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European Journal of Medicinal Chemistry 40 (2005) 249–257
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
www.elsevier.com/locate/ejmech

Original article

Synthesis and in vitro cytotoxic evaluation of 1,3-bisubstituted and 1,3,9-trisubstituted β-carboline derivatives

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Received 28 June 2004; received in revised form 8 November 2004; accepted 9 November 2004

Available online 13 January 2005

Abstract

A series of novel 1,3-bisubstituted and 1,3,9-trisubstituted β-carboline derivatives was synthesized from the starting material L-tryptophan. Cytotoxic activities of these compounds were investigated in vitro. The results showed that 1,3,9-trisubstituted β-carboline derivatives had higher cytotoxic activities in vitro than the corresponding 1,3-bisubstituted compounds. Among all the synthesized 1,3,9-trisubstituted β-carboline derivatives, the compounds with a methyl substituent at position-1 displayed more potent cytotoxic activities, furthermore compound **5e** having an ethoxycarbonyl substituent at position-3 and a pentafluorobenzyl at position-9, respectively, was found to be the most potent compounds of this series with IC_{50} value of 4 uM against BGC-823 cell lines. These data suggested that (1) the cytotoxic potencies of β-carboline derivatives were enhanced by the introduction of appropriate substituents into position-1 and position-9 in β-carboline; (2) the β-carboline structure might be an important basis for the design and synthesis of new antitumor drugs; (3) the methyl substituent at position-1, the pentafluorobenzyl group at position-9 and the ethoxycarbonyl substituent at position-3 were the optimal combination for the improvement of cytotoxic activity of the β-carboline derivatives.

Keywords: Synthesis; Cytotoxic; β-Carboline; Structure–activity relationships

1. Introduction

β-Carboline derivatives, containing planar polycyclic systems, represent a large number of naturally and synthetic indole alkaloids [1–8]. In the past several decades, these compounds attracted considerable attention owing to their biological and pharmaceutical importance [9–16]. The reported effects of this class of compounds comprise anticonvulsive, anxiolytic, sedative [9–11], antimicrobial [12], antithrombotic [13], anti-HIV [4], parasiticidal [14], intercalation DNA [15,16], CDK inhibition [17] as well as inhibition Topisomerase [18,19]. Recent work [20–23] had reported that this class compounds with β-carboline nucleus had potent cytotoxic activities in vitro against human tumor cell lines. However, many previous reports mainly concentrated on neurochemical and neuropharmacological roles of this class of compounds, so far few investigations have been reported on the antitumor activity of this class of compounds against human tumor cell lines in vitro, especially there was no information of systematic and detailed studies of structure–activity relationships on their antitumor activities and neurotoxic activities in vivo. In the present work, we reported such an investigation of synthesis and cytotoxicity evaluation of novel β-carboline derivatives on the basis of previous investigations and reports. This paper aims at elucidating the preliminary structure–activity relationships by simple chemical structural modification and probing structural requirement for the marked antitumor activity of these compounds. Another objective of the study was to search for and develop novel and more potent antitumor drugs with lower toxicity, based on this investigation.

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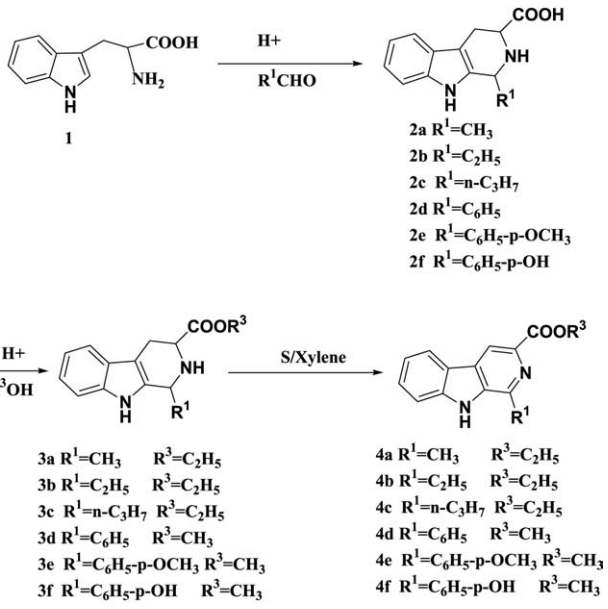
doi:10.1016/j.ejmech.2004.11.005

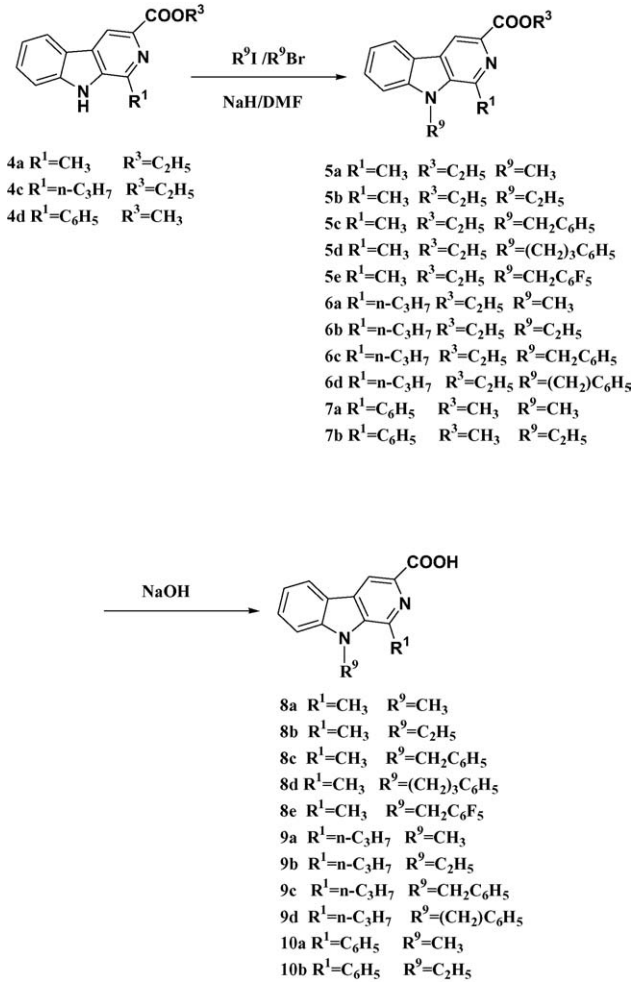
2. Chemistry

Pictet-Spengler cyclization [5,24,33] of L-tryptophan (**1**) in the presence of the appropriate aldehyde (R^1CHO) afforded the corresponding diastereoisomeric mixture 1,2,3,4-tetrahydro-βcarboline-3-carboxylic acid (**2a–f**). Esterification [5] of **2a–f** with ethanol or methanol in the presence of $SOCl_2$ yielded the respective 1,2,3,4-tetrahydro-β-carboline-3-carboxylate (**3a–f**), followed by dehydrogenation [25,29–31] with sulfur in xylene gave 1-substituted β-carboline-3-carboxylate (**4a–f**), respectively, as described in Scheme 1. All of the above tetrahydro-β-carbolines was devoid of cytotoxic activity against human tumor cell lines, therefore, in the present work the separation and purification of these compounds were not necessary.

Substitution of alkyl or benzyl groups at the N^9-position of β-carboline ring system would be expected to enhance cytotoxic activities, consequently a number of derivatives were designed and synthesized with substituents ranging in size from methyl group to phenylpropyl. The N^9-position of compounds **4a**, **4c** and **4d** were alkylated [26,27] or benzylated [27] by the action of sodium hydride in anhydrous DMF followed by addition of the relevant appropriate alkylating and benzylating agent to afford compounds **5a–e**, **6a–d**, **7a–b**, followed by hydrolyzation [5] in alkaline solution to provide the corresponding β-carboline-3-carboxylic acids (**8a–f**, **9a–d**, **10a–b**) as outlined in Scheme 2. The chemical structures of all the synthesized novel compounds were confirmed by FAB-MS, UV, IR, ^{1}H-NMR and elemental analyses data.



Scheme 1. Synthesis of 1,3-substituted β-carboline derivatives.



Scheme 2. Synthesis of 1,3,9-trisubstituted β-carboline derivatives.

3. Biological results

All the new synthesized compounds were tested for their cytotoxic activities in vitro against a panel of human tumor cell lines. Compounds were converted into their water-soluble hydrochlorides (**4a–f**, **5a–e**, **6a–d**, **7a–b**) or sodium salts (**8a–e**, **9a–d**, **10a–b**) by the usual methods before use. The results were summarized in Table 1.

As shown in Table 1, some compounds (e.g. **4a**, **5a**, **5b**, **5d**, **5e**) showed significant cytotoxic activities while others (e.g. **6a**, **6b**, **9c**, **9d**, **10a**, **10b**) displayed medium cytotoxic activities against human tumor cell lines, and others (e.g. **4b**, **4c**, **4f**) only had marginal or no cytotoxic effect in any cell lines. Among all the synthesized β-carboline derivatives, 1,3,9-trisubstituted-β-carboline-3-carboxylate demonstrated higher cytotoxic activities in vitro than the corresponding 1,3-bisubstituted compounds. Furthermore, the Lovo cell lines were more sensitive to these compounds than other cell lines. These data indicated that the cytotoxic potencies of β-carboline were enhanced by the introduction of appropriate substituent into N^9-position of β-carboline ring.

Of all 1,3-bisubstituted β-carboline derivatives, compound **4a**, having a methyl group at positon-1, displayed potent cytotoxic activity, however, the others were inactive against tumor cell lines tested. These results implied that the methyl group represented the most optimal structure at position-1 in β-carboline for these compounds class to exhibit remarkable cytotoxicity.

Table 1
Cytotoxicity of β-carboline derivatives in vitro[c] (μM)

	IC_{50} (μM)[a]					
Compound	PLA-801[b]	HepG2[b]	Bel-7402[b]	BGC-823[b]	Hela[b]	Lovo[b]
Harmine	45	46	54	68	60	66
4a	262	86	227	111	97	83
4b	>1000	>1000	>1000	>1000	>1000	>1000
4c	668	>1000	>1000	>1000	>1000	>1000
4d	>1000	>1000	>1000	>1000	318	>1000
4e	>1000	>1000	>1000	965	154	619
4f	>1000	>1000	>1000	>1000	>1000	>1000
5a	138	102	485	131	112	52
5b	158	83	84	73	63	50
5c	386	276	209	784	255	174
5d	111	40	78	43	42	25
5e	47	35	38	4	19	39
6a	142	103	462	109	>1000	61
6b	706	107	326	232	95	104
6c	157	79	>1000	>1000	807	>1000
6d	259	120	911	>1000	62	59
7a	>1000	>1000	624	>1000	>1000	>1000
7b	>1000	>1000	380	>1000	>1000	>70
8a	>1000	>1000	>1000	641	>1000	435
8b	>1000	>1000	>1000	>1000	967	594
8c	405	280	692	278	373	204
8d	208	144	560	270	222	53
8e	93	118	120	156	114	84
9a	>1000	>1000	>1000	545	955	210
9b	714	536	569	395	421	256
9c	270	168	184	267	158	137
9d	226	127	204	238	136	83
10a	652	163	282	210	173	110
10b	113	165	142	254	136	101

[a] Cytotoxicity as IC_{50} for each cell line, is the concentration of compound that causes a 50% growth inhibition to untreated cells using the MTT assay.
[b] Cell lines include non-small cell lung carcinoma (PLA-801), liver carcinoma (HepG2 and Bel-7402), gastric carcinoma (BGC-823), cervical carcinoma (Hula), colon carcinoma (Lovo).
[c] Data represent the mean values of three independent determinations.

Among all 1,3,9-trisubstituted β-carboline derivatives, compounds **5a**–**e** (except **5c**), which all had a methyl at position-1 demonstrated more cytotoxic activities than the other two series which had an *n*-propyl (**6a**–**d**) and a phenyl (**7a**–**b**) at position-1, respectively. Furthermore, compound **5e**, which has a methyl group at position-1, an ethoxycarbonyl substituent at position-3 and a pentafluorobenzyl group at position-9, respectively, was the most active compound with IC_{50} values of lower than 50 uM against all cell lines screened, and particularly exhibited the highest cytotoxic activity against BGC-823 cell lines with IC_{50} value of 4 uM. These data suggested that the cytotoxicity was maximal with a methyl group at position-1 and a pentafluorobenzyl substituent at position-9 of the β-carboline.

Unlike compounds **5a**–**e**, having an ethoxycarbonyl substituent at positon-3, their hydrolyzed congener **8a**–**e** (except **8c**), which had a free carboxylic acid group at position-3 exhibited comparatively weaker cytotoxic activities against all tested human tumor cell lines. Similarly, compared to compounds **6a**–**b**, compounds **9a**–**b** had no significant cytotoxicity in any cell lines. Conversely, **9c**–**d** and **10a**–**b** displayed significant cytotoxic activities, but their congener **6c**–**d** and **7a**–**b** exhibited weaker or no cytotoxic effect on tumor cell lines. In contrast to compounds with an ethoxycarbonyl at position-3, their hydrolyzed products having improved solubility in aqueous solution, presented a marked difference in the cytotoxic activity. These results implied that the cytotoxic potencies of these β-carboline derivatives depend upon the presence and location and nature of the subtituents, which were introduced into the β-carboline nucleus.

An overview of the cytotoxic activities data of all new synthesized β-carboline derivatives clearly indicated that substituents at position-1 and position-9 in β-carboline played a very vital role for exerting cytotoxic effect on human tumor cell lines. Meanwhile 3-ethoxycarbonyl substituent might be superior to 3-carboxylic acid for the series of compounds with a methyl group at position-1. The optimal combination of substituents in β-carboline nucleus was a methyl group at position-1, an ethoxycarbonyl at position-3 and a pentafluorobenzyl at position-9, respectively, which led to the most active compound **5e** with the lowest IC_{50} values of 4 uM against BGC-823.

A total analysis to the cytotoxic activities of β-carboline derivatives which had been reported above and of the previ-

ous investigation [26] clearly suggested that (1) the cytotoxic potencies of β-carboline derivatives were enhanced by the introduction of appropriate substituents into position-1 and position-9 in β-carboline nucleus; (2) the β-carboline structure might be an important basis for the design and synthesis of new antitumor drugs; (3) the methyl substituent at position-1, the pentafluorobenzyl group at position-9 and the ethoxycarbonyl substituent at position-3 were the optimal combination for enhancing cytotoxic activity of the β-carboline derivatives.

We have explored the influence of substituents at position-1, 3 and 9 in β-carboline nucleus on the ability of these compounds against human tumor cell lines. To acquire more information about the structural requirements for the possible improvement of the cytotoxic properties, synthesis of additional new β-carboline derivatives with various subtistuents at other positions of the β-carboline nucleus is desirable. Further biological evaluation, which are required to confirm these results in animal models on these β-carboline derivatives are in progress in our laboratories. Moreover, the molecular mechanisms of these compounds are ongoing to further design and develop more potent compounds.

4. Experimental

4.1. Chemistry

All reagents were purchased from commercial suppliers and were dried and purified when necessary. Harmine (purity 99.85%) was extracted from *Peganum multisectum Maxim*, a plant indigenous to western China, according to the method by Duan et al. [28]. Melting points (m.p.) were determined in capillary tubes on an electrothermal PIFYRT-3 apparatus and without correction. UV spectra were measured on Shimadzu UV 2501PC Spectrometer. FAB-MS spectra were obtained from VG ZAB-HS spectrometer. FT-IR spectra were run on a Bruker Equinox 55 Fourier Transformation Infared Spectrometer. ^{1}H-NMR spectra were recorded on a Varian INOVA 500NB spectrometer. Elemental analyses were carried out on an Elementar Vario EL CHNS Elemental Analyzer. Silica gel F_{254} was used in analytical thin-layer chromatography (TLC) and silica gel was used in column chromatography.

*4.1.1. General procedure for the preparation of 1-substituted 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acids (**2a–c**)*

A mixture of L-tryptophan (20.4 g, 100 mmol), aldehydes (300 mmol, acetaldehyde for **2a**, propionaldehyde for **2b**, *n*-butyraldehyde for **2c**), 0.5 M H_2SO_4 (2.5 ml) and H_2O (200 ml) was stirred at room temperature for about 10 h and detected by TLC. The precipitate was filtered and washed well with water, dried in vacuum. The material was used without further purification for the following steps.

*4.1.1.1. 1-Methyl-1,2,3,4-tetrahydro-β-arboline-3-carboxylic acid (**2a**).* Compound **2a** was obtained as white solid in 80% yield.

*4.1.1.2. 1-Ethyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (**2b**).* Compound **2b** was obtained as white solid in 82% yield.

*4.1.1.3. 1-*n*-Propyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (**2c**).* Compound **2c** was obtained as white solid in 75% yield.

*4.1.2. General procedure for the preparation of 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acids (**2d–f**)*

A mixture of L-tryptophan (10.2 g, 50 mmol), acetic acid (200 ml) and aldehyde (55 mmol, benzaldehyde for **2d**, homoanisaldehyde for **2e**, *p*-hydroxybenzaldehyde for **2f**) was refluxed for 2 h, then cooled and adjusted pH to 5 with concentrated ammonium hydroxide, the prepricitated product was collected by filtration and washed well with water and then dried. Further purification was not necessary and used directly for the next steps.

*4.1.2.1. 1-Phenyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (**2d**).* Compound **2d** was obtained as white solid in 87% yield.

*4.1.2.2. 1-(4-Methoxy) phenyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (**2e**).* Compound **2e** was obtained as white solid in 79% yield.

*4.1.2.3. 1-(4-Hydroxy) phenyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (**2f**).* Compound **2f** was obtained as white solid in 75% yield.

*4.1.3. General procedure for the preparation of 1,2,3,4-tetrahydro-β-carboline-3-carboxylates (**3a–f**)*

A solution of the corresponding 1,2,3,4-tetrahydro compounds **2a–f** (20 mmol) and alcohol (500 ml, ethanol for **2a–c** and methanol for **2d–f**) and $SOCl_2$ (20 ml) was heated at reflux for 2 h, then evaporated in reduced pressure. The resulting mixture was poured into H_2O (200 ml), and extracted with ethyl acetate (3 × 200 ml). The organic phase was washed with water and brine, then dried over anhydrous sodium sulfate, filtered and evaporated.

*4.1.3.1. Ethyl 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylate (**3a**).* Compound **3a** was obtained as yellow oil.

*4.1.3.2. Ethyl 1-ethyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylate (**3b**).* Compound **3b** was obtained as yellow oil.

*4.1.3.3. Ethyl 1-*n*-propyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylate (**3c**).* Compound **3c** was obtained as yellow oil.

*4.1.3.4. Ethyl 1-phenyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylate (**3d**).* Compound **3d** was obtained as yellow oil.

*4.1.3.5. Ethyl 1-(4-methoxy) phenyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylate (**3e**).* Compound **3e** was obtained as yellow oil.

*4.1.3.6. Ethyl 1-(4-hydroxy) phenyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylate (**3f**).* Compound **3f** was obtained as yellow oil.

*4.1.4. General procedure for the preparation of β-carboline-3-carboxylates (**4a–f**)*

A suspension of the corresponding 1,2,3,4-tetrahydro-β-carboline-3-carboxylate (**3a–f**) (10 mmol) and sulfur (25 mmol) in xylene (200 ml) was heated at reflux for 6 h, then cooled and stored at 4 °C for 3 h, and then filtered and washed generously with petroleum, the solid was dried and crystallized from ethyl acetate.

*4.1.4.1. Ethyl 1-methyl-β-carboline-3-carboxylate (**4a**).* Afforded white solid (15 g, 75%). M.p. 217–218 °C (lit [29] m.p. 248–249 °C); FAB-MS *m/z* (M + 1) 255; UV λ_{max} 345, 330, 303, 270, 236, 219 nm; IR (KBr) 3316, 3041, 2978, 1709, 1567, 1499, 1367, 1344, 1254, 1145, 1031 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 10.50 (1H, s, NH), 8.78 (1H, s, H-4), 8.15–8.17 (1H, d, J = 8 Hz, H-8), 7.58–7.60 (1H, d, J = 8 Hz, H-5), 7.52–7.55 (1H, m, H-6), 7.30–7.33 (1H, m, H-7), 4.44–4.48 (2H, m, OCH_2CH_3), 2.68 (3H, s, CH_3), 1.32–1.35 (3H, m, OCH_2CH_3). Anal. Calc. for $C_{15}H_{14}N_2O_2$: C, 70.87; H, 5.51; N, 11.02. Found: C, 70.65; H, 5.69; N, 11.12.

*4.1.4.2. Ethyl 1-ethyl-β-carboline-3-carboxylate (**4b**).* Afforded white solid (15 g, 75%). M.p. 209–210 °C (from ethyl acetate); FAB-MS *m/z* (M + 1) 269; UV λ_{max} 345, 331, 303, 270, 236 nm; IR (KBr) 3327, 2974, 2930, 1705, 1566, 1498, 1451, 1346, 1257, 1143, 1043 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 9.63 (1H, s, NH), 8.76 (1H, s, H-4), 8.16–8.17 (1H, d, J = 8 Hz, H-8), 7.60–7.62 (1H, d, J = 8.5 Hz, H-5), 7.54–7.57 (1H, m, H-6), 7.31–7.34 (1H, m, H-7), 4.47–4.51 (2H, m, OCH_2CH_3), 3.11–3.15 (2H, m, CH_2CH_3), 1.40–1.43 (3H, m, OCH_2CH_3), 1.29–1.32 (3H, m, CH_2CH_3). Anal. Calc. for $C_{16}H_{16}N_2O_2$: C, 71.64; H, 5.97; N, 10.45. Found: C, 71.52; H, 6.21; N, 10.36.

*4.1.4.3. Ethyl 1-*n*-propyl-β-carboline-3-carboxylate (**4c**).* Afforded white solid (15 g, 75%). M.p. 194–195 °C (from ethyl acetate); FAB-MS *m/z* (M + 1) 283; UV λ_{max} 346, 331, 302, 271, 237 nm; IR (KBr) 3329, 2963, 1706, 1567, 1498, 1367, 1344, 1252 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 11.14 (1H, s, NH), 8.79 (1H, s, H-4), 8.15–8.17 (1H, d, J = 7.5 Hz, H-8), 7.64–7.66 (1H, d, J = 8 Hz, H-5), 7.51–7.54 (1H, m, H-6), 7.25–7.31 (1H, m, H-7), 4.42–4.46 (2H, m, OCH_2CH_3), 2.76–2.79 (2H, m, $CH_2CH_2CH_3$), 1.45–1.51 (2H, m, $CH_2CH_2CH_3$), 1.25–1.32 (3H, m, OCH_2CH_3), 0.37–0.43 (3H, m, $CH_2CH_2CH_3$). Anal. Calc. for $C_{17}H_{18}N_2O_2$: C, 72.34; H, 6.38; N, 9.93. Found: C, 72.21; H, 6.58; N, 9.85.

*4.1.4.4. Methyl 1-phenyl-β-carboline-3-carboxylate (**4d**).* Afforded white solid (15 g, 75%). M.p. 257–258 °C (lit [30] m.p. 257–260 °C, [31] m.p. 253 °C); FAB-MS *m/z* (M + 1) 303; UV λ_{max} 355, 344, 279, 231 nm; IR (KBr) 3315, 1720, 1623, 1350, 1251, 1215, 1098 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.91 (1H, s, NH), 8.86 (1H, s, H-4), 8.20–8.21 (1H, d, J = 8 Hz, H-8), 7.90–7.91 (2H, m, H-5, H-6), 7.58–7.60 (2H, m, H-7, Ar–H), 7.54–7.57 (2H, m, Ar–H), 7.41–7.44 (1H, m, Ar–H), 7.35–7.37 (1H, m, Ar–H), 4.04 (3H, s, OCH_3). Anal. Calc. for $C_{19}H_{14}N_2O_2$: C, 75.50; H, 4.64; N, 9.27. Found: C, 75.36; H, 4.86; N, 9.18.

*4.1.4.5. Methyl 1-(4-methoxy) phenyl-β-carboline-3-carboxylate (**4e**).* Afforded white solid (15 g, 75%). M.p. 229–230 °C (lit [30] m.p. 229–231 °C); FAB-MS *m/z* (M + 1) 333; UV λ_{max} 357, 347, 284, 268, 230, 215 nm; IR (KBr) 3639, 3320, 1714, 1611, 1512, 1351, 1255, 1103, 1033 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 9.40 (1H, s, NH), 8.76 (1H, s, H-4), 8.15–8.16 (1H, d, J = 8 Hz, H-8), 7.69–7.70 (2H, d, J = 8.5 Hz, H-5, H-6), 7.54–7.56 (2H, m, H-7, Ar–H), 7.31–7.34 (1H, m, Ar–H), 6.75–6.7 (2H, d, J = 8.5 Hz, Ar–H), 4.00 (3H, s, OCH_3), 3.69 (3H, s, Ar–OCH_3). Anal. Calc. for $C_{20}H_{16}N_2O_3$: C, 72.29; H, 4.82; N, 8.43. Found: C, 72.08; H, 5.05; N, 8.29.

*4.1.4.6. Methyl 1-(4-hydroxy) phenyl-β-carboline-3-carboxylate (**4f**).* Afforded white solid (15 g, 75%). M.p. 267–269 °C (from ethyl acetate); FAB-MS *m/z* (M + 1) 319; UV λ_{max} 387, 340, 327, 285, 215 nm; IR (KBr) 3459, 3159, 1715, 1691, 1610, 1513, 1432, 1352, 1260 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 9.51 (1H, s, NH), 8.83 (1H, d, J = 8 Hz, H-4), 8.20–8.21 (1H, d, J = 8 Hz, H-8), 7.83–7.85 (2H, d, J = 8.5 Hz, H-5, H-6), 7.60–7.61 (2H, m, H-7, Ar–H), 7.38–7.39 (1H, m, Ar–H), 7.03–7.04 (2H, d, J = 8 Hz, Ar–H), 4.06 (3H, s, OCH_3). Anal. Calc. for $C_{19}H_{14}N_2O_3$: C, 71.70; H, 4.40; N, 8.81. Found: C, 71.56; H, 4.63; N, 8.72.

*4.1.4.7. Ethyl 1,9-dimethyl-β-carboline-3-carboxylate (**5a**).* A mixture of **4a** (2.54 g, 10 mmol) and anhydrous DMF (60 ml) was stirred at RT until clear, and then 60% NaH (0.6 g, 15 mmol) and Iodomethane (2 ml, 30 mmol) were added. The mixture was stirred at RT for 1 h. The resulting mixture was poured into H_2O (100 ml), and extracted with ethyl acetate (3 × 150 ml). The organic phase was washed with water and brine, then dried over anhydrous sodium sulfate, filtered and evaporated. The oil obtained was purified by silica column chromatography with ethyl acetate as the eluent. Upon recrystallization, white crystals of **2a** were obtained (2.2 g, 82%), m.p. 141–142 °C (lit [32] m.p. 145 °C); FAB-MS *m/z* (M + 1) 269; UV λ_{max} 354, 338, 307, 272, 239 nm; IR (KBr) 3044, 2978, 2903, 1713, 1620, 1557, 1456, 1369, 1249, 1215, 1138 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.71 (1H, s, H-4), 8.14–8.16 (1H, d, J = 8 Hz, H-8), 7.60–7.63 (1H, m, H-5), 7.45–7.47 (1H, d, J = 8.5 Hz, H-6), 7.31–7.34 (1H, m, H-7), 4.50–4.54 (2H, m, OCH_2CH_3), 4.16 (3H, s, NCH_3), 3.15 (3H, s, CH_3), 1.48–1.50 (3H, m, OCH_2CH_3). Anal. Calc. for $C_{16}H_{16}N_2O_2$: C, 71.64; H, 5.97; N, 10.45. Found: C, 71.39; H, 6.23; N, 10.35.

*4.1.4.8. Ethyl 9-ethyl-1-methyl-β-carboline-3-carboxylate (**5b**).* A mixture of **4a** (2.54 g, 10 mmol) and anhydrous DMF (60 ml) was stirred at RT until clear, and then 60% NaH (0.6 g,

15 mmol) and Iodoethane (2.5 ml, 30 mmol) were added. The mixture was stirred at RT for 2 h. The resulting mixture was poured into H_2O (100 ml), and extracted with ethyl acetate (3 × 150 ml). The organic phase was washed with water and brine, then dried over anhydrous sodium sulfate, filtered and evaporated. The oil obtained was purified by silica column chromatography with ethyl acetate as the eluent. Upon recrystallization, white crystals of **5b** were obtained (2.3 g, 81%), m.p. 96–98 °C (from ether); FAB-MS *m/z* (M + 1) 283; UV λ_{max} 354, 338, 307, 273, 240 nm; IR (KBr) 3359, 3057, 2974, 2931, 2902, 1687, 1620, 1556, 1449, 1368, 1342, 1275, 1243, 1133 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.74 (1H, s, H-4), 8.16–8.18 (1H, d, J = 7.5 Hz, H-8), 7.60–7.63 (1H, m, H-5), 7.48–7.50 (1H, d, J = 8.5 Hz, H-6), 7.31–7.35 (1H, m, H-7), 4.62–4.66 (2H, m, NCH_2CH_3), 4.50–4.55 (2H, m, OCH_2CH_3), 3.13 (3H, s, CH_3), 1.46–1.50 (6H, m, NCH_2CH_3, OCH_2CH_3). Anal. Calc. for $C_{17}H_{18}N_2O_2$: C, 72.34; H, 6.38; N, 9.93. Found: C, 72.21; H, 6.64; N, 9.84.

*4.1.4.9. Ethyl 9-benzyl-1-methyl-β-carboline-3-carboxylate (**5c**).* A mixture of **4a** (2.54 g, 10 mmol) and anhydrous DMF (60 ml) was stirred at RT until clear, and then 60% NaH (0.6 g, 15 mmol) and benzyl bromide (5 ml, 40 mmol) were added and heated at reflux for 3 h, and then treated in a manner similar to that described for **5a** to afford white crystals **5c** (2.5 g, 73%), m.p. 155–156 °C (lit [32] m.p. 149 °C); FAB-MS *m/z* (M + 1) 345; UV λ_{max} 351, 337, 305, 272, 239 nm; IR (KBr) 3441, 3059, 2967, 2929, 1694, 1622, 1561, 1455, 1342, 1272, 1238, 1136, 1029 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.80 (1H, s, H-4), 8.22–8.23 (1H, d, J = 7.5 Hz, H-8), 7.55–7.58 (1H, m, H-5), 7.40–7.41 (1H, d, J = 8.5 Hz, H-6), 7.34–7.37 (1H, m, H-7), 7.24–7.28 (3H, m, Ar–H), 6.94–6.96 (2H, m, Ar–H), 5.85 (2H, s, NCH_2Ar), 4.50–4.54 (2H, m, OCH_2CH_3), 2.96 (3H, s, CH_3), 1.47–1.50 (3H, m, OCH_2CH_3). Anal. Calc. for $C_{22}H_{20}N_2O_2$: C, 76.74; H, 5.81; N, 8.14. Found: C, 76.58; H, 6.05; N, 8.05.

*4.1.4.10. Ethyl 9-phenylpropyl-1-methyl-β-carboline-3-carboxylate (**5d**).* A mixture of **4a** (2.54 g, 10 mmol) and anhydrous DMF (60 ml) was stirred at RT until clear, and then 60% NaH (0.6 g, 15 mmol) and 1-bromo-3-phenylpropane (6 ml, 40 mmol) were added and refluxed for 4 h, and then treated in a manner similar to that described for **5a** to afford white crystals **5d** (2.8 g, 75%), m.p. 101–102 °C (from ether); FAB-MS *m/z* (M + 1) 373; UV λ_{max} 355, 339, 308, 273, 240, 220 nm; IR (KBr) 3059, 2977, 2930, 2852, 1694, 1620, 1557, 1454, 1366, 1341, 1257, 1135 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.73 (1H, s, H-4), 8.15–8.17 (1H, d, J = 7.5 Hz, H-8), 7.56–7.59 (1H, m, H-5), 7.18–7.35 (7H, m, H-6, H-7, Ar–H), 4.49–4.58 (4H, m, OCH_2CH_3, $NCH_2CH_2CH_2Ar$), 2.97 (3H, s, CH_3), 2.74–2.77 (2H, m, $NCH_2CH_2CH_2Ar$), 2.14–2.20 (2H, m, $NCH_2CH_2CH_2Ar$), 1.47–1.50 (3H, m, OCH_2CH_3). Anal. Calc. for $C_{24}H_{24}N_2O_2$: C, 77.42; H, 6.45; N, 7.53. Found: C, 77.36; H, 6.63; N, 7.42.

*4.1.4.11. Ethyl 9-(2′,3′,4′,5′,6′-pentafluoro)benzyl-1-methyl-β-carboline-3-carboxylate (**5e**).* A mixture of **4a** (2.54 g, 10 mmol) and anhydrous DMF (60 ml) was stirred at RT until clear, and then 60% NaH (0.6 g, 15 mmol) and α-bromo-2,3,4,5,6-pentafluorotoluene (3 ml, 20 mmol) were added, then reacted and treated in a manner similar to that described for **5a** to afford white crystals **5e** (3.0 g, 68%), m.p. 145–146 °C (from ether); FAB-MS *m/z* (M + 1) 435; UV λ_{max} 346, 332, 301, 271, 265, 238 nm; IR (KBr) 3398, 2974, 2931, 1708, 1629, 1503, 1454, 1341, 1268, 1122 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.75 (1H, s, H-4), 8.17–8.19 (1H, d, J = 8.5 Hz, H-8), 7.56–7.59 (1H, m, H-5), 7.34–7.38 (2H, m, H-6, H-7), 5.99 (2H, s, NCH_2Ar), 4.51–4.56 (2H, m, OCH_2CH_3), 3.16 (3H, s, CH_3), 1.48–1.51 (3H, m, OCH_2CH_3). Anal. Calc. for $C_{22}F_5H_{15}N_2O_2$: C, 60.83; H, 3.46; N, 6.45. Found: C, 60.73; H, 3.59; N, 6.38.

*4.1.4.12. Ethyl 9-methyl-1-*n*-propyl-β-carboline-3-carboxylate (**6a**).* A mixture of **4c** (2.82 g, 10 mmol) and anhydrous DMF (60 ml) was stirred at RT until clear, and then 60% NaH (0.6 g, 15 mmol) and Iodomethane (2 ml, 30 mmol) were added, then reacted and was treated in a manner similar to that described for **5a** to afford white crystals **6a** (2.2 g, 74%), m.p. 108–109 °C (from ether); FAB-MS *m/z* (M + 1) 297; UV λ_{max} 354, 339, 307, 272, 240 nm; IR (KBr) 2957, 2868, 1703, 1555, 1466, 1367, 1263, 1135 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.69 (1H, s, H-4), 8.14–8.16 (1H, d, J = 8 Hz, H-8), 7.59–7.62 (1H, m, H-5), 7.46–7.47 (1H, d, J = 8.5 Hz, H-6), 7.30–7.33 (1H, m, H-7), 4.49–4.53 (2H, m, OCH_2CH_3), 4.11 (3H, s, NCH_3), 3.36–3.39 (2H, m, $CH_2CH_2CH_3$), 1.87–1.92 (2H, m, $CH_2CH_2CH_3$), 1.46–1.49 (3H, m, OCH_2CH_3), 1.09–1.12 (3H, m, $CH_2CH_2CH_3$). Anal. Calc. for $C_{18}H_{20}N_2O_2$: C, 72.97; H, 6.76; N, 9.46. Found: C, 72.83; H, 6.97; N, 9.34.

*4.1.4.13. Ethyl 9-ethyl-1-*n*-propyl-β-carboline-3-carboxylate (**6b**).* A mixture of **4c** (2.82 g, 10 mmol) and anhydrous DMF (60 ml) was stirred at RT until clear, and then 60% NaH (0.6 g, 15 mmol) and Iodoethane (2.5 ml, 30 mmol) were added, then reacted and treated in a manner similar to that described for **5a** to afford white crystals **6b** (2.0 g, 65%), m.p. 86–87 °C (from ether); FAB-MS *m/z* (M + 1) 311; UV λ_{max} 355, 340, 307, 273, 240 nm; IR (KBr) 3052, 2960, 2868, 1694, 1555, 1446, 1344, 1243, 1132 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.73 (1H, s, H-4), 8.18–8.19 (1H, d, J = 8 Hz, H-8), 7.60–7.63 (1H, m, H-5), 7.50–7.52 (1H, d, J = 8.5 Hz, H-6), 7.32–7.35 (1H, m, H-7), 4.49–4.62 (4H, m, NCH_2CH_3, OCH_2CH_3), 3.30–3.34 (2H, m, $CH_2CH_2CH_3$), 1.87–1.95 (2H, m, $CH_2CH_2CH_3$), 1.45–1.49 (6H, m, NCH_2CH_3, OCH_2CH_3), 1.10–1.13 (3H, m, $CH_2CH_2CH_3$). Anal. Calc. for $C_{19}H_{22}N_2O_2$: C, 73.55; H, 7.10; N, 9.03. Found: C, 73.41; H, 7.38; N, 8.96.

*4.1.4.14. Ethyl 9-benzyl-1-*n*-propyl-β-carboline-3-carboxylate (β).* A mixture of **4c** (2.82 g, 10 mmol) and anhydrous DMF (60 ml) was stirred at RT until clear, and then 60% NaH (0.6 g, 15 mmol) and benzyl bromide (5 ml, 40 mmol) were added and heated at reflux for 3 h, and then treated in a manner similar to that described for **5a** to afford white crys-

tals **6c** (1.8 g, 64%), m.p. 158–159 °C (from ether); FAB-MS *m/z* (M + 1) 373; UV λ_{max} 352, 337, 304, 272, 240 nm; IR (KBr) 3430, 2960, 2934, 2868, 1727, 1619, 1556, 1462, 1339, 1259, 1223, 1147 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.78 (1H, s, H-4), 8.22–8.24 (1H, d, *J* = 8 Hz, H-8), 7.55–7.58 (1H, m, H-5), 7.41–7.42 (1H, d, *J* = 8.5 Hz, H-6), 7.34–7.37 (1H, m, H-7), 7.23–7.30 (3H, m, Ar–H), 6.94–6.95 (2H, m, Ar–H), 5.79 (2H, s, NCH_2Ar), 4.49–4.54 (2H, m, OCH_2CH_3), 3.12–3.16 (2H, m, $CH_2CH_2CH_3$), 1.78–1.84 (2H, m, $CH_2CH_2CH_3$), 1.46–1.52 (3H, m, OCH_2CH_3), 0.96–1.01 (3H, m, $CH_2CH_2CH_3$). Anal. Calc. for $C_{24}H_{24}N_2O_2$: C, 77.42; H, 6.45; N, 7.53. Found: C, 77.29; H, 6.65; N, 7.40.

*4.1.4.15. Ethyl 9-phenylpropyl-1-*n*-propy-β-carboline-3-carboxylate (**6d**).* A mixture of **4c** (2.82 g, 10 mmol) and anhydrous DMF (60 ml) was stirred at RT until clear, and then 60% NaH (0.6 g, 15 mmol) and 1-bromo-3-phenylpropane (6 ml, 40 mmol) were added and refluxed for 4 h, and then treated in a manner similar to that described for **5a** to afford white crystals **6d** (1.7 g, 57%), m.p. 92–93 °C (from ether); FAB-MS *m/z* (M + 1) 401; UV λ_{max} 355, 339, 307, 273, 241, 201 nm; IR (KBr) 3068, 2994, 2968, 2929, 2870, 1701, 1620, 1556, 1450, 1365, 1258, 1132 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.71 (1H, s, H-4), 8.15–8.17 (1H, d, *J* = 8 Hz, H-8), 7.55–7.59 (1H, m, H-5), 7.34–7.36 (1H, d, *J* = 8.5 Hz, H-6), 7.29–7.32 (3H, m, H-7, Ar–H), 7.18–7.25 (3H, m, Ar–H), 4.46–4.53 (4H, m, $NCH_2CH_2CH_2Ar$, OCH_2CH_3), 3.15–3.18 (2H, m, $CH_2CH_2CH_3$), 2.74–2.77 (2H, m, $NCH_2CH_2CH_2Ar$), 2.11–2.11 (2H, m, $NCH_2CH_2CH_2Ar$), 1.77–1.84 (2H, m, $CH_2CH_2CH_3$), 1.46–1.49 (3H, m, OCH_2CH_3), 0.97–1.00 (3H, m, $CH_2CH_2CH_3$). Anal. Calc. for $C_{26}H_{28}N_2O_2$: C, 78.00; H, 7.00; N, 7.00. Found: C, 77.89; H, 7.26; N, 6.92.

*4.1.4.16. Methyl 1-phenyl-9-methyl-β-carboline-3-carboxylate (**7a**).* A mixture of **4d** (3.02 g, 10 mmol) and anhydrous DMF (60 ml) was stirred at RT until clear, and then 60% NaH (0.6 g, 15 mmol) and Iodomethane (2 ml, 30 mmol) were added, then reacted and treated in a manner similar to that described for **5a** to afford white crystals **7a** (2.4 g, 76%), m.p. 205–206 °C (from ether); FAB-MS *m/z* (M + 1) 317; UV λ_{max} 360, 346, 309, 275, 233 nm; IR (KBr) 3426, 3051, 2944, 1723, 1622, 1557, 1493, 1435, 1356, 1262, 1223, 1129 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.91 (1H, s, H-4), 8.24–8.25 (1H, d, *J* = 7.5 Hz, H-8), 7.63–7.66 (3H, m, H-5, H-6, H-7), 7.45–7.53 (4H, m, Ar–H), 7.37–7.40 (1H, m, Ar–H), 4.03 (3H, s, NCH_3), 3.47 (3H, s, OCH_3). Anal. Calc. for $C_{20}H_{16}N_2O_2$: C, 75.95; H, 5.06; N, 8.86. Found: C, 75.74; H, 5.29; N, 8.78.

*4.1.4.17. Methyl 1-phenyl-9-ethyl-β-carboline-3-carboxylate (**7b**).* A mixture of **4d** (3.02 g, 10 mmol) and anhydrous DMF (60 ml) was stirred at RT until clear, and then 60% NaH (0.6 g, 15 mmol) and Iodoethane (2.5 ml, 30 mmol) were added, then reacted and treated in a manner similar to that described for **5a** to afford white crystals **7b** (2.3 g, 69%), m.p. 169–170 °C (from ether); FAB-MS *m/z* (M + 1) 331; UV λ_{max} 360, 345, 309, 275, 234 nm; IR (KBr) 3429, 3054, 2980, 1724, 1621, 1556, 1429, 1351, 1247, 1133, 1051 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.92 (1H, s, H-4), 8.23–8.26 (1H, d, *J* = 8 Hz, H-8), 7.60–7.64 (3H, m, H-5, H-6, H-7), 7.45–7.53 (4H, m, Ar–H), 7.35–7.39 (1H, m, Ar–H), 3.96–4.03 (5H, s, NCH_2CH_3, OCH_3), 0.98–1.01 (3H, m, NCH_2CH_3). Anal. Calc. for $C_{21}H_{18}N_2O_2$: C, 76.36; H, 5.45; N, 8.48. Found: C, 76.27; H, 5.68; N, 8.39.

*4.1.5. General procedure for the preparation of β-carboline-3-carboxylic acids **8a–e**, **9a–d** and **10a–b***

A mixture of the corresponding β-carboline-3-carboxylate (10 mmol), NaOH (40 mmol), ethanol (50 ml) and H_2O (100 ml) was refluxed for 1 h, and the ethanol was removed on the rotary evaporator. The mixture was neutralized (pH 5) with 5 M HCl and cooled. The precipitate was collected, washed well with H_2O and dried in vacuum.

*4.1.5.1. 1,9-Dimethyl-β-carboline-3-carboxylic acid (**8a**).* Yellow solid was obtained (2.32 g, 97%). M.p. 262–264 °C; FAB-MS *m/z* (M + 1) 241; UV λ_{max} 355, 340, 269, 239 nm; IR (KBr) 3558, 3332, 2250–3250, 1936, 1712, 1621, 1344, 1215 cm^{-1}; ^{1}H-NMR (500 MHz, DMSO-d_6) δ 12.25 (1H, s, COOH), 8.89 (1H, s, H-4), 8.44–8.45 (1H, d, *J* = 8 Hz, H-8), 7.82–7.83 (1H, d, *J* = 8.5 Hz, H-5), 7.70–7.73 (1H, m, H-6), 7.37–7.40 (1H, m, H-7), 4.50–4.54 (3H, s, NCH_3), 3.18 (3H, s, CH_3). Anal. Calc. for $C_{14}H_{12}N_2O_2$: C, 70.00; H, 5.00; N, 11.67. Found: C, 69.83; H, 5.21; N, 11.54.

*4.1.5.2. 9-Ethyl-1-methyl-β-carboline-3-carboxylic acid (**8b**).* Yellow solid was obtained (2.48 g, 97%). M.p. 243–245 °C; FAB-MS *m/z* (M + 1) 255; UV λ_{max} 355, 270, 240, 223 nm; IR (KBr) 3393, 2250–3250, 1714, 1621, 1589, 1366, 1233, 1130 cm^{-1}; ^{1}H-NMR (500 MHz, DMSO-d_6) δ 12.26 (1H, s, COOH), 8.89 (1H, s, H-4), 8.45–8.46 (1H, d, *J* = 7.5 Hz, H-8), 7.83–7.85 (1H, d, *J* = 8.5 Hz, H-5), 7.70–7.73 (1H, m, H-6), 7.37–7.41 (1H, m, H-7), 4.72–4.76 (2H, m, NCH_2CH_3), 3.14 (3H, s, CH_3), 1.40–1.42 (3H, m, NCH_2CH_3). Anal. Calc. for $C_{15}H_{14}N_2O_2$: C, 70.87; H, 5.51; N, 11.02. Found: C, 70.73; H, 5.69; N, 10.94.

*4.1.5.3. 9-Benzyl-1-methyl-β-carboline-3-carboxylic acid (**8c**).* Yellow solid was obtained (3.12 g, 98%). M.p. 246–248 °C; FAB-MS *m/z* (M + 1) 317; UV λ_{max} 352, 338, 268, 239 nm; IR (KBr) 2250–3750, 1720, 1615, 1340, 1206 cm^{-1}; ^{1}H-NMR (500 MHz, DMSO-d_6) δ 12.21 (1H, s, COOH), 8.80 (1H, s, H-4), 8.31–8.35 (1H, d, *J* = 7.5 Hz, H-8), 7.65–7.68 (1H, d, *J* = 8.0 Hz, H-5), 7.58–7.60 (1H, m, H-6), 7.15–7.30 (6H, m, H-7, Ar–H), 5.78 (2H, m, NCH_2Ar), 2.95 (3H, s, CH_3). Anal. Calc. for $C_{20}H_{16}N_2O_2$: C, 75.95; H, 5.06; N, 8.86. Found: C, 75.82; H, 5.24; N, 8.74.

*4.1.5.4. 9-Phenylpropyl-1-methyl-β-carboline-3-carboxylic acid (**8d**).* Yellow solid was obtained (3.4 g, 98%). M.p. 186–188 °C; FAB-MS *m/z* (M + 1) 345; UV λ_{max} 356, 269, 240 nm;

IR (KBr) 3417, 2500–3250, 1740, 1624, 1591, 1355, 1061 cm^{-1}; ^{1}H-NMR (500 MHz, DMSO-d_6) δ 12.16 (1H, s, COOH), 8.75 (1H, s, H-4), 8.36–8.37 (1H, d, J = 7.5 Hz, H-8), 7.68–7.69 (1H, d, J = 8.0 Hz, H-5), 7.61–7.64 (1H, m, H-6), 7.18–7.34 (6H, m, H-7, Ar–H), 4.64–4.67 (2H, m, $NCH_2CH_2CH_2Ar$), 2.90 (3H, s, CH_3), 2.73–2.76 (2H, m, $CH_2CH_2CH_2Ar$), 2.06–2.12 (2H, m, $NCH_2CH_2CH_2Ar$). Anal. Calc. for $C_{22}H_{20}N_2O_2$: C, 76.74; H, 5.81; N, 8.14. Found: C, 76.64; H, 5.98; N, 8.06.

*4.1.5.5. 9-(2′,3′,4′,5′,6′-Pentafluoro)benzyl-1-methyl-β-carboline-3-carboxylic acid (**8e**).* White solid was obtained (4.0 g, 98%). M.p. 191–193 °C; FAB-MS *m/z* (M + 1) 407; UV λ_{max} 349, 336, 268, 238 nm; IR (KBr) 3422, 2250–3250, 1754, 1652, 1625, 1592, 1491, 1360, 1131 cm^{-1}; ^{1}H-NMR (500 MHz, DMSO-d_6) δ 12.18 (1H, s, COOH), 8.80 (1H, s, H-4), 8.40–8.42 (1H, d, J = 8.0 Hz, H-8), 7.62–7.64 (2H, m, H-5, H-6), 7.34–7.37 (1H, m, H-7), 6.10 (2H, s, NCH_2Ar), 3.01–3.02 (3H, s, CH_3). Anal. Calc. for $C_{20}F_5H_{11}N_2O_2$: C, 59.11; H, 2.71; N, 6.90. Found: C, 58.92; H, 2.83; N, 6.83.

*4.1.5.6. 1-*n*-Propyl-9-methyl-β-carboline-3-carboxylic acid (**9a**).* Yellow solid was obtained (2.6 g, 97%). M.p. 181–183 °C; FAB-MS *m/z* (M + 1) 269; UV λ_{max} 356, 342, 269, 240 nm; IR (KBr) 3142, 3059, 2960, 2869, 1739, 1620, 1582, 1470, 1359, 1248, 1128 cm^{-1}; ^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.76 (1H, s, H-4), 8.36–8.37 (1H, d, J = 7.5 Hz, H-8), 7.75–7.77 (1H, d, J = 8.0 Hz, H-5), 7.64–7.67 (1H, m, H-6), 7.32–7.35 (1H, m, H-7), 4.17 (3H, s, NCH_3), 3.35–3.38 (2H, m, $CH_2CH_2CH_3$), 1.85–1.89 (2H, m, $CH_2CH_2CH_3$), 1.04–1.07 (3H, m, $CH_2CH_2CH_3$). Anal. Calc. for $C_{16}H_{16}N_2O_2$: C, 71.64; H, 5.97; N, 10.45. Found: C, 71.44; H, 6.19; N, 10.38.

*4.1.5.7. 1-*n*-Propyl-9-ethyl-β-carboline-3-carboxylic acid (**9b**).* Yellow solid was obtained (2.7 g, 97%). M.p. 172–174 °C; FAB-MS *m/z* (M + 1) 283; UV λ_{max} 356, 342, 268, 240 nm; IR (KBr) 3191, 2965, 2871, 1745, 1619, 1558, 1456, 1354, 1228, 1120 cm^{-1}; ^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.77 (1H, s, H-4), 8.37–8.39 (1H, d, J = 7.5 Hz, H-8), 7.77–7.79 (1H, d, J = 8.5 Hz, H-5), 7.64–7.67 (1H, m, H-6), 7.32–7.36 (1H, m, H-7), 4.63–4.68 (2H, m, NCH_2CH_3), 3.26–3.30 (2H, m, $CH_2CH_2CH_3$), 1.86–1.93 (2H, m, $CH_2CH_2CH_3$), 1.37–1.40 (3H, m, NCH_2CH_3), 1.05–1.08 (3H, m, $CH_2CH_2CH_3$). Anal. Calc. for $C_{17}H_{18}N_2O_2$: C, 72.34; H, 6.38; N, 9.93. Found: C, 72.11; H, 6.56; N, 9.82.

*4.1.5.8. 9-Benzyl-1-*n*-propyl-β-carboline-3-carboxylic acid (**9c**).* Yellow solid was obtained (3.4 g, 96%). M.p. 193–194 °C; FAB-MS *m/z* (M + 1) 345; UV λ_{max} 353, 339, 268, 240 nm; IR (KBr) 3064, 2957, 2923, 2866, 1752, 1643, 1589, 1456, 1353, 1209, 1130 cm^{-1}; ^{1}H-NMR (500 MHz, DMSO-d_6) δ 12.15 (1H, s, COOH), 8.83 (1H, s, H-4), 8.43–8.45 (1H, d, J = 8 Hz, H-8), 7.70–7.71 (1H, d, J = 8.0 Hz, H-5), 7.60–7.63 (1H, m, H-6), 7.35–7.38 (1H, m, H-7), 7.22–7.29 (3H, m, Ar–H), 6.93–6.95 (2H, m, Ar–H), 5.92 (2H, s, NCH_2Ar), 3.07–3.10 (2H, m, $CH_2CH_2CH_3$), 1.67–1.75 (2H, m, $CH_2CH_2CH_3$), 0.86–0.89 (3H, m, $CH_2CH_2CH_3$). Anal. Calc. for $C_{22}H_{20}N_2O_2$: C, 76.74; H, 5.81; N, 8.14. Found: C, 76.58; H, 5.98; N, 8.19.

*4.1.5.9. 9-Phenylpropyl-1-*n*-propyl-β-carboline-3-carboxylic acid (**9d**).* Yellow solid was obtained (3.6 g, 97%). M.p. 176–178 °C; FAB-MS *m/z* (M + 1) 373; UV λ_{max} 356, 343, 269, 240 nm; IR (KBr) 3163, 3068, 2963, 2933, 1745, 1620, 1583, 1460, 1362, 1245 cm^{-1}; ^{1}H-NMR (500 MHz, DMSO-d_6) δ 12.15 (1H, s, COOH), 8.75 (1H, s, H-4), 8.36–8.38 (1H, d, J = 7.5 Hz, H-8), 7.71–7.72 (1H, d, J = 8.0 Hz, H-5), 7.62–7.65 (1H, m, H-6), 7.19–7.35 (6H, m, H-7, Ar–H), 4.57–4.60 (2H, s, $NCH_2CH_2CH_2Ar$), 3.06–3.09 (2H, m, $CH_2CH_2CH_3$), 2.74–2.77 (2H, m, $NCH_2CH_2CH_2Ar$), 2.05–2.11 (2H, m, $CH_2CH_2CH_3$), 1.73–1.79 (2H, m, $NCH_2CH_2CH_2Ar$), 0.92–0.96 (3H, m, $CH_2CH_2CH_3$). Anal. Calc. for $C_{24}H_{24}N_2O_2$: C, 77.42; H, 6.45; N, 7.53. Found: C, 77.31; H, 6.56; N, 7.48.

*4.1.5.10. 1-Phenyl-9-methyl-β-carboline-3-carboxylic acid (**10a**).* Yellow solid was obtained (3.0 g, 98%). M.p. 223–224 °C; FAB-MS *m/z* (M + 1) 303; UV λ_{max} 360, 273, 237 nm; IR (KBr) 2000–3250, 1754, 1681, 1622, 1557, 1392, 1262, 1051 cm^{-1}; ^{1}H-NMR (500 MHz, DMSO-d_6) δ 12.18 (1H, s, COOH), 8.93 (1H, s, H-4), 8.45–8.46 (1H, d, J = 7.5 Hz, H-8), 7.65–7.70 (4H, m, H-5, H-6, H-7, Ar–H), 7.56–7.60 (3H, m, Ar–H), 7.36–7.39 (1H, m, Ar–H), 3.44–3.47 (3H, s, NCH_3). Anal. Calc. for $C_{19}H_{14}N_2O_2$: C, 75.50; H, 4.64; N, 9.27. Found: C, 75.39; H, 4.77; N, 9.16.

*4.1.5.11. 1-Phenyl-9-ethyl-β-carboline-3-carboxylic acid (**10b**).* Yellow solid was obtained (3.1 g, 97%). M.p. 194–195 °C; FAB-MS *m/z* (M + 1) 317; UV λ_{max} 360, 273, 239 nm; IR (KBr) 3283, 2974, 1730, 1620, 1559, 1451, 1355, 1299 cm^{-1}; ^{1}H-NMR (500 MHz, DMSO-d_6) δ 12.24 (1H, s, COOH), 8.98 (1H, s, H-4), 8.46–8.48 (1H, d, J = 8 Hz, H-8), 7.71–7.73 (1H, d, J = 8 Hz, H-5), 7.56–7.68 (6H, m, H-6, H-7, Ar–H), 7.36–7.39 (1H, m, Ar–H), 4.02–4.06 (2H, m, NCH_2CH_3), 0.86–0.89 (3H, m, NCH_2CH_3). Anal. Calc. for $C_{20}H_{16}N_2O_2$: C, 75.95; H, 5.06; N, 8.86. Found: C, 75.73; H, 5.23; N, 8.78.

4.2. 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 Al-Allaf and Rashan [20] with a slightly modification. Cells were grown in RPMI-1640 medium containing 10% (v/v) fetal calf serum and 100 ug ml^{-1} penicillin and 100 ug ml^{-1} streptomycin. Cultures were propagated at 37 °C in a humified atmosphere containing 5% CO_2. Cell lines were obtained from Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Science. 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 non-small cell lung carcinoma (PLA-

801), liver carcinoma (HepG2 and Bel-7402), gastric carcinoma (BGC-823), cervical carcinoma (Hela), colon carcinoma (Lovo). In all of these experiments, three replicate wells were used to determine each point.

Acknowledgements

This work was supported by grants from Xinjiang Huashidan Pharmaceutical Co. Ltd. and Guangzhou Commission of Science and Technology (97-Z-12-01). We also thank Center for Analysis and Test of Sun Yat-sen (Zhongshan) University for UV, FAB-MS, IR and ^{1}H-NMR spectra.

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