

RESEARCH PAPER



Synthesis and biological evaluation of novel N^9 -heterobivalent β -carbolines as angiogenesis inhibitors

Liang Guo^a, Qin Ma^b, Wei Chen^b, Wenxi Fan^b, Jie Zhang^a and Bin Dai^a

^aSchool of Chemistry and Chemical Engineering/Key Laboratory for Green Processing of Chemical Engineering of XinJiang Bingtuan, Shihezi University, Shihezi, China; ^bXinJiang Huashidan Pharmaceutical Research Co. Ltd., Urumqi, China

ABSTRACT

A series of novel N^9 -heterobivalent β -carbolines has been synthesized. All the novel compounds were tested for their anticancer activity against six tumour cell lines *in vitro*. Among these molecules, compounds **5b**, and **5w** exhibited strong cytotoxic activities with IC_{50} value of lower than $20 \mu\text{M}$. Acute toxicities and antitumor efficacies of the selected compounds in mice were also evaluated, compounds **5b** and **5w** exhibited that tumour inhibition rate of over 40% in the Sarcoma 180 and Lewis lung cancer animal models. Preliminary structure–activity relationships (SARs) analysis indicated that: (1) C^1 -methylation and C^7 -methoxylation were favorable for increased activities; (2) 3-Pyridyl or 2-thienyl group substituent into position-1 of the β -carboline core, and the aryl substituent into another β -carboline ring might be detrimental to cytotoxic effects of this class compounds. Investigation of the preliminary mechanism of action demonstrated that compound **5b** had obvious angiogenesis inhibitory effects in the chicken chorioallantoic membrane (CAM) assay.

ARTICLE HISTORY

Received 19 March 2018
Revised 21 June 2018
Accepted 3 July 2018

KEYWORDS

Heterobivalent β -carboline;
cytotoxic activities;
angiogenesis inhibitors;
structure–activity
relationship

Introduction

The population growth and aging associated with some risk factors leveraged the incidence of new cases of cancer and related deaths in developed countries and in developing countries¹. Cancer resistance to therapy is becoming a common phenomenon that threatens the current strategies adopted against this disease. For that reason we need to discover new anticancer agents. One of the successful and effective methods for the discovery of new anticancer drugs from natural products is to synthesize novel compounds through chemical structural modifications on the basis of leading compounds.

Peganum harmala L. have been traditionally used for hundreds of years to treat the alimentary tract cancers and malaria in Northwest China. Harmine, originally isolated from the seeds of *Peganum harmala* L. in 1847, is the most representative natural occurring β -carboline alkaloid, having a common tricyclic pyrindo [3,4-*b*] indole ring structure. In the past several decades, it has confirmed that harmine was the important active ingredients to treat the alimentary tract cancers^{2,3}. Recent reports demonstrated that harmine and its derivatives had remarkable antitumor activities, together with potential neurotoxicity^{2–5}. Moreover, it has been reported that harmine and its derivatives can exert antitumor activities through multiple mechanisms, such as DNA binding^{6–8}, inhibition topoisomerases I and II^{9,10}, CDK (cyclin-dependent kinase)^{11,12}, PLK1 (polo-like kinase)¹³, lipoxigenase^{14,15} and I κ B kinases¹⁶.

Previous investigations has shown that some dimer antitumor agents via an appropriate linker could lead to significantly improved antitumor activities (100- to 500-fold improvement over



the corresponding monomers)^{17–20}. Therefore, bivalent β -carbolines were expected to exhibit more potent antitumor efficacies than monomers. Inspired by this information, our group reported the synthesis, *in vitro* evaluation, *in vivo* efficacies and structure–activity relationships for the novel homobivalent β -carbolines with an alkyl spacer or alkylamino spacer in position-1, 3, 7, and 9 of the β -carboline nucleus, respectively (Figure 1)^{21–26}. In these compounds, 1-Methyl-9-[4-(1-methyl- β -carboline-9-yl)butyl]- β -carboline (B-9-3)^{21,27} exhibited potent antitumor activity. It was a symmetric dimeric β -carboline compound that contains two molecules of harman bound to each other by a tetramethylene group. The pharmacological mechanisms showed that the angiogenesis inhibitor B-9-3 selectively induces apoptosis of endothelial cells, in part through disruption of VEGF-A/VEGFR2 signaling²⁸.


Our strategy was based on the modification of the prototype B-9-3, following this previous work, we have continued our search for novel antitumor agents endowed with better antitumor activities, and we provide detailed studies of structure–activity relationships (SARs) on the antitumor efficacies *in vitro* and *in vivo* of this class of compounds. Here, we designed and synthesized a series of novel N^9 -heterobivalent β -carboline derivatives as potent antitumor agents.

Materials and methods

Chemistry

All reagents were purchased from commercial suppliers and were dried and purified when necessary. The following intermediates

CONTACT Bin Dai  db_tea@shzu.edu.cn  School of Chemistry and Chemical Engineering/Key Laboratory for Green Processing of Chemical Engineering of XinJiang Bingtuan, Shihezi University, Shihezi 832000, China

 Supplemental data for this article can be accessed [here](#).

© 2018 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

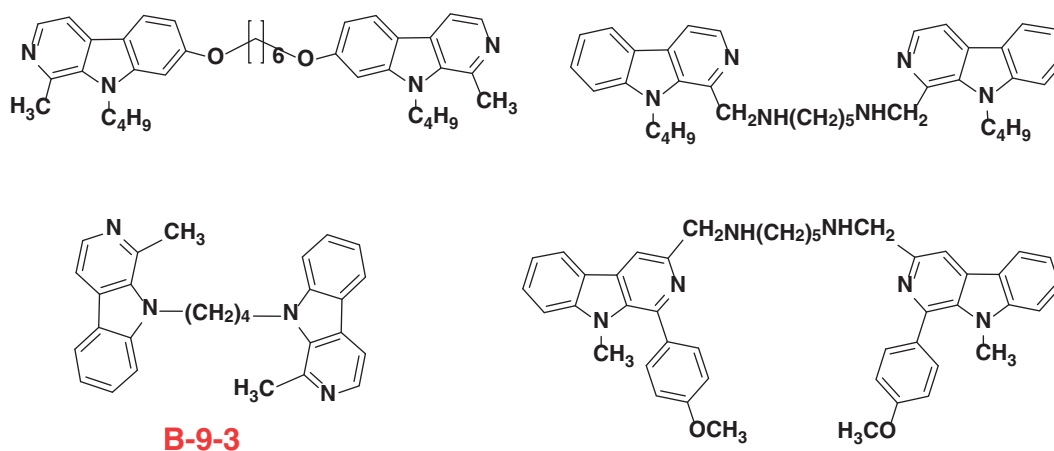


Figure 1. The chemical structure of the representative reported symmetric bivalent β -carbolines.

3a-3b²⁹, **3c-3e**²¹, **3f**³⁰, **3g**³¹, **3h**³², **4g-4h**³³ were prepared as previously described.

Melting points were determined in capillary tubes on an electrothermal X-5 apparatus and without correction. NMR spectra were recorded at room temperature on a Bruker Avance III HD 400 instrument at 400 MHz for ¹H NMR and 100 MHz for ¹³C NMR. Chemical shifts (δ) are reported in ppm relatively to the residual solvent peak and the multiplicity of each signal is designated by the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Coupling constants (J) are quoted in Hz. High resolution mass spectra (HRMS) were recorded on Bruker ultrafleXtreme MALDI-TOF/TOF-MS and HCCA (alpha-cyano-4-hydroxycinnamic acid) is used as matrix. Elemental analyses (C, H, and N) were carried out on an Elementar Vario ELIII CHNS Elemental Analyzer. Column chromatography was performed with silica gel (200–300 mesh) and analytical TLC on silica gel 60-F₂₅₄.

General procedure for the preparation of compounds 4a-h

A mixture of **3a** (1.68 g, 10 mmol) and anhydrous DMF (50 ml) was stirred at room temperature for 0.5 h, then NaH (0.50 g, 20 mmol) and the 1,4-dibromobutane (20 mmol) were added. The mixture was stirred at room temperature. After that period, the mixture was monitored via TLC and at the end of the reaction the mixture was poured into H₂O (150 ml) and extracted with ethyl acetate. The organic phase was collected and washed with water and brine, then dried under anhydrous sodium sulfate, filtered, and evaporated. The resulting oil was crystallized from ethyl ether or ethyl ether-petroleum ether to afford the compound **4a**. Products **4b-h** were prepared according to the same method of **4a**.

9-(4-bromobutyl)- β -carboline (**4a**)

Colorless crystals, yield 87%, m.p. 285.3–286.8 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ 9.10 (s, 1H), 8.40 (d, J = 5.2 Hz, 1H), 8.28 (d, J = 8.0 Hz, 1H), 8.14 (dd, J = 5.2, 0.8 Hz, 1H), 7.75 (d, J = 8.4 Hz, 1H), 7.60–7.64 (m, 1H), 7.27–7.31 (m, 1H), 4.56 (t, J = 6.8 Hz, 2H), 3.56 (t, J = 6.4 Hz, 2H), 1.90–1.97 (m, 2H), 1.81–1.88 (m, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 141.13, 138.88, 136.47, 133.13, 128.77, 127.66, 122.44, 120.80, 119.92, 115.04, 110.62, 42.15, 35.09, 30.23, 27.87.

9-(4-bromobutyl)-1-methyl- β -carboline (**4b**)

Colorless crystals, yield 88%, m.p. 213.9–215.6 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.71 (dd, J = 6.4, 1.2 Hz, 1H), 8.54 (dd, J = 8.0, 1.2 Hz, 1H), 8.49 (dd, J = 6.4, 2.0 Hz, 1H), 8.00 (d, J = 8.4 Hz, 1H), 7.83–7.87 (m, 1H), 7.46–7.50 (m, 1H), 4.75 (t, J = 7.6 Hz, 2H), 3.70 (t, J = 6.4 Hz, 2H), 3.27 (s, 3H), 2.04–1.77 (m, 4H). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 144.33, 139.00, 133.74, 133.26, 132.07, 129.29, 123.86, 122.14, 119.67, 116.15, 111.78, 45.35, 44.31, 29.63, 28.29, 18.31.

9-(5-bromopentyl)-1-methyl- β -carboline (**4c**)

Colorless crystals, yield 90%, m.p. 187.7–189.5 °C. ¹H NMR (400 MHz, CDCl₃): δ 8.42–8.33 (m, 2H), 8.29 (dt, J = 8.0, 1.2 Hz, 1H), 7.82–7.86 (m, 1H), 7.66 (dt, J = 8.4, 0.8 Hz, 1H), 7.48–7.51 (m, 1H), 4.69 (t, J = 7.6 Hz, 2H), 3.52 (s, 3H), 1.82–1.98 (m, 4H), 1.60–1.68 (m, 2H). ¹³C NMR (100 MHz, CDCl₃): δ 144.30, 137.12, 133.87, 133.76, 132.22, 128.57, 123.11, 122.38, 119.70, 115.13, 110.68, 45.35, 44.43, 31.86, 30.29, 24.04, 18.07.

9-(6-bromohexyl)-1-methyl- β -carboline (**4d**)

Colorless crystals, yield 69%, m.p. 73.9–74.8 °C. ¹H NMR (400 MHz, CDCl₃): δ 8.33 (d, J = 5.2 Hz, 1H), 8.12 (d, J = 7.6 Hz, 1H), 7.84 (d, J = 5.2 Hz, 1H), 7.56–7.60 (m, 1H), 7.45 (d, J = 8.4 Hz, 1H), 7.25–7.29 (m, 1H), 4.54 (t, J = 7.6 Hz, 2H), 3.38 (t, J = 6.4 Hz, 2H), 3.05 (s, 3H), 1.81–1.89 (m, 4H), 1.40–1.55 (m, 4H). ¹³C NMR (100 MHz, CDCl₃): δ 141.51, 141.13, 137.94, 135.09, 129.14, 128.17, 121.55, 121.33, 119.66, 112.99, 109.67, 44.76, 33.56, 30.70, 27.90, 26.13, 23.55.

9-(4-bromobutyl)-1-(3-pyridyl)- β -carboline (**4e**)

Yellow crystals, yield 71%, m.p. 135.0–136.7 °C. ¹H NMR (400 MHz, CDCl₃): δ 8.67 (d, J = 6.0 Hz, 1H), 8.53 (d, J = 6.0 Hz, 1H), 8.37 (d, J = 8.0 Hz, 1H), 7.87–7.81 (m, 2H), 7.68–7.64 (m, 2H), 7.54–7.49 (m, 1H), 7.36–7.33 (m, 1H), 4.19 (t, J = 8.0 Hz, 2H), 3.32 (dt, J = 55.2, 6.4 Hz, 2H), 1.77–1.69 (m, 2H), 1.60–1.44 (m, 2H). ¹³C NMR (100 MHz, CDCl₃): δ 149.60, 149.24, 142.21, 139.90, 138.50, 137.27, 131.21, 129.04, 128.23, 125.31, 123.53, 121.85, 121.25, 120.45, 114.54, 110.17, 43.77, 32.59, 29.44, 27.35.

9-(4-bromobutyl)-1-(2-thienyl)- β -carboline (**4f**)

Light yellow crystals, yield 60%, m.p. 197.6–199.8 °C. ¹H NMR (400 MHz, CDCl₃): δ 8.55 (d, J = 5.2 Hz, 1H), 8.19 (d, J = 7.6 Hz, 1H), 8.03 (d, J = 5.2 Hz, 1H), 7.62–7.54 (m, 3H), 7.54–7.51 (m, 1H),

7.49–7.41 (m, 3H), 7.30 (t, $J=7.2$ Hz, 1H), 4.03–3.78 (m, 2H), 3.20 (dt, $J=54.8, 6.4$ Hz, 2H), 1.71–1.49 (m, 2H), 1.44–1.24 (m, 2H). ^{13}C NMR (100 MHz, CDCl_3): δ 141.58, 140.89, 138.69, 138.31, 134.19, 131.47, 130.09, 129.47, 128.58, 126.95, 121.70, 121.26, 119.96, 114.31, 109.76, 43.23, 32.60, 29.77, 27.94.

General procedure for the preparation of compounds 5a-x

A solution of compound **3c** (2 mmol) in anhydrous DMF (6 ml) was added slowly with stirring to a solution of **4a** (3 mmol), NaH (0.25 g, 10 mmol), potassium iodide (1.68 g, 10 mmol) in anhydrous DMF (25 ml). The mixture was stirred at room temperature until the reaction is completed. Then the mixture was poured into ice-cold water. The reaction mixture was extracted with ethyl acetate (3 \times 30 ml), washed with water and brine, dried over anhydrous Na_2SO_4 , and filtered. Purification by column chromatography (DCM/MeOH 100:1 as the eluent) furnished the dimeric β -carboline **5a**. Compounds **5b-x** were synthesized using similar procedure as compound **5a**.

1-(3-pyridyl)-9-(4-(β -carboline-9-yl)butyl)- β -carboline (5a)

This compound was obtained as colourless crystals in 65% yield, m.p. 173.3–175.1 $^\circ\text{C}$. ^1H NMR (400 MHz, CDCl_3) δ : 8.77 (dd, $J=2.4, 0.8$ Hz, 1H), 8.65 (d, $J=0.8$ Hz, 1H), 8.61 (dd, $J=4.8, 1.6$ Hz, 1H), 8.50 (d, $J=5.2$ Hz, 1H), 8.47 (d, $J=5.2$ Hz, 1H), 8.15–8.18 (m, 2H), 8.00 (dd, $J=5.2, 0.8$ Hz, 1H), 7.98 (d, $J=5.2$ Hz, 1H), 7.56–7.63 (m, 3H), 7.30–7.34 (m, 3H), 7.24 (d, $J=8.4$ Hz, 1H), 7.11–7.15 (m, 1H), 4.14 (t, $J=7.2$ Hz, 2H), 3.96 (t, $J=7.2$ Hz, 2H), 1.38–1.41 (m, 4H). ^{13}C NMR (100 MHz, CDCl_3) δ : 150.00, 149.59, 142.09, 141.14, 140.25, 138.92, 138.05, 136.35, 136.06, 135.77, 134.09, 130.89, 128.85, 128.80, 123.04, 122.11, 121.81, 121.26, 120.90, 120.33, 120.02, 114.80, 114.22, 109.90, 109.37, 44.08, 42.65, 26.53, 26.01. HRMS calcd for $\text{C}_{31}\text{H}_{26}\text{N}_5$ [$\text{M} + \text{H}$] $^+$ 468.2183, found 468.2183. Anal. calcd for $\text{C}_{31}\text{H}_{25}\text{N}_5$: C, 79.63; H, 5.39; N, 14.98; found C 79.16, H 5.49, N 14.38.

1-(3-pyridyl)-9-(4-(1-methyl- β -carboline-9-yl)butyl)- β -carboline (5b)

This compound was obtained as slight yellow crystals in 54% yield, m.p. 201.2–202.2 $^\circ\text{C}$. ^1H NMR (400 MHz, CDCl_3) δ : 8.79 (dd, $J=2.4, 0.8$ Hz, 1H), 8.65 (dd, $J=4.8, 1.6$ Hz, 1H), 8.52 (d, $J=5.2$ Hz, 1H), 8.33 (d, $J=5.2$ Hz, 1H), 8.17–8.19 (m, 1H), 8.11 (d, $J=7.6$ Hz, 1H), 7.98 (d, $J=5.2$ Hz, 1H), 7.84 (d, $J=5.2$ Hz, 1H), 7.69–7.71 (m, 1H), 7.52–7.61 (m, 2H), 7.27–7.35 (m, 3H), 7.18–7.24 (m, 2H), 4.23 (t, $J=7.2$ Hz, 2H), 3.96 (t, $J=7.2$ Hz, 2H), 2.81 (s, 3H), 1.37–1.45 (m, 2H), 1.28–1.35 (m, 2H). ^{13}C NMR (100 MHz, CDCl_3) δ : 150.06, 149.64, 142.18, 141.40, 140.66, 140.31, 139.05, 136.41, 135.83, 134.70, 134.12, 130.97, 129.35, 128.86, 128.39, 123.11, 121.85, 121.63, 121.34, 121.17, 120.38, 119.91, 114.23, 113.07, 109.95, 109.55, 44.13, 43.90, 29.70, 27.71, 26.07. HRMS calcd for $\text{C}_{32}\text{H}_{28}\text{N}_5$ [$\text{M} + \text{H}$] $^+$ 482.2339, found 482.2342. Anal. calcd for $\text{C}_{32}\text{H}_{27}\text{N}_5$: C, 79.81; H, 5.65; N, 14.54; found C 79.39, H 5.37, N 14.75.

1-(3-pyridyl)-9-(5-(1-methyl- β -carboline-9-yl)pentyl)- β -carboline (5c)

This compound was obtained as yellow crystals in 62% yield, m.p. 189.4–190.5 $^\circ\text{C}$. ^1H NMR (400 MHz, CDCl_3) δ : 8.82 (d, $J=2.0$ Hz, 1H), 8.55 (d, $J=5.2$ Hz, 1H), 8.51 (dd, $J=4.8, 1.6$ Hz, 1H), 8.34 (d, $J=5.2$ Hz, 1H), 8.19 (d, $J=7.6$ Hz, 1H), 8.14 (d, $J=8.4$ Hz, 1H), 8.02 (d, $J=5.2$ Hz, 1H), 7.85–7.89 (m, 2H), 7.57–7.61 (m, 2H), 7.37

(d, $J=8.4$ Hz, 1H), 7.29–7.33 (m, 3H), 7.23–7.25 (m, 1H), 4.35 (t, $J=7.6$ Hz, 2H), 3.95 (t, $J=7.6$ Hz, 2H), 2.96 (s, 3H), 1.47–1.55 (m, 2H), 1.35–1.42 (m, 2H), 0.86–0.94 (m, 2H). ^{13}C NMR (100 MHz, CDCl_3) δ : 150.12, 149.51, 142.03, 141.61, 140.69, 140.38, 138.84, 137.29, 136.56, 135.99, 134.87, 134.27, 130.85, 129.51, 128.80, 128.52, 123.01, 121.76, 121.67, 121.30, 121.16, 120.27, 119.98, 114.26, 113.15, 110.01, 109.69, 44.48, 44.24, 30.29, 28.56, 23.87, 23.07. HRMS calcd for $\text{C}_{33}\text{H}_{30}\text{N}_5$ [$\text{M} + \text{H}$] $^+$ 496.2496, found 496.2503. Anal. calcd for $\text{C}_{33}\text{H}_{29}\text{N}_5$: C, 79.97; H, 5.90; N, 14.13; found C 79.11, H 5.88, N 13.90.

1-(3-pyridyl)-9-(6-(1-methyl- β -carboline-9-yl)hexyl)- β -carboline (5d)

This compound was obtained as yellow crystals in 66% yield, m.p. 141.3–142.9 $^\circ\text{C}$. ^1H NMR (400 MHz, CDCl_3) δ : 8.89 (dd, $J=2.0, 0.8$ Hz, 1H), 8.72 (dd, $J=4.8, 1.6$ Hz, 1H), 8.56 (d, $J=5.2$ Hz, 1H), 8.31 (d, $J=5.2$ Hz, 1H), 8.17–8.19 (m, 1H), 8.08–8.10 (m, 1H), 8.02 (d, $J=5.2$ Hz, 1H), 7.93–7.96 (m, 1H), 7.82 (d, $J=5.2$ Hz, 1H), 7.53–7.60 (m, 2H), 7.30–7.41 (m, 4H), 7.24–7.28 (m, 1H), 4.39 (t, $J=7.2$ Hz, 2H), 3.97 (t, $J=7.2$ Hz, 2H), 2.97 (s, 3H), 1.58–1.66 (m, 2H), 1.29–1.37 (m, 2H), 1.07–1.15 (m, 2H), 0.81–0.90 (m, 2H). ^{13}C NMR (100 MHz, CDCl_3) δ : 150.16, 149.54, 142.11, 141.45, 141.02, 140.44, 138.79, 137.84, 136.65, 136.07, 134.98, 134.35, 130.86, 129.13, 128.73, 128.15, 123.07, 121.72, 121.53, 121.31, 121.26, 120.19, 119.66, 114.23, 112.98, 110.10, 109.60, 44.54, 44.39, 30.59, 28.66, 26.33, 23.44. HRMS calcd for $\text{C}_{34}\text{H}_{32}\text{N}_5$ [$\text{M} + \text{H}$] $^+$ 510.2652, found 510.2656. Anal. calcd for $\text{C}_{34}\text{H}_{31}\text{N}_5$: C, 80.13; H, 6.13; N, 13.74; found C 79.72, H 6.14, N 13.32.

1-(2-thienyl)-9-(4-(β -carboline-9-yl)butyl)- β -carboline (5e)

This compound was obtained as yellow crystals in 56% yield, m.p. 141.6–143.1 $^\circ\text{C}$. ^1H NMR (400 MHz, CDCl_3) δ : 8.72 (d, $J=0.8$ Hz, 1H), 8.49 (d, $J=5.2$ Hz, 1H), 8.46 (d, $J=5.2$ Hz, 1H), 8.15–8.16 (m, 1H), 8.13–8.14 (m, 1H), 7.97 (dd, $J=5.2, 0.8$ Hz, 1H), 7.95 (d, $J=5.2$ Hz, 1H), 7.51–7.71 (m, 3H), 7.33–7.35 (m, 1H), 7.30–7.32 (m, 1H), 7.28–7.29 (m, 1H), 7.24–7.28 (m, 2H), 7.05 (dd, $J=4.0, 1.2$ Hz, 1H), 6.94 (dd, $J=5.2, 3.6$ Hz, 1H), 4.14 (t, $J=7.2$ Hz, 2H), 4.13 (t, $J=7.2$ Hz, 2H), 1.47–1.58 (m, 4H). ^{13}C NMR (100 MHz, CDCl_3) δ : 141.95, 141.05, 140.84, 138.62, 136.84, 136.22, 134.60, 132.44, 131.55, 130.86, 130.80, 128.78, 128.68, 128.54, 128.49, 128.21, 127.03, 126.58, 122.04, 121.73, 121.28, 121.02, 120.17, 119.79, 114.70, 114.23, 109.90, 109.34, 43.91, 42.68, 26.98, 26.28. HRMS calcd for $\text{C}_{30}\text{H}_{25}\text{N}_4\text{S}$ [$\text{M} + \text{H}$] $^+$ 473.1794, found 473.1792. Anal. calcd for $\text{C}_{30}\text{H}_{24}\text{N}_4\text{S}$: C, 76.24; H, 5.12; N, 11.86; S, 6.78; found C, 76.01; H, 5.61; N, 11.46; S, 6.34.

1-(2-thienyl)-9-(4-(1-methyl- β -carboline-9-yl)butyl)- β -carboline (5f)

This compound was obtained as yellow crystals in 47% yield, m.p. 214.8–216.0 $^\circ\text{C}$. ^1H NMR (400 MHz, CDCl_3) δ : 8.50 (d, $J=5.2$ Hz, 1H), 8.32 (d, $J=5.2$ Hz, 1H), 8.14–8.17 (m, 1H), 8.12–8.06 (m, 1H), 7.96 (d, $J=5.2$ Hz, 1H), 7.83 (d, $J=5.2$ Hz, 1H), 7.56–7.60 (m, 1H), 7.51–7.55 (m, 1H), 7.35–7.37 (m, 1H), 7.33–7.34 (m, 1H), 7.28–7.31 (m, 1H), 7.24–7.26 (m, 1H), 7.20–7.23 (m, 1H), 7.12 (dd, $J=4.0, 1.2$ Hz, 1H), 6.99 (dd, $J=5.2, 3.6$ Hz, 1H), 4.26 (t, $J=7.2$ Hz, 2H), 4.13 (t, $J=7.2$ Hz, 2H), 2.89 (s, 3H), 1.41–1.55 (m, 4H). ^{13}C NMR (100 MHz, CDCl_3) δ : 142.02, 141.36, 140.95, 140.86, 138.72, 137.86, 136.90, 134.85, 134.59, 130.89, 129.24, 128.70, 128.22, 127.07, 126.67, 121.76, 121.60, 121.36, 121.25, 120.22, 119.79, 114.23, 113.03, 109.94, 109.55, 44.04, 43.94, 27.94, 26.52, 23.35. HRMS calcd for $\text{C}_{31}\text{H}_{27}\text{N}_4\text{S}$ [$\text{M} + \text{H}$] $^+$ 487.1951, found 487.1950. Anal.

calcd for C₃₁H₂₆N₄S: C, 76.51; H, 5.39; N, 11.51; S, 6.59; found C, 76.27; H, 5.59; N, 10.93; S, 6.38.

1-(2-thienyl)-9-(5-(1-methyl-β-carboline-9-yl)pentyl)-β-carboline (5g)

This compound was obtained as yellow crystals in 62% yield, m.p. 158.4–159.2 °C. ¹H NMR (400 MHz, CDCl₃) δ: 8.51 (d, *J* = 5.2 Hz, 1H), 8.34 (d, *J* = 5.2 Hz, 1H), 8.15–8.17 (m, 1H), 8.11–8.14 (m, 1H), 7.97 (d, *J* = 5.2 Hz, 1H), 7.86 (d, *J* = 5.2 Hz, 1H), 7.54–7.60 (m, 2H), 7.40 (d, *J* = 8.4 Hz, 1H), 7.27–7.32 (m, 3H), 7.21 (dd, *J* = 5.2, 1.2 Hz, 1H), 7.16 (dd, *J* = 3.2, 1.2 Hz, 1H), 6.91 (dd, *J* = 5.2, 3.6 Hz, 1H), 4.37 (t, *J* = 7.6 Hz, 2H), 4.12 (t, *J* = 7.6 Hz, 2H), 2.96 (s, 3H), 1.53–1.60 (m, 2H), 1.42–1.50 (m, 2H), 0.98–1.06 (m, 2H). ¹³C NMR (100 MHz, CDCl₃) δ: 142.08, 141.45, 141.00, 140.94, 138.55, 137.76, 136.96, 134.97, 134.66, 130.82, 129.21, 128.62, 128.29, 128.23, 126.85, 126.51, 121.67, 121.55, 121.27, 121.25, 120.09, 119.75, 114.16, 113.02, 110.02, 109.66, 44.54, 44.06, 30.22, 28.89, 23.98, 23.40. HRMS calcd for C₃₂H₂₉N₄S [M + H]⁺ 501.2107, found 501.2113. Anal. calcd for C₃₂H₂₈N₄S: C, 76.71; H, 6.24; N, 10.84; S, 6.20; found C, 76.24; H, 5.92; N, 10.45; S, 6.07.

1-(2-thienyl)-9-(6-(1-methyl-β-carboline-9-yl)hexyl)-β-carboline (5h)

This compound was obtained as yellow crystals in 57% yield, m.p. 238.5–239.6 °C. ¹H NMR (400 MHz, CDCl₃) δ: 8.52 (d, *J* = 5.2 Hz, 1H), 8.32 (d, *J* = 5.2 Hz, 1H), 8.15–8.17 (m, 1H), 8.09–8.11 (m, 1H), 7.97 (d, *J* = 5.2 Hz, 1H), 7.83 (d, *J* = 5.2 Hz, 1H), 7.53–7.59 (m, 2H), 7.45 (dd, *J* = 5.2, 1.2 Hz, 1H), 7.40 (d, *J* = 8.4 Hz, 1H), 7.35 (d, *J* = 8.4 Hz, 1H), 7.24–7.30 (m, 3H), 7.13 (dd, *J* = 5.2, 3.6 Hz, 1H), 4.39 (t, *J* = 7.6 Hz, 2H), 4.14 (t, *J* = 7.6 Hz, 2H), 2.98 (s, 3H), 1.61–1.68 (m, 2H), 1.38–1.46 (m, 2H), 1.13–1.21 (m, 2H), 0.91–0.98 (m, 2H). ¹³C NMR (100 MHz, CDCl₃) δ: 142.18, 141.47, 141.21, 141.01, 138.54, 137.75, 137.09, 134.98, 134.77, 130.86, 129.14, 128.57, 128.27, 128.16, 126.98, 126.65, 121.66, 121.53, 121.34, 121.23, 120.06, 119.66, 114.12, 112.99, 110.20, 109.62, 44.58, 44.17, 30.61, 28.96, 26.43, 26.34, 23.40. HRMS calcd for C₃₃H₃₁N₄S [M + H]⁺ 515.2264, found 515.2268. Anal. calcd for C₃₃H₃₀N₄S: C, 77.01; H, 5.88; N, 10.89; S, 6.23; found C, 76.80; H, 5.76; N, 10.72; S, 5.97.

1-(2-chlorophenyl)-9-(4-(1-methyl-β-carboline-9-yl)butyl)-β-carboline (5i)

This compound was obtained as yellow crystals in 51% yield, m.p. 232.0–232.8 °C. ¹H NMR (400 MHz, CDCl₃) δ: 8.52 (d, *J* = 5.2 Hz, 1H), 8.34 (d, *J* = 5.2 Hz, 1H), 8.21–8.16 (m, 1H), 8.12–8.09 (m, 1H), 8.01 (d, *J* = 5.2 Hz, 1H), 7.85 (d, *J* = 5.2 Hz, 1H), 7.60–7.51 (m, 2H), 7.35–7.31 (m, 4H), 7.30–7.26 (m, 2H), 7.24–7.23 (m, 1H), 7.17–7.13 (m, 1H), 4.34–4.22 (m, 2H), 3.94–3.78 (m, 2H), 2.90 (s, 3H), 1.63–1.34 (m, 4H). ¹³C NMR (100 MHz, CDCl₃) δ: 141.57, 141.41, 140.87, 140.83, 138.62, 138.44, 137.97, 134.89, 134.02, 133.93, 131.34, 130.13, 130.05, 129.45, 129.30, 128.63, 128.27, 126.72, 121.81, 121.64, 121.31, 120.05, 119.86, 114.34, 113.08, 109.62, 109.58, 44.06, 43.78, 28.23, 26.73, 23.45. HRMS calcd for C₃₃H₂₈ClN₄ [M + H]⁺ 515.1997, found 515.2003. Anal. calcd for C₃₃H₂₇ClN₄: C, 76.96; H, 5.28; N, 10.88; found C 76.39, H 5.37, N 10.57.

1-(2-chlorophenyl)-9-(5-(1-methyl-β-carboline-9-yl)pentyl)-β-carboline (5j)

This compound was obtained as yellow crystals in 70% yield, m.p. 169.1–171.2 °C. ¹H NMR (400 MHz, CDCl₃) δ: 8.52 (d, *J* = 5.2 Hz, 1H), 8.36 (d, *J* = 5.2 Hz, 1H), 8.17–8.19 (m, 1H), 8.13–8.15 (m, 1H), 8.01 (d, *J* = 5.2 Hz, 1H), 7.87 (d, *J* = 5.2 Hz, 1H), 7.55–7.59 (m, 2H), 7.41 (dd, *J* = 7.6, 1.6 Hz, 1H), 7.36 (d, *J* = 8.4 Hz, 1H), 7.30–7.33 (m, 3H), 7.24–7.28 (m, 1H), 7.09–7.13 (m, 1H), 6.98–7.02 (m, 1H), 4.37 (t, *J* = 7.6 Hz, 2H), 3.76–3.93 (m, 2H), 2.97 (s, 3H), 1.35–1.56 (m, 4H), 0.88–1.02 (m, 2H). ¹³C NMR (100 MHz, CDCl₃) δ: 141.58, 141.41, 140.97, 140.86, 138.72, 138.27, 137.92, 134.99, 134.09, 133.94, 131.43, 130.06, 129.85, 129.30, 129.17, 128.54, 128.23, 126.56, 121.71, 121.59, 121.29, 121.22, 119.91, 119.82, 114.30, 113.04, 109.68, 109.65, 44.52, 43.80, 30.20, 28.98, 24.03, 23.50. HRMS calcd for C₃₄H₃₀ClN₄ [M + H]⁺ 529.2154, found 529.2161. Anal. calcd for C₃₄H₂₉ClN₄: C, 77.19; H, 5.53; N, 10.59; found C 76.74, H 5.32, N 10.82.

1-(4-methoxyphenyl)-9-(4-(1-(3-pyridyl)-β-carboline-9-yl)butyl)-β-carboline (5k)

This compound was obtained as yellow solid in 57% yield, m.p. 241.2–242.4 °C. ¹H NMR (400 MHz, CDCl₃) δ: 8.67 (d, *J* = 2.0 Hz, 1H), 8.62 (dd, *J* = 4.8, 1.6 Hz, 1H), 8.51 (d, *J* = 5.2 Hz, 1H), 8.47 (d, *J* = 5.2 Hz, 1H), 8.17 (m, 2H), 7.97 (d, *J* = 5.2 Hz, 1H), 7.91 (d, *J* = 5.2 Hz, 1H), 7.54–7.59 (m, 3H), 7.29–7.35 (m, 2H), 7.13–7.23 (m, 5H), 6.87 (s, 1H), 6.84 (s, 1H), 3.78 (s, 3H), 3.70–3.75 (m, 2H), 3.61–3.67 (m, 2H), 0.78–0.85 (m, 4H). ¹³C NMR (100 MHz, CDCl₃) δ: 159.68, 149.96, 149.45, 143.69, 142.07, 141.98, 140.22, 138.83, 138.42, 136.39, 135.75, 133.95, 133.70, 132.04, 130.74, 130.44, 130.40, 128.73, 128.40, 122.96, 121.75, 121.65, 121.34, 121.19, 120.20, 119.95, 114.16, 113.43, 113.32, 110.01, 109.95, 55.34, 43.73, 43.41, 25.96, 25.80. HRMS calcd for C₃₈H₃₂N₅O [M + H]⁺ 574.2601, found 574.2606. Anal. calcd for C₃₈H₃₁N₅O: C, 79.56; H, 5.45; N, 12.21; found C 79.51, H 5.66, N 11.72.

1-(3,4-dimethoxyphenyl)-9-(4-(1-(3-pyridyl)-β-carboline-9-yl)butyl)-β-carboline (5l)

This compound was obtained as yellow crystals in 63% yield, m.p. 239.8–240.5 °C. ¹H NMR (400 MHz, CDCl₃) δ: 8.68 (d, *J* = 2.0 Hz, 1H), 8.61 (dd, *J* = 4.8, 1.6 Hz, 1H), 8.51 (d, *J* = 5.2 Hz, 1H), 8.48 (d, *J* = 5.2 Hz, 1H), 8.18 (d, *J* = 7.6 Hz, 1H), 8.16 (d, *J* = 7.6 Hz, 1H), 7.97 (d, *J* = 5.2 Hz, 1H), 7.95 (d, *J* = 5.2 Hz, 1H), 7.55–7.60 (m, 3H), 7.31–7.35 (m, 2H), 7.13–7.20 (m, 3H), 6.96 (d, *J* = 2.0 Hz, 1H), 6.85 (d, *J* = 8.0 Hz, 1H), 6.79 (d, *J* = 8.0 Hz, 1H), 3.84 (s, 3H), 3.80 (s, 3H), 3.76 (t, *J* = 6.4 Hz, 2H), 3.66 (t, *J* = 6.4 Hz, 2H), 0.82–0.92 (m, 4H). ¹³C NMR (100 MHz, CDCl₃) δ: 149.95, 149.44, 149.20, 148.54, 143.62, 142.00, 141.87, 140.20, 138.83, 138.28, 136.36, 135.75, 133.98, 133.77, 132.17, 130.74, 130.46, 128.79, 128.49, 122.97, 121.73, 121.68, 121.34, 121.19, 120.23, 120.04, 114.19, 113.52, 112.45, 110.61, 110.09, 109.89, 55.99, 55.96, 43.82, 43.46, 26.05, 25.89. HRMS calcd for C₃₉H₃₄N₅O₂ [M + H]⁺ 604.2707, found 604.2705. Anal. calcd for C₃₉H₃₃N₅O₂: C, 77.59; H, 5.51; N, 11.60; found C 77.36, H 5.57, N 11.51.

1-(3,4,5-trimethoxyphenyl)-9-(4-(1-(3-pyridyl)-β-carboline-9-yl)butyl)-β-carboline (5m)

This compound was obtained as yellow crystals in 49% yield, m.p. 231.0–232.3 °C. ¹H NMR (400 MHz, CDCl₃) δ: 8.71 (dd, *J* = 2.0, 0.8 Hz, 1H), 8.60 (dd, *J* = 4.8, 1.6 Hz, 1H), 8.51 (d, *J* = 0.8 Hz, 1H), 8.49 (d, *J* = 0.8 Hz, 1H), 8.18 (d, *J* = 8.0 Hz, 1H), 8.14 (d, *J* = 8.0 Hz,

1H), 7.98 (d, $J=5.2$ Hz, 1H), 7.96 (d, $J=5.2$ Hz, 1H), 7.57–7.62 (m, 3H), 7.30–7.36 (m, 2H), 7.21 (d, $J=8.4$ Hz, 1H), 7.17 (d, $J=8.4$ Hz, 1H), 7.11–7.15 (m, 1H), 6.67 (s, 2H), 3.80 (s, 3H), 3.78 (s, 6H), 3.76 (t, $J=6.4$ Hz, 2H), 3.68 (t, $J=7.2$ Hz, 2H), 0.95–0.98 (m, 4H). ^{13}C NMR (100 MHz, CDCl_3) δ : 153.03, 149.94, 149.43, 143.58, 141.94, 141.68, 140.15, 138.78, 138.28, 138.24, 136.34, 135.79, 135.24, 133.98, 133.74, 130.72, 130.39, 128.97, 128.57, 122.98, 121.69, 121.67, 121.31, 121.11, 120.28, 120.11, 114.20, 113.79, 110.13, 109.86, 106.54, 60.93, 56.24, 43.91, 43.54, 26.09, 25.94. HRMS calcd for $\text{C}_{40}\text{H}_{36}\text{N}_5\text{O}_3$ $[\text{M} + \text{H}]^+$ 634.2813, found 634.2817. Anal. calcd for $\text{C}_{40}\text{H}_{35}\text{N}_5\text{O}_3$: C, 75.81; H, 5.57; N, 11.05; found C 75.79, H 5.56, N 11.32.

1-(2-chlorophenyl)-9-(4-(1-(3-pyridyl)- β -carboline-9-yl)butyl)- β -carboline (5n)

This compound was obtained as yellow crystals in 58% yield, m.p. 177.6–178.9 °C. ^1H NMR (400 MHz, CDCl_3) δ : 8.73 (dd, $J=2.4$, 0.8 Hz, 1H), 8.66 (dd, $J=4.8$, 1.6 Hz, 1H), 8.53 (d, $J=5.2$ Hz, 1H), 8.50 (d, $J=5.2$ Hz, 1H), 8.17–8.21 (m, 2H), 8.00 (d, $J=2.4$ Hz, 1H), 7.99 (d, $J=2.4$ Hz, 1H), 7.64 (d, $J=7.6$ Hz, 1H), 7.60–7.63 (m, 1H), 7.57–7.59 (m, 1H), 7.26–7.37 (m, 4H), 7.22–7.25 (m, 2H), 7.13–7.20 (m, 3H), 3.72 (t, $J=7.2$ Hz, 2H), 3.48–3.64 (m, 2H), 0.84–0.93 (m, 4H). ^{13}C NMR (100 MHz, CDCl_3) δ : 149.99, 149.55, 142.03, 141.51, 140.52, 140.19, 138.80, 138.34, 138.14, 136.48, 135.71, 134.02, 134.00, 133.71, 131.32, 130.86, 130.04, 129.92, 129.23, 128.76, 128.62, 126.60, 123.02, 121.81, 121.72, 121.24, 121.09, 120.31, 120.02, 114.31, 114.24, 109.97, 109.71, 43.72, 43.19, 26.30, 26.08. HRMS calcd for $\text{C}_{37}\text{H}_{29}\text{ClN}_5$ $[\text{M} + \text{H}]^+$ 578.2106, found 578.2111. Anal. calcd for $\text{C}_{37}\text{H}_{28}\text{ClN}_5$: C, 76.87; H, 4.88; N, 12.11; found C 76.38, H 4.85, N 11.75.

1-(4-methoxyphenyl)-9-(4-(1-(2-thienyl)- β -carboline-9-yl)butyl)- β -carboline (5o)

This compound was obtained as yellow crystals in 64% yield, m.p. 232.5–233.3 °C. ^1H NMR (400 MHz, CDCl_3) δ : 8.50 (d, $J=5.2$ Hz, 1H), 8.49 (d, $J=5.2$ Hz, 1H), 8.16 (t, $J=7.6$ Hz, 2H), 7.96 (d, $J=5.2$ Hz, 1H), 7.94 (d, $J=5.2$ Hz, 1H), 7.55–7.59 (m, 2H), 7.29–7.38 (m, 5H), 7.23 (d, $J=8.4$ Hz, 1H), 7.18 (d, $J=8.4$ Hz, 1H), 7.00–7.02 (m, 1H), 6.96–6.98 (m, 1H), 6.90–6.94 (m, 2H), 3.74–3.84 (m, 7H), 0.95–1.01 (m, 4H). ^{13}C NMR (100 MHz, CDCl_3) δ : 159.87, 142.11, 141.94, 140.82, 138.52, 136.77, 134.54, 133.87, 130.72, 130.55, 128.60, 128.52, 128.10, 126.92, 126.55, 121.77, 121.69, 121.38, 121.25, 120.10, 120.03, 114.18, 113.58, 113.48, 110.04, 109.93, 55.37, 43.60, 43.59, 26.18, 26.10. HRMS calcd for $\text{C}_{37}\text{H}_{31}\text{N}_4\text{O}_5$ $[\text{M} + \text{H}]^+$ 579.2213, found 579.2209. Anal. calcd for $\text{C}_{37}\text{H}_{30}\text{N}_4\text{O}_5$: C, 76.79; H, 5.23; N, 9.68; S, 5.54; found C, 76.10; H, 5.50; N, 9.29; S, 5.16.

1-(3,4-dimethoxyphenyl)-9-(4-(1-(2-thienyl)- β -carboline-9-yl)butyl)- β -carboline (5p)

This compound was obtained as yellow crystals in 49% yield, m.p. 217.2–218.4 °C. ^1H NMR (400 MHz, CDCl_3) δ : 8.51 (d, $J=5.2$ Hz, 1H), 8.47 (d, $J=5.2$ Hz, 1H), 8.18 (d, $J=7.6$ Hz, 1H), 8.14 (d, $J=7.6$ Hz, 1H), 7.96 (d, $J=5.2$ Hz, 1H), 7.94 (d, $J=5.2$ Hz, 1H), 7.55–7.59 (m, 2H), 7.22 (d, $J=8.4$ Hz, 1H), 7.19 (d, $J=8.4$ Hz, 1H), 7.03 (d, $J=2.0$ Hz, 1H), 6.98 (dd, $J=3.2$, 1.2 Hz, 1H), 6.96 (dd, $J=8.0$, 2.0 Hz, 1H), 6.92 (dd, $J=5.2$, 3.2 Hz, 1H), 6.85 (d, $J=8.4$ Hz, 1H), 3.86 (s, 3H), 3.82 (s, 3H), 3.78–3.85 (m, 4H), 0.98–1.08 (m, 4H). ^{13}C NMR (100 MHz, CDCl_3) δ : 149.24, 148.63, 143.72, 141.92, 141.90, 140.81, 138.53, 138.26, 136.75, 134.54, 133.98, 132.30, 130.69, 130.51, 128.65, 128.41, 128.04, 126.86, 126.50, 121.77, 121.73,

121.68, 121.46, 121.23, 120.10, 119.98, 114.20, 113.57, 112.47, 110.63, 110.11, 109.88, 55.99, 55.97, 43.66, 43.59, 26.29, 26.22. HRMS calcd for $\text{C}_{38}\text{H}_{33}\text{N}_4\text{O}_2\text{S}$ $[\text{M} + \text{H}]^+$ 609.2319, found 609.2314. Anal. calcd for $\text{C}_{38}\text{H}_{32}\text{N}_4\text{O}_2\text{S}$: C, 74.98; H, 5.30; N, 9.20; S, 5.27; found C, 74.47; H, 5.24; N, 9.36; S, 5.02.

1-(3,4,5-trimethoxyphenyl)-9-(4-(1-(2-thienyl)- β -carboline-9-yl)butyl)- β -carboline (5q)

This compound was obtained as yellow crystals in 65% yield, m.p. 226.5–227.8 °C. ^1H NMR (400 MHz, CDCl_3) δ : 8.50 (d, $J=5.2$ Hz, 1H), 8.46 (d, $J=5.2$ Hz, 1H), 8.19 (d, $J=7.6$ Hz, 1H), 8.12 (d, $J=7.6$ Hz, 1H), 7.99 (d, $J=5.2$ Hz, 1H), 7.92 (d, $J=5.2$ Hz, 1H), 7.56–7.60 (m, 2H), 7.28–7.36 (m, 2H), 7.17–7.24 (m, 3H), 6.96 (dd, $J=7.6$, 1.2 Hz, 1H), 6.88 (dd, $J=5.2$, 3.6 Hz, 1H), 6.71 (s, 2H), 3.80–3.87 (m, 4H), 3.82 (s, 3H), 3.79 (s, 6H), 1.03–1.12 (m, 4H). ^{13}C NMR (100 MHz, CDCl_3) δ : 153.07, 143.44, 141.91, 141.87, 140.71, 138.45, 138.40, 137.94, 136.61, 134.50, 133.86, 130.70, 130.66, 128.83, 128.62, 128.05, 126.82, 126.48, 121.80, 121.64, 121.39, 121.15, 120.17, 120.15, 114.22, 113.88, 110.19, 109.84, 106.65, 60.95, 56.24, 43.68, 26.36, 26.27. HRMS calcd for $\text{C}_{39}\text{H}_{35}\text{N}_4\text{O}_3\text{S}$ $[\text{M} + \text{H}]^+$ 639.2424, found 639.2422. Anal. calcd for $\text{C}_{39}\text{H}_{34}\text{N}_4\text{O}_3\text{S}$: C, 73.33; H, 5.37; N, 8.77; S, 5.02; found C, 73.37; H, 5.59; N, 8.65; S, 4.81.

1-(2-chlorophenyl)-9-(4-(1-(2-thienyl)- β -carboline-9-yl)butyl)- β -carboline (5r)

This compound was obtained as yellow crystals in 52% yield, m.p. 225.4–227.2 °C. ^1H NMR (400 MHz, CDCl_3) δ : 8.52 (s, 1H), 8.50 (s, 1H), 8.16–8.20 (m, 2H), 8.01 (d, $J=5.2$ Hz, 1H), 7.97 (d, $J=5.2$ Hz, 1H), 7.56–7.62 (m, 2H), 7.27–7.38 (m, 7H), 7.19–7.25 (m, 2H), 7.06 (dd, $J=3.6$, 1.2 Hz, 1H), 7.03 (dd, $J=5.2$, 3.2 Hz, 1H), 3.88 (t, $J=7.2$ Hz, 2H), 3.53–3.71 (m, 2H), 1.01–1.13 (m, 4H). ^{13}C NMR (100 MHz, CDCl_3) δ : 141.97, 141.52, 140.78, 140.60, 138.51, 138.41, 138.11, 136.77, 134.53, 134.06, 133.87, 131.34, 130.86, 130.07, 130.05, 129.33, 128.62, 128.54, 128.25, 127.03, 126.66, 126.63, 121.75, 121.29, 121.18, 120.19, 119.96, 114.33, 114.22, 109.97, 109.68, 43.59, 43.29, 26.42, 26.40. HRMS calcd for $\text{C}_{36}\text{H}_{28}\text{ClN}_4\text{S}$ $[\text{M} + \text{H}]^+$ 583.1718, found 583.1720. Anal. calcd for $\text{C}_{36}\text{H}_{27}\text{ClN}_4\text{S}$: C, 74.15; H, 4.67; N, 9.61; S, 5.50; found C, 73.73; H, 4.53; N, 9.63; S, 5.19.

7-methoxy-1-methyl-9-(4-(1-methyl- β -carboline-9-yl)butyl)- β -carboline (5s)

This compound was obtained as colorless crystals in 74% yield, m.p. 188.7–189.4 °C. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ : 8.23 (s, 1H), 8.21 (s, 1H), 8.16 (d, $J=5.2$ Hz, 1H), 8.08 (d, $J=8.8$ Hz, 1H), 7.99 (d, $J=5.2$ Hz, 1H), 7.87 (d, $J=5.2$ Hz, 1H), 7.70 (d, $J=8.8$ Hz, 1H), 7.54–7.58 (m, 1H), 7.22–7.26 (m, 1H), 7.20 (d, $J=2.0$ Hz, 1H), 6.86 (dd, $J=8.8$, 2.0 Hz, 1H), 4.58–4.62 (m, 4H), 3.87 (s, 3H), 2.94 (s, 3H), 2.89 (s, 3H), 1.82–1.86 (m, 4H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ : 160.38, 142.59, 141.14, 140.96, 140.39, 137.69, 137.49, 134.37, 134.23, 128.33, 127.97, 127.94, 122.27, 121.36, 120.36, 119.36, 114.09, 112.83, 112.12, 110.27, 108.91, 93.74, 55.48, 43.67, 43.57, 27.55, 27.37, 22.98, 22.86. HRMS calcd for $\text{C}_{29}\text{H}_{29}\text{N}_4\text{O}$ $[\text{M} + \text{H}]^+$ 449.2336, found 449.2340. Anal. calcd for $\text{C}_{29}\text{H}_{28}\text{N}_4\text{O}$: C, 77.65; H, 6.29; N, 12.49; found C 77.39, H 5.97, N 12.38.

7-methoxy-1-methyl-9-(5-(1-methyl- β -carboline-9-yl)pentyl)- β -carboline (5t)

This compound was obtained as colorless crystals in 67% yield, m.p. 176.8–177.6 °C. ^1H NMR (400 MHz, CDCl_3) δ : 8.33 (d, $J=5.2$ Hz, 1H), 8.29 (d, $J=5.2$ Hz, 1H), 8.11 (d, $J=7.6$ Hz, 1H), 7.97 (d, $J=8.4$ Hz, 1H), 7.84 (d, $J=5.2$ Hz, 1H), 7.74 (d, $J=5.2$ Hz, 1H), 7.52–7.56 (m, 1H), 7.36 (d, $J=8.4$ Hz, 1H), 7.25–7.29 (m, 1H), 6.89 (dd, $J=8.4, 2.0$ Hz, 1H), 6.77 (d, $J=2.0$ Hz, 1H), 4.49 (t, $J=7.2$ Hz, 2H), 4.42 (t, $J=7.2$ Hz, 2H), 3.89 (s, 3H), 2.99 (s, 3H), 2.97 (s, 3H), 1.81–1.91 (m, 4H), 1.41–1.50 (m, 2H). ^{13}C NMR (100 MHz, CDCl_3) δ : 160.92, 143.09, 141.45, 141.04, 140.26, 138.15, 138.09, 135.17, 135.01, 129.58, 129.14, 128.21, 122.48, 121.53, 121.29, 119.73, 115.21, 112.97, 112.31, 109.56, 108.60, 93.51, 55.72, 44.55, 30.83, 30.56, 24.42, 23.58, 23.33. HRMS calcd for $\text{C}_{30}\text{H}_{31}\text{N}_4\text{O}$ $[\text{M}+\text{H}]^+$ 463.2492, found 463.2497. Anal. calcd for $\text{C}_{30}\text{H}_{30}\text{N}_4\text{O}$: C, 77.89; H, 6.54; N, 12.11; found C, 77.42; H, 6.33; N, 12.07.

7-methoxy-1-methyl-9-(4-(1-(3-pyridyl)- β -carboline-9-yl)butyl)- β -carboline (5u)

This compound was obtained as colorless crystals in 70% yield, m.p. 163.8–164.5 °C. ^1H NMR (400 MHz, CDCl_3) δ : 8.79 (d, $J=2.0$ Hz, 1H), 8.65 (dd, $J=4.8, 1.6$ Hz, 1H), 8.51 (d, $J=5.2$ Hz, 1H), 8.29 (d, $J=5.2$ Hz, 1H), 8.15–8.17 (m, 1H), 7.94–7.96 (m, 2H), 7.71 (d, $J=5.2$ Hz, 1H), 7.65–7.68 (m, 1H), 7.56–7.60 (m, 1H), 7.30–7.34 (m, 2H), 7.23–7.25 (m, 1H), 6.89 (dd, $J=8.4, 2.0$ Hz, 1H), 6.60 (d, $J=2.0$ Hz, 1H), 4.16 (t, $J=7.2$ Hz, 2H), 3.95 (t, $J=7.2$ Hz, 2H), 3.90 (s, 3H), 2.77 (s, 3H), 1.48–1.35 (m, 2H), 1.35–1.27 (m, 2H). ^{13}C NMR (100 MHz, CDCl_3) δ : 160.85, 150.02, 149.66, 142.77, 142.20, 140.30, 140.13, 139.02, 138.36, 136.42, 135.80, 135.01, 134.09, 130.92, 129.43, 128.81, 123.15, 122.45, 121.79, 121.30, 120.33, 115.16, 114.17, 112.29, 109.94, 108.54, 93.55, 55.73, 44.12, 43.84, 27.37, 26.02, 23.30. HRMS calcd for $\text{C}_{33}\text{H}_{30}\text{N}_5\text{O}$ $[\text{M}+\text{H}]^+$ 512.2445, found 512.2453. Anal. calcd for $\text{C}_{33}\text{H}_{29}\text{N}_5\text{O}$: C, 77.47; H, 5.71; N, 13.69; found C, 76.67; H, 5.75; N, 13.05.

7-methoxy-1-methyl-9-(5-(1-(3-pyridyl)- β -carboline-9-yl)pentyl)- β -carboline (5v)

This compound was obtained as colorless crystals in 62% yield, m.p. 183.6–184.9 °C. ^1H NMR (400 MHz, CDCl_3) δ : 8.82 (dd, $J=2.0, 0.8$ Hz, 1H), 8.54 (d, $J=5.2$ Hz, 1H), 8.53 (dd, $J=5.2, 1.6$ Hz, 1H), 8.30 (d, $J=5.2$ Hz, 1H), 8.18 (d, $J=7.6$ Hz, 1H), 8.02 (d, $J=5.2$ Hz, 1H), 7.98 (d, $J=8.4$ Hz, 1H), 7.85–7.88 (m, 1H), 7.75 (d, $J=5.2$ Hz, 1H), 7.56–7.60 (m, 1H), 7.37 (d, $J=8.4$ Hz, 1H), 7.30–7.34 (m, 1H), 7.24–7.28 (m, 1H), 6.90 (dd, $J=8.4, 2.0$ Hz, 1H), 6.72 (d, $J=2.0$ Hz, 1H), 4.27 (t, $J=7.6$ Hz, 2H), 3.94 (t, $J=7.6$ Hz, 2H), 3.91 (s, 3H), 2.90 (s, 3H), 1.45–1.53 (m, 2H), 1.33–1.42 (m, 2H), 0.87–0.90 (m, 2H). ^{13}C NMR (100 MHz, CDCl_3) δ : 160.95, 150.08, 149.51, 143.06, 142.01, 140.36, 140.19, 138.81, 138.05, 136.60, 135.99, 135.11, 134.25, 130.83, 129.58, 128.81, 123.05, 122.48, 121.74, 121.26, 120.26, 115.15, 114.25, 112.34, 110.00, 108.66, 93.56, 55.76, 44.43, 44.25, 30.09, 28.61, 23.82, 23.22. HRMS calcd for $\text{C}_{34}\text{H}_{32}\text{N}_5\text{O}$ $[\text{M}+\text{H}]^+$ 526.2601, found 526.2609. Anal. calcd for $\text{C}_{34}\text{H}_{31}\text{N}_5\text{O}$: C, 77.69; H, 5.94; N, 13.32; found C, 77.76; H, 5.95; N, 13.74.

7-methoxy-1-methyl-9-(4-(1-(2-thienyl)- β -carboline-9-yl)butyl)- β -carboline (5w)

This compound was obtained as colorless crystals in 68% yield, m.p. 189.3–190.1 °C. ^1H NMR (400 MHz, CDCl_3) δ : 8.50 (d, $J=5.2$ Hz, 1H), 8.28 (d, $J=5.2$ Hz, 1H), 8.14–8.16 (m, 1H), 7.96 (d, $J=1.6$ Hz, 1H), 7.95 (d, $J=2.0$ Hz, 1H), 7.72 (d, $J=5.2$ Hz, 1H),

7.55–7.60 (m, 1H), 7.34–7.38 (m, 2H), 7.29–7.33 (m, 1H), 7.13 (dd, $J=3.6, 1.2$ Hz, 1H), 7.02 (dd, $J=5.2, 3.6$ Hz, 1H), 6.88 (dd, $J=8.4, 2.0$ Hz, 1H), 6.66 (d, $J=2.0$ Hz, 1H), 4.21 (t, $J=7.2$ Hz, 2H), 4.17–4.08 (t, $J=7.2$ Hz, 2H), 3.90 (s, 3H), 2.86 (s, 3H), 1.42–1.54 (m, 4H). ^{13}C NMR (100 MHz, CDCl_3) δ : 160.86, 142.92, 142.05, 140.94, 140.18, 138.72, 138.18, 136.91, 135.10, 134.60, 130.91, 129.54, 128.69, 128.26, 127.12, 126.70, 122.49, 121.75, 121.35, 120.22, 115.24, 114.21, 112.33, 109.94, 108.45, 93.68, 55.75, 43.94, 29.69, 27.70, 26.51, 23.28. HRMS calcd for $\text{C}_{32}\text{H}_{29}\text{N}_4\text{OS}$ $[\text{M}+\text{H}]^+$ 517.2057, found 517.2063. Anal. calcd for $\text{C}_{32}\text{H}_{28}\text{N}_4\text{OS}$: C, 74.39; H, 5.46; N, 10.84; S, 6.21; found C, 74.21; H, 5.42; N, 10.65; S, 6.17.

7-methoxy-1-methyl-9-(5-(1-(2-thienyl)- β -carboline-9-yl)pentyl)- β -carboline (5x)

This compound was obtained as yellow crystals in 71% yield, m.p. 196.1–197.4 °C. ^1H NMR (400 MHz, CDCl_3) δ : 8.50 (d, $J=5.2$ Hz, 1H), 8.30 (d, $J=5.2$ Hz, 1H), 8.13–8.16 (m, 1H), 7.94–7.98 (m, 2H), 7.74 (d, $J=5.2$ Hz, 1H), 7.55–7.59 (m, 1H), 7.39 (d, $J=8.4$ Hz, 1H), 7.30 (m, 1H), 7.23 (dd, $J=5.2, 1.2$ Hz, 1H), 7.16 (dd, $J=3.6, 1.2$ Hz, 1H), 6.92 (dd, $J=5.2, 3.6$ Hz, 1H), 6.89 (dd, $J=8.4, 2.0$ Hz, 1H), 6.72 (d, $J=2.0$ Hz, 1H), 4.28 (t, $J=7.6$ Hz, 2H), 4.10 (t, $J=7.6$ Hz, 2H), 3.90 (s, 3H), 2.92 (s, 3H), 1.50–1.58 (m, 2H), 1.40–1.48 (m, 2H), 0.96–1.04 (m, 2H). ^{13}C NMR (100 MHz, CDCl_3) δ : 160.86, 143.01, 142.10, 141.02, 140.33, 138.56, 138.18, 136.98, 135.20, 134.67, 130.84, 129.48, 128.66, 128.33, 126.89, 126.56, 122.44, 121.67, 121.28, 120.12, 115.23, 114.18, 112.32, 110.04, 108.52, 93.62, 55.73, 44.54, 44.09, 29.72, 28.94, 23.97, 23.38. HRMS calcd for $\text{C}_{33}\text{H}_{31}\text{N}_4\text{OS}$ $[\text{M}+\text{H}]^+$ 531.2213, found 531.2213. Anal. calcd for $\text{C}_{33}\text{H}_{30}\text{N}_4\text{OS}$: C, 74.69; H, 5.70; N, 10.56; S, 6.04; found C, 74.06; H, 6.08; N, 10.49; S, 6.44.

Biological evaluation**In vitro cell growth inhibition assay**

Target compounds were assayed by the MTT method for cytotoxic activity, as described previously⁵. The panel of cell lines included gastric carcinoma (BGC-823), liver carcinoma (HepG2), breast carcinoma (MCF-7), colon carcinoma (HT-29), esophageal carcinoma (Eca-109), and Lewis lung carcinoma (LLC). Growth inhibition rates were calculated with the following equation: Inhibition ratio (%) = $\frac{\text{OD}_{\text{compd}} - \text{OD}_{\text{blank}}}{\text{OD}_{\text{DMSO}} - \text{OD}_{\text{blank}}} \times 100\%$. Half maximal inhibitory concentration (IC_{50}) of each compound was calculated using software Graph-Pad Prism (version 6.0).

Assay of acute toxicities

Specific pathogen-free KM mice (6–8 weeks old) weighing 19–22 g were housed in a mouse room at 24 ± 2 °C and 60–70% humidity with 12 h light/dark cycles. The mice were provided rodent laboratory chow pellets and tap water for a week to adapt to the environment of the mouse room. The experimental protocol was approved by the Institutional Animal Ethical Committee, and all of the animals were provided by Laboratory Animal Center of Xinjiang Uygur Autonomous Region. Prior to each experiment, mice were fastened overnight and allowed free access to water. Various doses of the asymmetric dimeric β -carboline derivatives, ranging from 5.0 to 500 mg kg^{-1} dissolved in 0.5% carboxymethyl cellulose sodium (CMC-Na) salt solution, were given intraperitoneally to different groups of healthy KM mice, and each group contained 10 mice (5 males and 5 females). After the administration of the compounds, the mice were observed continuously for

the first 2 h for any gross behavioral changes and deaths, then intermittently for the next 24 h, and occasionally thereafter for 14 days, and for the onset of any delayed effects. All of the animals were killed on the 14th day after drug administration, and they were checked macroscopically for possible damage to the heart, liver, and kidneys. Mice that experienced immediate death following drug administration were also examined for any possible organ damage. LD₅₀ values were calculated graphically as described³⁴.

In vivo antitumor activity

Sarcoma180 and Lewis lung cancer cell lines were provided by Shanghai Institute of Pharmaceutical Industry. Mice were inoculated with Sarcoma180 and Lewis lung cancer tumour cells. After 7 days, the tumours were removed and the cells were harvested. Mice received subcutaneous injections of viable tumour cells (2×10^6 cells/mouse) in the armpit. Each compound was administered via i.p. injection to different groups of mice (each group contained 10 female mice) 24 h after the inoculation at a dosage about one-fifth of the LD₅₀ value once a day for seven consecutive days. This dose was the maximum tolerated dose for most of the compounds based on our preliminary studies. CTX at 30 mg kg⁻¹ was used as the positive control and the vehicle as the negative control. The weight of the animals was recorded every three days. All of the animals were killed on the 21st day after tumour inoculation, and the tumours were excised and weighed. The inhibition rate was calculated as follows:

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

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

CAM assay in vivo

Anti-angiogenic activity of the selected compounds **5b** and **5w** were investigated *in vivo* using a CAM assay. Five-day-old fertilized

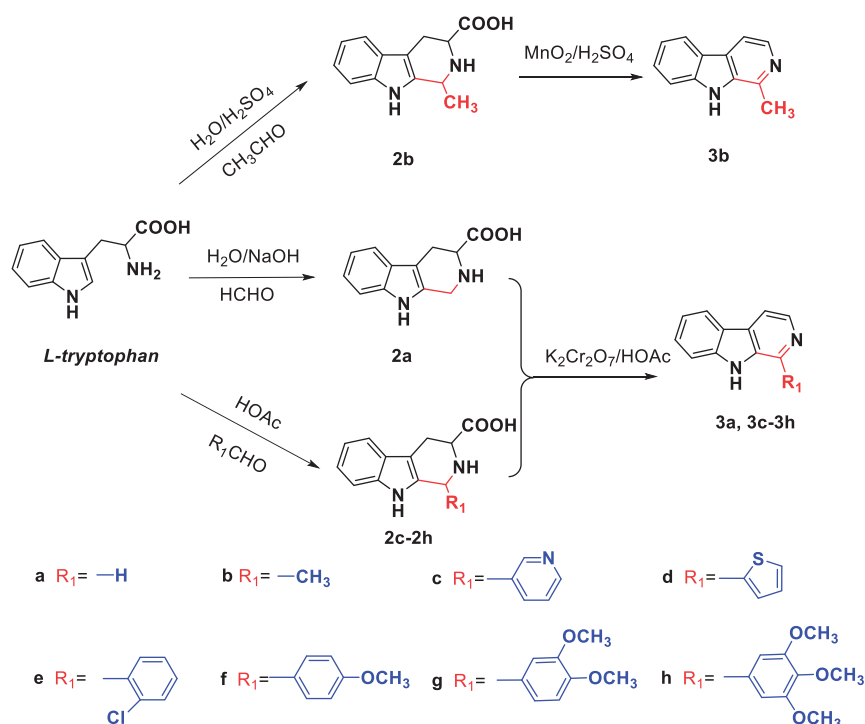
eggs were obtained from a local hatchery. We injected 5 ml of albumin, and the eggs were incubated horizontally to allow the CAM to detach from the shell to produce a sham chamber. Compounds **5b** and **5w** were prepared in gelatin sponge discs ($5 \times 5 \times 5$ mm³) at the concentration of 0.5, 5.0, and 50 μM/disc, respectively. CA4P was used as the positive control drug. Discs containing the vehicle only (DMSO) were used as the negative control. A small window opening was made in the shell, and the discs were directly applied onto the CAM. The square opening was covered with sterilized surgical tape and the embryos were incubated for 48 h at 38.5 °C. The CAMs were photographed under a dissecting microscope and blood vessels in each CAM were counted. The results are presented as a mean percentage of inhibition to the control ± SD, (*n* = 3).

Results and discussion

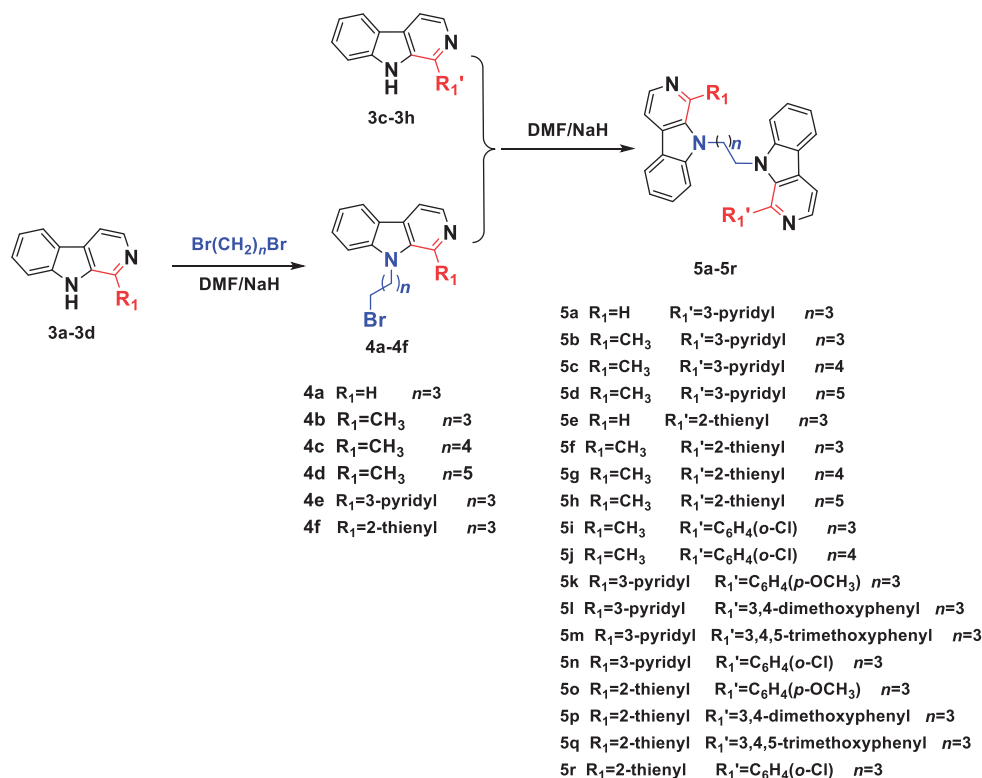
Chemistry

The synthesis of the desired *N*⁹-heterobivalent β-carbolines (**5a-r**) were performed in four steps starting from L-tryptophan as outlined in Scheme 1–2. The tetrahydro-β-carbolines **2a-h** was prepared by condensation of L-tryptophan with appropriate aldehyde via the Pictet-Spengler condensation, and followed by oxidation and decarboxylation to afford the intermediate 1-substituted-β-carbolines **3a-h**^{21,29}. Then the *N*⁹ of **3a-d** was alkylated by the action of sodium hydride (NaH) in *N,N*-dimethylformamide (DMF) followed by the addition of the appropriate dibromo alkane to obtain intermediates **4a-f**. Finally, **4a-f** with appropriate 1-substituted-β-carbolines **3c-h** in the presence of sodium hydride in DMF at room temperature to afford the target compounds **5a-r** in 47–74% yield.

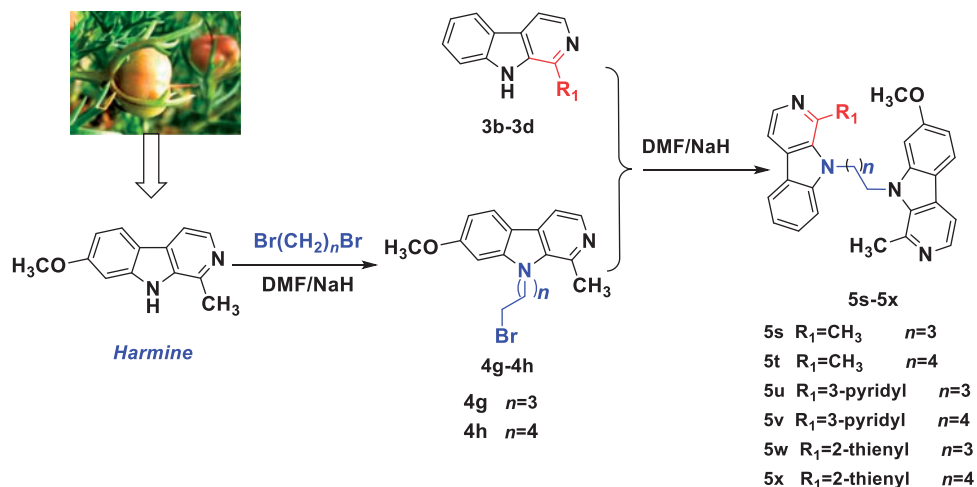
In Scheme 3, the intermediates **4g-f** were synthesized from harmine and 1,4-dibromobutane or 1,5-dibromopentane using the same method of compound **4a**, and finally compounds **5s-x** were obtained from the similar method of **5a**.



Scheme 1. Synthetic route to compounds **3a-h**.



Scheme 2. Synthesis of the N^9 -heterobivalent β -carbolines 5a-r.



Scheme 3. Synthesis of the N^9 -heterobivalent β -carbolines 5s-x.

The chemical structures of all the novel N^9 -heterobivalent β -carbolines were characterized by ^1H NMR, ^{13}C NMR, HRMS and the elemental analysis.

In vitro cytotoxicity and structure-activity relationships

All the 24 novel synthesized N^9 -heterobivalent β -carbolines (5a-x) were screened for their *in vitro* cytotoxic activities against six different cancer cell lines, namely BGC-823 (gastric carcinoma), HepG2 (liver carcinoma), MCF-7 (breast carcinoma), HT-29 (colon carcinoma), Eca-109 (esophageal carcinoma) and LLC (Lewis lung carcinoma). Cisplatin and B-9-3 were used as the reference control and the results were expressed as IC_{50} values and summarized in Table 1. The IC_{50} values were the average of at least three independent experiments.

As illustrated in Table 1, compounds 5b and 5w displayed a broad spectrum of cytotoxic activities with IC_{50} value of lower than $20\ \mu\text{M}$ against the tested six tumor cell lines, while compounds 5c, 5s, 5t and 5v only exhibited strong cytotoxic effects with IC_{50} value of lower than $20\ \mu\text{M}$ against three or four tumour cell lines. Interestingly, compounds 5d and 5j were selectively active against Eca-109 and LLC cell lines with IC_{50} value of lower than $20\ \mu\text{M}$ but fail to show cytotoxic effects in other cell lines. Similarly, compound 5u displayed selective activities against HT-29 and LLC cell lines. Moreover, compound 5f only exhibited strong cytotoxic effects with IC_{50} value of lower than $20\ \mu\text{M}$ against LLC cell lines. compounds 5g and 5i showed weak cytotoxic activities with IC_{50} values in the range of $18.8\text{--}97.6\ \mu\text{M}$. Unfortunately, compounds 5k-5r were weak or inactive against all tumour cell lines tested.

Table 1. Cytotoxic activity of N^9 -heterobivalent β -carbolines *in vitro*

Compd.	R ₁	R ₁ '	R ₇ '	N	IC ₅₀ (μM) ±SD ^a					
					BGC ^b	HepG2	MCF7	HT-29	Eca-109	LLC
5a		H	H	3	>100	39.1 ± 5.4	>100	>100	>100	>100
5b		CH ₃	H	3	12.1 ± 2.3	15.9 ± 1.4	8.4 ± 1.7	12.6 ± 1.2	10.5 ± 1.3	12.4 ± 2.1
5c		CH ₃	H	4	22.6 ± 1.9	10.5 ± 2.1	15.3 ± 2.6	13.3 ± 2.4	25.3 ± 3.2	5.7 ± 1.2
5d		CH ₃	H	5	>100	>100	>100	>100	18.9 ± 3.1	14.5 ± 0.9
5e		H	H	3	>100	>100	>100	18.1 ± 3.6	>100	32.3 ± 4.5
5f		CH ₃	H	3	38.8 ± 2.9	>100	46.6 ± 4.8	56.1 ± 4.2	64.1 ± 7.6	6.1 ± 0.3
5g		CH ₃	H	4	97.6 ± 12.4	18.8 ± 2.3	75.5 ± 10.7	32.3 ± 5.3	30.8 ± 3.2	22.7 ± 1.4
5h		CH ₃	H	5	>100	>100	75.9 ± 9.4	47.8 ± 6.5	30.2 ± 3.4	30.2 ± 1.5
5i		CH ₃	H	3	37.9 ± 4.2	23.9 ± 3.7	95.5 ± 14.8	28.3 ± 1.6	23.4 ± 4.1	44.7 ± 3.6
5j		CH ₃	H	4	>100	>100	46.8 ± 3.7	24.1 ± 2.2	18.6 ± 2.1	16.6 ± 3.1
5k			H	3	>100	>100	>100	>100	>100	>100
5l			H	3	>100	79.5 ± 12.3	>100	91.1 ± 10.6	93.2 ± 7.1	46.8 ± 6.4
5m			H	3	>100	85.1 ± 11.6	>100	>100	70.9 ± 8.4	>100
5n			H	3	>100	>100	86.9 ± 15.3	72.2 ± 5.2	>100	>100
5o			H	3	>100	>100	>100	>100	>100	96.8 ± 9.8
5p			H	3	>100	>100	>100	>100	>100	>100
5q			H	3	>100	>100	>100	>100	>100	>100
5r			H	3	>100	>100	60.7 ± 4.8	96.2 ± 5.1	96.7 ± 11.9	>100
5s	CH ₃	CH ₃	OCH ₃	3	17.9 ± 1.1	37.6 ± 5.8	36.4 ± 2.1	11.1 ± 0.8	11.3 ± 2.7	6.2 ± 0.6
5t	CH ₃	CH ₃	OCH ₃	4	42.7 ± 2.9	31.6 ± 2.2	17.6 ± 3.4	4.5 ± 0.6	51.9 ± 7.4	3.1 ± 0.4
5u		CH ₃	OCH ₃	3	39.1 ± 2.4	26.8 ± 2.8	23.9 ± 0.7	15.1 ± 3.1	25.2 ± 5.5	12.2 ± 2.3
5v		CH ₃	OCH ₃	4	23.2 ± 1.5	31.1 ± 3.4	18.7 ± 0.5	13.5 ± 2.2	13.2 ± 3.6	11.8 ± 1.8
5w		CH ₃	OCH ₃	3	16.5 ± 1.9	15.4 ± 2.7	14.1 ± 1.4	13.1 ± 3.5	15.4 ± 1.9	13.1 ± 2.4
5x		CH ₃	OCH ₃	4	>100	>100	41.6 ± 5.3	>100	46.7 ± 6.9	12.2 ± 3.7
B-9-3	CH ₃	CH ₃	H	3	22.3 ± 2.9	>100	13.2 ± 1.5	40.6 ± 5.8	14.5 ± 2.2	6.1 ± 0.9
Cisplatin					11.6 ± 0.7	14.8 ± 0.4	12.4 ± 0.7	26.8 ± 1.4	8.9 ± 0.6	7.6 ± 0.4

^aCytotoxicity as IC₅₀ for each cell line, is the concentration of compound which reduced by 50% the optical density of treated cells with respect to untreated cells using the MTT assay. The data represent the mean values ±SD of at least three independent determinations. Values >100 μM indicate less than 50% growth inhibition at >100 μM.

^bCell lines include gastric carcinoma (BGC), liver carcinoma (HepG2), breast carcinoma (MCF-7), colon carcinoma (HT-29), esophageal carcinoma (Eca-109), and Lewis lung carcinoma (LLC).

When N^9 -heterobivalent β -carbolines had the same linker, we examined the influence of the substituents in position-1 of the β -carboline core on the cytotoxic activities. In order to enhance the range of substituents, we designed compounds have methyl and different pattern of substitution with an aryl ring substituted by electron withdrawing (Cl) and donating (OCH₃) groups in position-1 of β -carboline. For example, compound **5a**, **5b**, and **5k–5n**, all have a 3-pyridyl group in R₁ of one β -carboline ring, while in another β -carboline core, unlike compound **5a**, the substituted group of the position-1' were methyl (**5b**), 4-methoxyphenyl (**5k**), 3,4-dimethoxyphenyl (**5l**), 3,4,5-trimethoxyphenyl (**5m**), 2-chlorophenyl (**5n**), respectively. Of these six dimeric β -carbolines, the compound **5b** displayed higher cytotoxic activities against BGC-823, HepG2, MCF-7, HT-29, Eca-109 and LLC with IC₅₀ values of 12.1, 15.9, 8.4, 12.6, 10.5, and 12.4 μ M, respectively. Additionally, among these six N^9 -heterobivalent β -carbolines **5e**, **5f**, and **5o–5r** bearing a 2-thienyl group in R₁ of one β -carboline core, compound **5f** (R₁'=methyl) showed the highest cytotoxic activities against the test cell lines except HepG2. These results suggested that 3-pyridyl or 2-thienyl group substituent into position-1 of the β -carboline core, and in another β -carboline ring, the methyl substituent into R₁' facilitated cytotoxic potency, and the aryl substituent into R₁' might be detrimental to cytotoxic effects.

Next, we examined the influence of the spacer length of N^9 -heterobivalent β -carbolines on cytotoxic activities. According the previous investigation, the synthesized N^9 -heterobivalent β -carbolines were connected at the indolo-N by an alkyl chain, which ranged from 4 to 6 carbon atoms. Comparing the structure of **5b–5d**, which bearing different carbon atoms linker and 3-pyridyl group in position-1 of the β -carboline core, compound **5b** bearing 4-carbon atoms showed a broad spectrum of cytotoxic activities with IC₅₀ value of lower than 20 μ M against six tumor cell lines, and all the three compounds show selective cytotoxicities with IC₅₀ value of lower than 20 μ M against LLC. Similarly, further introduction of 2-thienyl group in position-1 of the β -carboline ring resulted in dimeric derivatives **5f–5h**. Among these derivatives, the IC₅₀ values of compound **5f** against BGC, MCF7, and LLC cells is 38.8, 46.6, and 6.1 μ M, respectively, compound **5g** displayed IC₅₀ values for HepG2 and HT-29 of 18.8 and 32.3 μ M, respectively, and compound **5h** only exhibited higher cytotoxic effects with IC₅₀ value of 30.2 μ M against Eca-109 than other two compounds. Comparing the structure of **5i** with **5j**, compound **5i** showed weak or moderate cytotoxic activities against all tumor cell lines tested, and compound **5j** was selectively active against Eca-109 and LLC cell lines with IC₅₀ value of lower than 20 μ M but fail to show cytotoxic effects in BGC and HepG2 at the concentration of 100 μ M. Comparing the structure of **5u** with **5v**, there is an extra methoxyl group attached to position-7 of the β -carboline, compound **5v** demonstrated the higher cytotoxic activities against the

tested tumor cell lines than **5u** (except for HepG-2 cell line). Similarly, comparing the structure **5w** with **5x**, compound **5w** displayed the higher cytotoxic activities against the tested tumor cell lines than **5x** (except for LLC cell line). These results suggested that the length of the spacer had no obvious relationship with the cytotoxic activities against the tumor cell lines.

An overview of the cytotoxic activities data of all new synthesized N^9 -heterobivalent β -carbolines. 11 compounds were found to exhibit selective activity against LLC cell lines with IC₅₀ value of lower than 20 μ M. Moreover, Compound **5b**, **5s** displayed higher cytotoxic activities against at least four tumour cell lines than the prototype B-9-3, and compound **5c**, **5t**, **5v**, **5w** exhibited higher cytotoxic effects against three tumour cell lines than B-9-3.

In summary, a total analysis of the cytotoxic activities of N^9 -heterobivalent β -carbolines *in vitro* clearly suggest that: (1) C¹-methylation and C⁷-methoxylation were favorable for increased activities; (2) 3-Pyridyl or 2-thienyl group substituent into position-1 of the β -carboline core, and the aryl (electron withdrawing and donating groups) substituent into position-1' of another β -carboline ring might be detrimental to cytotoxic effects of this class of compounds.

Assessment of acute toxicity

The LD₅₀ values of the selected N^9 -heterobivalent β -carbolines in mice after intraperitoneal (i.p.) administration are shown in Table 2. All of the tested dimeric β -carbolines resulted in acute toxic manifestation but they did not cause any obvious neurotoxic effects, including tremors, twitch, jumping, and supination. The animals showed a decrease in locomotive activity after the administration of various bivalent β -carbolines. Death occurred mostly in the high dosage group within 4–8 h after injection. All of the surviving animals returned to normal within the next day. Autopsies of the animals that died during the course of experiment and the necropsy findings in the surviving animals at the end of the experimental period (14 days) revealed no obvious changes in any of the organs.

Of all of the investigated asymmetric dimeric β -carbolines, compounds **5b** and **5c**, which had no substituent in position-7, and bearing different carbon atoms linker and 3-pyridyl group in position-1 of the β -carboline core, demonstrated weaker acute toxicities with the LD₅₀ values of 150 mg kg⁻¹ and 175 mg kg⁻¹, respectively. Compounds **5t** and **5w**, which have a harmine molecular linked with another 1-substituted β -carboline ring, displayed remarkable acute toxicity with the LD₅₀ values of 35 mg kg⁻¹ and 50 mg kg⁻¹, respectively. These results suggested that the methoxy substituent in position-7 of β -carboline nucleus played a vital role in determining the remarkable neurotoxic effects.

Table 2. Acute toxic effects of N^9 -heterobivalent β -carbolines in mice and antitumor activities of these compounds against mice bearing Sarcoma 180 and Lewis lung cancer

Comp.	Acute toxicity		Dosage (mg kg ⁻¹)	Tumor inhibition rate (%) ^a	
	LD ₅₀ (mg kg ⁻¹)	Neurotoxic effect		Sarcoma 180	Lewis lung cancer
5b	150	– ^b	30	43.6 ± 8.9	41.9 ± 5.3
5c	175	–	35	33.6 ± 9.2	ND ^c
5t	35	+	7	37.2 ± 4.4	ND
5w	50	+	10	47.1 ± 6.2	42.3 ± 5.9
B-9-3 ²⁰	200	–	40	56.2	40.4
CTX			30	82.5 ± 3.4	80.7 ± 2.1

^aData are expressed as mean ± SD.

^bAcute neurotoxic manifestation were denoted by “+” and “–”. “+” represents toxic responses including tremble, twitch, jumping and supination, while “–” means no such reaction.

^cND = not determined.

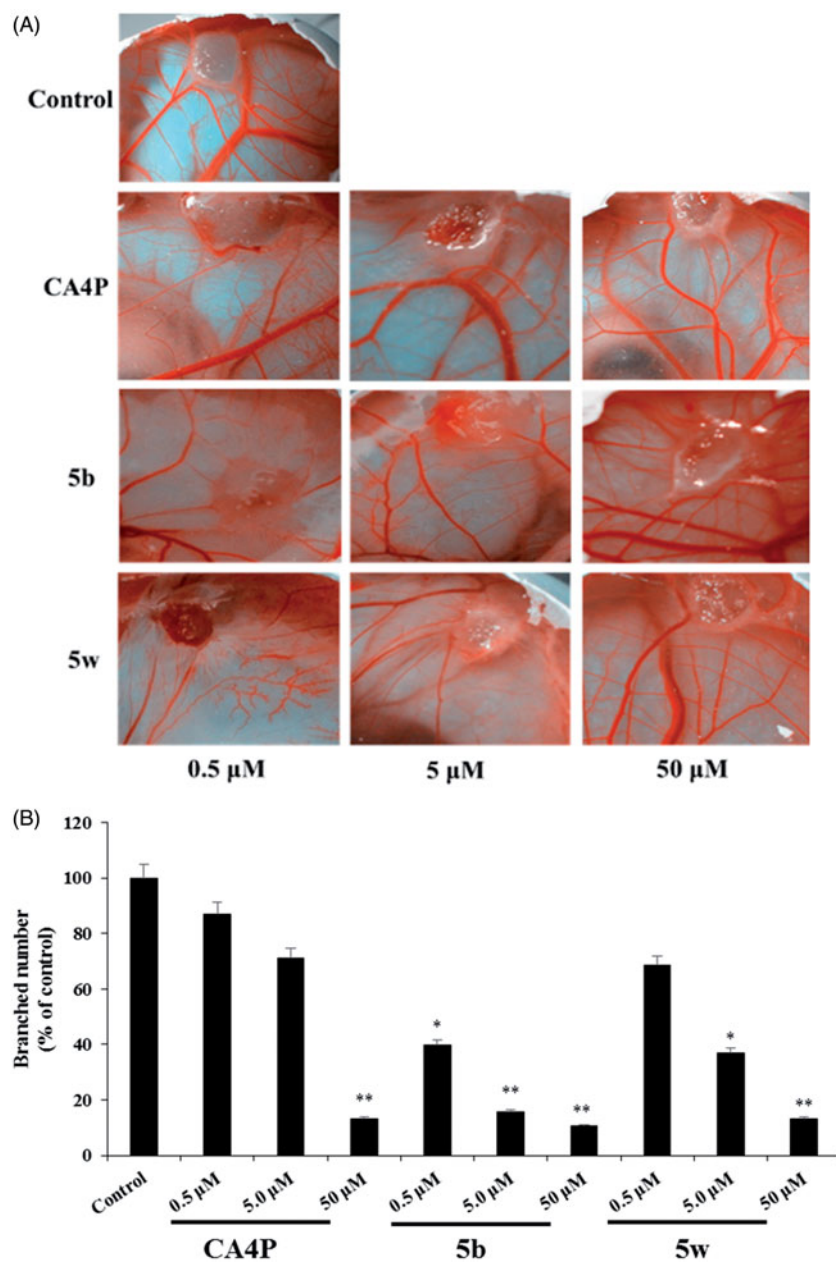


Figure 2. Compounds **5b** and **5w** inhibited angiogenesis. (A) *In vivo* anti-angiogenic effect of compounds **5b** and **5w** in chick chorioallantoic membrane (CAM) assay, positive control (CA4P, 0.5–50 mg/mL) and vehicle control (0.1% DMSO). (B) Quantification graphs of the inhibitory effects of compounds **5b** and **5w** on angiogenesis and migration. * $p < 0.05$, ** $p < 0.01$.

Evaluation of antitumor activity of N^9 -heterobivalent β -carbolines *in vivo*

Based on the *in vitro* assay results, we further tested the antitumor activity of four N^9 -heterobivalent β -carbolines *in vivo* against mice bearing Sarcoma 180 and Lewis lung cancer, respectively, and the reference drugs Cyclophosphamide (CTX). Our previous investigation demonstrated that mice bearing Lewis lung cancer were more susceptible to β -carbolines than other animal models; therefore, these animal models were selected and evaluated in the present investigation.⁵ The tumor inhibition rates of all of the investigated asymmetric dimeric β -carbolines were summarized in Table 2.

As shown in Table 2, all the tested N^9 -heterobivalent β -carbolines displayed moderate to strong antitumor activities in animal model. Interestingly, compounds **5b** and **5c**, which having the

same substituent and different carbon atoms linker. Compound **5b**, which bearing 4-carbon atoms, exhibited the potent antitumor agent with the tumor inhibition rate of 43.6 and 41.9% against Sarcoma 180-bearing mice and Lewis lung cancer-bearing mice, respectively, and compound **5c** displayed moderate antitumor activity with the tumor inhibition rate of 33.6% against Sarcoma 180-bearing mice at doses of 35 mg kg⁻¹. Compound **5t**, the harmine and harman linked with five methylene units, showed moderate antitumor activity against mice with Sarcoma 180 with the tumour inhibition rate of 37.2% at doses of 7 mg kg⁻¹. Particularly, compound **5w** was found to be the remarkable antitumor agent with the tumor inhibition rate of 47.1 and 42.3% against mice bearing Sarcoma 180 and Lewis lung cancer, respectively. These results implied that the harmine or harman linked with another of β -carboline nucleus (3-pyridyl or 2-thienyl substituent into position-1) enhanced their antitumor activities.

In vivo anti-angiogenic effect of compounds 5b and 5w

The most potent compounds, **5b** and **5w**, were selected to evaluate anti-angiogenic activity by chicken chorioallantoic membrane (CAM) assay. The inhibitory effects of compounds **5b** and **5w** on the angiogenesis of CAM are shown in Figure 2(A). The anti-angiogenic activity of compounds **5b** and **5w** were semi-quantitatively analyzed using Graph Pad Prism 5.0 (shown in Figure 2(B)). The results showed that compound **5b** ($p < 0.05$) could inhibit the angiogenesis of CAM. The anti-angiogenic activity of compound **5b** was comparable to the CA4P *in vivo* CAM assay at the same dose (50 μ M).

Conclusions

In this study, we synthesized 24 new, N^9 -heterodimeric β -carboline derivatives and focused on compounds with 4–6 carbon linkers between the indole nitrogen. All of the compounds were screened for their *in vitro* cytotoxic activity against BGC-823, HepG2, MCF-7, HT-29, Eca-109 and LLC cancer cell lines. The results showed that compounds **5b**, and **5w** exhibited strong cytotoxic activities with IC_{50} value of lower than 20 μ M against the six tested tumor cell lines. In addition, four asymmetric dimeric β -carbolines were selected for evaluation *in vivo* against mice bearing Sarcoma 180 and Lewis lung cancer, compounds **5b** and **5w** exhibited potent antitumor efficacies with tumour inhibition rate of over 40% in the tested animal models. Moreover, the pharmacological mechanisms showed that compound **5b** could retard in the CAM assay, and anti-angiogenic potency was more potent than the reference drug CA4P. Preliminary structure-activity analysis indicated that: (1) C^1 -methylation and C^7 -methoxylation were favorable for increased activities; (2) 3-Pyridyl or 2-thienyl group substituent into position-1 of the β -carboline core, and the aryl (electron withdrawing and donating groups) substituent into position-1' of another β -carboline ring might be detrimental to cytotoxic effects of this class of compounds. Although most N^9 -heterodimeric β -carbolines presented here showed modest cytotoxic activities, the investigations of these structural modifications and preliminary SARs would be helpful to further design and develop more potent compounds.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the scientific research innovation project in Xinjiang under Grant XJGRI2016052; and the Program for Outstanding Youth Science and Technology Innovation Talents Training in Xinjiang Uygur Region under Grant qn2015jc067.

References

- Smith BD, Smith GL, Hurria A, et al. Future of cancer incidence in the United States: burdens upon an aging, changing nation. *J Clin Oncol* 2009;27:2758–65.
- Ishida J, Wang HK, Bastow KF, et al. Antitumor agents 201. Cytotoxicity of harmine and beta-carboline analogs. *Bioorg Med Chem Lett* 1999; 9:3319–24.
- Cao RH, Chen Q, Hou XR, et al. Synthesis, acute toxicities and antitumor effects of novel 9-substituted β -carboline derivatives. *Bioorg Med Chem* 2004; 12:4613–23.
- Cao RH, Guan XD, Shi BX, et al. Design, synthesis and 3D-QSAR of beta-carboline derivatives as potent antitumor agents. *Eur J Med Chem* 2010; 45:2503–15.
- Cao RH, Fan WX, Guo L, et al. Synthesis and structure-activity relationships of harmine derivatives as potential antitumor agents. *Eur J Med Chem* 2013; 60:135–43.
- Shankaraiah N, Siraj KP, Nekkanti S, et al. DNA-binding affinity and anticancer activity of β -carboline–chalcone conjugates as potential DNA intercalators: molecular modelling and synthesis. *Bioorg Chem* 2015; 59:130–9.
- TAIRA Z, KANZAWA S, DOHARA C, et al. Intercalation of six β -carboline derivatives into DNA. *J Toxicol Environ Health* 1997; 43:83–91.
- Cao RH, Peng WL, Chen HS, et al. DNA binding properties of 9-substituted harmine derivatives. *Biochem Biophys Res Commun* 2005; 338:1557–63.
- Kamal A, Sathish M, Nayak VL, et al. Design and synthesis of dithiocarbamate linked β -carboline derivatives: DNA topoisomerase II inhibition with DNA binding and apoptosis inducing ability. *Bioorg Med Chem* 2015; 23:5511–26.
- Figueiredo PO, Perdomo RT, Garcez FR, et al. Further constituents of *Galianthe thalictroides* (Rubiaceae) and inhibition of DNA topoisomerases I and IIa by its cytotoxic β -carboline alkaloids. *Bioorg Med Chem Lett* 2014; 24:1358–61.
- Song YC, Kesuma D, Wang J, et al. Specific inhibition of cyclin-dependent kinases and cell proliferation by harmine. *Biochem Biophys Res Commun* 2004; 317:128–32.
- Li Y, Liang FS, Jiang W, et al. DH334, a beta-carboline anticancer drug, inhibits the CDK activity of budding yeast. *Cancer Biol Ther* 2007; 6:1193–9.
- Zhang J, Li Y, Guo L, et al. DH166, a beta-carboline derivative, inhibits the kinase activity of PLK1. *Cancer Biol Ther* 2009; 8:2374–83.
- Insaf F, Mohamed AB, Aymen BN, et al. Synthesis, cytotoxic, anti-lipoxygenase and anti-acetylcholinesterase capacities of novel derivatives from harmine. *J Enzyme Inhib Med Chem* 2016;31:23–33.
- Insaf F, Jalloul B, Mansour Z, et al. Synthesis of new isoxazoline derivatives from harmine and evaluation of their anti-Alzheimer, anti-cancer and anti-inflammatory activities. *J Enzyme Inhib Med Chem* 2015; 30:371–6.
- Castro AC, Dang LC, Soucy F, et al. Novel IKK inhibitors: β -carbolines. *Bioorg Med Chem Lett* 2003; 13:2419–22.
- Posner GH, D'Angelo J, O'Neill PM, et al. Anticancer activity of artemisinin-derived trioxanes. *Expert Opin Ther Pat* 2006; 16:1665–72.
- Alagbala AA, McRiner AJ, Borstnik K, et al. Biological mechanisms of action of novel C-10 non-acetal trioxane dimers in prostate cancer cell lines. *J Med Chem* 2006; 49:7836–42.
- Posner GH, McRiner AJ, Paik IH, et al. Anticancer and anti-malarial efficacy and safety of artemisinin-derived trioxane dimers in rodents. *J Med Chem* 2004; 47:1299–301.
- Jung M, Lee S, Ham J, et al. Antitumor activity of novel deoxoartemisinin monomers, dimers, and trimer. *J Med Chem* 2003; 46:987–94.
- Shi BX, Cao RH, Fan WX, et al. Design, synthesis and *in vitro* and *in vivo* antitumor activities of novel bivalent β -carbolines. *Eur J Med Chem* 2013;60:10–22.
- Wu QF, Bai ZS, Ma Q, et al. Synthesis and biological evaluation of novel bivalent β -carbolines as potential antitumor agents. *Med Chem Commun* 2014; 5:953–8.

23. Guo L, Cao RH, Fan WX, et al. Design, synthesis and *in vitro* antitumor activities of novel bivalent β -carbolines. *Chem J Chin Univer* 2013;60:10–99.
24. Guo L, Chen W, Fan WX, et al. Synthesis and preliminary evaluation of novel alkyl diamine linked bivalent β -carbolines as angiogenesis inhibitors. *Med Chem Commun* 2016; 7:2177–83.
25. Chen W, Zhang GX, Guo L, et al. Synthesis and biological evaluation of novel alkyl diamine linked bivalent β -carbolines as angiogenesis inhibitors. *Eur J Med Chem* 2016; 124:249–61.
26. Sun RQ, Liu R, Zhou C, et al. Synthesis and biological evaluation of piperazine group-linked bivalent β -carbolines as potential antitumor agents. *Med Chem Commun* 2015; 6:2170–4.
27. Daoud A, Song J, Xiao FY, et al. B-9-3, a novel β -carboline derivative exhibits anti-cancer activity via induction of apoptosis and inhibition of cell migration *in vitro*. *Eur J Pharmacol* 2014;724:219–30.
28. Ma Q, Chen W, Chen W. Anti-tumor angiogenesis effect of a new compound: B-9-3 through interference with VEGFR2 signaling. *Tumour Biol* 2016; 37:6107–16.
29. Xinjiang Huashidan Pharmaceutical Research Co., Ltd. EP 1634881 A1, 2006.
30. Reddy POV, Mishra S, Tantak MP, et al. Design, synthesis and *in vitro* cytotoxicity studies of novel β -carbolinium bromides. *Bioorg Med Chem Lett* 2017; 27:1379–84.
31. Hadjaz F, Yous S, Lebegue N, et al. A mild and efficient route to 2-benzyl tryptamine derivatives via ring-opening of β -carbolines. *Tetrahedron* 2008; 64:10004–8.
32. Wu QF, Cao RH, Feng MX, et al. Synthesis and *in vitro* cytotoxic evaluation of novel 3,4,5-trimethoxyphenyl substituted β -carboline derivatives. *Eur J Med Chem* 2009;44: 533–40.
33. Du HT, Tian S, Chen JC, et al. Synthesis and biological evaluation of N^9 -substituted harmine derivatives as potential anticancer agents. *Bioorg Med Chem Lett* 2016;26: 4015–9.
34. Kassa J, Vachek J. A comparison of the efficacy of pyridostigmine alone and the combination of pyridostigmine with anticholinergic drugs as pharmacological pretreatment of tabun-poisoned rats and mice. *Toxicology* 2002; 177:179–85.