European Journal of Medicinal Chemistry 45 (2010) 5513–5519

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

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

Short communication

Synthesis and cytotoxic evaluation of 1-carboxamide and 1-amino side chain substituted β-carbolines

Chunming Ma [a], Rihui Cao [a,*], Buxi Shi [a], Xiantai Zhou [a], Qin Ma [b], Jie Sun [b], Liang Guo [b], Wei Yi [a], Zhiyong Chen [a], Huacan Song [a,*]

[a] School of Chemistry and Chemical Engineering, Sun Yat-sen University, 135 Xin Gang West Road, Guangzhou 510275, PR China
[b] Xinjiang Huashidan Pharmaceutical Co. Ltd., 45 He Nan East Road, Urumqi 830011, PR China

ARTICLE INFO

Article history:
Received 13 March 2010
Received in revised form
24 July 2010
Accepted 29 August 2010
Available online 8 September 2010

Keywords:
Synthesis
β-Carboline
Cytotoxic
SARs

ABSTRACT

The condensation of alkylenediamine with ethyl β-carboline-1-carboxylate and 1-bromo-β-carboline gave β-carboline-1-carboxamides and 1-amino-β-carbolines, respectively. Some of these β-carbolines were active against a panel of human tumor cell lines, and 1-amino derivatives were more potent than their 1-carboxamide congeners. In particular, among the 1-amino-β-carbolines, the N^9-arylated alkyl substituted β-carbolines exhibited the most interesting cytotoxic activities with IC_{50} value of lower than 20 μM. The preliminary structure–activity relationships (SARs) analysis suggested that (1) 1-amino substituents were the advisable pharmacophoric group for enhanced cytotoxic activities; (2) the introduction of appropriate arylated alkyl groups into position-9 of β-carboline facilitated their cytotoxic potencies.

1. Introduction

The β-carboline nucleus is present in many natural and synthetic products associated with a wide spectrum of biochemical and pharmaceutical functions [1]. Recently, the β-carboline alkaloids have been characterized as a class of potential antitumor agents, and a large number of natural and synthetic β-carbolines acting as antitumor agents were reported [2–10]. Many studies suggested that these compounds exerted their antitumor activities through multiple mechanisms of action including intercalation into DNA [3,8,11], inhibition topoisomerase I and II [5,12,13], CDK (cyclin-dependent kinase) [14–16], MK-2 (mitogen activated protein kinase-activated protein kinase 2) [17,18], PLK1 (polo-like kinase) [19], kinesin-like protein Eg5 [20] and IKK (I-kappa-B kinase) [21].

Previous attempts were focused on incorporating substituents into position-1 and 3 of β-carboline nucleus. The β-carbolines bearing a flexible alkylamine side chain in position-3 showed potent DNA intercalating abilities resulting in remarkable antitumor potencies [3], and the β-carboline amino acid ester conjugates exhibited potent cytotoxic activities [6]. In addition, the complex polycyclic ring system in position-1 of β-carboline nucleus of manzamine A can be replaced with simpler amino substituents to give active compounds [9].

Our previous attention was focused on the incorporating various substituents into position-3 and 9 of β-carboline nucleus [22–27]. Structure-activity relationships (SARs) for *in vitro* and *in vivo* antitumor activities disclosed that (1) the antitumor potential of β-carbolines was correlated to both the planarity of the molecule and the presence of substituents in position-3 and 9 of β-carboline nucleus; (2) the introduction of appropriate substituents into position-9 of β-carboline nucleus played a vital role in the modulation of their antitumor efficacies.

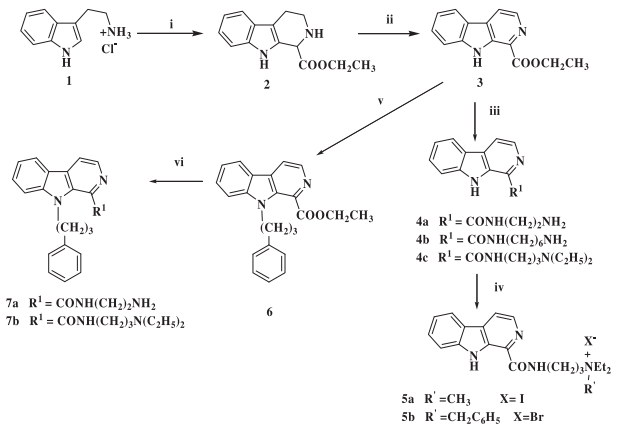
In continuing search for novel antitumor agents endowed with better pharmacological profiles and study in depth the influence of the substituent in position-1 and 9 of β-carboline nucleus, in the present investigation, we reported the design, synthesis and cytotoxic evaluation of novel antitumor agents bearing carboxamide or amino substituents in position-1 and alkyl or arylated alkyl groups in position-9 of β-carboline nucleus, respectively.

2. Chemistry

The ethyl 1,2,3,4-tetrahydro-β-carboline-1-carboxylate **2** employed in this investigation was accomplished *via* the well-known Pictet–Spengler reaction of tryptamine hydrochloride **1** with ethyl glyoxylate in ethanol [28]. Oxidative aromatization of **2** with

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

0223-5234/$ – see front matter © 2010 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2010.08.065



Scheme 1. [a] Synthesis of 1-carboxamide substituted β-carbolines **4a**–**c**, **5a**–**b** and **7a**–**b**. [a](i) OHC–CO_2Et, EtOH, reflux; (ii) MnO_2, toluene, reflux; (iii) $NH_2(CH_2)_2NH_2$ or $NH_2(CH_2)_6NH_2$ or $NH_2(CH_2)_3N(C_2H_5)_2$, stirred at RT or microwave irradiation, 170 °C, 30 min; (iv) CH_3I or C_6H_5Br, EA, reflux; (v) DMF, NaH, $C_6H_5(CH_2)_3Br$; (vi) $NH_2(CH_2)_2NH_2$ or $NH_2(CH_2)_3N(C_2H_5)_2$, microwave irradiation, 170 °C, 30 min.

MnO_2 in refluxing toluene afforded the desired ethyl β-carboline-1-carboxylate **3**. This sequence of reactions can be easily scaled up to give enough material **3** for further transformations. The reaction of 1-bromo-3-phenylpropane with compound **3** by the action of sodium hydride in anhydrous DMF furnished ethyl 9-(3-phenylpropyl)-β-carboline-1-carboxylate **6**. The condensation of compounds **3** and **6** with an excess of diamines by heating or subjecting to microwave irradiation (170 °C, 30 min) gave the amidated derivatives **4a**–**c** and **7a**–**b** in good yield. The methylated or benzylated quarternary β-carboline salts **5a**–**b** were prepared from the corresponding β-carboline **4c** by the addition of methyl iodide or benzyl bromide in refluxing ethyl acetate, respectively (Scheme 1).

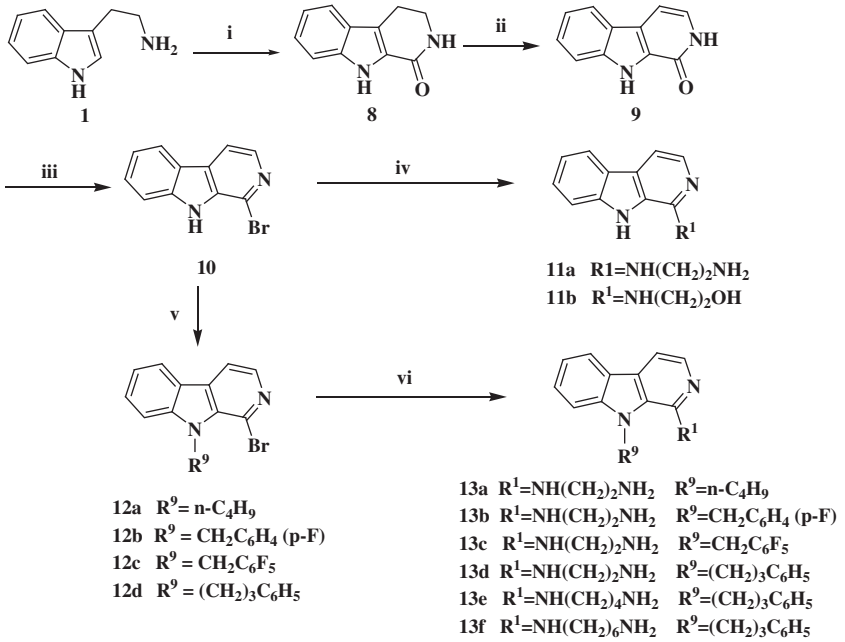
The intermediate 1-bromo-β-carboline **10** was prepared readily from tryptamine **1** in three steps according to already published methods [29]. The N^9-position of compound **10** was alkylated or arylated by the action of sodium hydride in dry DMF followed by addition of the relevant appropriate alkylating and arylating agents to provide **12a**–**d**. The reaction of **10** and **12a**–**d** with an access of various diamines without solvent by microwave-assisted heating (170 °C, 30min) provided the corresponding amines **11a**–**b** and **13a**–**f** in good yield (Scheme 2). The chemical structures of all the synthesized compounds were characterized by MS, IR, ^{1}H NMR and ^{13}C NMR spectra.

3. Results and discussion

The cytotoxic potential of all synthesized β-carbolines was evaluated *in vitro* against a panel of human tumor cell lines and compared with the reference drugs cisplatin that has been in wide clinical use. In order to enhance the solubility in aqueous solution, all compounds were prepared in the form of hydrochloride salt before use. As predicted, the hydrochloride salt of β-carbolines bearing carboxamide or amino substituents in position-1 showed excellent water-solubility (more than 200 mg/ml). The tumor cell line panel consisted of gastric carcinoma (BGC-823), liver carcinoma (HepG2), breast carcinoma (MCF-7), renal carcinoma (OS-RC-2, 786-0 and 769-P), melanoma (A375), colon carcinoma (HT-29), epidermoid carcinoma of the nasopharynx (KB) and prostate carcinoma (22RV1). The results were summarized in Table 1.

It was apparent that 1-amino derivatives **13a**–**f** showed more potent cytotoxic potencies than the corresponding 1-carboxamide derivatives **4a**–**c**, **5a**–**b** and **7a**–**b**. Of all 1-carboxamide derivatives, compounds **4a**–**c** exhibited moderate cytotoxic activities against human tumor cell lines with IC_{50} values range from 22.1 to 243 μM. As predicted, incorporating a 3-phenylpropyl group into position-9 of β-carboline nucleus of compounds **4a** and **4c** led to compounds **7a** and **7b**, which demonstrated significant cytotoxic potencies with IC_{50} values of lower than 50 μM against 10 human tumor cell lines. Unfortunately, the quarternary β-carboline salts **5a**–**b** were almost inactive at the concentration of 300 μM.

As shown in Table 1, compound **11a** bearing a aminoethylamino side chain group showed moderate cytotoxic activity, while replacement of aminoethylamino group of **11a** with



Scheme 2. [a] Synthesis of 1-amino substituted β-carbolines **11a**–**b** and **13a**–**f**. [a](i) toluene, triphosgene, Et_3N, 30% HBr/HAc, reflux; (ii) THF, DDQ, stirred at RT; (iii) $POBr_3$, $C_6H_5OCH_3$, 120 °C; (iv) $NH_2(CH_2)_2NH_2$ or $HO(CH_2)_2NH_2$, CuI, reflux; (v) DMF, NaH, alkyl halogenide, stirred at RT; (vi) $NH_2(CH_2)_nNH_2$, microwave irradiation, 170 °C, 30 min.

Table 1
Cell growth inhibitory activity (IC_{50}, μM) of compounds **4a**–**c**, **5a**–**b**, **7a**–**b**, **11a**–**b**, **12a**–**d** and **13a**–**f** against ten human cancer cell lines.

Compound	IC_{50} (μM)±SD[a]									
	BGC823	HepG2	MCF7	OS-RC-2	786–0	769-P	A375	HT-29	KB	22RV1
4a	77.7 ± 8.2	122 ± 15.6	80.4 ± 9.85	63.9 ± 5.82	86.1 ± 10.2	124 ± 18.6	65.3 ± 7.42	55.6 ± 4.85	118 ± 14.3	124 ± 20.5
4b	109 ± 13.6	85.5 ± 9.3	46.8 ± 6.20	58.5 ± 7.61	72.7 ± 6.89	75.3 ± 8.84	37.7 ± 4.25	73.2 ± 6.54	86.9 ± 10.2	33.6 ± 4.25
4c	174 ± 25.3	243 ± 26.8	164 ± 18.9	93.9 ± 14.3	111 ± 15.2	54.0 ± 4.86	107 ± 15.4	61.2 ± 5.86	62.3 ± 7.41	46.2 ± 4.89
5a	>300	>300	>300	>300	>300	>300	>300	>300	>300	>300
5b	>300	>300	51.1 ± 6.23	>300	137 ± 16.7	101 ± 13.6	>300	>300	>300	71.4 ± 8.62
7a	20.0 ± 1.56	15.9 ± 2.20	22.3 ± 1.87	15.7 ± 2.21	11.8 ± 1.04	32.5 ± 4.25	13.7 ± 1.19	17.9 ± 2.68	9.9 ± 0.96	10.8 ± 1.09
7b	22.3 ± 2.43	22.1 ± 3.12	31.4 ± 3.65	36.9 ± 4.20	31.4 ± 2.87	61.0 ± 8.62	33.8 ± 4.23	53.4 ± 6.59	14.0 ± 2.08	26.9 ± 2.26
11a	36.0 ± 3.81	30.6 ± 4.56	87.2 ± 12.1	40.0 ± 5.59	31.9 ± 3.56	31.5 ± 2.89	33.4 ± 3.26	33.4 ± 2.89	25.4 ± 3.26	41.3 ± 5.45
11b	>300	130 ± 20.1	>300	220 ± 30.5	>300	272 ± 25.2	>300	>300	>300	136 ± 15.6
12a	>300	>300	256 ± 35.2	136 ± 16.5	>300	>300	>300	186 ± 26.8	>300	236 ± 28.7
12b	>300	>300	>300	>300	>300	252 ± 32.6	>300	245 ± 30.5	>300	>300
12c	>300	>300	>300	>300	>300	>300	>300	>300	>300	>300
12d	>300	>300	>300	93.9 ± 12.8	>300	123 ± 15.6	>300	86.6 ± 10.3	>300	88.0 ± 10.3
13a	25.3 ± 3.64	6.6 ± 0.56	33.6 ± 4.58	9.3 ± 1.22	18.9 ± 2.32	68.7 ± 6.65	22.8 ± 3.21	13.3 ± 1.06	20.5 ± 2.87	14.2 ± 2.10
13b	9.7 ± 0.86	4.4 ± 0.39	21.4 ± 3.45	16.3 ± 2.62	18.8 ± 2.47	1.8 ± 0.16	15.6 ± 1.42	9.1 ± 0.87	19.1 ± 3.15	17.9 ± 2.36
13c	11.9 ± 1.23	12.8 ± 1.18	15.1 ± 2.26	12.0 ± 1.28	5.8 ± 0.63	10.4 ± 1.26	12.0 ± 1.54	9.9 ± 1.12	11.8 ± 1.28	12.5 ± 1.10
13d	9.6 ± 0.81	13.3 ± 2.30	9.3 ± 0.78	4.6 ± 0.36	6.9 ± 0.68	11.1 ± 1.43	7.3 ± 0.86	5.6 ± 0.48	4.9 ± 0.45	8.6 ± 0.78
13e	8.0 ± 0.75	4.0 ± 0.52	12.8 ± 1.12	4.2 ± 0.34	2.7 ± 0.32	2.2 ± 0.18	9.5 ± 1.03	17.5 ± 1.56	11.3 ± 1.42	2.9 ± 0.22
13f	12.4 ± 1.32	12.1 ± 1.08	17.9 ± 2.62	8.4 ± 0.75	11.3 ± 0.85	13.6 ± 1.45	16.3 ± 2.38	21.5 ± 2.56	13.0 ± 1.13	11.5 ± 0.98
Cisplatin	19.2 ± 1.62	4.6 ± 0.32	13.4 ± 1.45	4.9 ± 0.56	9.4 ± 1.28	3.4 ± 0.42	16.0 ± 2.36	85.7 ± 10.2	4.6 ± 0.40	12.4 ± 1.20

[a] Cytotoxicity as IC_{50} for each cell line is the concentration of compound, which reduced by 50% the optical density of treated cells with respect to untreated using the MTT assay. Values with standard deviations (SD) are averages of at least five independent determinations. Values >300 μM indicate less than 50% growth inhibition at 300 μM.

hydroxylethylamino group led to compound **11b**, which showed no cytotoxic efficacy at the concentration of 300 μM. 1-Bromo-β-carbolines **12a**–**d** failed to display cytotoxic activities, while compounds **13a**–**f**, the condensation products of compounds **12a**–**d** with various diamines, showed potent cytotoxic potencies with IC_{50} values of lower than 20 μM against most of tumor cell lines. Among 1-amino derivatives **13a**–**f**, compound **13b**–**f**, bearing an arylated alkyl substituents in position-9 of β-carboline nucleus exhibited more potent cytotoxic effects than compound **13a** having an n-butyl group in position-9. In addition, compounds **13d** and **13e** bearing a flexible amino side chain with two or four methylene spacer, respectively, displayed more potent cytotoxic potencies than compound **13f** having six methylene spacer. These results suggested that (1) the amino side chain substituents were the advisable pharmacophoric group for the enhanced cytotoxic activities; (2) the introduction of appropriate arylated alkyl groups into position-9 of β-carboline nucleus facilitated the cytotoxic potencies.

4. Conclusion

In the present investigation, two different types of 1-substituted β-carbolines were designed, synthesized and their cytotoxic potential against human tumor cell lines in culture were investigated. Some of these compounds had significant cytotoxic efficacies in comparable to the reference drug cisplatin. Preliminary structure–activity relationships analysis indicated that the 1-amino side chain substituents were more favorable than 1-carboxamide side chain groups for their enhanced cytotoxic potencies. In addition, the introduction of arylated alkyl groups into position-9 of β-carboline nucleus provided compounds with greatly enhanced cytotoxic potencies. These results were agreement with our previous observation that the N^9-arylated alkyl substitution facilitated the cytotoxic potencies. Moreover, our previous studies suggested that intercalation into DNA is a major cellular event of this class of molecules [31] and β-carbolines can pass through cell membrane and penetrate into nucleus quickly resulting in intercalating into DNA in cells [32]. Consequently, such compounds were expected to exert their tumor cell killing effects through similar mechanisms of action. Undoubtedly, to acquire more information about the structural requirements for the possible improvement of the cytotoxic potential and to elucidate SARs between substituent properties in position-1 of β-carboline nucleus and cytotoxic efficacies, design and synthesis of more novel β-carboline derivatives bearing various amino side chains are needed.

5. Experimental section

Reagents and general methods: All reagents were purchased from commercial suppliers and were dried and purified when necessary. Melting points were determined with a Kofler micromelting point apparatus without correction. ESI-MS spectra were obtained from VG ZAB-HS spectrometer. ^1H 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 $CDCl_3$ or DMSO-d_6 as solvent and chemical shifts (δ) were expressed in ppm. All reactions were monitored by TLC and spots were visualized with UV light or iodine. HPLC was performed on Agilent 1100 series, using a Waters C_{18} column (4.6 × 150 mm). All compounds (free bases) were greater than 95% pure. All commercially available reagents and solvents were used without further purification. The intermediate ethyl β-carboline-1-carboxylate (**3**) and ethyl 9-(3-phenylpropyl)-β-carboline-1-carboxylate (**6**) were prepared as previously described [22,28]. The intermediate 1-oxo-tetrahydro-β-carboline (**8**), 1-oxo-1,2-dihydro-β-carboline (**9**) and 1-bromo-β-carboline (**10**) were prepared according to literature methods [29].

5.1. *N*-(2-aminoethyl)-β-carboline-1-carboxamide (**4a**) [30]

A mixture of ethyl β-carboline-1-carboxylate **3** (0.24 g, 1.0 mmol) and ethylenediamine (10 mL) was stirred at room temperature for 2 h. After completion of the reaction as indicated by TLC, the reaction mixture was evaporated under reduced pressure and the residue dissolved in EA (50 mL), washed with H_2O. The organic phase was dried over anhydrous Na_2SO_4, filtered and evaporated to give the crude product, and then washed with anhydrous ethyl ether to afford **4a** as yellow solid (0.21 g, 85%), mp 118–120 °C. ESI-MS *m*/*z* 255 $[M + H]^+$; IR (KBr) ν 3379, 3269, 3055, 2927, 2859, 1646, 1624, 1528, 1321, 724 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 10.32 (1H, s, NH), 8.42 (1H, s, NH), 8.37 (1H, d, J = 5.1 Hz, ArH), 8.13 (1H, d, J = 7.8 Hz, ArH), 8.07 (1H, d, J = 5.1 Hz, ArH), 7.61–7.53 (2H, m, 2ArH), 7.30 (1H, t,

J = 7.5 Hz, ArH); 3.62 (2H, q, J = 6.0 Hz, CH_2), 3.03 (2H, t, J = 6.0 Hz, CH_2).

5.2. N-(6-Aminohexyl)-β-carboline-1-carboxamide hydrochloride salt (**4b**)

In a sealed microwave vial, ethyl β-carboline-1-carboxylate **3** (0.24 g, 1 mmol) and 1, 6-hexanediamine (1.16 g, 10 mmol) were heated with stirring at 210 °C under microwave irradiation for 30 min. The reaction tube was then cooled to room temperature and the mixture was poured into H_2O (100 mL) and extracted with CH_2Cl_2. The organic phase was dried over anhydrous Na_2SO_4, filtered and evaporated. The residue obtained was purified by silica column chromatography with CH_2Cl_2/MeOH (30:1) as the eluent to give yellow oil **4b** (0.27 g, 85%). The hydrochloride salt of **4b**, prepared with dry HCl gas in MeOH, had mp 237–239 °C. ESI-MS m/z 311 $[M + H]^+$; IR (KBr) ν 3330, 3050, 2932, 1659, 1625, 1575, 1537, 1498, 1246, 788, 753 cm^{-1}; ^1H NMR (300 MHz, D_2O) δ 7.72 (1H, d, J = 5.7 Hz, ArH), 7.68 (1H, d, J = 5.7 Hz, ArH), 7.36 (1H, d, J = 8.1 Hz, ArH), 7.23 (1H, t, J = 7.8 Hz, ArH), 6.92 (1H, d, J = 8.1 Hz, ArH), 6.85 (1H, t, J = 7.8 Hz, ArH), 3.22 (2H, t, J = 7.2 Hz, CH_2), 2.90 (2H, t, J = 7.5 Hz, CH_2), 1.64–1.50 (4H, m, $2CH_2$), 1.37–1.32 (4H, m, $2CH_2$); ^{13}C NMR (75 MHz, D_2O) δ 160.7, 142.6, 134.8, 132.0, 130.1, 125.2, 121.9, 121.6, 117.9, 117.8, 112.0, 40.4, 39.7, 28.2, 27.0, 26.1, 25.6.

5.3. N-[(3-diethylamino)propyl]-β-carboline-1-carboxamide (**4c**)

A mixture of ethyl β-carboline-1-carboxylate **3** (0.24 g, 1 mmol) and N, N-diethyl-1, 3-propanediamine (10 mL) was heated with stirring to 170 °C under microwave irradiation for 30 min. The mixture was poured into H_2O (100 mL) and extracted with CH_2Cl_2. The organic phase was dried over anhydrous Na_2SO_4, filtered and evaporated. The residue obtained was purified by silica column chromatography with CH_2Cl_2/MeOH (30:1) as the eluent to give yellow oil **4c** (0.29 g, 88%). ESI-MS m/z 325 $[M + H]^+$; IR (KBr) ν 3427, 3049, 2943, 2774, 1664, 1626, 1529, 1452, 1246, 753 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 10.37 (1H, s, NH), 8.87 (1H, s, NH), 8.35 (1H, d, J = 5.1 Hz, ArH), 8.12 (1H, d, J = 7.8 Hz, ArH), 8.04 (1H, d, J = 5.1 Hz, ArH), 7.59–7.52 (2H, m, 2ArH), 7.30 (1H, td, J = 6.8, 1.8 Hz, ArH), 3.62 (2H, q, J = 6.3 Hz, CH_2), 2.65–2.56 (6H, m, $3CH_2$), 1.90–1.81 (2H, m, CH_2), 1.09 (6H, t, J = 7.2 Hz, $2CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.7, 141.2, 137.3, 135.6, 132.6, 131.3, 129.1, 121.9, 120.8, 120.2, 117.6, 112.1, 51.9, 47.1, 38.9, 27.0, 12.0. The hydrochloride salt of **4c**, prepared with dry HCl gas in MeOH, had mp 203–205 °C.

5.4. 3-(β-Carboline-1-carbonyl)aminopropyl diethyl methyl ammonium iodide (**5a**)

A mixture of **4c** (0.32 g, 1.0 mmol), methyl iodide (1 mL) and ethyl acetate (20 mL) was stirred under reflux for 4 h, and then cooled and filtered under reduced pressure and washed with ethyl ester to give yellow solid, which could be recrystallized from ethanol, dried in vacuum to afford yellow crystal **5a** (0.40 g, 86%). mp 192–193 °C. ESI-MS m/z 339 $[M - I]^+$; IR (KBr) ν 3368, 3314, 3035, 2922, 2873, 1658, 1623, 1526, 1460, 734 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 11.68 (1H, s, NH), 9.15 (1H, t, J = 6.3 Hz, CONH), 8.39 (1H, d, J = 4.8 Hz, ArH), 8.34 (1H, d, J = 4.8 Hz, ArH), 8.26 (1H, d, J = 7.8 Hz, ArH), 7.78 (1H, d, J = 8.1 Hz, ArH), 7.56 (1H, t, J = 7.5 Hz, ArH), 7.26 (1H, t, J = 7.5 Hz, ArH), 4.48 (2H, s, N^+CH_2), 3.48 (2H, q, J = 6.3 Hz, CH_2), 3.32–3.26 (6H, m, $3CH_2$), 2.92 (3H, s, CH_3), 2.06–1.96 (2H, m, CH_2), 1.20 (6H, t, J = 6.9 Hz, $2CH_3$); ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.4 (CO), 142.1, 137.2, 135.0, 132.9, 131.3, 129.4, 122.4, 120.5, 120.4, 118.6, 113.6, 58.4, 56.4, 47.4, 36.6, 23.0, 8.3. Anal. Calcd for $C_{20}H_{27}IN_4O$: C, 51.51; H, 5.84; N, 12.01. Found: C, 51.68; H, 5.88; N, 12.12.

5.5. 3-(β-Carboline-1-carbonyl)aminopropyl diethyl benzyl ammonium bromide (**5b**)

A mixture of **4c** (0.32 g, 1.0 mmol), benzyl bromide (10 mmol) and ethyl acetate (20 mL) was stirred under reflux for 4 h, and then cooled, filtered and washed with ethyl ester to give yellow solid, which could be recrystallized from ethanol, dried in vacuum to afford yellow crystal **5b** (0.41 g, 83%), mp 214–215 °C. ESI-MS m/z 415 $[M - Br]^+$; IR (KBr) ν 3292, 3034, 2974, 2905, 2821, 1667, 1628, 1591, 1530, 1228, 754 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 11.68 (1H, s, NH), 9.22 (1H, t, J = 6.0 Hz, CONH), 8.41 (1H, d, J = 5.1 Hz, ArH), 8.37 (1H, d, J = 5.1 Hz, ArH), 8.27 (1H, d, J = 7.8 Hz, ArH), 7.77 (1H, d, J = 8.1 Hz, ArH), 7.55 (1H, t, J = 7.2 Hz, ArH), 7.47 (2H, d, J = 7.2 Hz, 2ArH), 7.36–7.23 (4H, m, 4ArH), 4.48 (2H, s, N^+CH_2), 3.48 (2H, q, J = 6.0 Hz, CH_2), 3.22–3.10 (6H, m, $3CH_2$), 2.20–2.10 (2H, m, CH_2), 1.29 (6H, t, J = 6.9 Hz, $2CH_3$); ^{13}C NMR (75 MHz, DMSO-d_6) δ 165.9 (CO), 142.4, 136.6, 134.9, 133.2, 132.6, 131.8, 130.7, 129.8, 129.5, 128.3, 122.6, 120.6, 120.4, 118.8, 113.7, 60.8, 55.5, 53.4, 36.5, 23.1, 8.5. Anal. Calcd for $C_{26}H_{31}BrN4O$: C, 63.03; H, 6.31; N, 11.31. Found: C, 63.23; H, 6.28; N, 11.37.

5.6. N-(2-Aminoethyl)-9-(3-phenylpropyl)-β-carboline-1-carboxamide hydrochloride salt (**7a**)

A mixture of ethyl 9-(3-phenylpropyl)-β-carboline-1-carboxylate **6** (0.36 g, 1 mmol) and ethylenediamine (10 mL) was stirred at room temperature for 2 h. After completion of the reaction as indicated by TLC, the reaction mixture was evaporated under reduced pressure and the residue was dissolved in ethyl acetate, washed with water. The organic phase was dried over anhydrous Na_2SO_4, filtered and evaporated to give the crude product, and then washed with anhydrous ethyl ether to afford pure product **7a** as yellow solid (0.31g, 83%), mp 102–103 °C. The hydrochloride salt of **7a**, prepared with dry HCl gas in methanol, had mp 221–223 °C. ESI-MS m/z 373 $[M + H]^+$; IR (KBr) ν 3432, 3054, 2929, 1625, 1496, 1454, 749 cm^{-1}; ^1H NMR (300 MHz, D_2O) δ 8.32 (1H, d, J = 6.0 Hz, ArH), 8.22 (1H, d, J = 6.0 Hz, ArH), 8.10 (1H, d, J = 8.1 Hz, ArH), 7.65 (1H, t, J = 7.5 Hz, ArH), 7.46 (1H, t, J = 7.5 Hz, ArH), 7.30 (1H, t, J = 7.5 Hz, ArH), 7.02–6.84 (5H, m, 5ArH), 4.27 (2H, t, J = 7.2 Hz, CH_2), 3.61 (2H, t, J = 6.3 Hz, CH_2), 3.20 (2H, t, J = 6.6 Hz, CH_2), 2.47 (2H, t, J = 7.2 Hz, CH_2), 1.98–1.87 (2H, m, CH_2).

5.7. N-[(3-diethylamino)propyl]-9-(3-phenylpropyl)-β-carboline-1-carboxamide hydrochloride salt (**7b**)

A mixture of ethyl 9-(3-phenylpropyl)-β-carboline-1-carboxylate **6** (0.36 g, 1 mmol) and N, N-diethyl-1, 3-propanediamine (10 mL) was heated with stirring to 170 °C under microwave irradiation for 30 min. The mixture was poured into H_2O (100 mL) and extracted with CH_2Cl_2. The organic phase was dried over anhydrous Na_2SO_4, filtered and evaporated. The residue obtained was purified by silica column chromatography with CH_2Cl_2/MeOH (30:1) as the eluent to give compound **7b** as yellow oil (0.36 g, 81%). The hydrochloride salt of **7b**, prepared with dry HCl gas in methanol, had mp 201–203 °C. ESI-MS m/z 443 $[M + H]^+$; IR (KBr) ν 3159, 3026, 2941, 2650, 1672, 1625, 1553, 1496, 1302, 750, 704 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 8.66 (1H, d, J = 5.7 Hz, ArH), 8.52 (1H, d, J = 5.7 Hz, ArH), 8.46 (1H, d, J = 8.1 Hz, ArH), 7.82–7.73 (2H, m, 2ArH), 7.41 (1H, t, J = 8.1 Hz, ArH), 7.25–7.12 (5H, m, 5ArH), 4.60 (2H, t, J = 7.2 Hz, CH_2), 3.47 (2H, q, CH_2), 3.27–3.17 (2H, m, CH_2), 3.14–1.02 (4H, m, $2CH_2$), 2.58 (2H, t, J = 7.2 Hz, CH_2), 2.07–1.95 (4H, m, $2CH_2$), 1.22 (6H, t, J = Hz, $2CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$)

δ 166.9, 142.8, 141.6, 136.7, 134.6, 132.5, 129.0, 128.5, 126.1, 121.4, 121.1, 120.2, 116.9, 110.7, 51.9, 47.1, 45.8, 39.6, 33.4, 31.3, 26.8, 11.9.

5.8. 1-(2-Aminoethylamino)-β-carboline (**11a**)

A mixture of 1-bromo-β-carboline **10** (0.50 g, 2.0 mmol), cuprous iodide (0.19 g, 1.0 mmol) and ethylenediamine (10 mL) was stirred under reflux for 12 h. After completion of the reaction as indicated by TLC, the mixture was evaporated under reduced pressure and the residue was added H_2O (50 mL) and extracted with CH_2Cl_2. The organic phase was dried over anhydrous Na_2SO_4, filtered and evaporated. The crude product obtained was purified by silica column chromatography with CH_2Cl_2/MeOH (30:1) as the eluent to give the target product **11a** (0.28 g, 62%). The hydrochloride salt of **11a**, prepared with dry HCl gas in MeOH, had mp 253–255 °C. ESI-MS *m/z* 227 $[M + H]^+$; IR (KBr) ν 3432, 3229, 3057, 2965, 1660, 1628, 1563, 1112, 1067, 739 cm^{-1}; ^1H NMR (300 MHz, D_2O) δ 7.99 (1H, t, *J* = 7.5 ArH), 7.51–7.47 (3H, m, 3ArH), 7.39 (1H, t, *J* = 7.5 Hz, ArH), 7.27 (1H, t, *J* = 7.2 Hz, ArH), 3.81–3.73 (2H, m, CH_2), 3.31 (2H, t, *J* = 6.0 Hz, CH_2).

5.9. 1-(2-Hydroxylethylamino)-β-carboline (**11b**) [9]

A mixture of 1-bromo-β-carboline **10** (0.50 g, 2.0 mmol) and ethanolamine (10 mL) were subjected to the same procedure which was used to produce **11a** afford the target product **11b** with 56% yield. The hydrochloride salt of **11b**, prepared with dry HCl gas in MeOH, had mp 187–189 °C. ESI-MS *m/z* 228 $[M + H]^+$; IR (KBr) ν 3254, 3027, 2921, 2847, 1649, 1603, 1531, 1506, 1304, 1069, 753 cm^{-1}; ^1H NMR (300 MHz, D_2O) δ 7.36 (1H, d, *J* = 7.8 Hz, ArH), 7.25 (1H, t, *J* = 7.2 Hz, ArH), 7.09 (H, d, *J* = 6.3 Hz, 2ArH), 6.96–6.90 (3H, m, 3ArH), 4.04 (2H, t, *J* = 4.2 Hz, CH_2), 3.86 (2H, t, *J* = 4.2 Hz, CH_2).

5.10. 1-Bromo-9-butyl-β-carboline (**12a**)

To the solution of 1-iodobutane (0.74 g, 3.0 mmol) in anhydrous DMF (10 mL), 60% NaH (0.08 g, 2.0 mmol) was added. The mixture was stirred at room temperature for 10 min, and then 1-bromo-β-carboline **10** (0.25 g, 1.0 mmol) was added and stirred for 30 min. After completion of the reaction as indicated by TLC, the mixture was evaporated and the residue was added H_2O (50 mL) and extracted with EA. The organic phase was washed with H_2O and brine, then dried over anhydrous Na_2SO_4, filtered and evaporated. The residue obtained was purified by silica column chromatography with ethyl acetate/petroleum ether as the eluent to give yellow needle crystal **12a** (0.28 g, 92%), mp 63–65 °C. ESI-MS *m/z* 302, 304 $[M + H]^+$; IR (KBr) ν 3440, 3058, 3034, 2982, 2956, 2926, 2863, 1621, 1565, 1561, 1533, 1464, 1202, 745 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.18 (1H, d, *J* = 5.1 Hz, ArH), 8.11 (1H, d, *J* = 7.8 Hz, ArH), 7.93 (1H, d, *J* = 5.1 Hz, ArH), 7.63 (1H, t, *J* = 7.5 Hz, ArH), 7.51 (1H, d, *J* = 8.4 Hz, ArH), 7.32 (1H, t, *J* = 7.5 Hz, ArH), 4.78 (2H, t, *J* = 7.8 Hz, CH_2), 1.90 (2H, m, CH_2), 1.53–1.46 (2H, m, CH_2), 1.01 (3H, t, *J* = 7.5 Hz, CH_3). Anal. Calcd for $C_{15}H_{15}BrN_2$: C, 59.42; H, 4.99; N, 9.24. Found: C, 59.62; H, 5.03; N, 9.18.

5.11. 1-Bromo-9-(4-fluorobenzyl)-β-carboline (**12b**)

A mixture of 4-fluorobenzyl bromide (0.38g, 2.0 mmol), potassium iodide (0.17g, 1.0 mmol) and anhydrous DMF (10 mL) was stirred at room temperature for 10 min, and then 60% NaH (0.06 g, 1.5 mmol) was added. After 10 min, the 1-bromo-β-carboline **10** (0.25 g, 1.0 mmol) was added and stirred for 15 min. Later the mixture was treated in a manner similar to that described for **12a** to afford pale yellow needle crystal **12b** (0.32 g, 90%), mp 130–131. ESI-MS *m/z* 354, 356 $[M + H]^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 8.23 (1H, d, *J* = 4.8 Hz, ArH), 8.15 (1H, d, *J* = 7.8 Hz, ArH), 7.98 (1H, d, *J* = 4.8 Hz, ArH), 7.58 (1H, t, *J* = 7.8 Hz, ArH), 7.39 (1H, d, *J* = 7.8 Hz, ArH), 7.34 (1H, t, *J* = 7.8 Hz, ArH), 7.08–6.93 (4H, m, 4ArH), 6.04 (2H, s, CH_2). Anal. Calcd for $C_{18}H_{12}BrFN_2$: C, 60.86; H, 3.41; N, 7.89. Found: C, 60.93; H, 3.46; N, 7.94.

5.12. 1-Bromo-9-perfluorobenzyl-β-carboline (**12c**)

A mixture of perfluorobenzyl bromide (0.31g, 1.2 mmol), potassium iodide (0.17g, 1.0 mmol) and anhydrous DMF (10 mL) was stirred at room temperature for 10 min, and 60% NaH (0.06 g, 1.5 mmol) was added. After 5 min, the 1-bromo-β-carboline **10** (0.25 g, 1.0 mmol) was added and stirred for 5 min. Later the mixture was treated in a manner similar to that described for **12a** to afford pale yellow needle crystal **12c** (0.36 g, 85%), mp 145–146. ESI-MS *m/z* 426, 428 $[M + H]^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 8.25 (1H, d, *J* = 5.1 Hz, ArH), 8.12(1H, dt, *J* = 7.5, 0.9 Hz, ArH), 7.96 (1H, d, *J* = 5.1 Hz, ArH), 7.59 (1H, td, *J* = 7.8, 1.2 Hz, ArH), 7.35 (2H, t, *J* = 7.8 Hz, 2ArH), 6.35–6.27 (1H, m, CH_2). Anal. Calcd for $C_{18}H_8BrF_5N_2$: C, 50.61; H, 1.89; N, 6.56. Found: C, 50.74; H, 1.84; N, 6.63.

5.13. 1-Bromo-9-(3-phenylpropyl)-β-carboline (**12d**)

A mixture of 1-bromo-3-phenylpropane (0.60 g, 3.0 mmol), potassium iodide (0.17 g, 1.0 mmol) and anhydrous DMF (10 mL) was stirred at room temperature for 1 h, and 60% NaH (0.06 g, 1.5 mmol) was added. After 15 min, 1-bromo-β-carboline **10** (0.25 g, 1.0 mmol) was added and stirred for 1 h. Later the mixture was treated in a manner similar to that described for **12a** to afford pale yellow needle crystal **12d** (0.34 g, 93%), mp 113–114 °C. ESI-MS *m/z* 364, 366 $[M + H]^+$; IR (KBr) ν 3424, 3050, 3031, 2930, 2860, 1620, 1533, 1437, 1178, 834, 748, 701 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.18 (1H, d, *J* = 5.1 Hz, ArH), 8.10 (1H, d, *J* = 7.8 Hz, ArH), 7.92 (1H, d, *J* = 5.1 Hz, ArH), 7.59 (1H, t, *J* = 7.5 Hz, ArH), 7.36–7.18 (7H, m, 7ArH), 4.79 (2H, t, *J* = 7.8 Hz, CH_2), 2.80 (2H, t, *J* = 7.8 Hz, CH_2), 2.56–2.48 (2H, m, CH_2). Anal. Calcd for $C_{20}H_{17}BrN_2$: C, 65.76; H, 4.69; N, 7.67. Found: C, 75.89; H, 4.60; N, 7.73.

5.14. 1-(2-Aminoethylamino)-9-butyl-β-carboline hydrochloride salt (**13a**)

A mixture of 1-bromo-9-butyl-β-carboline **12a** (0.30 g, 1.0 mmol), cuprous iodide (0.19 g, 1.0 mmol) and ethylenediamine (2 mL) was heated with stirring at 170 °C under microwave irradiation for 30 min. Then the mixture was added H_2O (50 mL) and extracted with dichloromethane. The organic phase was dried over anhydrous Na_2SO_4, filtered and evaporated. The residue obtained was purified by silica column chromatography with CH_2Cl_2/MeOH (30:1) as the eluent to afford yellow oil **13a** (0.23 g, 82%). The hydrochloride salt of **13a**, prepared with dry HCl gas in MeOH, had mp 224–225 °C. ESI-MS *m/z* 283 $[M + H]^+$; IR (KBr) ν 3388, 3257, 2952, 2924, 2854, 1639, 1604, 1535, 1498, 1459, 1343, 754 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 8.30 (1H, d, *J* = 7.8 Hz, ArH), 8.24 (3H, s, N^+H_3), 7.89 (1H, d, *J* = 7.8 Hz, ArH), 7.86 (1H, d, *J* = 6.6 Hz, ArH), 7.77 (1H, d, *J* = 6.6 Hz, ArH), 7.67 (1H, t, *J* = 7.5 Hz, ArH), 7.35 (1H, t, *J* = 7.5 Hz, ArH), 4.88 (2H, t, *J* = 6.9 Hz, NCH_2), 4.07–3.98 (2H, m, CH_2), 3.24–3.15 (2H, m, CH_2), 1.64–1.58 (2H, m, CH_2), 1.19–1.11 (2H, m, CH_2), 0.80 (3H, t, *J* = 7.2 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 146.8, 141.3, 136.7, 128.7, 127.2, 124.8, 122.0, 121.4, 119.5, 109.9, 106.2, 45.5, 44.2, 34.0, 30.1, 20.6, 14.3.

5.15. 1-(2-Aminoethylamino)-9-(4-fluorobenzyl)-β-carboline (**13b**)

A mixture of 1-bromo-9-(4-fluorobenzyl)-β-carboline **12b** (0.36 g, 1.0 mmol) and ethylenediamine (20 mL) was subjected to the same procedure which was used to prepare **13a** afforded the target product **13b** in 65% yield. ESI-MS *m/z* 335 $[M + H]^+$; IR (KBr) *v* 3424, 3057, 2927, 2870, 1620, 1598, 1560, 1508, 1453, 741 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.07 (1H, d, $J = 7.8$ Hz, ArH), 7.97 (1H, d, $J = 5.4$ Hz, ArH), 7.49 (1H, t, $J = 7.8$ Hz, ArH), 7.38 (1H, d, $J = 5.4$ Hz, ArH), 7.34 (1H, d, $J = 8.4$ Hz, ArH), 7.27 (1H, t, $J = 7.8$ Hz, ArH), 7.15–7.10 (2H, m, 2ArH), 7.00 (2H, t, $J = 8.4$ Hz, ArH), 5.67 (2H, s, CH_2), 4.87 (1H, s, NH), 3.46 (2H, t, $J = 5.1$ Hz, CH_2), 2.83 (2H, t, $J = 5.1$ Hz, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.1, 160.8, 146.9, 141.7, 137.5, 133.6, 129.0, 127.7, 127.7, 127.7, 125.1, 122.2, 121.5, 120.2, 116.5, 116.2, 109.6, 106.3, 47.9, 44.8, 41.5. The hydrochloride salt of **13b**, prepared with dry HCl gas in MeOH, had mp 253–255 °C.

5.16. 1-(2-Aminoethylamino)-9-perfluorobenzyl-β-carboline (**13c**)

A mixture of 1-bromo-9-perfluorobenzyl-β-carboline **12c** (0.43 g, 1.0 mmol) and ethylenediamine (20 mL) was subjected to the same procedure which was used to prepare **13a** afforded the target product **13c** in 37% yield. ESI-MS *m/z* 407 $[M + H]^+$; IR (KBr) *v* 3303, 3036, 2971, 2914, 1637, 1532, 1504, 1464, 753 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 9.01 (1H, s, NH), 8.46 (1H, d, $J = 5.4$ Hz, ArH), 8.12 (1H, dt, $J = 7.8$, 0.9 Hz, ArH), 7.94 (1H, d, $J = 5.4$ Hz, ArH), 7.62–7.60 (2H, m, 2ArH), 7.32–7.27 (1H, m, ArH), 5.53 (2H, s, CH_2), 4.46 (1H, s, NH), 3.41–3.35 (2H, m, CH_2), 2.89 (2H, t, $J = 6.0$ Hz, CH_2). The hydrochloride salt of **13c**, prepared with dry HCl gas in MeOH, had mp >270 °C.

5.17. 1-(2-Aminoethylamino)-9-(3-phenylpropyl)-β-carboline hydrochloride salt (**13d**)

A mixture of 1-bromo-9-(3-phenylpropyl)-β-carboline **12d** (0.37 g, 1.0 mmol) and ethylenediamine (20 mL) was subjected to the same procedure which was used to prepare **13a** afforded the target product **13d** with 87% yield. The hydrochloride salt of **13d**, prepared with dry HCl gas in MeOH, had mp 256–258 °C. ESI-MS *m/z* 345 $[M + H]^+$; IR (KBr) *v* 3407, 3269, 3027, 2921, 2853, 1638, 1605, 1537, 1405, 1460, 1344, 752, 699 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 8.29 (1H, d, $J = 7.8$ Hz, ArH), 8.19 (3H, s, N^+H_3), 7.84 (1H, d, $J = 5.4$ Hz, ArH), 7.78–7.75 (2H, m, 2ArH), 7.65 (1H, t, $J = 7.8$ Hz, ArH), 7.36 (1H, t, $J = 7.8$ Hz, ArH), 7.21–7.05 (5H, m, 5ArH), 4.91 (2H, t, $J = 7.2$ Hz, NCH_2), 4.09–4.03 (2H, m, CH_2), 3.22–3.16 (2H, m, CH_2), 2.56 (2H, t, $J = 7.2$ Hz, CH_2), 2.01–1.91 (2H, m, CH_2); ^{13}C NMR (75 MHz, DMSO-d_6) δ 143.6, 143.0, 141.6, 134.9, 130.4, 130.0, 128.8, 128.7, 126.4, 123.0, 122.8, 121.9, 120.9, 112.0, 107.3, 45.2, 38.2, 33.1, 32.7.

5.18. 1-(4-Aminobutylamino)-9-(3-phenylpropyl)-β-carboline (**13e**)

A mixture of 1-bromo-9-(3-phenylpropyl)-β-carboline **12d** (0.37 g, 1.0 mmol) and 1, 4-diaminobutane (2 mL) was heated with stirring at 150 °C under microwave irradiation for 30 min. Then the mixture was treated in a manner similar to that described for **13a** to afford yellow oil **13e** (0.26 g, 71%). ESI-MS *m/z* 373 $[M + H]^+$; IR (KBr) *v* 3403, 3056, 3027, 2929, 2860, 1620, 1590, 1560, 1454, 740 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.01 (1H, d, $J = 7.8$ Hz, ArH), 7.96 (1H, d, $J = 5.4$ Hz, ArH), 7.47 (1H, t, $J = 7.8$ Hz, ArH), 7.34–7.15 (8H, m, 8ArH), 4.40–4.31 (3H, m, NH, CH_2), 3.47 (2H, t, $J = 6.9$ Hz, CH_2), 2.76 (2H, t, $J = 6.6$ Hz, CH_2), 2.69 (2H, t, $J = 6.6$ Hz, CH_2), 2.20–2.10 (2H, m, CH_2), 1.66–1.53 (4H, m, $2CH_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 146.5, 141.0, 140.7, 137.0, 128.9, 128.7, 128.6, 127.2, 126.6, 124.4, 122.1, 121.4, 119.6, 109.8, 106.1, 44.9, 42.3, 42.2, 33.3, 32.9, 31.6, 27.5. The hydrochloride salt of **13e**, prepared with dry HCl gas in MeOH, had mp 220–222 °C.

5.19. 1-(6-Aminohexylamino)-9-(3-phenylpropyl)-β-carboline hydrochloride salt (**13f**)

A mixture of 1-bromo-9-(3-phenylpropyl)-β-carboline **12d** (0.37 g, 1.0 mmol) and 1, 6-hexanediamine (1.16g, 10 mmol) was heated with stirring at 210 °C under microwave irradiation for 30 min. Then the mixture was treated in a manner similar to that described for **13a** to afford yellow oil **13f** (0.23 g, 56%). The hydrochloride salt of **13f**, prepared with dry HCl gas in MeOH, had mp 186–188 °C. ESI-MS *m/z* 401 $[M + H]^+$; IR (KBr) *v* 3170, 3030, 2937, 1642, 1618, 1535, 1487, 754, 700 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 13.63 (1H, s, NH), 10.60 (1H, s, NH), 8.29 (1H, d, $J = 8.1$ Hz, ArH), 8.25 (1H, s, NH), 7.83–7.74 (3H, m, 3ArH), 7.65 (1H, t, $J = 7.8$ Hz, ArH), 7.36 (1H, d, $J = 7.5$ Hz, ArH), 7.22–7.05 (5H, m, 5ArH), 4.91 (2H, t, $J = 6.6$ Hz, CH_2), 3.86–3.80 (2H, m, CH_2), 3.22–3.16 (2H, m, CH_2), 2.75–2.70 (6H, m, $3CH_2$), 2.56 (2H, t, $J = 7.8$ Hz, CH_2), 2.16–2.07 (2H, m, CH_2), 1.98–1.90 (2H, m, CH_2).

5.20. Cytotoxicity assays in vitro

Cytotoxicity assays *in vitro* were carried out using 96 microtitre plate cultures and MTT staining according to the procedures described by our group [22]. Briefly, cells were grown in RPMI-1640 medium containing 10% (v/v) fetal calf serum and 100 μg ml^{-1} penicillin and 100 μg ml^{-1} streptomycin. Cultures were propagated at 37 °C in a humidified atmosphere containing 5% CO_2. Drug stock solutions were prepared in DMSO. The final concentration of DMSO in the growth medium was 2% (v/v) or lower, concentration without effect on cell replication. The tumor cell line panel consisted of gastric carcinoma (BGC823), liver carcinoma (HepG2), breast carcinoma (MCF-7), renal carcinoma (OS-RC-2, 786-0 and 769-P), melanoma (A375), colon carcinoma (HT-29), epidermoid carcinoma of the nasopharynx (KB), prostate carcinoma (22RV1). In all of these experiments, five replicate wells were used to determine each point.

Acknowledgements

This work was supported by MEGA-Project (2009ZX09103-015) and the Science and Technology Project of Guangdong Province, China (2009B060700048) and the Fundamental Research Funds for the Central Universities and Xinjiang Huashidan Pharmaceutical Co. Ltd.

References

[1] R. Cao, W. Peng, Z. Wang, A. Xu, Curr. Med. Chem. 14 (2007) 479–500.
[2] J. Ishida, H.K. Wang, K.F. Bastow, C.Q. Hu, K.H. Lee, Bioorg. Med. Chem. Lett. 9 (1999) 3319–3324.
[3] S. Xiao, W. Lin, C. Wang, M. Yang, Bioorg. Med. Chem. Lett. 11 (2001) 437–441.
[4] Y.C. Shen, C.Y. Chen, P.W. Hsieh, C.Y. Duh, Y.M. Lin, C.L. Ko, Chem. Pharm. Bull. 53 (2005) 32–36.
[5] A.M. Deveau, M.A. Labroli, C.M. Dieckhaus, M.T. Barthen, K.S. Smith, T.L. Macdonald, Bioorg. Med. Chem. Lett. 11 (2001) 1251–1255.
[6] M. Zhao, L. Bi, W. Wang, C. Wang, M. Baudy-Floc'h, J. Ju, S. Peng, Bioorg. Med. Chem. 14 (2006) 6998–7010.
[7] A.S.N. Formagio, L.T.D. Dusman, M.A. Foglio, C. Madjarof, J.E. Carvalho, W.F. Costa, F.P. Cardoso, M.H. Sarragiotto, Bioorg. Med. Chem. 16 (2008) 9660–9667.
[8] J. Wu, M. Zhao, K. Qian, K.-H. Lee, S. Morris-Natschke, S. Peng, Eur. J. Med. Chem. 44 (2009) 4153–4161.
[9] Y. Boursereau, I. Coldham, Bioorg. Med. Chem. Lett. 14 (2004) 5841–5844.
[10] W. Jiang, C. Charlet-Fagnere, J. Sapi, J.-Y. Laronze, P. Renard, B. Pfeiffer, S. Leonce, J. Enzyme Inhib. Med. Chem. 17 (2002) 369–374.
[11] K. Hayashi, M. Nagao, T. Sugimura, Nucleic Acids Res. 4 (1977) 3679–3685.

[12] Y. Funayama, K. Nishio, K. Wakabayashi, M. Nagao, K. Shimoi, T. Ohira, S. Hasegawa, M. Saijo, Mutat. Res. 349 (1996) 183–191.
[13] A.M. Sobhani, S.A. Ebrahimi, M. Mahmoudian, J. Pharm. Pharm. Sci. 5 (2002) 19–23.
[14] Y. Song, J. Wang, S.F. Teng, D. Kesuma, Y. Deng, J. Duan, J.H. Wang, R.Z. Qi, M.M. Sim, Bioorg. Med. Chem. Lett. 12 (2002) 1129–1132.
[15] Y. Song, D. Kesuma, J. Wang, Y. Deng, J. Duan, J.H. Wang, R.Z. Qi, Biochem. Biophys. Res. Commun. 317 (2004) 128–132.
[16] Y. Li, F. Liang, W. Jiang, F. Yu, R. Cao, Q. Ma, X. Dai, J. Jiang, Y. Wang, S. Si, Cancer Biol. Ther. 6 (2007) 1193–1199.
[17] A.C. Castro, L.C. Dang, F. Soucy, L. Grenier, H. Mazdiyasni, M. Hottelet, L. Parent, C. Pien, V. Palombella, J. Adams, Bioorg. Med. Chem. Lett. 13 (2003) 2419–2422.
[18] 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, Bioorg. Med. Chem. Lett. 17 (2007) 4657–4663.
[19] 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, Cancer Biol. Ther. 8 (2009) 2374–2383.
[20] P.A. Barsanti, W. Wang, Z. Ni, D. Duhl, N. Brammeier, E. Martin, Bioorg. Med. Chem. Lett. 20 (2010) 157–160.
[21] A.C. Castro, L.C. Dang, F. Soucy, L. Grenier, H. Mazdiyasni, M. Hottelet, L. Parent, C. Pien, V. Palombella, J. Adams, Bioorg. Med. Chem. Lett. 13 (2003) 2419–2422.
[22] R. Cao, Q. Chen, X. Hou, H. Chen, H. Guan, Y. Ma, W. Peng, A. Xu, Bioorg. Med. Chem. 12 (2004) 4613–4623.
[23] R. Cao, W. Peng, H. Chen, X. Hou, H. Guan, Q. Chen, Y. Ma, A. Xu, Eur. J. Med. Chem. 40 (2005) 249–257.
[24] R. Cao, H. Chen, W. Peng, Y. Ma, X. Hou, H. Guan, X. Liu, A. Xu, Eur. J. Med. Chem. 40 (2005) 991–1001.
[25] H. Guan, H. Chen, W. Peng, Y. Ma, R. Cao, X. Liu, A. Xu, Eur. J. Med. Chem. 41 (2006) 1167–1179.
[26] Q. Wu, R. Cao, M. Feng, X. Guan, C. Ma, J. Liu, H. Song, W. Peng, Eur. J. Med. Chem. 44 (2009) 533–540.
[27] C. Ma, R. Cao, B. Shi, S. Li, Z. Chen, W. Yi, W. Peng, Z. Ren, H. Song, Eur. J. Med. Chem. 45 (2010) 1515–1523.
[28] K. Takasu, T. Shimogama, C. Saiin, H.-S. Kim, Y. Wataya, R. Brun, M. Ihara, Chem. Pharm. Bull. 53 (2005) 653–661.
[29] F. Bracher, D. Hildebrand, Tetrahedron 50 (1994) 12329–12336.
[30] R. Wen, L. Huang, W. Jiang, X. Dong, H. Wang, P. Zhou. Faming Zhuanli Shenqing Gongkai Shuomingshu (2004), CNo 1,472,208 A.
[31] R. Cao, W. Peng, H. Chen, Y. Ma, X. Liu, X. Hou, H. Guan, A. Xu, Biochem. Biophys. Res. Commun. 338 (2005) 1557–1563.
[32] H. Guan, X. Liu, W. Peng, R. Cao, Y. Ma, H. Chen, A. Xu, Biochem. Biophys. Res. Commun. 342 (2006) 894–901.