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Synthesis, acute toxicities, and antitumor effects of novel 9-substituted β-carboline derivatives

Rihui Cao, Qi Chen, Xuerui Hou, Hongsheng Chen, Huaji Guan, Yan Ma, Wenlie Peng and Anlong Xu*

Department of Biochemistry and Center for Biopharmaceutical Research, College of Life Sciences, Sun Yat-sen (Zhongshan) University, 135 Xin Gang Xi Road, Guangzhou 510275, PR China

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Abstract—A series of novel 9-substituted β-carboline derivatives was synthesized from harmine and L-tryptophan, respectively. Cytotoxic activities of these compounds in vitro were investigated. The results showed that most compounds of 9-substituted β-carboline derivatives had more remarkable cytotoxic activities in vitro than their corresponding parent compounds. Acute toxicities and antitumor effects of the selected β-carboline derivatives in mice were also examined. The results demonstrated that a short alkyl or benzyl substituent at position-9 increased the antitumor activities significantly and a ethoxycarbonyl or carboxyl substituent at position-3 reduced the acute toxicity and neurotoxicity of these β-carboline derivatives dramatically. Moreover the compounds both with an alkoxycarbonyl or carboxyl substituent at position-3 and a short alkyl or benzyl substituent at positon-9 exhibited more significant antitumor activities and lower acute toxicities and neurotoxicities than the other compounds. The compound **8c**, having an *n*-butyl and a carboxyl substituent at position-9 and 3, respectively, was found to have the highest antitumor effect and the lowest acute toxicity and neurotoxicity. These data suggested that (**1**) appropriate substituents at both position-9 and 3 of β-carboline derivatives might play a crucial role in determining their enhanced antitumor activities and decreased acute toxicities and neurotoxic effects; (**2**) the β-carboline derivatives have the potential to be used as antitumor drug leads.

1. Introduction

β-Carbolines are a large group of naturally occurring and synthetic indole alkaloids with different degrees of aromaticity, some of which are widely distributed in nature, including various plants,[1–3] marine creatures,[4] insects,[5] mammalians as well as human tissues and body fluids.[6–9] These compounds are of great interest due to their various biological activities such as intercalating into DNA,[10,11] inhibiting CDK,[12] and Topisomerase,[13,14] inhibiting monoamine oxidase[15,16] as well as interacting with benzodiazepine receptors[17–19] and 5-hydroxy serotonin receptors,[20] and their broad spectrum pharmacological properties including anxiolytic, hypnotic, anticonvulsant,[21–23] parasiticidal,[24] antiviral[25] as well as antimicrobial activities.[30] Recent work[26–28] and our preliminary investigation results demonstrated that the compounds with β-carboline nucleus have potential antitumor activities. Yet we also found that this class of compounds caused remarkable acute neurotoxicity characterized by tremble, twitch, and jumping in experimental mice models.

Many previous reports focused on the effects of these compounds on the central nervous system (CNS), such as their affinity with benzodiazepine receptors,[17–19] 5-HT_{2A} and 5-HT_{2C} receptors[20] and imidazoline receptor[32], and so on. However so far there have been few such literatures about their cytotoxic activities of these compounds in vitro, even no reports are available dealing with systematic and detailed studies of structure–activity relationships on both antitumor activities and neurotoxic activities in vivo. A probable reason for this is that many of the β-carboline derivatives of interest are not readily available. One goal of the present investigations was to synthesize a series of novel β-carboline derivatives and elucidate their preliminary relationships between structures and antitumor activities as well as

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* Corresponding author. Tel.: +86-20-84113655; fax: +86-20-84038377; e-mail: ls36@zsu.edu.cn

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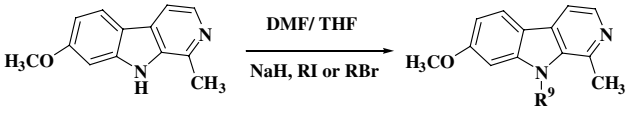
neurotoxicities, and the other aim of this study was to increase the antitumor activities and decrease the neurotoxic activities of these compounds. The work would help search for new antitumor leading compounds with significant antitumor activities and lower neurotoxicities by simple chemical structural modifications on the basis of β-carboline ring. To the best of our knowledge, all 9-substituted β-carboline derivatives except **2a–c** are novel compounds, and this is the first time to report the antitumor activities and acute toxicities as well as neurotoxicities of this class of compounds in mice.

2. Results and discussion

2.1. Synthetic approach

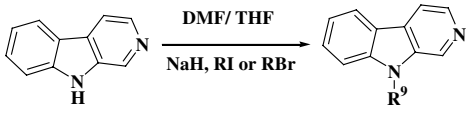
N-alkylation and N-benzylation derivatives of harmine **1** could be easily obtained in a good yield through reacting with alkyl or benzyl bromide in the presence of sodium hydride in DMF and THF (see Scheme 1). The starting material L-tryptophan reacted with formaldehyde via well-known Pictet–Spengler condensation and then the products were esterified with relevant alcohol to produce 1,2,3,4-tetrahydro-β-carboline-3-carboxylates, respectively. The 1,2,3,4-tetrahydro-β-carboline-3-carboxylates reacted with SeO_2 in acetic acid solution by oxidative decarboxylation to produce β-carboline **3** and reacted with sulfur in anhydrous xylene by dehydrogenation to afford β-carboline-3-carboxylate **5**, **6**, **7**, respectively. The N-9 position of compound **3**, **5**, **6**, **7** was further alkylated or benzylated by the action of sodium hydride in anhydrous DMF followed by addition of the relevant appropriate alkylating and benzylating agents (see Schemes 2 and 3) to produce various 9-substituted β-carboline-3-carboxylate (**5a–d**, **6a–e**, **7a–d**). The compounds **6** and **6a–f** were hydrolyzed in sodium hydroxide solution to afford the compounds **8** and **8a–f** (see Scheme 4). In our studies, we found that N-alkylation and N-benzylation of β-carboline-3-carboxylate would be accompanied by ester exchange and ester hydrolysis unexpectedly. As a result, separating and purifying the products would become difficult. Consequently, the key step was to use anhydrous DMF so as to guarantee the dryness of reaction system of alkylation and benzylation of β-carboline-3-carboxylate **5**, **6**, **7**. The chemical structures of all the synthesized novel compounds were confirmed by FAB-MS, UV, IR, ^{1}H NMR, and elemental analyses data.



1

2a $R^9=CH_3$
2b $R^9=CH_2CH_3$
2c $R^9=n\text{-}C_4H_9$
2d $R^9=CH_2CH_2OH$
2e $R^9=CH_2C_6H_5$
2f $R^9=CH_2C_6F_5$
2g $R^9=(CH_2)_3C_6H_5$

Scheme 1. Synthesis of 9-substituted harmine derivatives.



3

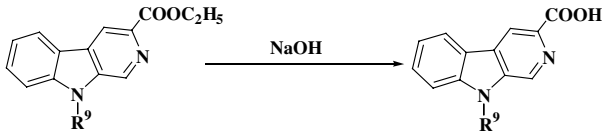
4a $R^9=CH_3$
4b $R^9=CH_2CH_3$
4c $R^9=n\text{-}C_4H_9$
4d $R^9=CH_2C_6H_5$

Scheme 2. Synthesis of 9-substituted β-carbolines.

5 $R^3=CH_3$
6 $R^3=CH_2CH_3$
7 $R^3=n\text{-}C_4H_9$

5a $R^3=CH_3$ $R^9=CH_3$
5b $R^3=CH_3$ $R^9=CH_2CH_3$
5c $R^3=CH_3$ $R^9=n\text{-}C_4H_9$
5d $R^3=CH_3$ $R^9=CH_2C_6H_5$
6a $R^3=CH_2CH_3$ $R^9=CH_3$
6b $R^3=CH_2CH_3$ $R^9=CH_2CH_3$
6c $R^3=CH_2CH_3$ $R^9=n\text{-}C_4H_9$
6d $R^3=CH_2CH_3$ $R^9=CH_2C_6H_5$
6e $R^3=CH_2CH_3$ $R^9=CH_2C_6F_5$
6f $R^3=CH_2CH_3$ $R^9=(CH_2)_3C_6H_5$
7a $R^3=n\text{-}C_4H_9$ $R^9=CH_3$
7b $R^3=n\text{-}C_4H_9$ $R^9=CH_2CH_3$
7c $R^3=n\text{-}C_4H_9$ $R^9=n\text{-}C_4H_9$
7d $R^3=n\text{-}C_4H_9$ $R^9=CH_2C_6H_5$

Scheme 3. Synthesis of 9-substituted β-carboline-3-carboxylate derivatives.



6, 6a-6f

8 $R^9=H$
8a $R^9=CH_3$
8b $R^9=CH_2CH_3$
8c $R^9=n\text{-}C_4H_9$
8d $R^9=CH_2C_6H_5$
8e $R^9=CH_2C_6F_5$
8f $R^9=(CH_2)_3C_6H_5$

Scheme 4. Synthesis of 9-substituted β-carboline-3-carboxylic acid derivatives.

2.2. Cytotoxicity assays

Thirty-seven β-carboline derivatives were examined for cytotoxic activities against a panel of human tumor cell lines. In order to enhance the solubility in aqueous solution, compounds **8** and **8a–f** were converted into their water-soluble sodium salts and the other compounds

Table 1. Cytotoxicity of β-carboline derivatives in vitro[c] (IC_{50},[a] μM)

Compounds	PLA-801[b]	HepG2[b]	Bel-7402[b]	BGC-823[b]	Hela[b]	Lovo[b]
Harmine	45	46	54	68	60	66
2a	29	16	63	52	8	43
2b	22	14	28	45	17	170
2c	111	62	58	69	28	87
2d	78	313	244	204	25	27
2e	65	85	57	95	270	52
2f	42	46	43	22	28	31
2g	50	36	44	46	24	27
3	17	228	379	274	353	35
4a	219	102	302	204	327	220
4b	130	167	311	171	234	159
4c	133	99	164	101	88	134
4d	124	107	77	73	48	123
5	295	241	293	278	100	160
5a	125	119	195	181	181	175
5b	25	117	124	79	17	107
5c	136	464	433	310	289	166
5d	>1000	>1000	>1000	>1000	>1000	312
6	275	>1000	>1000	>1000	567	>1000
6a	170	108	260	141	136	69
6b	87	109	107	108	137	77
6c	71	476	784	760	487	173
6d	78	38	45	38	13	122
6e	47	116	9	52	78	37
6f	>1000	>1000	387	>1000	>1000	>1000
7	>1000	>1000	>1000	>1000	>1000	>1000
7a	675	266	313	394	295	104
7b	109	70	74	82	67	83
7c	>1000	>1000	>1000	72	954	45
7d	994	>1000	>1000	>1000	>1000	>1000
8	327	354	369	330	262	198
8a	267	147	226	126	89	88
8b	212	179	130	163	183	62
8c	92	73	92	116	60	11
8d	100	102	112	52	86	41
8e	86	31	61	35	44	13
8f	57	31	53	28	100	5

[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 cells using the MTT assay.

[b]Cell lines include nonsmall cell lung carcinoma (PLA-801), liver carcinoma (HepG2 and Bel-7402), gastric carcinoma (BGC-823), cervical carcinoma (Hela), colon carcinoma (Lovo).

[c]Data represent the mean values of three independent determinations.

investigated were all prepared in the form of hydrochloride by the usual methods before use. The results were summarized in Table 1.

As shown in Table 1, most studied compounds showed significant cytotoxic activities against several human tumor cell lines. 9-Substituted harmines, β-carbolines, β-carboline-3-carboxylates, and β-carboline-3-carboxylic acids (except **5d**, **6f**, and **7d**) displayed more remarkable cytotoxic activities than their parent compounds, respectively, indicating that the introduction of an appropriate alkyl or benzyl group into 9-position of β-carboline ring system facilitates the increase of their cytotoxic activities.

Among all the compounds investigated, 9-substituted harmine series demonstrated the highest cytotoxicities while 9-substituted butyl β-carboline-3-carboxylates **7a**, **7c**, **7d** (except **7b**), and their corresponding parent compound **7** were found to be less active with IC_{50} values of more than 100 μM against tumor cell lines, suggesting that the substitution of 7-methoxy and 1-methyl groups might contribute to their increased cytotoxic activities while a long chain alkoxy at 3-position might not be preferable. Compared with the IC_{50} values of other compounds, the IC_{50} values of the compounds **2a**, **2b**, **2f**, and **2g** was lower than 50 μM, which implicated that the alkyl and benzyl substituents had almost equal potency to enhance their cytotoxic activities in vitro.

As for the harmine series, the compound **2b** with an ethyl group at 9-position exhibited the highest cytotoxic activities (except for Lovo cell line). The compound **2a** having a methyl at position-9 showed selective cytotoxic activities against the screened tumor cell lines with the lowest IC_{50} value (8 μM) against Hela. The compounds **2f** and **2g** with a pentafluorobenzyl and a phenylpropyl at position-9, respectively, demonstrated the prominent

and broader spectrum of cytotoxic activities against all six human tumor cell lines with IC_{50} values of all lower than 50 μM.

In contrast to the harmine series, 9-substituted β-carboline series failed to exhibit obviously increased cytotoxicities. The compound **3** having no substituent on ring system showed a good cytotoxic activity against PLA-801 and Lovo cell lines. Compounds **4a**–**d** displayed almost equal cytotoxic activities while the compound **4d** showed higher and selective cytotoxic activity against Lovo cells with IC_{50} values of 48 μM.

Of all 9-substituted β-carboline-3-carboxylate derivatives, the compound **6d** having a benzyl at position-9 displayed strong cytotoxic activities against HepG2, Bel-7402, BGC-823, and Hela with IC_{50} values of 38, 45, 38, and 13 μM, respectively. However compounds **5d** and **7d**, having a benzyl at 9-position, had little or even no cytotoxic effects on the tested cell lines, indicating that ethoxycarbonyl substituent was preferable for improving cytotoxic activities of this type of compounds. The compounds **5b**, **6b**, and **7b** with an ethyl at position-9, respectively, displayed higher cytotoxic activities against human tumor cell lines tested. Meanwhile the compound **5b** showed highly selective cytotoxic effects against PLA-801 and Hela with IC_{50} values 25 and 17 μM, respectively. All these results, together with the cytotoxic activity of the compound **2b**, confirmed that the ethyl group at position-9 might play a crucial role in eliciting distinct cytotoxic activities of these compounds.

For the β-carboline-3-carboxylic acid series (**8** and **8a**–**e**), the cytotoxic activities were also enhanced by introduction of short alkyl or benzyl substituent into position-9 of the β-carboline ring. The compounds **8c** and **8e**, having an *n*-butyl and a benzyl at positon-9, respectively, showed remarkable cytotoxic activities against Lovo cell lines with IC_{50} values of 11 and 13 μM, respectively, furthermore the compound **8f** exhibited the most significant cytotoxic activity against Lovo cell lines with IC_{50} values 5 μM.

A total analysis of the cytotoxic activities of β-carboline derivatives in vitro clearly suggested that (1) the β-carboline nucleus might be an important basis for the design and synthesis of new antitumor drugs; (2) the cytotoxic potencies of β-carboline derivatives depended upon the presence and location and nature of the subtituents, which were introduced into the β-carboline ring. (3) The cytotoxic activities of β-carboline derivatives were enhanced by the introduction of an appropriate substituent into position-9 of β-carboline ring; (4) the Lovo cell lines were more sensitive to the tested compounds than other tumor cells.

2.3. Assessment of acute toxicity

The LD_{50} values and scores for neurotoxicity of the selected β-carboline derivatives in mice after administration were summarized in Table 2. Neurotoxicities were the principal acute toxic effects observed in mice after receiving the β-carboline derivatives via intraperitoneal (i.p.) route. All the tested compounds resulted in acute toxic manifestation. Of all the compounds investigated, **1**, **2b**, **2c**, **2e**, and **5a** caused remarkable acute toxic effects including tremor, twitch, jumping, tetanus, and supination. Death occurred mostly in high dosage group within 1 min after administration and then reached a peak 1 h later. For survived animals, the tremor and jumping lasted for about 20 min and then relieved gradually and returned to normal in the next day. Animals were drowsy and exhibited a decrease in locomotor activity after the administration of the compound **8**, **6d**, **8d**, and **8f**. Death only occurred in high dosage group 10–20 min after administration and reached a peak 1 h later. However, the compounds **8c** caused no obvious neurotoxic reaction and no death at tested dosage. Autopsy of the animals that died in the course of experiment and the necropsy findings in surviving animals at the end of experimental period (14 days) revealed no apparent changes in any organs. The results of toxicities suggested that an alkoxycarbonyl or carboxyl substituent at position-3 of the β-carboline ring might play a vital role in determining their decreased acute toxicities and neurotoxicities, and perhaps the carboxyl substituent is more favorable.

Table 2. Acute toxic effects of β-carboline derivatives in mice

Compound	Acute toxicity	
	LD_{50} (mg/kg, 95% CL)	Neurotoxic effect
1	59.00 (43.50–110.00)	++[a]
2b	24.25 (22.24–26.44)	++
2c	26.45 (23.91–29.26)	++
2e	147.82 (132.55–164.85)	++
5a	70.61 (64.17–77.70)	+
6d	240.38 (211.38–273.50)	–
8	135.22 (121.62–150.34)	–
8c	>500	–
8d	163.48 (141.56–188.76)	–
8e	219.19 (193.25–248.61)	–

[a] Acute neurotoxic manifestation were denoted by '+' and '–'. A '+' represents toxic reactions including tremble, twitch, jumping, tetanus, and supination, '++' means the same reactions with more severity, while '–' means no such reaction.

2.4. Evaluation of antitumor activity

The tumor inhibition rates of the selected β-carboline derivatives in mice bearing Lewis lung cancer and Sarcoma180 (S180) were summarized in Table 3. Harmine (compound **1**) only showed a moderate antitumor effect (34.1% and 15.3% against Lewis lung cancer and S180, respectively), however the selected 9-substituted β-carboline derivatives all exhibited more potent antitumor activities. Compounds **2c**, **2d**, **2e**, **6d**, and **8c** displayed remarkable antitumor activities with the tumor inhibition rate of more than 40% against mice bearing Lewis lung cancer and S180. Moreover the compounds **2e** and **8c** demonstrated the highest antitumor effect with the tumor inhibition rate of 46.9% against mice bearing Lewis lung cancer among all the tested compounds. The results indicated that short alkyl or benzyl substituent at

Table 3. Antitumor effects of β-carboline derivatives against mice bearing Sarcoma 180 and Lewis lung carcinoma

Compound	Tumor inhibition rate (%)	
	Lewis lung carcinoma	Sarcoma180
1	34.1	15.3
2b	42.0	37.6
2c	44.0	40.9
2e	46.9	45.2
5a	35.0	31.1
6d	43.3	42.1
8	33.4	32.2
8c	46.9	43.1
8d	43.2	34.4
8e	39.6	32.2
CTX	88.6	87.5

position-9 of the β-carboline ring facilitated the increase of their antitumor activities.

3. Conclusions

The present investigation reported for the first time that β-carboline derivatives had significant antitumor activities in mice bearing Lewis lung cancer and Sarcoma 180 but also exhibited remarkable acute neurotoxicity in experimental mice models. Alkyl or benzyl substituent at position-9 of β-carboline ring might facilitate their antitumor activities and minimize their acute toxicities but not neurotoxicities. However alkoxycarbonyl or carboxyl substituent at position-3 played a crucial role in determining their decreased acute toxicities and neurotoxicities. Furthermore, the compounds with appropriate substituents both at positon-3 and 9 displayed enhanced antitumor activities and decreased neurotoxicities simultaneously. The compound **8c**, having an *n*-butyl and a carboxyl at position-9 and 3, respectively, exhibited the highest antitumor effect and the lowest acute neurotoxicity and was found to be the most promising leading compound for further investigation. These data suggested that (1) appropriate substituents at both position-9 and 3 of β-carboline derivatives might play a crucial role in determining their enhanced antitumor activities and decreased acute toxicities and neurotoxic effects; (2) the β-carboline derivatives have the potential to be used as antitumor drug leads.

Although the antitumor effects of the studied β-carboline derivatives presented here remained relatively modest, it should be noted that the acute neurotoxicities of some compounds decreased dramatically by introduction of an alkoxycarbonyl or carboxyl substituent into position-3 of the β-carboline ring. This investigation and finding would be helpful to further design and develop more potent antitumor agents together with low neurotoxicities. Further investigations are in progress in our laboratory to elucidate the molecular mechanisms involved in antitumor activities and neurotoxicities of β-carboline derivatives. To acquire more information about the structural requirements for enhancing antitumor activities and minimizing neurotoxicities, the synthesis of more new β-carboline derivatives with different substituents at other positions is needed.

4. Experimental

4.1. Materials

All reagents were purchased from commercial suppliers and were dried and purified when necessary. Compound **1** (Harmine) was extracted from *Peganum multisectum Maxim*, a plant indigenous to western China, according to the method by Duan et al.[2] Compounds **3** and **5**–**8** were synthesized from the starting material L-tryptophan according to the procedures described by Hagen et al.[19] and Lippke et al.[18] with a slight modification, respectively.

Melting points were determined in capillary tubes on an electrothermal PIF YRT-3 apparatus and without correction. UV spectra were measured on Shimadzu UV 2501PC Spectrometer. FAB-MS spectra were obtained from VG ZAB-HS spectrometer. FTIR were run on a Bruker Equinox 55 Fourier Transformation Infarred 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 F254 were used in analytical thin-layer chromatography (TLC) and silica gel were used in column chromatography, respectively.

4.1.1. 7-Methoxy-1,9-dimethyl-β-carboline (2a). A mixture of Harmine **1** (2.12 g, 10 mmol), anhydrous DMF (50 mL), and anhydrous THF (50 mL) was stirred at rt until clear, and then 60% NaH (0.6 g, 15 mmol) and iodomethane (2 mL, 30 mmol) were added and stirred at rt for 30 min. Later the mixture was evaporated in reduced pressure. The resulting solution 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 (1.8 g, 80%), mp 121–123 °C (from ether); FAB-MS *m/e* 227 (M+1); UV λ_{max} 345, 332, 302, 264, 244, 214 nm; IR (KBr): 3380, 2744, 1628, 1570, 1469, 1348, 1249, 1152, 1045, 810 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): 8.24–8.25 (1H, m, H-5), 7.93–7.96 (1H, m, H-3), 7.69–7.71 (1H, m, H-4), 6.86–6.89 (1H, m, H-8), 6.81–6.83 (1H, m, H-6), 4.04–4.07 (3H, m, NCH_3), 3.94–3.95 (3H, m, OCH_3), 3.04–3.05 (3H, m, CH_3). Anal. Calcd for $C_{14}H_{14}N_2O$: C, 74.33; H, 6.19; N, 12.39. Found: C, 74.21; H, 6.37; N, 12.31.

4.1.2. 7-Methoxy-9-ethyl-1-methyl-β-carboline (2b). A mixture of Harmine **1** (2.12 g, 10 mmol), anhydrous DMF (50 mL), and anhydrous THF (50 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. Later the mixture was treated in a manner similar to that described for **2a** to afford **2b** (2.0, 83%), mp 99–101 °C (from ether); FAB-MS *m/e* 241 (M+1); UV λ_{max} 345,

332, 303, 265, 244, 213 nm; IR (KBr): 3362, 3128, 1622, 1565, 1451, 1346, 1217, 1136, 812 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): 8.26–8.27 (1H, d, J = 5 Hz, H-5), 7.96–7.98 (1H, d, J = 5 Hz, H-3), 7.74–7.75 (1H, d, J = 4.5 Hz, H-4), 6.86–6.90 (2H, m, H-8, H-6), 4.52–4.57 (2H, m, CH_2CH_3), 3.95 (3H, s, OCH_3), 3.05 (3H, s, CH_3), 1.43–1.46 (3H, m, CH_2CH_3). Anal. Calcd for $C_{15}H_{16}N_2O$: C, 75.00; H, 6.67; N, 11.67. Found: C, 74.89; H, 6.87; N, 11.59.

4.1.3. 7-Methoxy-9-butyl-1-methyl-β-carboline (2c). A mixture of Harmine **1** (2.12 g, 10 mmol), anhydrous DMF (50 mL), and anhydrous THF (50 mL) was stirred at rt until clear, and then 60% NaH (0.6 g, 15 mmol) and *n*-butyl iodide (6 mL, 50 mmol) were added and refluxed for 1 h. Later the resulting mixture was treated in a manner similar to that described for **2a** to afford **2c** (2.1, 78%), mp 104–105 °C (from ether); FAB-MS *m/e* 269 (M+1); UV λ_{max} 346, 334, 303, 265, 244, 213 nm; IR (KBr): 3428, 2959, 2927, 1621, 1563, 1497, 1448, 1356, 1243, 1197, 1137, 812 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): 8.26–8.28 (1H, d, J = 5.5 Hz, H-5), 7.95–7.97 (1H, d, J = 9 Hz, H-3), 7.71–7.72 (1H, d, J = 5 Hz, H-4), 6.85–6.88 (2H, m, H-8, H-6), 4.43–4.46 (2H, m, J = 8 Hz, $CH_2CH_2CH_2CH_3$), 3.94 (3H, s, OCH_3), 3.01 (3H, s, CH_3), 1.78–1.84 (2H, m, $CH_2CH_2CH_2CH_3$), 1.41–1.48 (2H, m, $CH_2CH_2CH_2CH_3$), 0.97–1.00 (3H, m, $CH_2CH_2CH_2CH_3$). Anal. Calcd for $C_{17}H_{20}N_2O$: C, 76.12; H, 7.46; N, 10.45. Found: C, 75.96; H, 7.69; N, 10.53.

4.1.4. 7-Methoxy-9-hydroxyethyl-1-methyl-β-carboline (2d). A mixture of Harmine **1** (1.05 g, 5.0 mmol) and anhydrous DMF (25 mL), and anhydrous THF (25 mL) was stirred at rt until clear, and then 60% NaH (0.3 g, 7.5 mmol) and 2-iodoethanol (3 mL, 40 mmol) were added and refluxed for 5 h. Then the resulting mixture was treated in a manner similar to that described for **2a** to afford **2d** (0.7 g, 54%), mp 204–206 °C (from ether); FAB-MS *m/e* 257 (M+1); UV λ_{max} 328, 304, 249, 211 nm; IR (KBr) 3295, 2696, 1629, 1569, 1467, 1352, 1155, 1051, 810 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): 8.15–8.16 (1H, d, J = 4 Hz, H-5), 7.93–7.94 (1H, d, J = 3.5 Hz, H-3), 7.63–7.64 (1H, d, J = 5.0 Hz, H-4), 6.88–6.95 (2H, m, H-8, H-6), 4.66–4.71 (2H, t, J = 5.5 Hz, NCH_2CH_2OH), 4.06–4.08 (2H, m, NCH_2CH_2OH), 3.94 (3H, s, OCH_3), 2.99 (3H, s, CH_3). Anal. Calcd for $C_{15}H_{16}N_2O_2$: C, 70.31; H, 6.25; N, 10.94. Found: C, 70.13; H, 6.46; N, 10.87.

4.1.5. 9-Benzyl-7-methoxy-1-methyl-β-carboline (2e). A mixture of Harmine **1** (2.12 g, 10 mmol), anhydrous DMF (50 mL), and anhydrous THF (50 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. Later the mixture was treated in a manner similar to that described for **2a** to afford **2e** (2.2, 67%), mp 131–133 °C (from ether); FAB-MS *m/e* 303 (M+1); UV λ_{max} 343, 330, 301, 244, 210 nm; IR (KBr): 3421, 2958, 1620, 1565, 1498, 1447, 1361, 1256, 1197, 1172, 1044, 825 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): 8.29–8.30 (1H, d, J = 5.5 Hz, H-5), 8.01–8.02 (1H, d, J = 8.5 Hz, H-3), 7.80–7.81 (1H, d, J = 5.5 Hz, H-4), 7.23–7.30 (3H, m, H-8, H-6, Ar–H), 6.98–7.00 (1H, d, J = 7.0 Hz, Ar–H), 6.90–6.92 (1H, m, Ar–H), 6.76 (1H, s, Ar–H), 5.75 (2H, s, NCH_2Ar), 3.85 (3H, s, OCH_3), 2.88 (3H, s, CH_3). Anal. Calcd for $C_{20}H_{18}N_2O$: C, 79.47; H, 5.96; N, 9.27. Found: C, 79.29; H, 6.19; N, 9.21.

4.1.6. 9-(2′,3′,4′,5′,6-Pentafluoro)benzyl-7-methoxy-1-methyl-β-carboline (2f). A mixture of Harmine **1** (0.53 g, 2.5 mmol), anhydrous DMF (15 mL), and anhydrous THF (15 mL) was stirred at rt until clear, and then 60% NaH (0.15 g, 3.8 mmol) and α-bromo-2,3,4,5,6-pentafluorotoluene (0.7 mL, 4.5 mmol) were added. Later the mixture was treated in a manner similar to that described for **2a** to afford **2f** (0.64 g, 65%), mp 173–174 °C (from ether); FAB-MS *m/e* 393 (M+1); UV λ_{max} 338, 325, 300, 241, 209 nm; IR (KBr): 2961, 1622, 1502, 1446, 1256, 1026, 816 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): 8.32–8.33 (1H, d, J = 5 Hz, H-5), 7.94–7.95 (1H, d, J = 7.5 Hz, H-3), 7.72–7.73 (1H, d, J = 5.5 Hz, H-4), 7.26 (1H, s, H-8), 6.87–6.89 (1H, m, H-6), 5.85 (2H, s, CH_2Ar), 3.88 (3H, s, OCH_3), 3.05 (3H, s, CH_3). Anal. Calcd for $C_{20}H_{13}F_5N_2O$: C, 61.22; H, 3.32; N, 7.14. Found: C, 61.09; H, 3.46; N, 7.06.

4.1.7. 9-Phenylpropyl-7-methoxy-1-methyl-β-carboline (2g). A mixture of Harmine **1** (1.05 g, 5 mmol), anhydrous DMF (25 mL), and anhydrous THF (25 mL) were stirred at rt until clear, and then 60% NaH (0.3 g, 7.5 mmol) and 1-bromo-3-phenylpropane (3 mL, 20 mmol) were added and refluxed for 3 h. Later the mixture was treated in a manner similar to that described for **2a** to afford **2g** (0.84 g, 51%), mp 117–118 °C (from ether); FAB-MS *m/e* 331 (M+1); UV λ_{max} 346, 332, 302, 265, 244, 211 nm; IR (KBr): 2995, 2931, 1623, 1563, 1449, 1238, 1156, 1043, 801 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): 8.25–8.26 (1H, d, J = 5 Hz, H-5), 7.92–7.94 (1H, d, J = 8.5 Hz, H-3), 7.69–7.70 (1H, d, J = 5 Hz, H-4), 7.29–7.32 (2H, m, H-8, H-6), 7.20–7.25 (3H, m, Ar–H), 6.84–6.86 (1H, m, Ar–H), 6.63–6.64 (1H, m, Ar–H), 4.42–4.45 (2H, m, $NCH_2CH_2CH_2Ar$), 3.84–3.85 (3H, s, OCH_3), 2.88 (3H, s, CH_3), 2.74–2.77 (2H, m, $NCH_2CH_2CH_2Ar$), 2.12–2.18 (2H, m, $NCH_2CH_2CH_2Ar$). Anal. Calcd for $C_{22}H_{22}N_2O$: C, 80.00; H, 6.67; N, 8.48. Found: C, 79.87; H, 6.82; N, 8.39.

4.1.8. 9-Methyl-β-carboline (4a). A mixture of norharman **3** (1.68 g, 10 mmol) and anhydrous DMF (50 mL) was stirred at rt till clear. Then 60% NaH (0.6 g, 15 mmol) and iodomethane (2 mL, 30 mmol) were added, and the mixture was stirred at rt for 30 min. Later the resulting mixture was poured into H_2O (150 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 petroleum ether–acetone (2:1) as the eluent. Upon recrystallization, white crystals of **4a** were obtained (1.4 g, 77%), mp 108–109 °C (from petroleum ether–acetone); FAB-MS *m/e* 183 (M+1); UV λ_{max} 360, 346, 289, 236, 216 nm; IR (KBr): 2509, 1638, 1504, 1335, 1259, 835 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): 8.88 (1H, s, H-4), 8.46–8.47 (1H, d, J = 5 Hz,

H-1), 8.13–8.14 (1H, d, J=6 Hz, H-8), 7.94–7.95 (1H, d, J=5 Hz, H-3), 7.59–7.62 (1H, m, H-5), 7.45–7.46 (1H, d, J=8.5 Hz, H-6), 7.25–7.30 (1H, m, H-7), 3.93 (3H, s, CH_3). Anal. Calcd for $C_{12}H_{10}N_2$: C, 79.12; H, 5.49; N, 15.38. Found: C, 78.93; H, 5.71; N, 15.45.

4.1.9. 9-Ethyl-β-carboline (4b). A mixture of norharman **3** (1.68 g, 10 mmol) and anhydrous DMF (50 mL) was stirred at rt till clear, and then 60% NaH (0.6 g, 15 mmol) and iodoethane (2.5 mL, 30 mmol) were added. Later the mixture was treated following the method described for **4a** to afford yellow oil **4b** (1.5 g, 76%), FAB-MS *m/e* 197 (M+1); UV λ_{max} 365, 342, 291, 239, 218 nm; IR(KBr): 2485, 2022, 1633, 1500, 1462, 1355, 1239, 831 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): 8.84 (1H, s, H-4), 8.42–8.43 (1H, d, J=5 Hz, H-1), 8.04–8.05 (1H, d, J=8 Hz, H-8), 7.85–7.86 (1H, d, J=5 Hz, H-3), 7.50–7.53 (1H, m, H-5), 7.34–7.36 (1H, d, J=8 Hz, H-6), 7.20–7.23 (1H, m, H-7), 4.26–4.30 (2H, m, CH_2CH$_3$), 1.35–1.38 (3H, m, CH$_2$CH_3). Anal. Calcd for $C_{13}H_{12}N_2$: C, 79.59; H, 6.12; N, 14.29. Found: C, 79.32; H, 6.43; N, 14.13.

4.1.10. 9-Butyl-β-carboline (4c). A mixture of norharman **3** (1.68 g, 10 mmol) and anhydrous DMF (50 mL) was stirred at rt till clear, and then 60% NaH (0.6 g, 15 mmol) and *n*-butyl iodide (6 mL, 50 mmol) were added and refluxed for 1 h. Later the mixture was treated following the method described for **4a** to afford white crystals **4c** (1.6 g, 71%), mp 108–109 °C (from light petroleum ether–acetone); FAB-MS *m/e* 225 (M+1); UV λ_{max} 362, 348, 290, 237, 217 nm; IR (KBr): 2525, 2030, 1632, 1500, 1458, 1355, 1235, 823 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): 8.86 (1H, s, H-4), 8.43–8.44 (1H, d, J=5.5 Hz, H-1), 8.06–8.08 (1H, d, J=8 Hz, H-8), 7.88–7.89 (1H, d, J=4.5 Hz, H-3), 7.52–7.55 (1H, m, H-5), 7.38–7.40 (1H, d, J=8.5 Hz, H-6), 7.21–7.25 (1H, m, H-7), 4.25–4.28 (2H, m, CH_2CH$_2$CH$_2$CH$_3$), 1.79–1.85 (2H, m, CH$_2$CH_2CH$_2$CH$_3$), 1.30–1.38 (2H, m, CH$_2$CH$_2$CH_2CH$_3$), 0.86–0.91 (3H, m, CH$_2$CH$_2$-CH$_2$CH_3). Anal. Calcd for $C_{15}H_{16}N_2$: C, 80.36; H, 7.14; N, 12.50. Found: C, 80.15; H, 7.35; N, 12.39.

4.1.11. 9-Benzyl-β-carboline (4d). A mixture of norharman **3** (1.68 g, 10 mmol) and anhydrous DMF (50 mL) was stirred at rt till clear, and then 60% NaH (0.6 g, 15 mmol) and benzyl bromide (5 mL, 40 mmol) were added and refluxed for 3 h. Later the mixture was treated following the method described for **4a** to afford white crystals **4d** (1.8 g, 69%), mp 118–120 °C (from ether); FAB-MS *m/e* 259 (M+1); UV λ_{max} 358, 344, 289, 237, 213 nm; IR (KBr): 3023, 1619, 1448, 1332, 1258, 821 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): 8.84 (1H, s, H-4), 8.48 (1H, s, H-1), 8.15–8.16 (1H, d, J=8 Hz, H-8), 7.98 (1H, s, H-3), 7.53–7.56 (1H, m, H-5), 7.41–7.43 (1H, d, J=8 Hz, H-6), 7.13–7.31 (6H, m, J=8 Hz, H-7, Ar–H), 5.55 (2H, s, CH_2Ar). Anal. Calcd for $C_{18}H_{14}N_2$: C, 83.72; H, 5.43; N, 10.85. Found: C, 83.57; H, 5.69; N, 10.76.

4.1.12. Methyl 9-methyl-β-carboline-3-carboxylate (5a). A mixture of **5** (2.26 g, 10 mmol) and anhydrous DMF (60 mL) was stirred at rt for 10 min, then 60% NaH (0.6 g, 2 mmol) and iodomethane (2 mL, 30 mmol) were added. Later the mixture was stirred at rt for 30 min. The resulting mixture was poured into iced-water (150 mL) and extracted with ethyl acetate (3×100 mL). The combined organic phase was washed with water and brine. Then dried over anhydrous sodium sulfate, filtered, and evaporated. The yellow oil obtained was purified by silica column chromatography with ethyl acetate as the eluent. After that the solid was collected, it was recrystallized from ethyl ether to give a white crystals (1.8 g, 75%), mp 215–216 °C (from ether); FAB-MS *m/e* 241 (M+1); UV λ_{max} 358, 343, 307, 272, 236, 219 nm; IR (KBr): 3387, 2548, 2056, 1730, 1631, 1334, 1282, 1207 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): 8.94 (1H, s, H-4), 8.88 (1H, s, H-1), 8.20–8.21 (1H, d, J=7.5 Hz, H-8), 7.65–7.68 (1H, m, Ar–H), 7.50–7.52 (1H, d, J=8 Hz, Ar–H), 7.36–7.39 (1H, m, J=8 Hz, Ar–H), 4.06 (3H, s, OCH_3), 4.00 (3H, s, NCH_3). Anal. Calcd for $C_{14}H_{12}N_2O_2$: C, 70.00; H, 5.00; N, 11.67. Found: C, 69.83; H, 5.23; N, 11.59.

4.1.13. Methyl 9-ethyl-β-carboline-3-carboxylate (5b). A mixture of **5** (2.26 g, 10 mmol) and anhydrous DMF (60 mL) was stirred at rt for 10 min, then 60% NaH (0.6 g, 2 mmol) and iodoethane (2.5 mL, 30 mmol) were added. Later the mixture was treated in a manner similar to that described for **5a** to afford white crystals **5b** (2.0 g, 79%), mp 155–156 °C (from ether); FAB-MS *m/e* 255 (M+1); UV λ_{max} 358, 345, 306, 273, 236, 205 nm; IR (KBr): 3248, 1694, 1629, 1337, 1250 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): 8.90–9.00 (2H, m, H-4, H-1), 8.21–8.23 (1H, d, J=8 Hz, H-8), 7.65–7.68 (1H, m, H-5), 7.52–7.54 (1H, d, J=8 Hz, H-6), 7.36–7.39 (1H, m, H-7), 4.51 (2H, s, NCH_2CH$_3$), 4.07 (3H, s, OCH_3), 1.52 (3H, s, NCH$_2$CH_3). Anal. Calcd for $C_{15}H_{14}N_2O_2$: C, 70.87; H, 5.51; N, 11.02. Found: C, 70.65; H, 5.68; N, 10.93.

4.1.14. Methyl 9-butyl-β-carboline-3-carboxylate (5c). A mixture of **5** (2.26 g, 10 mmol) and anhydrous DMF (60 mL) was stirred at rt for 10 min, then 60% NaH (0.6 g, 2 mmol) and *n*-butyl iodide (6 mL, 50 mmol) were added and refluxed for 1 h. Later the mixture was treated in a manner similar to that described for **5a** to afford white crystals **5c** (2.3 g, 82%), mp 181–183 °C (from petroleum ether–ethyl ether 1:2); FAB-MS *m/e* 283 (M+1); UV λ_{max} 358, 344, 306, 273, 236, 222 nm; IR (KBr): 2959, 1735, 1625, 1361, 1246 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): 8.94 (1H, s, H-4), 8.89 (1H, s, H-1), 8.19–8.21 (1H, d, J=7 Hz, H-8), 7.62–7.65 (1H, m, H-5), 7.50-7.52 (1H, d, J=7.5 Hz, H-6), 7.34–7.37 (1H, m, H-7), 4.41–4.44 (2H, m, CH_2CH$_2$CH$_2$CH$_3$), 4.06 (3H, s, OCH_3), 1.87–1.93 (2H, m, CH$_2$CH_2CH$_2$CH$_3$), 1.35–1.42 (2H, m, CH$_2$CH$_2$CH_2CH$_3$), 0.93–0.96 (3H, m, CH$_2$CH$_2$CH$_2$CH_3). Anal. Calcd for $C_{17}H_{18}N_2O_2$: C, 72.34; H, 6.38; N, 9.93. Found: C, 72.21; H, 6.57; N, 9.86.

4.1.15. Methyl 9-benzyl-β-carboline-3-carboxylate (5d). A mixture of **5** (2.26 g, 10 mmol) and anhydrous DMF (60 mL) was stirred at rt for 10 min, then 60% NaH (0.6 g, 2 mmol) and benzyl bromide (5 mL, 40 mmol) were added and refluxed for 3 h. Later the mixture was treated in a manner similar to that described for **5a** to afford white crystals **5d** (2.3 g, 73%), mp 187–188 °C (from petroleum

ethyl ether); FAB-MS *m/e* 317 (M+1); UV λ_{max} 356, 341, 304, 272, 235, 205 nm; IR (KBr): 3026, 2945, 1731, 1622, 1335, 1242 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): 8.89–8.90 (2H, d, J=4 Hz, H-4, H-1), 8.21–8.22 (1H, d, J=8 Hz, H-8), 7.58–7.61 (1H, m, H-5), 7.47–7.48 (1H, d, J=8.5 Hz, H-6), 7.35–7.38 (1H, m, H-7), 7.24–7.28 (3H, m, Ar–H), 7.13–7.15 (2H, m, Ar–H), 5.60 (2H, s, CH_2Ar), 4.05 (3H, s, OCH_3). Anal. Calcd for $C_{20}H_{16}N_2O_2$: C, 75.95; H, 5.06; N, 8.86. Found: C, 75.75; H, 5.29; N, 8.78.

4.1.16. Ethyl 9-methyl-β-carboline-3-carboxylate (6a). A mixture of **6** (2.4 g, 10 mmol) and anhydrous DMF (60 mL) was stirred at rt for 10 min, then 60% NaH (0.6 g, 15 mmol) and iodomethane (2 mL, 30 mmol) were added. Later the mixture was treated in a manner similar to that described for **5a** to afford white crystals **6a** (1.9 g, 75%), mp 139–140 °C (from ethyl ether); FAB-MS *m/e* 255 (M+1); UV λ_{max} 358, 342, 306, 272, 236, 219 nm; IR (KBr): 3446, 3398, 2603, 1721, 1628, 1328, 1280 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): 8.94 (1H, s, H-4), 8.86 (1H, s, H-1), 8.19–8.20 (1H, d, J=7.5 Hz, H-8), 7.66–7.67 (1H, m, H-5), 7.49–7.51 (1H, d, J=8.5 Hz, H-6), 7.35–7.37 (1H, m, H-7), 4.52–4.56 (2H, m, OCH_2CH_3), 3.98 (3H, s, NCH_3), 1.48–1.51 (3H, s, OCH_2CH_3). Anal. Calcd for $C_{15}H_{14}N_2O_2$: C, 70.87; H, 5.51; N, 11.02. Found: C, 70.73; H, 5.73; N, 11.09.

4.1.17. Ethyl 9-ethyl-β-carboline-3-carboxylate (6b). A mixture of **6** (2.4 g, 10 mmol) and anhydrous DMF (60 mL) was stirred rt for 10 min, then 60% NaH (0.6 g, 2 mmol) and iodoethane (2.5 mL, 30 mmol) were added. Later the mixture was treated in a manner similar to that described for **5a** to afford white crystals **6b** (1.8 g, 67%), mp 117–118 °C (from ethyl ether); FAB-MS *m/e* 269 (M+1); UV λ_{max} 359, 344, 306, 273, 237, 221 nm; IR (KBr): 3413, 2984, 1717, 1633, 1334, 1257 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): 8.93 (1H, s, H-4), 8.86 (1H, s, H-1), 8.18–8.21 (1H, d, J=8 Hz, H-8), 7.62–7.64 (1H, m, H-5), 7.48–7.50 (1H, d, J=8 Hz, H-6), 7.33–7.36 (1H, m, H-7), 4.42–4.56 (4H, m, OCH_2CH_3, NCH_2CH_3), 1.46–1.52 (6H, m, OCH_2CH_3, NCH_2CH_3). Anal. Calcd for $C_{16}H_{16}N_2O_2$: C, 71.64; H, 5.97; N, 10.45. Found: C, 71.41; H, 6.21; N, 10.38.

4.1.18. Ethyl 9-butyl-β-carboline-3-carboxylate (6c). A mixture of **6** (2.4 g, 10 mmol) and anhydrous DMF (60 mL) was stirred rt for 10 min, then 60% NaH (0.6 g, 15 mmol) and *n*-butyl iodide (6 mL, 50 mmol) were added and refluxed for 1 h. Later the mixture was treated in a manner similar to that described for **5a** to afford white crystals **6c** (2.3 g, 78%), mp 76–77 °C (from petroleum ether–ethyl ether 1:2); FAB-MS *m/e* 297 (M+1); UV λ_{max} 359, 343, 307, 273, 235, 221 nm; IR (KBr): 3438, 2956, 1731, 1623, 1333, 1242 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): 8.98 (1H, s, H-4), 8.89 (1H, s, H-1), 8.21–8.23 (1H, d, J=8 Hz, H-8), 7.63–7.66 (1H, m, H-5), 7.51–7.53 (1H, d, J=8.5 Hz, H-6), 7.36–7.38 (1H, m, H-7), 4.52–4.56 (2H, m, OCH_2CH_3), 4.42–4.44 (2H, m, $CH_2CH_2CH_2CH_3$), 1.88–1.94 (2H, m, $CH_2CH_2CH_2CH_3$), 1.49–1.52 (3H, m, OCH_2CH_3), 1.35–1.42 (2H, m, $CH_2CH_2CH_2CH_3$), 0.93–0.96 (3H, m, $CH_2CH_2CH_2CH_3$). Anal. Calcd for $C_{18}H_{20}N_2O_2$: C, 72.97; H, 6.76; N, 9.46. Found: C, 72.78; H, 6.98; N, 9.37.

4.1.19. Ethyl 9-benzyl-β-carboline-3-carboxylate (6d). A mixture of **5** (2.4 g, 10 mmol) and anhydrous DMF (60 mL) was stirred rt for 10 min, then 60% NaH (0.6 g, 2 mmol) and benzyl bromide (5 mL, 40 mmol) were added and refluxed for 3 h. Later the mixture was treated in a manner similar to that described for **5a** to afford white crystals **6d** (2.4 g, 70%), mp 126–127 °C (from ethyl ether); FAB-MS *m/e* 331 (M+1); UV λ_{max} 356, 342, 305, 273, 234 nm; IR (KBr): 3425, 3060, 3027, 2974, 1723, 1622, 1335, 1214 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): 8.91 (2H, m, H-4, H-1), 8.23–8.25 (1H, d, J=8 Hz, H-8), 7.59–7.62 (1H, m, H-5), 7.49–7.50 (1H, d, J=8 Hz, H-6), 7.36–7.39 (1H, m, H-7), 7.25–7.27 (3H, s, Ar–H), 7.14–7.16 (2H, m, Ar–H), 5.62 (2H, s, CH_2Ar), 4.51–4.55 (2H, m, OCH_2CH_3), 1.47–1.50 (3H, m, OCH_2CH_3). Anal. Calcd for $C_{21}H_{18}N_2O_2$: C, 76.36; H, 5.45; N, 8.48. Found: C, 76.19; H, 5.67; N, 8.38.

4.1.20. Ethyl 9-(2′,3′,4′,5′,6′-pentafluoro)benzyl-β-carboline-3-carboxylate (6e). A mixture of **5** (2.4 g,10 mmol) and anhydrous DMF(60 mL) was stirred at rt for 10 min, then 60% NaH (0.6 g, 15 mmol) and α-bromo-2,3,4,5,6-pentafluorotoluene (3 mL, 20 mmol) were added. Later the mixture was treated in a manner similar to that described for **5a** to afford white crystals **6d** (2.6 g, 62%), mp 153–154 °C (from ethyl ether); FAB-MS *m/e* 421 (M+1); UV λ_{max} 350, 335, 299, 271, 236, 217 nm; IR (KBr): 3399, 3065, 2987, 1709, 1627, 1337, 1245 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): 9.07 (1H, s, H-4), 8.86 (1H, s, H-1), 8.19–8.21 (1H, d, J=8 Hz, H-8), 7.65–7.68 (1H, m, H-5), 7.59–7.61 (1H, d, J=8.5 Hz, H-6), 7.38–7.41 (1H, m, H-7), 5.67 (2H, s, CH_2Ar), 4.52–4.56 (2H, m, OCH_2CH_3), 1.49–1.51 (3H, m, OCH_2CH_3). Anal. Calcd for $C_{21}F_5H_{13}N_2O_2$: C, 60.00; H, 3.10; N, 6.67. Found: C, 59.87; H, 3.29; N, 6.58.

4.1.21. Ethyl 9-phenylpropyl-β-carboline-3-carboxylate (6f). A mixture of **5** (2.4 g, 10 mmol) and anhydrous DMF (60 mL) was stirred at rt for 10 min, then 60% NaH (0.6 g, 2 mmol) and 1-bromo-3-phenylpropane (6 mL, 40 mmol) were added and refluxed for 3 h. Later the mixture was treated in a manner similar to that described for **5a** to afford white crystals **6e** (2.0 g, 56%), mp 140–142 °C (from ethyl ether); FAB-MS *m/e* 359 (M+1); UV λ_{max} 358, 343, 306, 273, 236, 217 nm; IR (KBr): 3026, 2983, 2933, 1724, 1622, 1333, 1246 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): 8.89 (2H, m, H-4, H-1), 8.20–8.22 (1H, d, J=7.5 Hz, H-8), 7.61–7.64 (1H, m, H-5), 7.41–7.42 (1H, m, H-6), 7.34–7.37 (1H, m, H-7), 7.26–7.30 (2H, m, Ar–H), 7.19–7.22 (1H, m, Ar–H), 7.13–7.15 (2H, m, Ar–H), 4.52–4.57 (2H, m, $NCH_2CH_2CH_2Ar$), 4.41–4.44 (2H, m, $NCH_2CH_2CH_2Ar$), 2.70–2.73 (2H, m, OCH_2CH_3), 2.24–2.30 (2H, m, $NCH_2CH_2CH_2Ar$), 1.49–1.52 (3H, m, OCH_2CH_3). Anal. Calcd for $C_{23}H_{22}N_2O_2$: C, 77.09; H, 6.15; N, 7.82. Found: C, 76.88; H, 6.38; N, 7.75.

4.1.22. Butyl 9-methyl-β-carboline-3-carboxylate (7a). A mixture of **7** (2.68 g, 10 mmol) and anhydrous DMF (80 mL) was stirred at rt for 10 min, then 60% NaH (0.6 g, 15 mmol) and iodomethane (2 mL, 30 mmol) were added and stirred at rt for 30 min. Later the mixture was

treated in a manner similar to that described for **5a** to afford white crystals **7a** (2.0 g, 70%), mp 235–238 °C (from ethyl ether); FAB-MS *m/e* 283 (M+1); UV λ_{max} 358, 343, 305, 272, 236, 219 nm; IR (KBr): 3402, 2956, 2869, 1726, 1625, 1333, 1238 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): 8.94 (1H, s, H-4), 8.83 (1H, s, H-1), 8.18–8.20 (1H, d, J = 7.5 Hz, H-8), 7.63–7.66 (1H, m, H-5), 7.48–7.50 (1H, d, J = 8.5 Hz, H-6), 7.34–7.37 (1H, m, H-7), 4.46–4.49 (2H, m, $OCH_2CH_2CH_2CH_3$), 3.97 (3H, s, NCH_3), 1.84–1.90 (2H, m, J = 7.0 Hz, $OCH_2CH_2CH_2CH_3$), 1.48–1.56 (2H, m, $OCH_2CH_2CH_2CH_3$), 0.99–1.02 (3H, m, $OCH_2CH_2CH_2CH_3$). Anal. Calcd for $C_{17}H_{18}N_2O_2$: C, 72.34; H, 6.38; N, 9.93. Found: C, 72.25; H, 6.58; N, 9.85.

4.1.23. Butyl 9-ethyl-β-carboline-3-carboxylate (7b). A mixture of **6** (2.4 g, 10 mmol) and anhydrous DMF (60 mL) was stirred at rt for 10 min, then 60% NaH (0.6 g, 15 mmol) and iodoethane (2.5 mL, 30 mmol) were added. Later the mixture was treated in a manner similar to that described for **5a** to afford white crystals **6b** (2.0 g, 65%), mp 76–77 °C (from ethyl ether); FAB-MS *m/e* 297 (M+1); UV λ_{max} 360, 345, 306, 273, 240, 220 nm; IR (KBr): 3053, 2963, 2401, 1999, 1722, 1627, 1337, 1269 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): 9.02 (1H, s, H-4), 8.86 (1H, s, H-1), 8.20–8.22 (1H, d, J = 8 Hz, H-8), 7.63–7.67 (1H, m, H-5), 7.51–7.53 (1H, d, J = 7.5 Hz, H-6), 7.35–7.38 (1H, m, H-7), 4.47–4.51 (4H, m, $OCH_2CH_2CH_2CH_3$, NCH_2CH_3), 1.84–1.90 (2H, m, $OCH_2CH_2CH_2CH_3$), 1.49–1.56 (2H, m, $OCH_2CH_2CH_2CH_3$), 0.99–1.02 (3H, m, $OCH_2CH_2CH_2CH_3$). Anal. Calcd 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, 9.39.

4.1.24. Butyl 9-butyl-β-carboline-3-carboxylate (7c). A mixture of **6** (2.4 g, 10 mmol) and anhydrous DMF (60 mL) was stirred at rt for 10 min, then 60% NaH (0.6 g, 15 mmol) and *n*-butyl iodide (6 mL, 50 mmol) were added and refluxed for 1 h. Later the mixture was treated in a manner similar to that described for **5a** to afford white crystals **6c** (2.2 g, 74%), mp 94–95 °C (from petroleum ether–ethyl ether 1:2); FAB-MS *m/e* 325 (M+1); UV λ_{max} 359, 344, 307, 274, 238, 221 nm; IR (KBr): 3061, 2956, 2866, 1728, 1624, 1358, 1247 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): 8.95 (1H, s, H-4), 8.85 (1H, s, H-1), 8.19–8.20 (1H, d, J = 7 Hz, H-8), 7.60–7.64 (1H, m, H-5), 7.49–7.50 (1H, d, J = 8.5 Hz, H-6), 7.32–7.36 (1H, m, H-7), 4.47–4.49 (2H, m, $OCH_2CH_2CH_2CH_3$), 4.37–4.42 (2H, m, $NCH_2CH_2CH_2CH_3$), 1.84–1.92 (4H, m, $OCH_2CH_2CH_2CH_3$, $NCH_2CH_2CH_2CH_3$), 1.49–1.56 (2H, m, $OCH_2CH_2CH_2CH_3$), 1.34–1.40 (2H, m, $NCH_2CH_2CH_2CH_3$), 0.99–1.02 (3H, m, $OCH_2CH_2CH_2CH_3$), 0.92–0.95 (3H, m, $NCH_2CH_2CH_2CH_3$). Anal. Calcd for $C_{20}H_{24}N_2O_2$: C, 74.07; H, 7.41; N, 8.64. Found: C, 73.88; H, 7.65; N, 8.57.

4.1.25. Butyl 9-benzyl-β-carboline-3-carboxylate (7d). A mixture of **5** (2.4 g, 10 mmol) and anhydrous DMF (60 mL) was stirred at rt for 10 min, then 60% NaH (0.6 g, 15 mmol) and benzyl bromide (5 mL, 40 mmol) were added and refluxed for 3 h. Later the mixture was treated in a manner similar to that described for **5a** to afford white crystals **7d** (2.4 g, 67%), mp 105–106 °C (from ethyl ether); FAB-MS *m/e* 359 (M+1); UV λ_{max} 356, 342, 305, 273, 235, 205 nm; IR (KBr): 3051, 2959, 2930, 2869, 1700, 1620, 1362, 1246 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): 8.89 (1H, s, H-4), 8.85 (1H, s, H-1), 8.19–8.20 (1H, d, J = 8 Hz, H-8), 7.56–7.59 (1H, m, H-5), 7.45–7.46 (1H, d, J = 8.5 Hz, H-6), 7.33–7.36 (1H, m, H-7), 7.22–7.26 (3H, m, ArH), 7.11–7.13 (2H, m, ArH), 5.56 (2H, s, CH_2Ar), 4.45–4.48 (2H, m, $OCH_2CH_2CH_2CH_3$), 1.82–1.88 (2H, m, $OCH_2CH_2CH_2CH_3$), 1.47–1.54 (2H, m, $OCH_2CH_2CH_2CH_3$), 0.98–1.01 (3H, m, $OCH_2CH_2CH_2CH_3$). Anal. Calcd for $C_{23}H_{22}N_2O_2$: C, 77.09; H, 6.15; N, 7.82. Found: C, 76.89; H, 6.35; N, 7.75.

4.1.26. General procedure for the preparation of β-carboline-3-carboxylic acids 8a–e. A mixture of the corresponding β-carboline-3-carboxylate (**6a–f**, 10 mmol), NaOH (50 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 vacuo.

4.1.27. 9-Methyl-β-carboline-3-carboxylic acid (8a). Yellow solid was obtained (2.32 g, 99%). Mp 267–269 °C; FAB-MS *m/e* 227 (M+1); UV λ_{max} 384, 360, 273, 241, 217 nm; IR (KBr): 3250–2100, 1716, 1630, 1405, 1200 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 12.15 (1H, s, COO*H*), 9.19 (1H, s, H-4), 9.12 (1H, s, H-1), 8.42–8.44 (1H, d, J = 8.0 Hz, H-8), 7.81–7.88 (2H, m, H-5, H-6), 7.50–7.53 (1H, m, H-7), 4.16 (1H, s, NCH_3). Anal. Calcd for $C_{13}H_{10}N_2O_2$: C, 69.03; H, 4.42; N, 12.39. Found: C, 68.96; H, 4.57; N, 12.31.

4.1.28. 9-Ethyl-β-carboline-3-carboxylic acid (8b). Yellow solid was obtained (2.41 g, 98%). Mp 201–202 °C; FAB-MS *m/e* 241 (M+1); UV λ_{max} 387, 359, 273, 239, 220 nm; IR (KBr): 3250–2250, 1713, 1630, 1407, 1336, 1198 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 12.10 (1H, s, COO*H*), 9.16 (1H, s, H-4), 8.97 (1H, s, H-1), 8.31–8.33 (1H, d, J = 8.0 Hz, H-8), 7.75–7.81(2H, m, H-5, H-6), 7.43–7.46 (1H, m, H-7), 4.63–4.67 (2H, s, NCH_2CH_3), 1.46–1.52 (3H, s, NCH_2CH_3). Anal. Calcd for $C_{14}H_{12}N_2O_2$: C, 70.00; H, 5.00; N, 11.67. Found: C, 69.89; H, 5.19; N, 11.59.

4.1.29. 9-*n*-Butyl-β-carboline-3-carboxylic acid (8c). Yellow solid was obtained (2.71 g, 99%). Mp 182–184 °C; FAB-MS *m/e* 269 (M+1); UV λ_{max} 387, 359, 272, 238, 221 nm; IR (KBr): 3250–2250, 1710, 1630, 1334, 1213 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 12.11 (1H, s, COO*H*), 9.14 (1H, s, H-4), 8.94 (1H, s, H-1), 8.42–8.44 (1H, d, J = 8.0 Hz, H-8), 7.77–7.79 (1H, d, J = 8.5 Hz, H-5), 7.65–7.68 (1H, m, H-6), 7.34–7.37 (1H, m, H-7), 4.56–4.59 (2H, m, $NCH_2CH_2CH_2CH_3$), 1.79–1.85 (2H, m, $NCH_2CH_2CH_2CH_3$), 1.28–1.32 (2H, m, $NCH_2CH_2CH_2CH_3$), 0.87–0.90 (3H, m, $NCH_2CH_2CH_2CH_3$). Anal. Calcd for $C_{16}H_{16}N_2O_2$: C, 71.64; H, 5.97; N, 10.45. Found: C, 71.45; H, 6.19; N, 10.36.

4.1.30. 9-Benzyl-β-carboline-3-carboxylic acid (8d). Yellow solid was obtained (2.96 g, 98%). Mp 261–262 °C;

FAB-MS *m/e* 303 (M+1); UV λ_{max} 355, 342, 267, 239 nm; IR (KBr): 3457, 3409, 3250–2250, 1683, 1624, 1334, 1225 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 12.18 (1H, s, COO*H*), 9.15 (1H, s, H-4), 8.96 (1H, s, H-1), 8.44–8.45 (1H, d, J = 8.0 Hz, H-8), 7.79–7.80 (1H, d, J = 8.0 Hz, H-5), 7.63–7.66 (1H, m, H-6), 7.35–7.38 (1H, m, H-7), 7.22–7.31 (5H, m, Ar–H), 5.85 (2H, s, NCH_2Ar). Anal. Calcd for $C_{19}H_{14}N_2O_2$: C, 75.50; H, 4.64; N, 9.27. Found: C, 75.38; H, 4.85; N, 9.18.

4.1.31. 9-(2′,3′,4′,5′,6′-Pentafluoro)benzyl-β-carboline-3-carboxylic acid (8e). White solid was obtained (3.84 g, 98%). Mp >270 °C; FAB-MS *m/e* 393 (M+1); UV λ_{max} 351, 338, 261, 239, 218 nm; IR (KBr): 3500–2250, 1714, 1628, 1335 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 12.14 (1H, s, COO*H*), 9.10 (1H, s, H-4), 8.83 (1H, s, H-1), 8.45–8.47 (1H, d, J = 8 Hz, H-8), 7.65–7.68 (1H, m, H-5), 7.59–7.61 (1H, d, J = 8.5 Hz, H-6), 7.38–7.41 (1H, m, H-7), 5.67 (2H, s, CH_2Ar). Anal. Calcd for $C_{19}F_5H_9N_2O_2$: C, 58.16; H, 2.30; N, 7.14. Found: C, 58.03; H, 2.39; N, 7.18.

4.1.32. 9-Phenylpropyl-β-carboline-3-carboxylic acid (8f). Yellow solid was obtained (3.2 g, 97%). Mp 213–215 °C; FAB-MS *m/e* 331 (M+1); UV λ_{max} 358, 346, 268, 239, 218 nm; IR ν_{max} 3500–2250, 1692, 1629, 1335, 1215 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 12.09 (1H, s, COO*H*), 9.13 (1H, s, H-4), 9.00 (1H, s, H-1), 8.46–8.48 (1H, d, J = 7.5 Hz, H-8), 7.76–7.78 (1H, d, J = 8.0 Hz, H-5), 7.69–7.72 (1H, m, H-6), 7.38–7.41 (1H, m, H-7), 7.21–7.26 (2H, m, ArH), 7.13–7.17 (3H, m, Ar–H), 4.63–4.66 (2H, m, NCH_2CH_2CH_2Ar), 2.49–2.51 (2H, m, NCH$_2$CH_2CH$_2$Ar), 2.14–2.20 (2H, m, NCH$_2$CH$_2$CH_2Ar). Anal. Calcd for $C_{21}H_{18}N_2O_2$: C, 76.36; H, 5.45; N, 8.48. Found: C, 76.22; H, 5.69; N, 8.38.

4.2. In vitro cytotoxicity assays

Cytotoxicity assays in vitro were carried out using 96 microtiter plate cultures and MTT staining according to the procedures described by Al-Allaf and Rashan[26] with a slight modification. Cells were grown in RPMI-1640 medium containing 10% (v/v) fetal calf serum and 100 μg/mL penicillin and 100 μg/mL streptomycin. Cultures were propagated at 37 °C in a humified atmosphere containing 5% CO_2. Cell lines were obtained from Shanghai Cell Institute, 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 effects on cell replication. The human tumor cell line panel consisted of nonsmall 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.

4.3. Assay of acute toxicities

Healthy C57BL/6 mice (9–12 weeks) weighing 18–22 g were housed in rooms where the temperature was approximately 24 ± 2 °C, with a relative humidity 60–70%, and in 12 h light-dark cycle. The sterile food and water were provided according to institutional guidelines. All animals were provided by Shanghai Laboratory Animal Center of Chinese Academy of Science. All animal procedures were approved by the Animal Ethical Committee of the Sun Yat-sen University. Prior to each experiment, mice were fastened overnight and allowed free access to water. Various doses of the β-carboline derivatives ranging from 10 to 300 mg/kg dissolved in 0.5% carboxymethyl cellulose sodium (CMC-Na) salt solution were given via intraperitoneal (i.p.) to different groups of healthy C_{57}BL/6 mice, and each group contained 10 mice (five males and five females). After the administration of the compounds, 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 animals were sacrificed at the 14th day after drug administration and checked macroscopically for possible damage to the heart, liver, and kidneys. Mice of immediate death following drug administration were also examined for any possible organ damage. LD_{50} values were calculated graphically as described.[31]

4.4. Assay of antitumor activity

Lewis lung cancer and S180 sarcoma cell lines were provided by Shanghai Institute of Pharmaceutical Industry. Tumor cells of Lewis lung cancer and S180 sarcoma were inoculated to mice. After 7 days, tumors were taken out and cells harvested. Viable tumor cells (2×10^6 cells/mouse) were inoculated to the armpit of mice by subcutaneous injection. Each compound was injected by intraperitoneal (i.p.) to different group mice (each group containing 10 female mice) 24 h after the inoculation at a dosage of 7.5 mg/kg once a day for consecutive 7 days. This dose was the maximum tolerated dose for most compounds based on our preliminary studies. Cyclophosphane (CTX) at 30 mg/kg was used as a positive control and vehicle as negative control. The weights of animals were recorded every 3 days. All animals were sacrificed at the 21st day after tumor inoculation and the tumors were excised and weighed. The inhibition rate was calculated as follows:

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

T: average tumor weight of treated group; C: average tumor weight of negative control group.

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