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Synthesis and structure–activity relationships of harmine derivatives as potential antitumor agents

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ABSTRACT

Harmine, a naturally occurring β-carboline alkaloid, showed good antitumor activities together with remarkable neurotoxic effects in animal models. In order to search for novel leading compounds endowed with better antitumor activities and less neurotoxicities, a series of harmine derivatives were designed and synthesized by modification of position-2, 7 and 9 of β-carboline nucleus, and their cytotoxic activities against human tumor cell lines were investigated. Acute toxicities and antitumor activities of the selected compounds in mice were also evaluated. Structure–activity relationships studies confirmed that (1) the 7-methoxy structural moiety was the pharmacophore responsible for the neurotoxic effects of this class of compounds; (2) the substituents in position-2 and 9 played a vital role in modulation of their antitumor activities.

1. Introduction

Harmine, the most representative naturally occurring β-carboline alkaloid, was originally isolated from *Peganum harmala* which is being widely used as a traditional herbal drug as an emmenagogue and abortifacient in the Middle East and North Africa [1]. In Northwest China, the extracts of the seeds of *P. harmala* have been traditionally used for hundreds of years to treat the alimentary tract cancers and malaria [2], and subsequent investigation confirmed that harmine was the most important active ingredients [3,4]. So far, numerous previous studies demonstrated that harmine possessed a wide spectrum of biochemical activities including intercalation into DNA [5–8], inhibition of topoisomerase I (Topo I) [8,9] and cyclin-dependent kinases (CDKs) [10,11], inhibition of monoamine oxidase A (MAO-A) [12,13] and 5-hydroxytryptamine (5-HT) uptake of human platelet [13]. Moreover, harmine was reported to exhibit a diverse range of pharmacological properties such as hallucinogenic [14], antitumor [2–4,8], antiviral [15] and antiparasitic [16] activities.

Previous investigations were focused on the neuropharmacological effects of harmine on the central nervous system (CNS) such as hallucination, tremor, anxiolytic and sedation. However, recent interest in harmine has been attracted to its antitumor activity. Ishida et al. [3] reported the incorporation of various substituents into position-1, 2, 6, 7 and 9 of harmine and the evaluation of their biological activities as antitumor agents. Structure–activity relationships (SARs) analysis demonstrated that (1) introducing alkoxy substituents into position-7 of harmine led to enhanced cytotoxic activities; (2) the length of alkoxy chain affected both cytotoxicity and cell line specificity; (3) N^9-alkylated harmine derivatives exhibited strong cytotoxic effects; (4) N^2-alkylated β-carboline derivatives displayed specific cytotoxic activities. Our previous investigation [4] also indicated that N^9-alkyl and aryl alkyl substituted harmine derivatives had significant antitumor in mice bearing both Lewis lung carcinoma and Sarcoma 180, while their exhibited remarkable neurotoxic effects including tremor, twitch and jumping in experimental animal models. SARs studies suggested that (1) the introduction of appropriate substituents into position-9 of harmine remarkably enhanced the antitumor activities *in vitro* and *in vivo*; (2) the methoxy group at position-7 of harmine might play a very crucial role in determining their remarkable neurotoxic effects. More recently, our group investigation [17] on the syntheses of harmine derivatives bearing various

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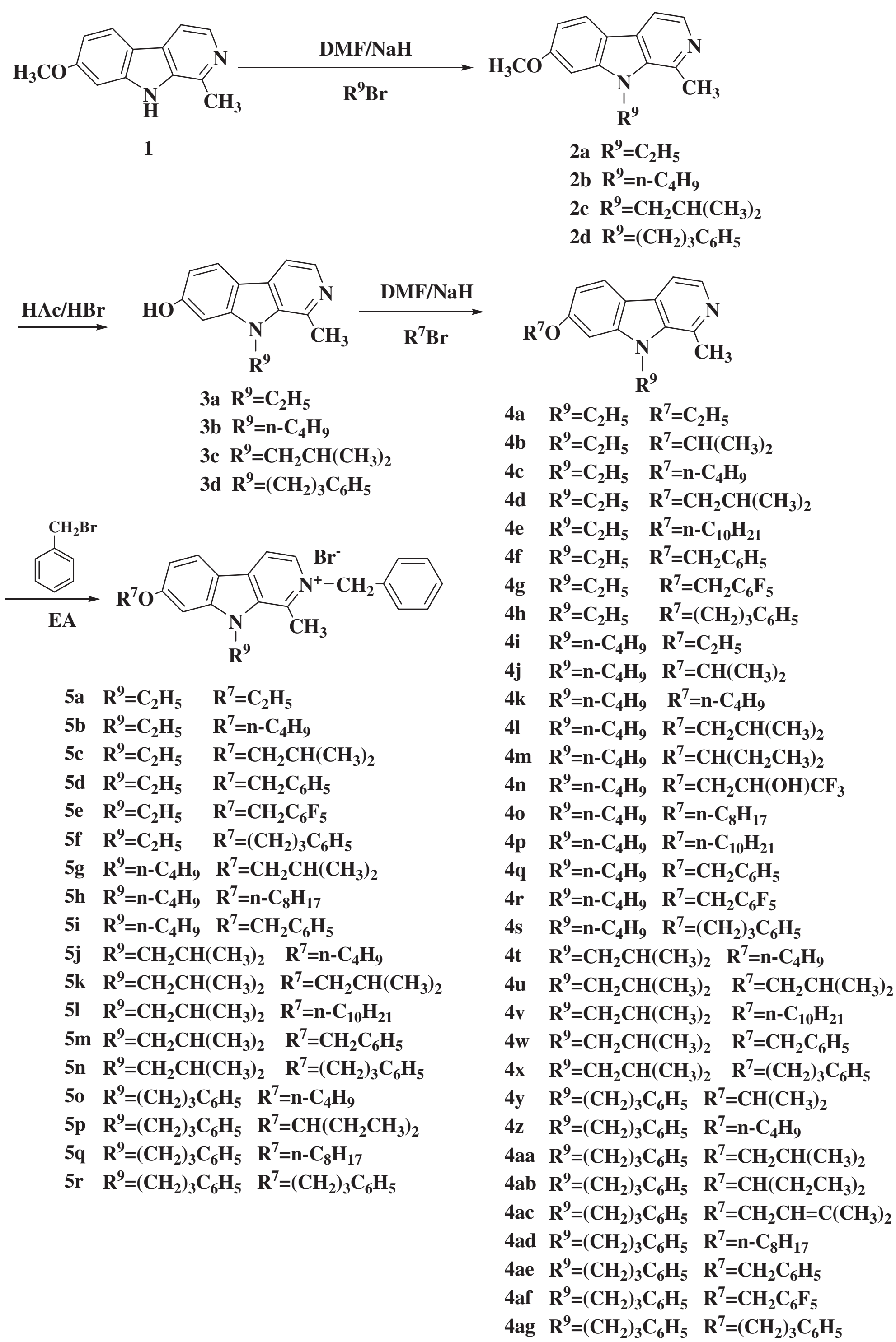
substituents at postion-2, 7 and 9 of β-carboline nucleus and the evaluation of their antitumor activities *in vitro* disclosed that the N^2-benzyl substituent on the β-carboline ring played an important role in the modulation of the cytotoxic activities.

In continuing search for novel antitumor agents endowed with better pharmacological profiles and elucidate the antitumor structure–activity relationships (SARs) of harmine derivatives in finer detail, in the present investigation, we reported the design, synthesis and structure–activity relationships of harmine derivatives as antitumor agents.

2. Chemistry

The synthetic routes of harmine derivatives **4a**–**ag** and **5a**–**r** are outlined in Scheme 1. The N^9-alkylated harmine derivatives **2a**–**d** were prepared according to the synthetic protocol described by



Scheme 1. Synthesis of harmine derivatives **4a**–**ag** and **5a**–**r**.

our group [4]. The preparation of compounds **3a–d** followed a common synthetic scheme, characterized by demethylation of compounds **2a–d** using acetic acid and hydrobromic acid as reaction solvent [17]. Compounds **4a–ag**, bearing alkoxy in postion-7 of β-carboline ring, were synthesized from compounds **3a–d** by the action of sodium hydride in dry DMF followed by addition of the appropriate alkylating and arylating agents in 63–87% yield. The N^2-benzylated β-carbolinium bromates **5a–r** were prepared from compounds **4** by the addition of benzyl bromide in refluxing ethyl acetate [17]. The chemical structures of all the newly synthesized compounds were characterized by MS, IR, ^{1}H NMR, ^{13}C NMR and elemental analysis.

3. Results and discussion

3.1. Cytotoxicity in vitro

The cytotoxic potencies of harmine derivatives **4a–ag** and **5a–r** against a panel of human tumor cell lines were investigated and compared with the reference drugs cisplatin. The human tumor cell line panel consisted of cervical carcinoma (Hela), liver carcinoma (Bel-7402 and HepG2), gastric carcinoma (BGC-823), breast carcinoma (MCF-7), renal carcinoma (769-P, 786-0 and OS-RC-2), epidermoid carcinoma of the nasopharynx (KB), non-small cell lung carcinoma (A549), malignant melanoma (A375), colon carcinoma (HT-29), bladder squamous carcinoma (SCaBER), malignant bladder carcinoma (Blu-87), malignant glioma (U251). Compounds **4a–ag** were converted into their water-soluble hydrochloride salt by the usual methods before use. The results were summarized in Table 1.

The cytotoxic potency of most 7,9-disubstituted harmine derivatives **4a–ag** showed no distinct difference and the IC_{50} values of this class of compounds ranged from 10 to 100 μM. Exceptionally, compounds **4e–g**, **4n–p**, **4r**, **4aa**, **4ac**, **4v** and **4af–ag** bearing relatively large and bulky alkoxy substituent in position-7 displayed weak or no cytotoxic activities against several human tumor cell lines, and the poor water-soluble properties might be responsible for their weak activities.

Interestingly, compounds **5a–r** having a benzyl substituent in position-2 of β-carboline nucleus exhibited the most interesting cytotoxic potencies with IC_{50} values of lower than 10 μM against most of human tumor cell lines.

Of all 2,7,9-trisubstituted harmine derivatives **5a–r**, compounds **5o–r** bearing a 3-phenylpropyl substituent in position-9 of β-carboline nucleus showed more potent cytotoxic activities than compounds **5a–f**, **5g–i** and **5j–n**, which having a ethyl, butyl and isobutyl group in position-9, respectively. The influence of substituent in position-9 on cytotoxic activities followed the tendency of 3-phenylpropyl > isobutyl > butyl > ethyl group. Particularly, compound **5p** bearing a benzyl group in position-2, an isobutoxy group in position-7 and a 3-phenylpropyl group in position-9, was found to be the most potent cytotoxic agent with IC_{50} values of lower than 5.0 μM against all human tumor cell lines. These results suggested that the large and bulky substituent in position-9 might be advisable pharmacophoric group for enhanced cytotoxic activities.

An overview of the cytotoxic activities data of all newly synthesized harmine derivatives and of the earlier reports [4,17] clearly confirmed that (1) the arylated alkyl substituent in position-9 of β-carboline nucleus was the suitable pharmacophore giving rise to significant cytotoxic agents; (2) the introduction of benzyl group into position-2 of β-carboline nucleus facilitated significantly cytotoxic potencies.

3.2. Assessment of acute toxicity

The LD_{50} values and scores for neurotoxicity of the selected harmine derivatives in mice after administration by i.p. route were summarized in Table 2. All the tested harmine derivatives resulted in acute toxic manifestation but caused no obvious neurotoxic effects including tremor, twitch, jumping and supination just like harmine **1**. Animals were drowsy and exhibited a decrease in locomotor activity after the administration of harmine derivatives. Of all investigated compounds, compound **5r** exhibited the highest acute toxicity with LD_{50} value of 3.75 mg/kg. Compounds **5c**, **5e**, **5f**, **5m** and **5p** also displayed remarkable acute toxicity with LD_{50} value of 12.5, 12.5, 15.0, 5.0 and 6.25 mg/kg, respectively, while for the compounds **4a**, **4h** and **4ab**, acute toxicities were much less with LD_{50} value of 200, 200 and 100 mg/kg, respectively. 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 obvious changes in any organs.

A total analysis to the acute toxicity and neurotoxic effect of harmine derivatives investigated above and of our previous report [4] confirmed that (1) the methoxy substituent in position-7 of β-carboline nucleus played a vital role in determining the remarkable neurotoxic effects; (2) replacing the methoxy substituent with a bulky alkoxy group led to eliminating neurotoxic effect of these compounds; (3) the acute toxicity increased remarkably by the introduction of a benzyl substituent into position-2 of β-carboline nucleus.

3.3. Evaluation of antitumor activity

Nine harmine derivatives were selected for evaluation *in vivo* against mice bearing Lewis lung cancer and Sarcoma 180 and compared with the reference drugs harmine **1** and Cyclophosphamide (CTX). The tumor inhibition rates of these compounds were summarized in Table 2. All the tested compounds showed potent antitumor activities. Harmine **1** exhibited almost equal antitumor activity against mice both bearing Sarcoma 180 and Lewis lung cancer with the tumor inhibition rate of 30.8 and 33.7%, respectively. Compounds **5c**, **5e**, **5f**, **5m**, **5p** and **5r** exhibited remarkable antitumor activities with the tumor inhibition rate of over 40% against mice bearing Lewis lung cancer and Sarcoma 180 at dose 2.5, 3.0, 1.0, 1.2 and 0.63 mg/kg, respectively. Particularly, compounds **5f** and **5m** were found to be the most potent antitumor agents with the tumor inhibition rate of 53.1 and 52.6% against mice bearing Sarcoma 180, respectively. The other compounds tested showed moderate antitumor activities with the tumor inhibition rate ranging from 20.8 to 36.9% at dose ranging from 2.5 to 40.0 mg/kg. Interestingly, the Sarcoma 180 was more susceptible to all tested compounds than the Lewis lung cancer, and the results contradicted with our previous report [4]. The analysis of the structure–activity relationships indicated that (1) introducing a benzyl substituent into position-2 of β-carboline nucleus improved significantly their antitumor activities; (2) the arylated alkoxy group in position-7 of β-carboline nucleus was the advisable pharmacophoric group for their enhanced antitumor activities.

4. Conclusions

In the present investigation, a series of harmine derivatives bearing an alkyl substituent in position-9, a benzyl group in position-2 and an alkoxy chain in position-7 were synthesized and evaluated as potential antitumor agents. The results corroborated the previous observations that the antitumor activities and acute toxicities as well as neurotoxic effects of harmine derivatives were substituent-dependent. An overview of the present study and of the previous reports, we arrived at the following conclusions: (1) the methoxy substituent in position-7 of β-carboline nucleus played a vital role in determining the remarkable neurotoxic effects; (2) replacing the methoxy substituent with a bulky alkoxy group led to eliminating neurotoxic effects of these compounds; (3)

Table 1
Cytotoxic activities of harmine derivatives **4a**–**ag** and **5a**–**r** *in vitro*[c] (IC_{50}, μM[a]).

Compd	Hela[b]	Bel-7402	BGC-823	HepG2	MCF	OS-RC-2	A549	A375	786-0	HT-29	SCaBER	Blu-87	769-P	U251	KB	22RV1
1[e]	60	54	68	46	ND[d]	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4a	30.7	27.4	57.1	68.9	14.4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4b	27.6	43.6	59.7	52.6	>100	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4c	29.2	52.9	15.6	16.3	18.5	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4d	23.3	21.9	29.8	20.8	13.2	32.0	30.3	ND	24.3	16.9	16.4	14.2	27.0	44.6	20.3	ND
4e	>100	>100	15.2	14.1	45.9	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4f	>100	>100	17.2	15.5	>100	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4g	>100	>100	>100	>100	>100	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4h	30.2	13.5	11.9	11.4	>100	ND	22.6	19.7	13.2	11.8	6.5	16.7	22.6	28.0	22.6	ND
4i	32.1	36.1	37.2	43.3	12.0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4j	21.2	26.8	19.1	30.2	19.5	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4k	17.4	69.9	15.8	15.5	17.3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4l	40.7	25.6	23.4	15.3	21.6	30.5	30.0	18.4	14.4	20.1	15.9	17.4	4.0	32.3	19.4	ND
4m	28.2	21.5	16.7	15.6	5.4	26.9	21.0	18.8	13.4	25.1	43.8	16.0	22.2	25.1	19.8	ND
4n	>100	62.0	96.4	>100	33.1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4o	>100	>100	15.4	13.6	13.6	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4p	>100	18.8	28.4	>100	62.7	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4q	47.0	>100	17.2	17.8	16.9	ND	18.1	28.6	10.1	26.8	39.7	32.7	17.1	ND	45.0	ND
4r	>100	>100	18.3	13.6	>100	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4s	24.7	>100	13.8	15.6	16.0	ND	28.1	21.5	12.9	51.2	20.3	26.9	21.3	29.9	41.6	40.5
4t	20.8	42.5	12.1	12.1	7.2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4u	32.3	42.6	26.6	36.8	29.4	30.5	32.4	22.2	18.1	35.3	28.8	32.3	30.8	49.6	27.5	ND
4v	2.0	>100	74.1	>100	>100	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4w	50.5	26.8	36.0	22.2	54.1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4x	27.6	>100	18.5	27.7	19.5	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4y	48.6	36.2	16.3	14.6	45.3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4z	88.7	34.8	14.0	12.4	68.9	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4aa	>100	39.3	23.0	12.9	>100	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4ab	70.1	34.3	18.3	7.3	41.9	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4ac	>100	24.7	32.3	21.1	>100	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4ad	69.6	21.8	17.4	>100	45.9	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4ae	72.6	29.4	19.5	15.7	63.3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4af	73.5	18.6	7.9	>100	38.7	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4ag	>100	>100	19.8	16.7	>100	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
5a	2.2	12.6	9.2	14.3	8.1	7.8	11.2	6.8	9.6	10.2	19.8	23.4	6.4	15.2	4.6	ND
5b	2.4	2.9	10.9	10.0	5.9	8.8	13.0	6.9	7.8	6.4	21.8	22.9	4.6	14.2	<1.7	ND
5c	4.9	3.2	10.6	9.6	11.1	10.6	11.6	7.8	8.5	4.0	34.1	25.9	5.2	17.3	<1.7	ND
5d	56.3	3.6	66.1	49.9	67.6	15.9	19.6	12.3	15.4	10.9	46.3	22.1	8.9	16.2	8.7	ND
5e	0.93	4.0	4.6	1.6	5.7	1.8	1.8	3.8	2.9	1.6	6.8	17.7	13.0	6.6	13.8	<1.4
5f	2.2	2.4	7.6	7.6	2.8	2.2	2.3	2.4	1.5	4.2	16.2	17.3	5.0	10.5	<1.5	ND
5g	3.9	2.7	4.6	4.2	10.4	3.2	3.8	4.2	2.5	3.1	18.2	12.3	4.6	12.1	2.0	6.7
5h	4.0	0.86	3.7	2.7	4.1	<1.5	<1.5	<1.5	<1.5	3.2	4.6	2.0	<1.5	2.0	<1.5	2.9
5i	2.2	3.6	21.4	2.7	15.7	3.6	4.4	4.0	<1.5	3.7	8.5	4.6	20.0	5.5	4.0	2.1
5j	2.6	5.3	6.8	1.8	5.3	5.8	2.2	2.1	2.4	4.0	9.7	8.9	2.3	1.8	6.0	<1.6
5k	2.1	4.3	5.4	5.7	2.9	8.3	4.3	3.9	3.4	4.2	12.8	5.2	<1.5	4.5	<1.5	2.6
5l	1.6	5.8	2.0	2.4	1.8	4.0	<1.5	<1.5	1.9	1.7	7.8	3.2	<1.5	1.5	<1.5	<1.5
5m	12.4	5.0	5.2	1.9	3.9	4.6	3.5	3.3	<1.5	3.4	4.6	6.9	2.4	<1.5	<1.5	<1.5
5n	1.4	1.3	2.5	4.0	9.2	3.5	<1.5	<1.5	2.3	4.9	3.6	3.8	3.7	2.5	<1.5	2.8
5o	3.9	2.5	4.1	4.1	3.8	6.8	<1.5	1.5	2.6	3.4	4.7	4.4	2.9	3.7	<1.4	3.4
5p	1.5	0.81	2.5	3.1	1.8	2.5	2.0	<1.5	<1.5	2.0	4.4	2.1	<1.5	2.1	<1.5	<1.5
5q	2.2	2.0	3.9	3.9	5.3	1.7	1.6	<1.5	<1.5	3.4	6.1	1.9	1.8	2.9	<1.5	<1.5
5r	3.1	2.0	2.5	3.8	6.6	3.1	3.3	<1.5	2.2	2.0	7.7	1.5	2.9	0.74	<1.5	2.3
Cisplatin	7.6	8.9	10.2	6.4	11.3	5.6	16.0	5.6	3.9	8.7	12.3	6.8	9.8	4.6	12.1	6.3

[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 cervical carcinoma (Hela), liver carcinoma (Bel-7402 and HepG2), gastric carcinoma (BGC-823), breast carcinoma (MCF-7), renal carcinoma (769-P, 786-0 and OS-RC-2), epidermoid carcinoma of the nasopharynx (KB), non-small cell lung carcinoma (A549), malignant melanoma (A375), colon carcinoma (HT-29), bladder squamous carcinoma (SCaBER), malignant bladder carcinoma (Blu-87), malignant glioma (U251).
[c] Data represent the mean values of three independent determinations.
[d] ND = not tested.
[e] See Ref. [4].

the acute toxicity increased remarkably by introducing a benzyl substituent into position-2 of β-carboline nucleus; (4) the antitumor activities improved greatly by the introduction of appropriate substituents into position-2, 7 and 9.

5. Experimental section

5.1. Reagents and general methods

All reagents were purchased from commercial suppliers and were dried and purified when necessary. Harmine **1** was extracted from *Peganum multisectum Maxim*, a plant indigenous to western China according to the method by Duan et al. [18]. The following intermediates, 7-methoxy-9-ethyl-1-methyl-β-carboline **2a** [4], 7-methoxy-9-*n*-butyl-1-methyl-β-carboline **2b** [4], 7-methoxy-9-isobutyl-1-methyl-β-carboline **2c** [17], 7-methoxy-9-(3-phenylpropyl)-1-methyl-β-carboline **2d** [4], 9-ethyl-1-methyl-β-carboline7-ol **3a** [17], 9-*n*-butyl-1-methyl-β-carboline-7-ol **3b** [17], 9-isobutyl-1-methyl-β-carboline7-ol **3c** [17] and 1-methyl-9-(3-phenyl-propyl)-β-carboline-7-ol **3d** [17], were prepared according to the described procedures. Compounds **4a**, **4g**, **4i**–**k**, **4p**, **4t**, **4w**, **4y**, **4ad**, **4ae**, **4af**, **5a**, **5e**, **5j**, **5m** and **5q** are known compounds [17].

Table 2
Acute toxic effects of harmine derivatives in mice and antitumor activities of these compounds against mice bearing sarcoma 180 and Lewis lung cancer.

Compds	Acute toxicity		Dosage (mg/kg)	Tumor inhibition rate (%)	
	LD_{50} (mg/kg)	Neurotoxic effect		Sarcoma 180	Lewis lung carcinoma
4d	200	−	40.0	27.8	20.8
4h	200	−	40.0	26.0	21.7
4ab	100	−	20.0	30.1	27.1
5c	12.5	−	2.5	44.2	30.0
5e	12.5	−	2.5	36.9	23.7
5f	15.0	−	3.0	53.1	27.2
5m	5.0	−	1.0	52.6	23.7
5p	6.25	−	1.2	41.5	28.0
5r	3.75	−	0.63	46.1	33.7
1	59.0	+[a]	7.5	30.8	33.7
CTX		−	30	88.7	85.6

[a] Acute neurotoxic manifestation was denoted by "+" and "−". A "+" represents toxic responses including tremble, twitch, jumping and supination, while "−" means no such reaction.

Melting points were determined in capillary tubes on an electrothermal PIF YRT-3 apparatus and without correction. FAB-MS spectra were obtained from VG ZAB-HS spectrometer. FT-IR spectra were run as KBr pellets on a Bruker Equinox 55 Fourier Transformation Infrared Spectrometer. ^{1}H NMR spectra were recorded on a Varian INOVA 500NB spectrometer. Chemical shifts are reported in δ (ppm) downfield from an internal solvent peak and coupling constants, J in hertz. Elemental analyses (C, H and N) were carried out on an Elementar Vario EL CHNS Elemental Analyzer. Silica gel F254 was used in analytical thin-layer chromatography (TLC) and silica gel was used in column chromatography respectively.

5.2. General procedure for the preparation of 7-alkoxyl-β-carboline derivatives

A mixture of 7-hydroxyl-β-carbolines **3a–d** (5 mmol) and anhydrous DMF (50 ml) was stirred at room temperature until clear, and then 60% NaH (0.3 g, 7.5 mmol) and alkyl halogenide (15 mmol) were added. The mixture was stirred at room temperature for 0.5–2 h. After completion of the reaction as indicated by TLC, the solution was poured into H_2O (150 ml), and extracted with ethyl acetate. The organic phase was made acidic with concentrated hydrochloric acid. Upon removal of solvent, the residue was crystallized from acetone to afford yellow solid. The solid was dissolved in water and made basic with sodium bicarbonate, and the aqueous mixture extracted with ethyl acetate. The organic phase was washed with water and brine, then dried over anhydrous sodium sulfate, filtered and evaporated. The resulting oil was crystallized from ethyl ether or ethyl ether–petroleum ether.

5.2.1. 7-Isopropoxy-9-ethyl-1-methyl-β-carboline (*4b*)

White crystals (1.0 g, 79%) were obtained, mp 114–115 °C; FAB-MS m/z (M + 1) 269; IR (KBr) 2973, 2926, 1620, 1565, 1446, 1373, 1209, 1108, 1038, 978, 813 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.26 (1H, d, J = 5.5 Hz); 7.95 (1H, d, J = 9.0 Hz); 7.70 (1H, d, J = 5.5 Hz); 6.85–6.87 (2H, m); 4.68–4.73 (1H, m); 4.53 (2H, q, J = 7.5 Hz); 3.01 (3H, s); 1.40–1.45 (9H, m). ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.2, 142.8, 140.6, 138.3, 135.2, 129.5, 122.5, 115.5, 112.3, 110.2, 95.9, 70.8, 39.7, 23.6, 22.5, 15.8. Anal. Calcd for $C_{17}H_{20}N_2O$: C, 76.09; H, 7.51; N, 10.44. Found: C, 75.95; H, 7.48; N, 10.37.

5.2.2. 7-n-Butoxy-9-ethyl-1-methyl-β-carboline (*4c*)

White crystals (1.2 g, 85%) were obtained, mp 109–110 °C; FAB-MS m/z (M + 1) 283; IR (KBr) 2973, 2938, 2875, 1623, 1560, 1453, 1409, 1343, 1260, 1215, 1140, 1047, 1007, 810 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.26 (1H, d, J = 6.0 Hz); 7.95 (1H, d, J = 8.5 Hz); 7.73 (1H, d, J = 6.0 Hz); 6.86–6.90 (2H, m); 4.54 (2H, q, J = 7.0 Hz); 4.10 (2H, t, J = 6.5 Hz); 3.05 (3H, s); 1.84–1.87 (2H, m); 1.54–1.58 (2H, m); 1.45 (3H, t, J = 7.5 Hz); 1.01 (3H, t, J = 7.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.6, 142.8, 140.6, 138.4, 135.3, 129.6, 122.5, 115.5, 112.3, 109.3, 94.1, 68.5, 39.8, 31.8, 23.6, 19.7, 15.9, 14.3. Anal. Calcd for $C_{18}H_{22}N_2O$: C, 76.56; H, 7.85; N, 9.92. Found: C, 76.48; H, 7.83; N, 9.98.

5.2.3. 7-Isobutoxy-9-ethyl-1-methyl-β-carboline (*4d*)

White crystals (0.97 g, 76%) were obtained, mp 127–128 °C. FAB-MS m/z (M + 1) 283; IR (KBr) 3042, 2961, 2925, 2871, 1622, 1566, 1450, 1349, 1263, 1214, 1142, 1101, 1044, 808 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.26 (1H, d, J = 5.5 Hz); 7.95 (1H, d, J = 8.5 Hz); 7.72 (1H, d, J = 5.5 Hz); 6.85–6.89 (2H, m); 4.54 (2H, q, J = 7.0 Hz); 3.85 (2H, d, J = 6.5 Hz); 3.03 (3H, s); 2.14–2.19 (1H, m); 1.44 (3H, t, J = 7.5 Hz), 1.09 (6H, d, J = 6.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.7, 142.8, 140.4, 138.0, 135.1, 129.6, 122.4, 115.2, 112.3, 109.5, 93.9, 75.1, 39.7, 28.8, 23.4, 19.7, 15.9. Anal. Calcd for $C_{18}H_{22}N_2O$: C, 76.56; H, 7.85; N, 9.92. Found: C, 76.58; H, 7.90; N, 9.97.

5.2.4. 7-Decyloxy-9-ethyl-1-methyl-β-carboline (*4e*)

White crystals (1.52 g, 83%) were obtained, mp 76–77 °C. FAB-MS m/z (M + 1) 367; IR (KBr) 3047, 2923, 2850, 1628, 1561, 1443, 1345, 1296, 1207, 1147, 1027, 859, 821, 786 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.29 (1H, d, J = 5.0 Hz); 7.98 (1H, d, J = 9.0 Hz); 7.74 (1H, d, J = 5.0 Hz); 6.88–6.91 (2H, m); 4.57 (2H, q, J = 7.5 Hz); 4.12 (2H, t, J = 7.5 Hz); 3.06 (3H, s); 1.85–1.99 (2H, m), 1.51–1.56 (2H, m), 1.47(3H, t, J = 7.5 Hz), 1.30–1.44 (12H, m); 0.91 (3H, t, J = 7.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.6, 142.8, 140.6, 138.4, 135.2, 129.6, 122.4, 115.4, 112.3, 109.3, 94.1, 68.8, 39.7, 32.3, 30.0(2C), 29.8(2C), 29.7, 26.5, 23.6, 23.1, 15.9, 14.5. Anal. Calcd for $C_{24}H_{34}N_2O$: C, 78.64; H, 9.35; N, 7.64. Found: C, 78.48; H, 9.43; N, 7.56.

5.2.5. 7-Benzyloxy-9-ethyl-1-methyl-β-carboline (*4f*)

White crystals (1.37 g, 87%) were obtained, mp 177–178 °C; FAB-MS m/z (M + 1) 317; IR (KBr) 3032, 2967, 2925, 2871, 1621, 1564, 1446, 1261, 1213, 1134, 1001, 837, 810 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.27 (1H, d, J = 5.5 Hz); 7.98 (1H, d, J = 8.5 Hz); 7.73 (1H, d, J = 5.5 Hz); 7.26–7.50 (5H, m); 6.95–6.97 (2H, m); 5.16 (2H, s); 4.53 (2H, q, J = 7.5 Hz); 3.03 (3H, s); 1.40 (3H, t, J = 5.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.1, 142.8, 140.7, 138.5, 137.1, 135.3, 129.5, 128.8, 128.2, 127.7, 122.6, 115.9, 112.4, 109.5, 94.9, 70.9, 39.8, 23.6, 15.8. Anal. Calcd for $C_{21}H_{20}N_2O$: C, 79.72; H, 6.37; N, 8.85. Found: C, 79.78; H, 6.35; N, 8.91.

5.2.6. 7-(3-Phenylpropoxy)-9-ethyl-1-methyl-β-carboline (*4h*)

White crystals (1.31 g, 76%) were obtained, mp 110–111 °C. FAB-MS m/z (M + 1) 345; IR (KBr) 3030, 2950, 2924, 2871, 1623, 1561, 1447, 1392, 1210, 1136, 1089, 1040, 966, 805 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.27 (1H, d, J = 5.5 Hz); 7.96 (1H, d, J = 8.5 Hz); 7.72 (1H, d, J = 5.5 Hz); 7.19–7.31 (5H, m); 6.83–6.89 (2H, m); 4.52 (2H, q, J = 7.0 Hz); 4.10 (2H, t, J = 8.0 Hz); 3.02 (3H, s); 2.88 (2H, t, J = 7.5 Hz); 2.13–2.21 (2H, m); 1.43 (2H, t, J = 7.0 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.5, 142.8, 141.7, 140.7, 138.4, 135.2, 129.5, 128.7(2C), 126.2, 122.5, 115.5, 112.4, 109.4, 94.0, 67.7, 39.7, 32.6, 31.3, 23.7, 15.9. Anal. Calcd for $C_{23}H_{24}N_2O$: C, 80.20; H, 7.02; N, 8.13. Found: C, 80.03; H, 6.98; N, 8.18.

5.2.7. 7-Isobutoxy-9-n-butyl-1-methyl-β-carboline (*4l*)

White crystals (1.19 g, 77%) were obtained, mp 91–92 °C. FAB-MS m/z (M + 1) 311; IR (KBr) 3423, 2962, 2928, 2868, 1622, 1564, 1447, 1411, 1366, 1242, 1198, 1139, 1043, 810 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.27 (1H, d, J = 5.5 Hz); 7.96 (1H, d, J = 9.0 Hz);

7.76 (1H, d, *J* = 5.5 Hz); 6.85–6.91 (2H, m); 4.47 (2H, t, *J* = 7.5 Hz); 3.87 (2H, d, *J* = 6.5 Hz); 3.07 (3H, s); 2.14–2.19 (1H, m); 1.79–1.85 (2H, m); 1.43–1.48 (2H, m); 1.09 (6H, d, *J* = 4.5 Hz); 1.00 (3H, t, *J* = 7.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.6, 143.3, 140.7, 138.4, 135.5, 129.6, 122.4, 115.4, 112.3, 109.3, 94.5, 75.2, 45.0, 33.1, 28.8, 23.8, 20.6, 19.7, 14.2. Anal. Calcd for $C_{20}H_{26}N_2O$: C, 77.38; H, 8.44; N, 9.02. Found: C, 77.29; H, 8.40; N, 8.98.

*5.2.8. 9-n-Butyl-1-methyl-7-(pentan-3-yloxy)-β-carboline (**4m**)*

Yellow oil (1.1 g, 68%) was obtained. FAB-MS *m/z* (M + 1) 325; IR (KBr) 3412, 2959, 2873, 2529, 1620, 1570, 1462, 1336, 1242, 1197, 1140, 1111, 1035, 980, 954, 822 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.26 (1H, d, *J* = 5.0 Hz); 7.94 (1H, d, *J* = 9.0 Hz); 7.71 (1H, d, *J* = 5.0 Hz); 6.86–6.88 (2H, m); 4.43 (2H, t, *J* = 7.5 Hz); 4.24–4.29 (1H, m); 3.01 (3H, s); 1.73–1.82 (6H, m); 1.42–1.47 (2H, m); 0.97–1.03 (9H, m). ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.6, 143.4, 140.3, 137.8, 135.3, 129.8, 122.4, 115.2, 112.4, 109.5, 94.3, 75.2, 44.9, 33.0, 28.8, 23.3, 20.5, 19.7, 14.2.

*5.2.9. 7-(1,1,1-Trifluoro-2-hydroxyl-propoxy)-9-n-butyl-1-methyl-β-carboline (**4n**)*

White crystals (1.24 g, 68%) were obtained, mp 162–164 °C. FAB-MS *m/z* (M + 1) 367; IR (KBr) 3061, 2961, 2932, 2837, 1624, 1568, 1497, 1453, 1410, 1351, 1241, 1134, 1048, 810 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.24 (1H, d, *J* = 5.0 Hz); 7.93 (1H, d, *J* = 8.5 Hz); 7.72 (1H, d, *J* = 5.0 Hz); 6.87–6.89 (2H, m); 6.64 (1H, s); 4.50–4.53 (1H, m), 4.35–4.37 (1H, m), 4.20–4.28 (3H, m); 2.94 (3H, s); 1.66–1.73 (2H, m); 1.36–1.41 (2H, m); 0.98 (3H, t, *J* = 7.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.7, 143.2, 140.7, 137.5, 135.4, 129.7, 122.5, 115.7, 115.6, 112.6, 109.4, 94.6, 69.7, 69.3, 67.7, 44.9, 33.0, 22.7, 22.6, 20.4, 14.1. Anal. Calcd for $C_{19}H_{21}F_3N_2O_2$: C, 62.29; H, 5.78; N, 7.65. Found: C, 62.23; H, 5.76; N, 7.74.

*5.2.10. 7-Octyloxy-9-n-butyl-1-methyl-β-carboline (**4o**)*

White crystals (1.37 g, 75%) were obtained, mp 75–76 °C. FAB-MS *m/z* (M + 1) 367; IR (KBr) 2927, 2855, 1622, 1563, 1446, 1410, 1373, 1244, 1198, 1140, 1042, 806 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.27 (1H, d, *J* = 5.5 Hz); 7.96 (1H, d, *J* = 8.5 Hz); 7.74 (1H, d, *J* = 5.5 Hz); 6.85–6.90 (2H, m); 4.46 (2H, t, *J* = 7.5 Hz); 4.10 (2H, t, *J* = 8.0 Hz); 3.05 (3H, s); 1.80–1.89 (4H, m); 1.29–1.53 (12H, m); 0.99 (3H, t, *J* = 7.5 Hz); 0.89 (3H, t, *J* = 7.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.6, 143.3, 140.3, 137.9, 135.3, 129.6, 122.3, 115.1, 112.3, 109.3, 94.3, 68.7, 44.8, 33.0, 32.2, 30.1, 29.8 (2C), 29.6, 26.5, 23.4, 23.0, 20.5, 14.5, 14.2. Anal. Calcd for $C_{24}H_{34}N_2O$: C, 78.64; H, 9.35; N, 7.64. Found: C, 78.58; H, 9.33; N, 7.68.

*5.2.11. 7-Benzyloxy-9-n-butyl-1-methyl-β-carboline (**4q**)*

White crystals (1.43 g, 83%) were obtained, mp 121–122 °C. FAB-MS *m/z* (M + 1) 345; IR (KBr) 3424, 3036, 2957, 2929, 2866, 1622, 1565, 1495, 1448, 1408, 1377, 1349, 1240, 1192, 1139, 1009, 813, 731 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.27 (1H, d, *J* = 5.5 Hz); 7.98 (1H, d, *J* = 8.5 Hz); 7.76 (1H, d, *J* = 5.5 Hz); 7.26–7.50 (5H, m); 6.92–6.99 (2H, m); 5.22 (2H, s); 4.43 (2H, t, *J* = 8.0 Hz); 3.05 (3H, s); 1.75–1.78 (2H, m); 1.38–1.43 (2H, m); 0.95 (3H, t, *J* = 8.0 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.0, 143.2, 140.8, 138.4, 137.1, 135.6, 129.5, 128.9, 128.2, 127.7, 122.5, 115.7, 112.4, 109.4, 95.3, 70.9, 45.1, 33.0, 23.7, 20.5, 14.2. Anal. Calcd for $C_{23}H_{24}N_2O$: C, 80.20; H, 7.02; N, 8.13. Found: C, 80.16; H, 6.99; N, 8.19.

*5.2.12. 7-(Perfluorobenzyloxy)-9-n-butyl-1-methyl-β-carboline (**4r**)*

White crystals (1.58 g, 73%) were obtained, mp 121–122 °C. FAB-MS *m/z* (M + 1) 435; IR (KBr) 3424, 3036, 2957, 2929, 2866, 1622, 1565, 1495, 1448, 1408, 1377, 1349, 1240, 1192, 1139, 1009, 813, 731 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.29 (1H, d, *J* = 5.5 Hz); 8.00 (1H, d, *J* = 8.5 Hz); 7.76 (1H, d, *J* = 5.5 Hz); 6.93–6.96 (2H, m); 5.28 (2H, s); 4.47 (2H, t, *J* = 7.5 Hz); 3.05 (3H, s); 1.80–1.83(2H, m); 1.44–1.48 (2H, m); 1.00 (3H, t, *J* = 7.5 Hz). Anal. Calcd for $C_{23}H_{19}F_5N_2O$: C, 63.59; H, 4.41; N, 6.45. Found: C, 63.48; H, 4.36; N, 6.38.

*5.2.13. 7-(3-Phenylpropoxy)-9-n-butyl-1-methyl-β-carboline (**4s**)*

White crystals (1.4 g, 75%) were obtained, mp 89–90 °C. FAB-MS *m/z* (M + 1) 373; IR (KBr) 2954, 2926, 2869, 1622, 1561, 1494, 1445, 1410, 1366, 1355, 1244, 1190, 1139, 1040, 809, 758 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.27 (1H, d, *J* = 5.5 Hz); 7.95 (1H, d, *J* = 8.5 Hz); 7.72 (1H, d, *J* = 5.5 Hz); 7.20–7.31 (5H, m); 6.83–6.89 (2H, m); 4.43 (2H, t, *J* = 7.5 Hz); 4.10 (2H, t, *J* = 8.0 Hz); 3.02 (3H, s); 2.86–2.89 (2H, m); 2.17–2.20 (2H, m); 1.78–1.84 (2H, m); 1.42–1.51 (2H, m); 0.97 (3H, t, *J* = 7.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.4, 143.2, 141.6, 140.7, 138.4, 135.4, 129.4, 128.7(2C), 126.2, 122.4, 115.4, 112.4, 109.2, 94.4, 67.7, 44.8, 33.1, 32.6, 31.3, 23.8, 20.6, 14.3. Anal. Calcd for $C_{25}H_{28}N_2O$: C, 80.61; H, 7.58; N, 7.52. Found: C, 80.50; H, 7.55; N, 7.56.

*5.2.14. 7-Isobutoxy-9-isobutyl-1-methyl-β-carboline (**4u**)*

Yellow solid (1.27 g, 82%) was obtained, mp 93–95 °C. FAB-MS *m/z* (M + 1) 311; IR (KBr) 2956, 2869, 2480, 1624, 1575, 1470, 1432, 1337, 1256, 1204, 1138, 1043, 806 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.28 (1H, d, *J* = 5.5 Hz); 7.97 (1H, d, *J* = 8.5 Hz); 7.80 (1H, d, *J* = 5.5 Hz); 6.86–6.92 (2H, m); 4.29 (2H, d, *J* = 8.5 Hz); 3.85 (2H, d, *J* = 7.5 Hz); 3.08 (3H, s); 2.13–2.29 (2H, m); 1.10 (6H, d, *J* = 7.0 Hz); 0.94 (6H, d, *J* = 6.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.6, 143.9, 140.3, 137.7, 135.5, 129.8, 122.2, 114.9, 112.3, 109.6, 95.0, 75.1, 51.9, 30.8, 28.7, 23.5, 20.4, 19.7.

*5.2.15. 7-Dceyloxy-9-isobutyl-1-methyl-β-carboline (**4v**)*

White crystals (1.48 g, 75%) were obtained, mp 62–64 °C. FAB-MS *m/z* (M + 1) 395; IR (KBr) 3431, 2950, 2922, 2849, 1625, 1567, 1447, 1335, 1254, 1202, 1142, 809 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.27 (1H, d, *J* = 5.0 Hz); 7.96 (1H, d, *J* = 8.5 Hz); 7.75 (1H, d, *J* = 5.5 Hz); 6.86–6.89 (2H, m); 4.27 (2H, d, *J* = 8.5 Hz); 4.08 (2H, t, *J* = 7.5 Hz); 3.03 (3H, s); 2.23–2.29 (1H, m); 1.82–1.88 (2H, m); 1.48–1.54 (2H, m); 1.28–1.40 (12H, m); 0.93 (6H, d, *J* = 6.5 Hz); 0.88 (3H, t, *J* = 7.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.3, 143.7, 140.7, 138.4, 135.8, 129.6, 122.2, 115.2, 112.3, 109.2, 95.2, 68.8, 52.1, 32.3, 30.8, 29.9(2C), 29.8(2C), 29.7, 26.5, 24.0, 23.0, 20.5, 14.5. Anal. Calcd for $C_{26}H_{38}N_2O$: C, 79.14; H, 9.71; N, 7.10. Found: C, 79.28; H, 9.76; N, 7.15.

*5.2.16. 7-(3-Phenylpropoxy)-9-isobutyl-1-methyl-β-carboline (**4x**)*

White crystals (1.54 g, 83%) were obtained, mp 94–95 °C. FAB-MS *m/z* (M + 1) 373; IR (KBr) 3041, 2958, 1621, 1567, 1448, 1405, 1339, 1253, 1196, 1139, 1050, 977, 817 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.28 (1H, d, *J* = 5.5 Hz); 7.97 (1H, d, *J* = 8.5 Hz); 7.77 (1H, d, *J* = 5.5 Hz); 7.19–7.31 (5H, m); 6.88–6.91 (1H, m); 6.84–6.85 (1H, m); 4.26 (2H, d, *J* = 7.5 Hz); 4.10 (2H, t, *J* = 8.0 Hz); 3.04 (3H, s); 2.88 (2H, t, *J* = 7.5 Hz); 2.21–2.28 (1H, m), 2.16–2.20 (2H, m); 0.92 (6H, d, *J* = 6.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.2, 143.7, 141.6, 140.8, 138.5, 135.8, 129.6, 128.7(2C), 126.2, 122.4, 115.3, 112.4, 109.3, 95.2, 67.7, 52.1, 32.6, 31.3, 30.9, 24.1, 20.6. Anal. Calcd for $C_{25}H_{28}N_2O$: C, 80.61; H, 7.58; N, 7.52. Found: C, 80.48; H, 7.63; N, 7.58.

*5.2.17. 7-n-Butoxy-9-(3-phenylpropyl)-1-methyl-β-carboline (**4z**)*

White crystals (1.52 g, 82%) were obtained, mp 92–93 °C. FAB-MS *m/z* (M + 1) 373; IR (KBr) 3024, 2955, 2868, 1622, 1565, 1497, 1447, 1409, 1368, 1241, 1161, 810, 738 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.25 (1H, d, *J* = 5.5 Hz); 7.93 (1H, d, *J* = 8.5 Hz); 7.72 (1H, d, *J* = 5.5 Hz); 7.20–7.33 (5H, m); 6.86 (1H, dd, *J* = 2.0 Hz, 8.5 Hz); 6.64 (1H, d, *J* = 2.0 Hz); 4.44 (2H, t, *J* = 8.0 Hz); 3.99 (2H, t, *J* = 8.5 Hz); 2.90 (3H, s); 2.76 (2H, t, *J* = 7.5 Hz); 2.12–2.19 (2H, m); 1.80–1.86

(2H, m); 1.52–1.59(2H, m); 1.03 (3H, t, *J* = 7.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.5, 143.1, 140.9, 140.6, 138.4, 135.4, 129.5, 128.8, 128.6, 126.5, 122.4, 115.2, 112.3, 109.6, 93.9, 68.4, 44.3, 33.3, 32.0, 31.8, 23.5, 19.8, 14.4. Anal. Calcd for $C_{25}H_{28}N_2O$: C, 80.61; H, 7.58; N, 7.52. Found: C, 80.68; H, 7.63; N, 7.56.

5.2.18. 7-Isobutoxy-9-(3-phenylpropyl)-1-methyl-β-carboline (*4aa*)

White crystals (1.34 g, 76%) were obtained, mp 123–124 °C. FAB-MS *m/z* (M + 1) 373; IR (KBr) 3415, 2961, 2934, 2869, 1622, 1565, 1495, 1447, 1411, 1365, 1242, 1205, 1161, 1039, 817, 744 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.27 (1H, d, *J* = 5.5 Hz); 7.94–7.97 (1H, d, *J* = 8.5 Hz); 7.72–7.73 (1H, d, *J* = 5.5 Hz); 7.23–7.34 (5H, m); 6.87–6.90 (1H, m); 6.66–6.67 (1H, m); 4.46 (2H, t, *J* = 7.5 Hz); 3.77 (2H, d, *J* = 8.0 Hz); 2.91 (3H, s); 2.77–2.81 (2H, m); 2.16–2.20 (3H, m); 1.11 (6H, d, *J* = 7.0 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.8, 143.2, 140.8, 140.5, 138.1, 135.3, 129.7, 128.8, 128.6, 126.5, 122.4, 115.1, 112.4, 109.8, 93.9, 75.0, 44.2, 33.2, 32.0, 28.9, 23.3, 19.8. Anal. Calcd for $C_{25}H_{28}N_2O$: C, 80.61; H, 7.58; N, 7.52. Found: C, 80.54; H, 7.55; N, 7.53.

5.2.19. 7-(Pentan-3-yloxy)-9-(3-phenylpropyl)-1-methyl-β-carboline (*4ab*)

White crystals (1.21 g, 63%) were obtained, mp 93–94 °C. FAB-MS *m/z* (M + 1) 387; IR (KBr) 2967, 2934, 2874, 1621, 1564, 1494, 1449, 1409, 1238, 1202, 1158, 974, 815 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.25 (1H, d, *J* = 5.5 Hz); 7.93 (1H, d, *J* = 8.5 Hz); 7.72 (1H, d, *J* = 5.5 Hz); 7.20–7.33 (5H, m); 6.86–6.88 (1H, m); 6.71–6.72 (1H, m); 4.44 (2H, t, *J* = 7.5 Hz); 4.19–4.21 (1H, m); 2.90 (3H, s); 2.76 (2H, t, *J* = 7.5 Hz); 2.12–2.18 (2H, m); 1.71–1.76 (4H, m); 1.01 (6H, t, *J* = 7.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.1, 143.2, 140.9, 140.7, 138.5, 135.5, 129.6, 128.8, 128.6, 126.5, 122.5, 115.4, 112.3, 110.5, 96.1, 81.0, 44.5, 33.4, 32.1, 26.6,23.6, 10.1. Anal. Calcd for $C_{26}H_{30}N_2O$: C, 80.79; H, 7.82; N, 7.25. Found: C, 80.68; H, 7.89; N, 7.28.

5.2.20. 7-(3-Methylbut-2-enyloxy)-9-(3-phenylpropyl)-1-methyl-β-carboline (*4ac*)

White crystals (1.25 g, 65%) were obtained, mp 95–96 °C. FAB-MS *m/z* (M + 1) 385; IR (KBr) 2962, 2927, 2856, 1622, 1567, 1446, 1409, 1239, 1159, 988, 817, 753 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.25 (1H, d, *J* = 5.5 Hz); 7.96 (1H, d, *J* = 8.5 Hz); 7.73 (1H, d, *J* = 5.5 Hz); 7.19–7.33 (5H, m); 6.88–6.90 (1H, m); 6.70–6.71 (1H, m); 5.52–5.56 (1H, m); 4.55 (2H, d, *J* = 8.0 Hz); 4.44 (2H, t, *J* = 6.5 Hz); 2.91 (3H, s); 2.76 (2H, t, *J* = 7.5 Hz); 2.13–2.19 (2H, m); 1.80 (6H, d, *J* = 13.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.3, 143.1, 140.8, 140.6, 138.4, 138.3, 135.4, 129.6, 128.8, 128.6, 126.5, 122.5, 119.8, 115.4, 112.4, 109.7, 94.4, 65.6, 44.5, 33.4, 32.1, 26.2, 23.4, 18.7. Anal. Calcd for $C_{26}H_{28}N_2O$: C, 81.21; H, 7.34; N, 7.29. Found: C, 81.28; H, 7.43; N, 7.23.

5.2.21. 7-(3-Phenylpropoxyl)-9-(3-phenylpropyl)-1-methyl-β-carboline (*4ag*)

White crystals (1.84 g, 85%) were obtained, mp 118–119 °C. FAB-MS *m/z* (M + 1) 435; IR (KBr) 2932, 2867, 1623, 1567, 1495, 1449, 1411, 1366, 1240, 1161, 1042, 813, 752 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.25 (1H, d, *J* = 5.5 Hz); 7.96 (1H, d, *J* = 8.5 Hz); 7.77 (1H, d, *J* = 5.5 Hz); 7.18–7.32 (10H, m); 6.89–6.92 (1H, m); 6.24–6.28 (1H, m); 4.44 (2H, t, *J* = 8.0 Hz); 3.99 (2H, t, *J* = 6.5 Hz); 2.92 (3H, s); 2.88 (2H, t, *J* = 8.0 Hz); 2.76 (2H, t, *J* = 7.0 Hz); 2.12–2.20 (4H, m). ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.4, 143.1, 141.7, 140.9, 140.7, 138.5, 135.5, 129.6, 128.8 (2C), 128.7, 128.6, 126.5, 126.2, 122.5, 115.4, 112.4, 109.6, 94.1, 67.6, 44.4, 33.3, 32.6, 32.1, 31.3, 23.6. Anal. Calcd for $C_{30}H_{30}N_2O$: C, 82.91; H, 6.96; N, 6.45. Found: C, 82.78; H, 7.01; N, 6.48.

5.3. General procedure for the preparation of β-carbolinium bromides

A mixture of β-carboline (2 mmol) and benzyl bromide (30–50 mmol) in ethyl acetate (50 ml) was refluxed for 5–10 h. After completion of the reaction as indicated by TLC, the solution was cooled and filtered to afford yellow solid. The solid was recrystallized from ethanol.

5.3.1. 7-Butoxy-2-benzyl-9-ethyl-1-methyl-β-carbolinium bromide (*5b*)

Yellow crystals (0.68 g, 75%) were obtained, mp 215–216 °C. FAB-MS *m/z* 373; IR (KBr) 3386, 3045, 2958, 2931, 2869, 1622, 1577, 1454, 1374, 1256, 1221, 1132, 1032, 809, 735 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.80 (1H, d, *J* = 6.5 Hz); 8.62 (1H, d, *J* = 6.5 Hz); 8.38 (1H, d, *J* = 8.5 Hz); 7.36–7.44 (4H, m); 7.20 (2H, d, *J* = 7.0 Hz); 7.08–7.10 (1H, m); 6.06 (2H, s); 4.70–4.75 (2H, q, *J* = 7.0 Hz); 4.22 (2H, t, *J* = 7.5 Hz); 3.11 (3H, s); 1.78–1.83 (2H, m); 1.48–1.55 (2H, m); 1.39 (3H, t, *J* = 8.0 Hz); 0.98 (3H, t, *J* = 7.5 Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ 163.6, 147.5, 139.5, 135.9, 135.1, 134.9, 133.7, 129.9, 129.2, 127.3, 125.1, 114.8, 114.0, 113.1, 94.4, 69.0, 60.6, 41.1, 31.3, 19.5, 16.7, 15.9, 14.4. Anal. Calcd for $C_{25}H_{29}BrN_2O$: C, 66.22; H, 6.45; N, 6.15. Found: C, 66.35; H, 6.49; N, 6.13.

5.3.2. 7-Isobutoxy-2-benzyl-9-ethyl-1-methyl-β-carbolinium bromide (*5c*)

Yellow crystals (0.72 g, 79%) were obtained, mp 222–224 °C. FAB-MS *m/z* 373; IR (KBr) 3420, 2959, 1622, 1454, 1372, 1259, 1223, 1134, 1034, 1010, 826, 736 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.78 (1H, d, *J* = 6.5 Hz); 8.61–8.62 (1H, d, *J* = 6.5 Hz); 8.38 (1H, d, *J* = 9.0 Hz); 7.35–7.43 (4H, m); 7.20 (2H, d, *J* = 7.0 Hz); 7.09–7.11 (1H, m); 6.05 (2H, s); 4.74 (2H, t, *J* = 7.5 Hz); 4.00 (2H, d, *J* = 7.5 Hz); 3.11 (3H, s); 2.11–2.14 (1H, m); 1.39 (3H, t, *J* = 7.5 Hz); 1.06 (6H, d, *J* = 6.5 Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ 168.4, 152.3, 144.3, 140.6, 140.0, 139.7, 138.5, 134.6, 134.0, 132.0, 129.8, 119.5, 118.8, 117.9, 99.3, 73.8, 65.4, 36.0, 24.2, 21.5, 20.7, 19.1. Anal. Calcd for $C_{25}H_{29}BrN_2O$: C, 66.22; H, 6.45; N, 6.18. Found: C, 66.38; H, 6.42; N, 6.23.

5.3.3. 7-Benzyloxy-2-benzyl-9-ethyl-1-methyl-β-carbolinium bromide (*5d*)

Yellow crystals (0.81 g, 82%) were obtained, mp 240–242 °C. FAB-MS *m/z* 407; IR (KBr) 3437, 3005, 2969, 1620, 1577, 1452, 1335, 1260, 1210, 1133, 1034, 994, 823, 731 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.81 (1H, *J* = 7.0 Hz); 8.64 (1H, d, *J* = 6.5 Hz); 8.41 (1H, d, *J* = 9.0 Hz); 7.53–7.56 (3H, m); 7.35–7.45 (6H, m); 7.17–7.21 (3H, m); 6.06 (2H, s); 5.36 (2H, s); 4.73 (2H, q, t, *J* = 6.5 Hz); 3.11 (3H, s); 1.38 (3H, t, *J* = 7.5 Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ 163.1, 147.4, 139.6, 136.7, 135.8, 135.3, 134.7, 133.7, 129.7, 129.0, 128.9, 128.5, 128.2, 127.1, 125.0, 114.7, 114.0, 113.4, 95.3, 70.7, 60.4, 40.9, 16.3, 15.3. Anal. Calcd for $C_{28}H_{27}BrN_2O$: C, 68.99; H, 5.58; N, 5.75. Found: C, 68.86; H, 5.61; N, 5.80.

5.3.4. 7-(3-Phenylpropoxy)-2-benzyl-9-ethyl-1-methyl-β-carbolinium bromide (*5f*)

Yellow crystals (0.8 g, 78%) were obtained, mp 189–191 °C. FAB-MS *m/z* 435; IR (KBr) 3410, 2989, 2933, 2876, 1623, 1453, 1370, 1341, 1260, 1220, 1134, 1035, 824, 732 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.81 (1H, d, *J* = 6.5 Hz); 8.63 (1H, d, *J* = 6.5 Hz); 8.40 (1H, d, *J* = 9.0 Hz); 7.11–7.43 (12H, m); 6.06 (2H, s); 4.71 (2H, q, *J* = 7.5 Hz); 4.25 (2H, t, *J* = 6.5 Hz); 3.11 (3H, s); 2.82 (2H, t, *J* = 7.5 Hz); 2.10–2.16 (2H, m); 1.38 (3H, t, *J* = 7.5 Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ 163.5, 147.5, 141.8, 139.5, 135.9, 135.1, 134.9, 133.7, 130.0, 129.2, 128.9, 128.8, 127.3, 126.5, 125.1, 114.8, 113.9, 113.2, 94.5, 68.5, 60.7, 41.1, 32.2, 30.9, 16.8, 15.9. Anal. Calcd for $C_{30}H_{31}BrN_2O$: C, 69.90; H, 6.06; N, 5.43. Found: C, 70.03; H, 6.12; N, 5.47.

*5.3.5. 7-Isobutoxy-2-benzyl-9-n-butyl-1-methyl-β-carbolinium bromide (**5g**)*

Yellow crystals (0.8 g, 78%) were obtained, mp 247–249 °C. FAB-MS *m/z* 401; IR (KBr) 3401, 3020, 2957, 2869, 1620, 1578, 1456, 1377, 1247, 1207, 1137, 1012, 821, 721 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.80 (1H, d, *J* = 6.5 Hz); 8.62 (1H, d, *J* = 6.5 Hz); 8.38 (1H, d, *J* = 9.5 Hz); 7.36–7.43 (4H, m); 7.18–7.20 (2H, d, *J* = 7.5 Hz); 7.09–7.11 (1H, m); 6.05 (2H, s); 4.67 (2H, t, *J* = 7.5 Hz); 4.00 (2H, d, *J* = 7.5 Hz); 3.09 (3H, s); 2.10–2.15 (1H, m); 1.70–1.76 (2H, m); 1.30–1.38 (2H, m); 1.05–1.06 (6H, d, *J* = 6.5 Hz); 0.87 (3H, t, *J* = 7.5 Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ 163.8, 148.1, 139.6, 135.9, 135.5, 134.8, 133.9, 129.7, 129.1, 127.1, 125.0, 114.6, 113.8, 113.2, 95.0, 75.3, 60.5, 45.5, 32.4, 28.1, 19.6, 19.3, 16.5, 13.8. Anal. Calcd for $C_{27}H_{33}BrN_2O$: C, 67.35; H, 6.91; N, 5.82. Found: C, 67.49; H, 6.96; N, 5.79.

*5.3.6. 7-n-Octyloxy-2-benzyl-9-n-butyl-1-methyl-β-carbolinium bromate (**5h**)*

Yellow crystals (0.92 g, 86%) were obtained, mp 196–198 °C. FAB-MS *m/z* 457; IR (KBr) 2926, 2857, 1621, 1560, 1458, 1374, 1349, 1247, 1136, 1034, 819, 727 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.80 (1H, d, *J* = 6.5 Hz); 8.63 (1H, d, *J* = 6.5 Hz); 8.38 (1H, d, *J* = 9.0 Hz); 7.36–7.44 (4H, m); 7.19–7.20 (2H, m); 7.08–7.10 (1H, m); 6.06 (2H, s); 4.66 (2H, t, *J* = 7.5 Hz); 4.21 (2H, t, *J* = 7.5 Hz); 3.09 (3H, s); 1.71–1.82 (2H, m); 1.46–1.49 (2H, m); 1.26–1.37 (12H, m); 0.85–0.94 (6H, m). ^{13}C NMR (75 MHz, DMSO-d_6) δ 163.6, 148.1, 139.6, 136.2, 135.4, 134.9, 133.9, 129.9, 129.2, 127.3, 125.1, 114.8, 113.9, 113.1, 94.9, 69.2, 60.7, 45.7, 32.8, 31.9, 29.4, 29.2, 29.1, 26.2, 22.7, 20.0, 16.9, 14.6, 14.3. Anal. Calcd for $C_{31}H_{41}BrN_2O$: C, 69.26; H, 7.69; N, 5.21. Found: C, 69.30; H, 7.72; N, 5.18.

*5.3.7. 7-Benzyloxy-2-benzyl-9-n-butyl-1-methyl-β-carbolinium bromide (**5i**)*

Yellow crystals (0.84 g, 82%) were obtained, mp 229–230 °C. FAB-MS *m/z* 435; IR (KBr) 3423, 3028, 2957, 2927, 2868, 1622, 1579, 1495, 1454, 1373, 1247, 1200, 1135, 1028, 819, 733 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.80 (1H, d, *J* = 6.5 Hz); 8.63 (1H, d, *J* = 6.5 Hz); 8.41 (1H, d, *J* = 9.0 Hz); 7.17–7.55 (12H, m); 6.05 (2H, s); 5.37 (2H, s); 4.65 (2H, t, *J* = 7.5 Hz); 3.09 (3H, s); 1.68–1.71 (2H, m); 1.30–1.34 (2H, m); 0.87 (3H, t, *J* = 7.5 Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ 162.8, 147.8, 139.8, 136.8, 136.0, 135.3, 134.9, 133.7, 129.9, 129.2, 128.8, 128.5, 127.2, 125.2, 114.9, 114.3, 113.3, 95.6, 70.7, 60.7, 45.8, 32.8, 20.0, 16.8, 14.4. Anal. Calcd for $C_{30}H_{31}BrN_2O$: C, 69.90; H, 6.06; N, 5.43. Found: C, 69.82; H, 6.01; N, 5.49.

*5.3.8. 7-Isobutoxy-2-benzyl-9-isobutyl-1-methyl-β-carbolinium bromide (**5k**)*

Yellow crystals (0.76 g, 79%) were obtained, mp 255–257 °C. FAB-MS *m/z* 401; IR (KBr) 3422, 2992, 2959, 2894, 1619, 1578, 1454, 1376, 1252, 1212, 1135, 1003, 825 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.82 (1H, d, *J* = 6.5 Hz); 8.65 (1H, d, *J* = 6.5 Hz); 8.39 (1H, d, *J* = 9.0 Hz); 7.37–7.44 (4H, m); 7.18 (2H, d, *J* = 7.0 Hz); 7.09–7.11 (1H, m); 6.05 (2H, s); 4.54 (2H, d, *J* = 7.5 Hz); 3.99 (2H, d, *J* = 7.5 Hz); 3.07(3H, s); 1.98–2.14 (2H, m); 1.05 (6H, d, *J* = 6.5 Hz); 0.83 (6H, d, *J* = 6.5 Hz). Anal. Calcd for $C_{27}H_{33}BrN_2O$: C, 67.35; H, 6.91; N, 5.82. Found: C, 67.43; H, 6.97; N, 5.90.

*5.3.9. 7-Decyloxy-2-benzyl-9-isobutyl-1-methyl-β-carbolinium bromide (**5l**)*

Yellow crystals (0.92 g, 78%) were obtained, mp 202–203 °C. FAB-MS *m/z* 485; IR (KBr) 3409, 2957, 2924, 2852, 1621, 1579, 1456, 1375, 1252, 1219, 1137, 1030, 820, 726 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.83 (1H, d, *J* = 6.5 Hz); 8.65 (1H, d, *J* = 6.5 Hz): 8.39 (1H, d, *J* = 9.0 Hz); 7.37–7.43 (4H, m); 7.18–7.20 (2H, d, *J* = 7.5 Hz); 7.08 (1H, d, *J* = 8.5 Hz); 6.06 (2H, s); 4.54 (2H, d, *J* = 8.5 Hz); 4.21 (2H, t, *J* = 7.5 Hz); 3.07 (3H, s); 2.04–2.10 (1H, m); 1.77–1.83 (2H, m); 1.44–1.50 (2H, m); 1.26–1.36 (12H, m); 0.83–0.86 (9H, m). ^{13}C NMR (75 MHz, DMSO-d_6) δ 163.4, 148.6, 139.7, 136.1, 135.6, 134.7, 134.0, 129.7, 129.0, 127.1, 124.8, 114.8, 113.9, 113.0, 95.6, 69.1, 60.5, 52.2, 31.5, 30.5, 29.2, 29.1, 29.0, 28.8, 28.7, 25.8, 22.3, 19.8, 16.6, 14.0. Anal. Calcd for $C_{33}H_{45}BrN_2O$: C, 70.07; H, 8.02; N, 4.95. Found: C, 70.21; H, 8.04; N, 4.90.

*5.3.10. 7-(3-Phenylpropoxy)-2-benzyl-9-isobutyl-1-methyl-β-carbolinium bromide (**5n**)*

Yellow crystals (0.78 g, 72%) were obtained, mp 204–206 °C. FAB-MS *m/z* 463; IR (KBr) 3410, 3023, 2957, 2871, 1621, 1579, 1454, 1253, 1216, 1137, 1032, 821, 728 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.82 (1H, d, *J* = 6.5 Hz); 8.65 (1H, d, *J* = 6.5 Hz); 8.40 (1H, d, *J* = 9.0 Hz); 7.11–7.44 (12H, m); 6.05 (2H, s); 4.53 (2H, d, *J* = 7.5 Hz); 4.22 (2H, t, *J* = 7.5 Hz); 3.06 (3H, s); 2.80–2.83 (2H, m); 2.10–2.14 (2H, m); 2.03–2.09(1H, m); 0.82–0.83 (6H, d, *J* = 6.5 Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ 163.2, 148.6, 141.8, 139.7, 136.2, 135.3, 134.9, 133.9, 129.9, 129.2, 129.0, 128.9, 127.2, 126.5, 125.0, 114.8, 114.0, 112.9, 95.5, 68.3, 60.7, 52.2, 32.1, 31.1, 30.9, 20.1, 16.9. Anal. Calcd for $C_{32}H_{35}BrN_2O$: C, 70.71; H, 6.49; N, 5.15. Found: C, 70.63; H, 6.45; N, 5.20.

*5.3.11. 7-n-Butoxy-9-(3-phenylpropyl)-2-benzyl-1-methyl-β-carbolinium bromide (**5o**)*

White crystals (0.88 g, 82%) were obtained, mp 204–205 °C. FAB-MS *m/z* 463; IR (KBr) 3401, 3024, 2931, 2868, 1621, 1579, 1453, 1372, 1243, 1255, 1135, 1025, 827, 755 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.78 (1H, d, *J* = 6.5 Hz); 8.60 (1H, d, *J* = 6.5 Hz); 8.36 (1H, d, *J* = 8.5 Hz); 7.07–7.43 (12H, m); 6.03 (2H, s); 4.67 (2H, t, *J* = 7.5 Hz); 4.15 (2H, t, *J* = 7.5 Hz); 2.97 (3H, s); 2.68–2.71 (2H, m); 2.04–2.11 (2H, m); 1.77–1.83 (2H, m); 1.48–1.56 (2H, m); 0.99 (3H, t, *J* = 7.5 Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ 163.5, 147.9, 141.2, 139.6, 136.1, 135.4, 134.9, 134.2, 133.9, 129.9, 129.2, 128.9, 128.8, 127.2, 126.6, 125.1, 114.8, 114.1, 94.5, 68.9, 60.6, 45.3, 32.4, 31.9, 31.2, 19.5, 16.6, 14.4. Anal. Calcd for $C_{32}H_{35}BrN_2O$: C, 70.71; H, 6.49; N, 5.15. Found: C, 70.85; H, 6.53; N, 5.21.

*5.3.12. 7-(3-Pentyloxy)-9-(3-phenylpropyl)-2-benzyl-1-methyl-β-carbolinium bromide (**5p**)*

Yellow crystals (0.86 g, 78%) were obtained, mp 216–217 °C. FAB-MS *m/z* 477; IR (KBr) 3405, 2964, 2874, 1620, 1579, 1456, 1374, 1247, 1223, 1137, 1107, 1030, 979, 935, 826 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.78 (1H, d, *J* = 6.5 Hz); 8.60 (1H, d, *J* = 6.5 Hz); 8.37 (1H, d, *J* = 9.0 Hz); 7.09–7.43 (12H, m); 6.03 (2H, s); 4.67 (2H, t, *J* = 7.5 Hz); 4.57–4.61 (1H, m); 2.97 (3H, s); 2.70 (2H, t, *J* = 6.5 Hz); 2.03–2.10 (2H, m); 1.65–1.78 (4H, m); 0.96 (6H, t, *J* = 7.5 Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ 163.4, 148.0, 141.0, 139.4, 136.0, 135.5, 134.8, 134.0, 129.7, 129.0, 128.7, 128.5, 127.1, 126.4, 125.2, 114.6, 113.2, 95.9, 80.8, 60.5, 45.1, 32.2, 31.5, 26.1, 16.3, 9.5. Anal. Calcd for $C_{33}H_{37}BrN_2O$: C, 71.09; H, 6.69; N, 5.02. Found: C, 70.98; H, 6.75; N, 5.06.

*5.3.13. 7-(3-Phenylpropoxy)-9-(3-phenylpropyl)-2-benzyl-1-methyl-β-carbolinium bromide (**5r**)*

Yellow crystals (1.06 g, 87%) were obtained, mp 207–208 °C. FAB-MS *m/z* 525; IR (KBr) 3401, 3022, 2938, 1620, 1579, 1453, 1373, 1348, 1248, 1135, 1028, 825, 737 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.80 (1H, d, *J* = 6.0 Hz); 8.61 (1H, d, *J* = 6.0 Hz); 8.38 (1H, d, *J* = 9.0 Hz); 7.30–7.43 (3H, m), 7.10–7.29 (14H, m); 6.04 (2H, s); 4.65 (2H, t, *J* = 7.5 Hz); 4.16 (2H, t, *J* = 7.5 Hz); 2.98 (3H, s); 2.83 (2H, t, *J* = 7.5 Hz); 2.69 (2H, t, *J* = 7.5 Hz); 2.06–2.16 (4H, m). ^{13}C NMR (75 MHz, DMSO-d_6) δ 163.5, 147.9, 141.7, 141.0, 139.5, 136.0, 135.5, 134.7, 133.9, 129.7, 129.1, 128.8, 128.7, 128.6, 128.5, 127.1, 126.4, 126.3, 125.0, 114.6, 113.9, 113.3, 94.7, 68.2, 60.5, 45.1, 32.2, 31.8, 31.4, 30.4, 16.3. Anal. Calcd for $C_{37}H_{37}BrN_2O$: C, 73.38; H, 6.16; N, 4.63. Found: C, 73.28; H, 6.20; N, 4.67.

5.4. 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 Cao et al. [4]. Briefly, cells were grown in RPMI-1640 medium containing 10% (v/v) fetal calf serum and 100 μg ml^{-1} penicillin and 100 μg ml^{-1} streptomycin. Cultures were propagated at 37 °C in a humified atmosphere containing 5% CO_2. Cell lines were obtained from Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Science. DMSO was used as the solution for drugs. Final concentration of DMSO in the growth medium was 2% (v/v) or lower, concentration without effect on cell replication. In all of these experiments, three replicate wells were used to determine each point.

5.5. Assay of acute toxicities

Acute toxicity assay was performed according to the method described by Cao et al. [4]. Briefly, 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 harmine derivatives ranging from 1.0 to 500 mg/kg dissolved in 0.5% carboxymethyl cellulose sodium (CMC-Na) salt solution were given via intraperitoneal (i.p.) to different groups of healthy C57BL/6 mice, and each group contained 10 mice (5 males and 5 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 [19].

5.6. Assay of antitumor activity

Antitumor activity against Lewis lung cancer and Sarcoma 180 was performed as described by Cao et al. [4] with a slightly modification. Briefly, 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 about one fifth of LD_{50} value once a day for consecutive 7 days. Cyclophosphamide (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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