma, J. Sun, L. Guo, W. Yi, Z. Chen, H. Song, Synthesis and cytotoxic evaluation of 1-carboxamide and 1-amino side chain substituted β-carbolines, Eur. J. Med. Chem. 45 (2010) 5513-5519.

[34] Z. Chen, R. Cao, B. Shi, L. Guo, J. Sun, Q. Ma, W. Fan, H. Song, Synthesis and biological evaluation of 1,9-disubstituted β-carbolines as potent DNA intercalating and cytotoxic agents, Eur. J. Med. Chem. 46 (2011) 5127-5137.

[35] R. Cao, W. Fan, L. Guo, Q. Ma, G. Zhang, J. Li, X. Chen, Z. Ren, L. Qiu, Synthesis and structure-activity relationships of harmine derivatives as potential antitumor agents, Eur. J. Med. Chem. 60 (2013) 135-143.

[36] G. Zhang, R. Cao, L. Guo, Q. Ma, W. Fan, X. Chen, J. Li, G. Shao, L. Qiu, Z. Ren, Synthesis and structure–activity relationships of N^2-alkylated quaternary β-carbolines as novel antitumor agents, Eur. J. Med. Chem. 65 (2013) 21-31.

[37] R. Cao, W. Peng, H. Chen, Y. Ma, X. Liu, X. Hou, H. Guan, A. Xu, DNA binding properties of 9-substituted harmine derivatives, Biochem. Biophys. Res. Commun. 338 (2005) 1557-1563.

[38] Q. Chen, R. Chao, H. Chen, X. Hou, H. Yan, S. Zhou, W. Peng, A. Xu, Antitumor

and neurotoxic effects of novel harmine derivatives and structure-activity relationship analysis, Int J. Cancer. 114 (2004) 675-682.

[39] J. Zhang, Y. Li, L. Guo, R. Cao, P. Zhao, W. Jiang, Q. Ma, H. Yi, Z. Li, J. Jiang, J. Wu, Y. Wang, S. Si, DH166, a beta-carboline derivative, inhibits the kinase activity of PLK1, Cancer Biol. Ther. 8 (2009), 2374-2383.

[40] X. Han, J. Zhang, L. Guo, R. Cao, Y. Li, N. Li, Q. Ma, J. Wu, Y. Wang, S. Si, A series of beta-carboline derivatives inhibit the kinase activity of PLKs, PLOS ONE 7 (2012) e46546.

[41] G. H. Posner, J. D'Angelo, P. M O'Neill, A. Mercer, Anticancer activity of artemisinin-derived trioxanes, Expert. Opin. Ther. Pat. 16 (2006) 1665-1672.

[42] M. Jung, S. Lee, J. Ham, K. Lee, H. Kim, S. K. Kim, Antitumor activity of novel deoxoartemisinin monomers, dimers, and trimer, J. Med. Chem. 46 (2003) 987-994.

[43] G. H. Posner, P. Ploypradith, M. H. Parker, H. O'Dowd, S. H. Woo, J. Northrop, M. Krasavin, P. Dolan, T. W. Kensler, S. Xie, T. A. Shapiro, Antimalarial, antiproliferative, and antitumor activities of artemisinin-derived, chemically robust, trioxane dimers, J. Med. Chem. 42 (1999) 4275-4280.

[44] G. H. Posner, A. J. McRiner, I. H. Paik, S. Sur, K. Borstnik, S. Xie, T. A. Shapiro, A. A. Alagbala, B. Foster, Anticancer and antimalarial efficacy and safety of artemisininderived trioxane dimers in rodents, J. Med. Chem. 47 (2004) 1299-1301.

[45] A. A. Alagbala, A. J. McRiner, K. Borstnik, T. Labonte, W. Chang, J. G.

D'Angelo, G. H. Posner, B. A. Foster. Biological mechanisms of action of novel C-10 non-acetal trioxane dimers in prostate cancer cell lines, J. Med. Chem. 49 (2006) 7836-7842.

[46] B. Shi, R. Cao, W. Fan, L. Guo, Q. Ma, X. Chen, G. Zhang, L. Qiu, H. Song, Design, synthesis and in vitro and in vivo antitumor activities of novel bivalent β-carbolines, Eur. J. Med. Chem. 60 (2013) 10-22.

[47] Q. Wu, Z. Bai, Q. Ma, W. Fan, L. Guo, G. Zhang, L. Qiu, H. Yu, G. Shao, R. Cao, Synthesis and biological evaluation of novel bivalent β-carbolines as potential antitumor agents, Med. Chem. Commun. 5(2014) 953-957.

[48] R. Sun, R. Liu, C. Zhou, Z. Ren, L. Guo, Q. Ma, W. Fan, L. Qiu, H. Yu, G. Shao and R. Cao, Synthesis and biological evaluation of piperazine group linked bivalent β-carbolines as potential antitumor agents, Med. Chem. Commun. 6 (2015) 2170-2174.

[49] H. R. Snyder, S. M. Parmerter, L. Katz, The synthesis of derivatives of β-carboline. III. The nitration of Harman, J. Am. Chem. Soc. 70 (1948) 222-225.

[50] V. A. Machado, D. Peixoto, R. Costa, H. J. Froufe, R. C. Calhelha, R. M. Abreu, I. C. Ferreira, R. Soares and M. J. Queiroz, Synthesis, antiangiogenesis evaluation and molecular docking studies of 1-aryl-3-[(thieno[3,2-b]pyridin-7-ylthio) phenyl]ureas: Discovery of a new substitution pattern for type II VEGFR-2 Tyr kinase inhibitors, Bioorg. Med. Chem. 23 (2015) 6497-6509.

[51]C. Meinguet, C. Bruyère, R. Frédérick, V. Mathieu, C. Vancraeynest, L. Pochet, J. Laloy, J. Mortier, G. Wolber, R. Kiss, B. Masereel and J. Wouters, 3D-QSAR,

design, synthesis and characterization of trisubstituted harmine derivatives with in vitro antiproliferative properties, Eur. J. Med. Chem. 94 (2015) 45-55.

Table and Figure captions

Table 1 Inhibitory effect of novel bivalent β-carbolines on endothelial cell proliferation

Figure 1 The chemical structure of the representative reported bivalent β-carbolines.

Figure 2 The effect of inhibiting EA.HY926 cells migration by compound **4m**.

Figure 3 The effect of inhibiting tube formation of EA.HY926 cells by compound **4m**.

Figure 4 Inhibitory effects of compound **4m** on angiogenesis of CAM.

Table 1 Inhibitory effect of bivalent β-carbolines on endothelial cell proliferation

Compd	IC_{50} (μM)	Compd	IC_{50} (μM)
4a	25.8	**4q**	5.16
4b	14.5	**4r**	8.20
4c	17.0	**4s**	9.33
4d	11.5	**4t**	8.00
4e	7.77	**4u**	8.89
4f	5.36	**4v**	3.81
4g	9.73	**4w**	8.87
4h	8.56	**4x**	4.96
4i	8.06	**4y**	10.8
4j	5.55	**4z**	4.69
4k	8.39	**4aa**	9.35
4l	4.37	**4ab**	4.78
4m	2.16	**4ac**	2.76
4n	2.92	**4ad**	3.22
4o	9.23	**CA_4P**	6.40
4p	8.93	**Endostar**	1.76

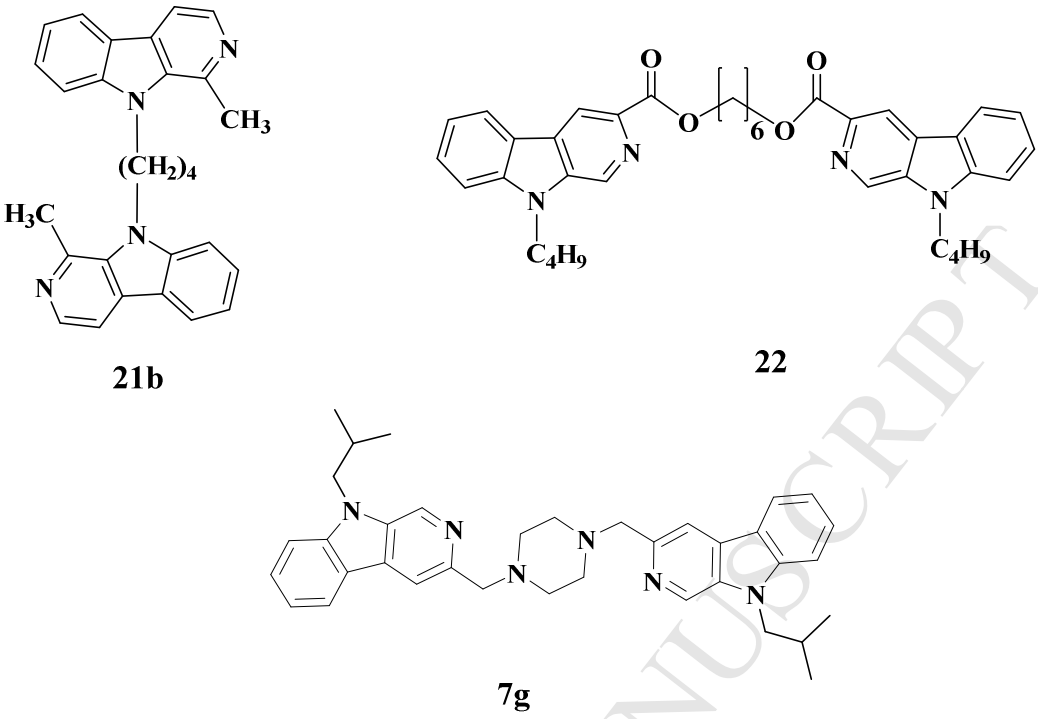


Figure 1 The chemical structure of the representative reported bivalent β-carbolines.

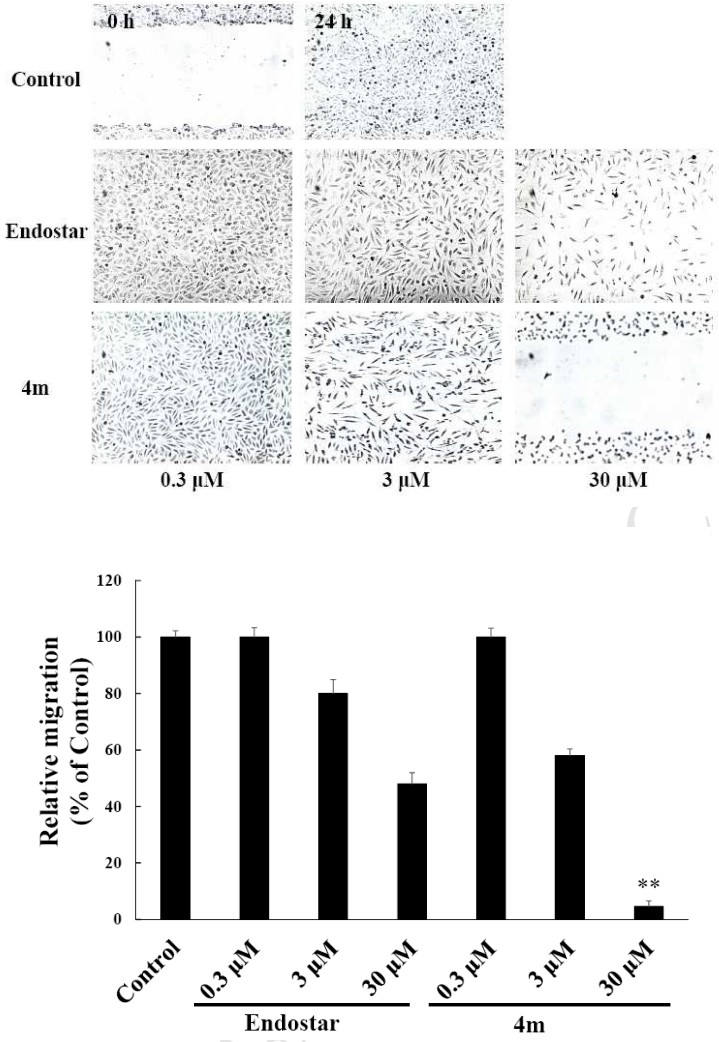


Figure 2 The effect of inhibiting EA.HY926 cells migration by compound **4m**.

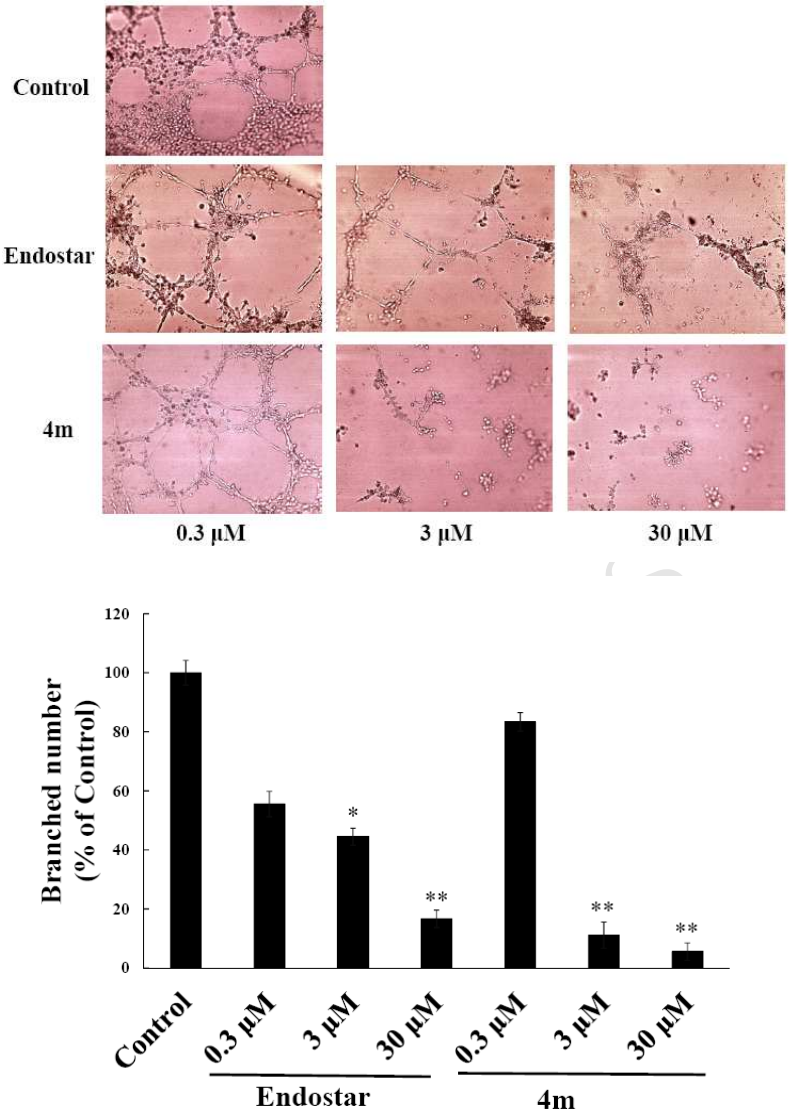


Figure 3 The effect of inhibiting tube formation of EA.HY926 cells by compound **4m**.

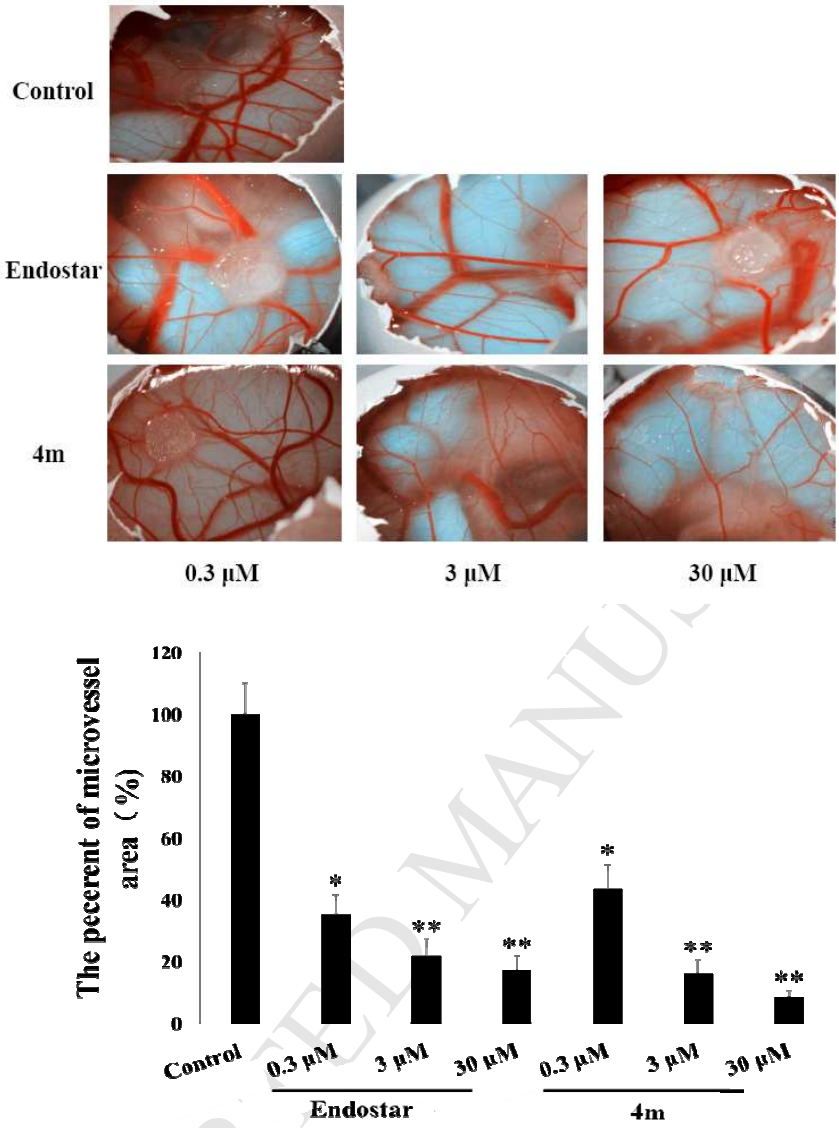
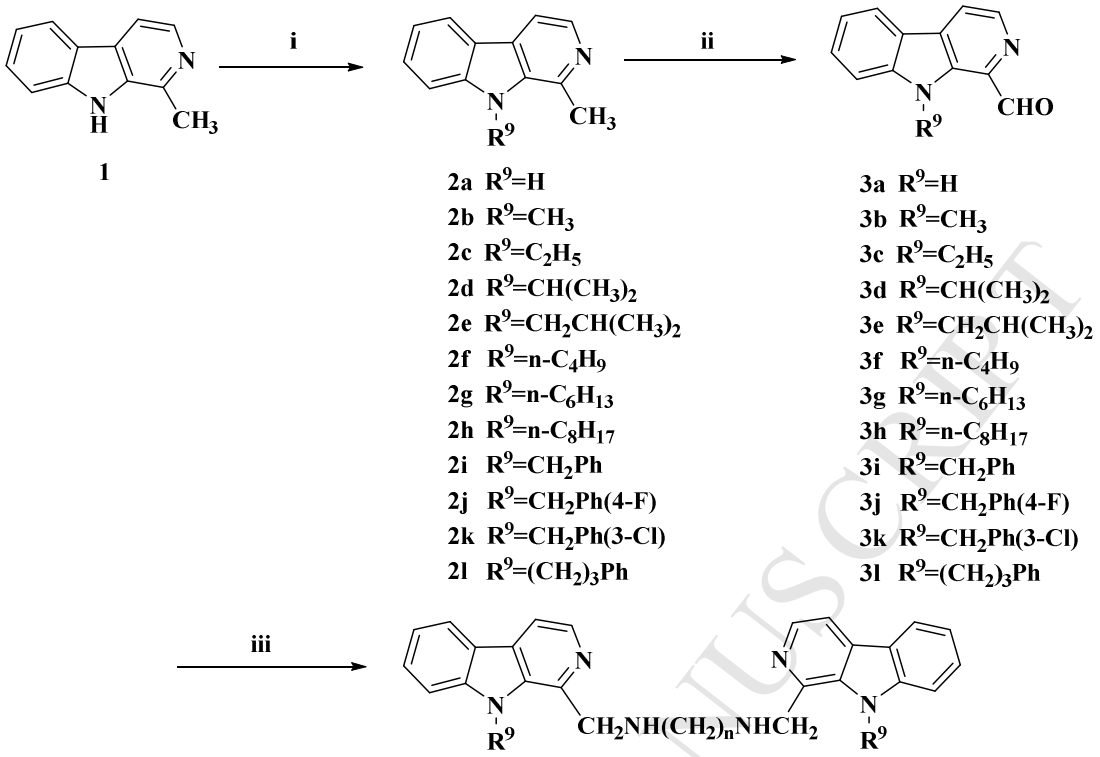


Figure 4 Inhibitory effects of compound **4m** on angiogenesis of CAM.



Scheme 1 Synthesis of the bivalent β-carbolines **4a-ad.** Reagents and conditions: (i) DMF, NaH, alkyl halogenide, stirred at RT; (ii) SeO_2, dioxane, reflux, 2 h; (iii) sym-diamine, $NaBH_3CN$, stirred at RT.

Research highlights

- ✓ A series of novel bivalent β-carbolines was prepared and evaluated as angiogenesis inhibitors.
- ✓ Compound **4m** was found to be the most potent antiangiogenetic agent.
- ✓ The length of the linker affected antiangiogenetic potency of bivalent β-carbolines.