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ScienceDirect
European Journal of Medicinal Chemistry 44 (2009) 533–540
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
http://www.elsevier.com/locate/ejmech

Original article

Synthesis and *in vitro* cytotoxic evaluation of novel 3,4,5-trimethoxyphenyl substituted β-carboline derivatives

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Received 11 January 2008; received in revised form 25 March 2008; accepted 27 March 2008
Available online 4 April 2008

Abstract

To elucidate further our SARs' study on the chemistry and cytotoxic activity and probe the structural requirement for the potent antitumor activity of β-carbolines, a series of novel 1,9-disubstituted and 1,3,9-trisubstituted β-carboline derivatives were designed and synthesized from the starting material L-tryptophan and 3,4,5-trimethoxybenezaldehyde. Cytotoxic activities of these compounds *in vitro* were investigated, and the SARs associated with position-1, 3 and 9 substituents in β-carbolines have also been discussed. It has been observed that these compounds only displayed moderate to weak cytotoxic activities. Interestingly, most of the investigated compounds displayed selectively cytotoxic activities to human BCG-823 cell lines with IC_{50} value lower than 100 μM. In addition, the short alkyl substituents in position-9 increased the cytotoxic activities with the tendency of *n*-butyl > ethyl > methyl. These data confirmed that (1) an alkyl substituent at position-9 of β-carboline nucleus plays an important role in determining their antitumor activities; (2) different β-carbolines bearing various substituents in β-carboline nucleus interacted selectively with specific targets leading to the difference of biochemical and pharmacological effects.

Keywords: β-Carboline; Synthesis; Antitumor; Cytotoxic activities; SARs

1. Introduction

The β-carboline alkaloids are a large group of natural and synthetic indole alkaloids that possess a common tricyclic pyrido[3,4-*b*]indole ring structure [1,2]. These compounds possess a wide diversity of important biochemical effects and pharmacological properties. Numerous previous reports investigated the effects of β-carboline alkaloids on the central nervous system (CNS), such as their affinity with benzodiazepine receptors (BZRs) [3–12] and 5-hydroxy serotonin receptors [13–16]. Recently, interests in these alkaloids were stimulated by their potent antitumor activities.

Previous reports [17–22] and our recent investigations [23–30] on the synthesis of a variety of β-carboline derivatives and the evaluation of their antitumor activities unraveled that β-carbolines demonstrated potent antitumor activities and the activity was correlated to both the planarity of the molecule and the presence of the ring substituents. Subsequently, a systematic structure–activity relationships (SARs) analysis was carried out to determine the influence on antitumor activity of various substituents in β-carboline ring, and preliminary SARs were also depicted [31]. Clearly, to acquire more information about the structural requirements for the possible improvement of the cytotoxic potential and to elucidate SARs between substituent properties in β-carboline and antitumor activities, design and synthesis of more novel β-carboline derivatives with various substituents at different positions of the β-carboline nucleus are needed.

Inspired by these results of previous and our recent investigation, in the present investigation, we designed and synthesized

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doi:10.1016/j.ejmech.2008.03.030

several series of novel β-carboline derivatives bearing 3,4,5-trimethoxyphenyl in position-1 on the basis of harmine chemical structure, it is possible to discover and develop novel antitumor agents. The design of substituents in position-9 was based on the previous experience [24–27], and the choice of substituents in position-3 was limited to carboxylate, carboxyl, acyl, hydroxymethyl and aldehyde. The focus of this investigation was to probe the optimal structural requirement of these compounds with regard to antitumor activities and further elucidate the SARs. To the best of our knowledge, all β-carboline derivatives are novel. We report here the preparation of novel β-carboline derivatives and their cytotoxic activities.

2. Chemistry

1-(3,4,5-Trimethoxy)phenyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid **3** was prepared by the condensation of L-tryptophan with 3,4,5-trimethoxybenzaldehyde *via* well-known Pictet–Spengler condensation in acid media, and subsequently oxidated and decarboxylated in a single step according to the method previously described by Snyder et al. [32] to afford 1-(3,4,5-trimethoxy)phenyl-β-carboline **4**. Esterification of compound **3** with ethanol in the presence of $SOCl_2$ by heating yielded the corresponding ethyl 1-(3,4,5-trimethoxy)phenyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylate **5**, and followed by dehydrogenation with sulfur in refluxing xylene to afford fully unsaturated compound **6**. This sequence of reactions can be easily scaled up to give enough material **6** for further transformation.

Substitution of alkyl or benzyl groups at the N^9-position of β-carboline ring system would be expected to enhance cytotoxicity, consequently a number of derivatives were designed and synthesized with substituents ranging in size from methyl group to phenylpropyl in position-9 of β-carboline ring system. The N^9-position of compounds **4** and **6** was alkylated or arylated by the action of sodium hydride in dry DMF followed by addition of the relevant appropriate alkylating and arylating agents to provide various 9-substituted β-carboline **4a–c** and β-carboline-3-carboxylate **6a–d**, respectively. Compounds **6a–c** were further hydrolyzed in alkaline solution to produce the corresponding β-carboline-3-carboxylic acids **7a–c**.

The amidation of carboxylate in position-3 of compounds **6a–c** with ethanolamine could be easily reacted in dry ethanol followed by addition of sodium hydride to give the corresponding amidated derivatives **8a–c** in good yield. The ester group in position-3 of compounds **6a–d** was reduced to its corresponding alcohols by lithium borohydride ($LiBH_4$) in dry THF according to the method reported by Cain et al. [4] to provide **9a–d**, and further oxidized by MnO_2 in CH_3CN to afford 3-carboxaldehyde derivatives **10a–d**. Unfortunately, in our studies, compounds **6a–d** failed to be reduced to alcohols by $LiAlH_4$ as described by Srivastava et al. [33], whereas, they could be easily reduced to alcohols by $LiBH_4$ in good yield (see Scheme 1). The chemical structures of all the synthesized novel compounds were confirmed by FAB-MS, IR, ^1H NMR and elemental analyses data.

3. Results and discussion

The cytotoxic potential of all newly synthesized β-carboline derivatives was evaluated *in vitro* against a panel of human tumor cell lines. Compounds **7a–c** were converted into its water-soluble sodium salts, and the other compounds examined were all prepared in the form of hydrochloride in order to enhance the solubility in aqueous solution by the usual methods before use. The results are summarized in Table 1.

As shown in Table 1, compound **4** and its N^9-alkyl substituted derivatives **4a–c** showed moderate cytotoxic activities against human tumor cell lines. Interestingly, the compounds bearing a carboxylate (**6a–d**), carboxyl (**7a–c**), and acylamide (**8a–c**) substituents in position-3 of β-carboline ring system displayed selectively cytotoxic activities against human BGC-823 cell lines but failed to show cytotoxic effect in other tumor cell lines at the concentration of 100 μM. In addition, the compounds with a hydroxymethyl (**9a–d**) and carboxyaldehyde (**10a–d**) substituents in position-3 were almost inactive to all tumor cell lines investigated at the concentration of 100 μM.

As predicted, introducing a short alkyl substituent into position-9 of compounds **4** and **6** led to compounds **4a** and **6a** (methyl), **4b** and **6b** (ethyl), **4c** and **6c** (butyl), which all showed more potent cytotoxic activities than their parent compounds **4** and **6** with the tendency of methyl > ethyl > butyl. Similarly, the cytotoxic potency of compounds **7a–c** and **8a–c** followed the sequence of **7c** > **7b** > **7a** and **8c** > **8b** > **8a**.

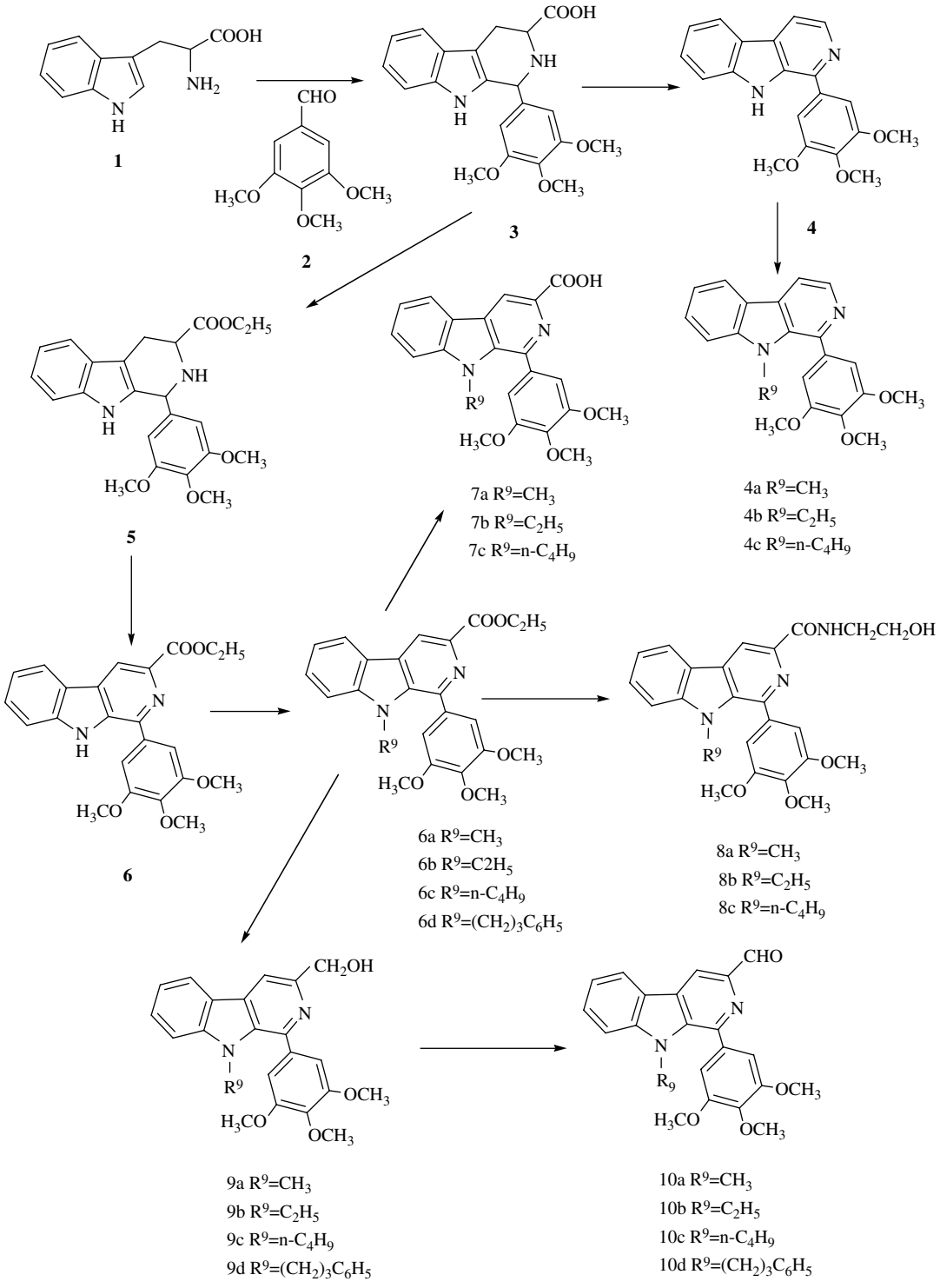
As a whole, the present results showed that 9-position short alkyl substituents might be a favorable group to exploit in searching for new antitumor leading compounds, and these data further substantiated our previous observations, indicating that position-9 substituted with short alkyl led to derivatives with enhanced cytotoxic acitivities. In addition, substituents at position-3 of β-carboline were detrimental to cytotoxic activity of β-carboline derivatives, and these results also confirmed our previous reports that substituents in position-3 were unfavorable for their cytotoxic activities. Although the β-carboline derivatives presented here showed modest cytotoxic activities, the investigations of these structural modifications and preliminary SARs would be helpful to further design and develop more potent compounds.

In this paper we reported only preliminary results on the relationship between structure and cytotoxic activities. To acquire more information about the structural requirements for improving cytotoxic activities, the synthesis of more new β-carboline derivatives with different substituents at other positions is needed. Further investigations and biological evaluations on these and other unreported β-carboline derivatives in animal models are in progress in our laboratories, and the data will be reported in a separated paper.

4. Experimental

4.1. Chemistry

All reagents were purchased from commercial suppliers and were dried and purified when necessary. Harmine was



Scheme 1. Synthesis of 1,9 and 1,3,9-substituted β-carboline derivatives.

extracted from *Peganum multisectum Maxim*, a plant indigenous to western China, according to the method by Duan et al. [34]. Melting points were determined in capillary tubes on an electrothermal PIF YRT-3 apparatus without correction. FAB-MS spectra were obtained from VG ZAB-HS spectrometer. FT-IR was run on a Bruker Equinox 55 Fourier Transformation Infrared Spectrometer. ^{1}H NMR spectra were recorded on a Varian INOVA 500NB spectrometer. Elemental analyses were carried out on an ElementarVario EL CHNS Elemental Analyzer. Silica gel F254 was used in analytical thin-layer chromatography (TLC) and silica gel was used in column chromatography.

Table 1
Cytotoxicity of β-carboline derivatives *in vitro*[c]

IC_{50} (μM)[a]					
Compounds	Hela[b]	Bel-7402[b]	BGC-823[b]	HepG2[b]	MCF-7[b]
Harmine	60.0	54.0	68.0	46.0	72.0
4	19.6	43.8	43.1	63.2	31.7
4a	71.8	91.1	43.2	86.8	74.2
4b	50.9	43.9	35.2	>100	72.7
4c	44.2	42.5	27.5	51.9	51.8
6	>100	>100	96.6	>100	92.3
6a	>100	>100	56.6	>100	>100
6b	>100	>100	53.2	>100	>100
6c	>100	>100	27.3	>100	43.6
6d	>100	>100	95.4	>100	>100
7a	>100	>100	91.1	>100	>100
7b	>100	61.4	66.5	>100	>100
7c	>100	>100	51.1	>100	>100
7d	>100	>100	36.2	79.1	36.3
8a	>100	>100	90.9	>100	95.5
8b	>100	>100	74.7	>100	53.8
8c	>100	>100	37.2	87.4	31.6
9a	>100	>100	>100	>100	>100
9b	>100	>100	>100	>100	>100
9c	>100	47.5	>100	>100	>100
9d	>100	>100	>100	>100	>100
10a	>100	>100	>100	>100	>100
10b	>100	>100	>100	>100	>100
10c	>100	>100	>100	>100	>100
10d	>100	>100	>100	>100	>100

[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 (HepG2 and Bel -7402), gastric carcinoma (BGC-823), and breast carcinoma (MCF-7).
[c] Data represent the mean values of three independent determinations.

*4.1.1. 1-(3,4,5-Trimethoxy)phenyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (**3**)*

A mixture of L-tryptophan (40.8 g, 200 mmol), 3,4,5-trimethoxybenzaldehyde (39.2 g, 200 mmol) and acetic acid (300 ml) was refluxed for 3 h, then cooled and adjusted to pH 5 with concentrated ammonium hydroxide, the precipitated product was collected by filtration and washed well with water and then dried. White solid (74.0 g, 97%) was obtained. Further purification was not necessary and used directly for the next steps.

*4.1.2. 1-(3,4,5-Trimethoxy)phenyl-β-carboline (**4**)*

Compound **3** (38.2 g, 100 mmol) was diluted to 3000 ml with water, and then heated to boiling. To the hot solution was added potassium dichromate (150 g) and glacial acetic acid (200 ml). The brown suspension was heated for 10 min and then cooled under the tap. After treating the cold solution with sodium sulfite to remove the excess oxidizing agent, the mixture was made definitely alkaline with sodium hydroxide. The solution was extracted exhaustively with ethyl acetate (10 L), the extracts dried over anhydrous sodium sulfate, and the solvent removed. The residue was crystallized from anhydrous ethanol, and white crystals of **4** were obtained (20 g, 60%), mp 167–169 °C. FAB-MS *m/z* (M + 1): 335; IR (KBr): 3556, 3309, 2937, 2832, 1624, 1583, 1502, 1456, 1404, 1346, 1232, 1126, 1000, 827, 751; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.79 (1H, s, NH); 8.52–8.53 (1H, d, J = 5.5 Hz, H-3); 8.14–8.16 (1H, d, J = 8.0 Hz, H-5); 7.91–7.92 (1H, d, J = 5.5 Hz, H-8); 7.52–7.57 (2H, m, H-6, H-7); 7.29–7.32 (1H, m, H-4); 7.13 (2H, s, PhH); 3.90 (9H, s, OCH_3); Anal. Calcd for $C_{20}H_{18}N_2O_3$: C, 71.84; H, 5.43; N, 8.38. Found: C, 71.70; H, 5.48; N, 8.32.

*4.1.3. General procedure for the preparation of 9-substituted 1-(3,4,5-trimethoxy)-phenyl-β-car-boline (**4a–c**)*

A mixture of **4** (1.67 g, 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 halogenated alkane (10–15 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 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.

*4.1.3.1. 1-(3,4,5-Trimethoxy)phenyl-9-methyl-β-carboline (**4a**).* Afforded white crystals (1.4 g, 80%), mp 147–148 °C. FAB-MS *m/z* (M + 1): 349; IR (KBr): 3005, 2938, 2836, 1618, 1581, 1502, 1460, 1401, 1353, 1229, 1123, 1006, 840, 752; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.50–8.51 (1H, d, J = 5.5 Hz, H-3); 8.18–8.19 (1H, d, J = 7.0 Hz, H-8); 7.98–7.99 (1H, d, J = 5.0 Hz, H-5); 7.61–7.64 (1H, m, H-7); 7.43–7.45 (1H, d, J = 8.0 Hz, H-6); 7.31–7.34 (1H, m, H-4); 6.86 (2H, s, PhH); 3.91–3.93 (9H, m, OCH_3); 3.55 (3H, s, NCH_3); Anal. Calcd for $C_{21}H_{20}N_2O_3$: C, 72.40; H, 5.79; N, 8.04. Found: C, 72.53; H, 5.86; N, 7.99.

*4.1.3.2. 1-(3,4,5-Trimethoxy)phenyl-9-ethyl-β-carboline (**4b**).* Afforded white crystals (1.5 g, 83%), mp 144–145 °C. FAB-MS *m/z* (M + 1): 363; IR (KBr): 2962, 2931, 2826, 1617, 1584, 1504, 1447, 1402, 1352, 1229, 1129, 1001, 832, 748; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.49–8.50 (1H, d, J = 5.0 Hz, H-3); 8.18–8.19 (1H, d, J = 8.0 Hz, H-8); 7.98–7.99 (1H, d, J = 5.0 Hz, H-5); 7.58–7.61 (1H, m, H-7); 7.45–7.46 (1H, d, J = 8.5 Hz, H-6); 7.30–7.33 (1H, m, H-4); 6.83 (2H, s, PhH); 4.08–4.09 (2H, m, NCH_2CH_3); 3.89–3.93 (9H, m, OCH_3); 1.05–1.08 (3H, s, NCH_2CH_3); Anal. Calcd for $C_{22}H_{22}N_2O_3$: C, 72.91; H, 6.12; N, 7.73. Found: C, 72.67; H, 6.21; N, 7.64.

*4.1.3.3. 1-(3,4,5-Trimethoxy)phenyl-9-n-butyl-β-carboline (**4c**).* Afforded white crystals (1.4 g, 83%), mp 118–119 °C. FAB-MS *m/z* (M + 1): 391; IR (KBr): 3013, 2959, 2931, 2835, 1617, 1582, 1505, 1450, 1410, 1362, 1238, 1123, 997, 831, 751; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.49–8.50 (1H, d, J = 5.0 Hz, H-3); 8.17–8.19 (1H, d, J = 7.5 Hz, H-8); 7.98–7.99 (1H, d, J = 5.0 Hz, H-5); 7.57–7.61 (1H, m, H-7); 7.43–7.45 (1H, d, J = 8.0 Hz, H-6); 7.30–7.32 (1H, m, H-4); 6.83 (2H, s, PhH); 3.98–4.01 (2H, m, $NCH_2(CH_2)_2CH_3$); 3.89–3.92 (9H, m, OCH_3); 1.44–1.47 (2H, m, $NCH_2CH_2CH_2CH_3$); 0.94–1.01 (2H, m, $NCH_2CH_2CH_2CH_3$);

0.70–0.73 (3H, m, $NCH_2CH_2CH_2CH_3$); Anal. Calcd for $C_{24}H_{26}N_2O_3$: C, 73.82; H, 6.71; N, 7.17. Found: C, 73.72; H, 6.79; N, 7.11.

*4.1.4. Ethyl 1-(3,4,5-trimethoxy)phenyl-1,2,3, 4-tetrahydro-β-carboline-3-carboxylate (**5**)*

A mixture of compound **3** (76.4 g, 200 mmol), anhydrous ethanol (500 ml) and $SOCl_2$ (20 ml) was heated at reflux for 4 h, and then evaporated in reduced pressure. The resulting mixture was poured into H_2O (300 ml) and neutralized with sodium hydrogen carbonate. The solution was extracted with ethyl acetate (3 × 300 ml). The organic phase was washed with water and brine, then dried over anhydrous sodium sulfate, filtered and evaporated. The residue was crystallized from ethyl acetate to afford white solid (73 g, 91%). Further purification was not necessary and used directly for the next steps.

*4.1.5. Ethyl 1-(3,4,5-trimethoxy)phenyl-β-carboline-3-carboxylate (**6**)*

A suspension of compound **5** (41 g, 100 mmol) and sulfur (9.6 g, 300 mmol) in xylene (250 ml) was heated at reflux for 8 h. The solution was cooled and stored at 4 °C for 3 h, and then filtered and washed generously with petroleum ether, the solid was dried and recrystallized from ethyl acetate to afford white crystals (27 g, 68%), mp 229–230 °C. FAB-MS *m/z* (M + 1): 407; IR (KBr): 3252, 2991, 2940, 2832, 1711, 1625, 1588, 1501, 1455, 1391, 1248, 1127, 1006, 840, 745; ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.28 (1H, s, N*H*); 8.80 (1H, s, H-4); 8.20–8.22 (1H, d, *J* = 7.5 Hz, H-8); 7.58–7.63 (2H, m, H-5, H-6); 7.36–7.39 (1H, m, H-7); 6.82 (2H, s, Ph*H*); 4.51–4.55 (2H, m, OCH_2CH_3); 3.87–3.89 (9H, m, OCH_3); 1.47–1.50 (3H, m, OCH_2CH_3); Anal. Calcd for $C_{23}H_{22}N_2O_5$: C, 67.97; H, 5.46; N, 6.89. Found: C, 67.79; H, 5.52; N, 6.83.

*4.1.6. General procedure for the preparation of ethyl 1-(3,4,5-trimethoxy)-phenyl-9-substituted β-carboline-3-carboxylate (**6a–d**)*

A mixture of compound **6** (2.0 g, 5 mmol) and anhydrous DMF (50 ml) was stirred at RT until clear, and then 60% NaH (0.3 g, 7.5 mmol) and halogenated alkane (10–15 mmol) were added. The mixture was stirred at RT 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 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.

*4.1.6.1. Ethyl 1-(3,4,5-trimethoxy)phenyl-9-methyl-β-carboline-3-carboxylate (**6a**).* Afforded white crystals (1.5 g, 71%), mp 173–175 °C. FAB-MS *m/z* (M + 1): 421; IR (KBr): 2939, 2834, 1702, 1622, 1583, 1504, 1461, 1369, 1319, 1259, 1128, 1005, 736; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.87 (1H, s, H-4); 8.24–8.26 (1H, d, *J* = 7.5 Hz, H-8); 7.64–7.67 (1H, m, H-5); 7.47–7.48 (1H, d, *J* = 8.5 Hz, H-6); 7.37–7.40 (1H, m, H-7); 6.86 (2H, s, Ph*H*); 4.50–4.54 (2H, m, OCH_2CH_3); 3.90–3.92 (9H, m, OCH_3); 3.55 (3H, s, NCH_3); 1.46–1.49 (3H, m, OCH_2CH_3); Anal. Calcd for $C_{24}H_{24}N_2O_5$: C, 68.56; H, 5.75; N, 6.66. Found: C, 68.39; H, 5.86; N, 6.61.

*4.1.6.2. Ethyl 1-(3,4,5-trimethoxy)phenyl-9-ethyl-β-carboline-3-carboxylate (**6b**).* Afforded white crystals (1.3 g, 60%), mp 170–171 °C. FAB-MS *m/z* (M + 1): 435; IR (KBr): 3059, 2977, 2939, 2826, 1730, 1695, 1619, 1583, 1505, 1465, 1369, 1246, 1123, 1005, 747; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.89 (1H, s, H-4); 8.25–8.27 (1H, d, *J* = 7.0 Hz, H-8); 7.63–7.66 (1H, m, H-5); 7.49–7.50 (1H, d, *J* = 8.5 Hz, H-6); 7.37–7.40 (1H, m, H-7); 6.84 (2H, s, Ph*H*); 4.51–4.55 (2H, m, OCH_2CH_3); 4.05–4.07 (2H, m, NCH_2CH_3); 3.89–3.92 (9H, m, OCH_3); 1.46–1.49 (3H, m, NCH_2CH_3); 1.07–1.10 (3H, m, OCH_2CH_3); Anal. Calcd for $C_{25}H_{26}N_2O_5$: C, 69.11; H, 6.03; N, 6.45. Found: C, 69.00; H, 6.13; N, 6.42.

*4.1.6.3. Ethyl 1-(3,4,5-trimethoxy)phenyl-9-n-butyl-β-carboline-3-carboxylate (**6c**).* Afforded white crystals (1.6 g, 69%), mp 138–139 °C. FAB-MS *m/z* (M + 1): 463; IR (KBr): 3065, 2933, 1711, 1621, 1583, 1505, 1450, 1413, 1368, 1260, 1237, 1128, 1006, 739; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.89 (1H, s, H-4); 8.25–8.26 (1H, d, *J* = 8.5 Hz, H-8); 7.62–7.65 (1H, m, H-5); 7.47–7.49 (1H, d, *J* = 8.0 Hz, H-6); 7.36–7.39 (1H, m, H-7); 6.83 (2H, s, Ph*H*); 4.51–4.55 (2H, m, OCH_2CH_3); 3.95–3.98 (2H, m, $NCH_2CH_2CH_2CH_3$); 3.89–3.91 (9H, m, OCH_3); 1.46–1.49 (5H, m, OCH_2CH_3, $NCH_2CH_2CH_2CH_3$); 0.95–0.99 (2H, m, $NCH_2CH_2CH_2CH_3$); 0.70–0.73 (3H, m, $NCH_2CH_2CH_2CH_3$); Anal. Calcd for $C_{27}H_{30}N_2O_5$: C, 70.11; H, 6.54; N, 6.06. Found: C, 69.83; H, 6.65; N, 5.98.

*4.1.6.4. Ethyl 1-(3,4,5-trimethoxy)phenyl-9-phenylpropyl-β-carboline-3-carboxylate (**6d**).* Afforded white crystals (1.8 g, 68%), mp 158–159 °C. FAB-MS *m/z* (M + 1): 525; IR (KBr): 3061, 3025, 2938, 2840, 1729, 1704, 1621, 1583, 1504, 1452, 1412, 1367, 1242, 1124, 1008,745; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.94 (1H, s, H-4); 8.47–8.49 (1H, d, *J* = 10.0 Hz, H-6); 7.72–7.74 (1H, d, *J* = 10.5 Hz, H-8); 7.64–7.68 (1H, m, H-5); 7.36–7.39 (1H, m, H-7); 7.01–7.29 (7H, m, Ph*H*); 4.34–4.41 (2H, m, $NCH_2CH_2CH_2Ph$); 4.02–4.06 (2H, m, OCH_2CH_3); 3.79 (9H, m, OCH_3); 2.16–2.20 (2H, m, $NCH_2CH_2CH_2Ph$); 1.74–1.76 (2H, m, $NCH_2CH_2CH_2Ph$); 1.34–1.38 (3H, m, OCH_2CH_3); Anal. Calcd for $C_{32}H_{32}N_2O_5$: C, 73.26; H, 6.15; N, 5.34. Found: C, 73.43; H, 6.26; N, 5.29.

*4.1.7. General procedure for the preparation of 1-(3,4,5-trimethoxy)phenyl-9-substituted-β-carboline-3-carboxylic acid (**7a–c**)*

A mixture of compound **6a–c** (5 mmol), NaOH (25 mmol), ethanol (50 ml) and H_2O (50 ml) was refluxed for 1 h, and ethanol was removed on the rotary evaporator. The mixture was neutralized (pH 5) with 5 M HCl and cooled. The precipitate was collected, washed well with H_2O and dried in vacuum.

*4.1.7.1. 1-(3,4,5-Trimethoxy)phenyl-9-methyl-β-carboline-3-carboxylic acid (**7a**).* Yellow solid was obtained (1.9 g,

97%), mp 246–248 °C. FAB-MS *m/z* (M + 1): 393; IR (KBr): 3192, 2944, 2826, 1747, 1619, 1585, 1501, 1466, 1421, 1362, 1239, 1126, 1004, 751; ^{1}H NMR (500 MHz, DMSO): δ 8.93 (1H, s, H-4); 8.46–8.47 (1H, d, *J* = 7.5 Hz, H-8); 7.66–7.71 (2H, m, H-5, H-6); 7.37–7.40 (1H, m, H-7); 6.94 (2H, s, Ph*H*); 3.78–3.84 (9H, m, OCH_3); 3.56 (3H, s, NCH_3); Anal. Calcd for $C_{22}H_{20}N_2O_5$: C, 67.34; H, 5.14; N, 7.14. Found: C, 67.26; H, 5.16; N, 7.10.

*4.1.7.2. 1-(3,4,5-Trimethoxy)phenyl-9-ethyl-β-carboline-3-carboxylic acid (**7b**).* Yellow solid was obtained (1.9 g, 94%), mp 215–216 °C. FAB-MS *m/z* (M + 1): 407; IR (KBr): 3525, 3368, 2942, 2356, 1707, 1620, 1584, 1502, 1455, 1413, 1362, 1234, 1126, 993, 786; ^{1}H NMR (500 MHz, DMSO): δ 9.05 (1H, s, H-4); 8.53–8.54 (1H, d, *J* = 7.5 Hz, H-8); 7.70–7.78 (2H, m, H-5, H-6); 7.40–7.43 (1H, m, H-7); 6.99 (2H, s, Ph*H*); 4.07–4.11 (2H, m, NCH_2CH$_3$); 3.79–3.82 (9H, m, OCH_3); 1.03–1.06 (3H, s, NCH$_2$CH_3); Anal. Calcd for $C_{23}H_{22}N_2O_5$: C, 67.97; H, 5.46; N, 6.89. Found: C, 67.87; H, 5.50; N, 6.83.

*4.1.7.3. 1-(3,4,5-Trimethoxy)phenyl-9-n-butyl-β-carboline-3-carboxylic acid (**7c**).* Yellow solid was obtained (2.0, 97%), mp 203–204 °C. FAB-MS *m/z* (M + 1): 435; IR (KBr): 3300, 2962, 2873, 1736, 1620, 1584, 1506, 1456, 1410, 1362, 1304, 1238, 1125, 1002, 957, 824, 737; ^{1}H NMR (500 MHz, DMSO): δ 8.93 (1H, s, H-4); 8.45–8.47 (1H, d, *J* = 7.5 Hz, H-8); 7.64–7.73 (2H, m, H-5, H-6); 7.35–7.38 (1H, m, H-7); 6.91 (2H, s, Ph*H*); 3.99–4.02 (2H, m, NCH_2CH$_2$CH$_2$CH$_3$); 3.78–3.82 (9H, m, OCH_3); 1.39–1.45 (2H, m, NCH$_2$CH_2CH$_2$CH$_3$); 0.91–0.95 (2H, m, NCH$_2$CH$_2$CH_2CH$_3$); 0.65–0.68 (3H, m, NCH$_2$CH$_2$CH$_2$CH_3); Anal. Calcd for $C_{25}H_{26}N_2O_5$: C, 69.11; H, 6.03; N, 6.45. Found: C, 68.98; H, 6.13; N, 6.42.

*4.1.8. General procedure for the preparation of N-(2-hydroxyethyl)-1-(3,4,5-trimethoxy)-phenyl-9-substituted-β-carboline-3-formamide (**8a–c**)*

A solution of compounds **6a–c** (5 mmol) in ethanol (50 ml) containing ethanolamine (40 mmol) and 60% NaH (0.4 g, 10 mmol) was refluxed for 15–30 min. After completion of the reaction, as indicated by TLC, the solution was cooled and poured into ice-water (200 ml), and 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 obtained was purified by silica column chromatography with ethyl acetate as the eluent, and crystallized from ethyl acetate or ethyl acetate-petroleum ether to give white crystals.

*4.1.8.1. N-(2-Hydroxyethyl)-1-(3,4,5-trimethoxy)phenyl-9-methyl-β-carboline-3-formamide (**8a**).* White crystals were obtained (1.6 g, 74%), mp 206–207 °C. FAB-MS *m/z* (M + 1): 436; IR (KBr): 3387, 2935, 2835, 1658, 1583, 1538, 1500, 1457, 1363, 1320, 1232, 1125, 1053, 1004, 842, 742; ^{1}H NMR (500 MHz, CDCl$_3$): δ 8.98 (1H, s, H-4); 8.26–8.27 (1H, d, *J* = 6.5 Hz, H-8); 7.65–7.68 (1H, m, H-5); 7.46–7.48 (1H, d, *J* = 8.5 Hz, H-6); 7.37–7.40 (1H, m, H-7); 6.83 (2H, s, Ph*H*); 3.92–3.97 (9H, m, OCH_3); 3.88–3.90 (2H, m, NHCH$_2$CH_2OH); 3.68–3.70 (2H, m, NHCH_2CH$_2$OH); 3.55 (3H, s, NCH_3); Anal. Calcd for $C_{24}H_{25}N_3O_5$: C, 66.19; H, 5.79; N, 9.65. Found: C, 66.01; H, 5.86; N, 9.68.

*4.1.8.2. N-(2-Hydroxyethyl)-1-(3,4,5-trimethoxy)phenyl-9-ethyl-β-carboline-3-formamide (**8b**).* White crystals were obtained (1.8 g, 80%), mp 168–169 °C. FAB-MS *m/z* (M + 1): 450; IR (KBr): 3400, 2966, 2933, 1666, 1583, 1533, 1503, 1464, 1368, 1234, 1125, 1005, 750, 728; ^{1}H NMR (500 MHz, CDCl$_3$): δ 8.97 (1H, s, H-4); 8.25–8.27 (1H, d, *J* = 6.5 Hz, H-8); 7.62–7.66 (1H, m, H-5); 7.47–7.49 (1H, d, *J* = 8.0 Hz, H-6); 7.36–7.39 (1H, m, H-7); 6.81 (2H, s, Ph*H*); 4.03–4.08 (2H, m, NCH_2CH$_3$); 3.91–3.98 (9H, m, OCH_3); 3.85–3.87 (2H, m, NHCH$_2$CH_2OH); 3.68–3.69 (2H, m, NHCH_2CH$_2$OH); 1.08–1.11 (3H, s, NCH$_2$CH_3); Anal. Calcd for $C_{25}H_{27}N_3O_5$: C, 66.80; H, 6.05; N, 9.35. Found: C, 66.89; H, 6.14; N, 9.32.

*4.1.8.3. N-(2-Hydroxyethyl)-1-(3,4,5-trimethoxy)phenyl-9-n-butyl-β-carboline-3-formamide (**8c**).* White crystals were obtained (1.8 g, 76%), mp 197–198 °C. FAB-MS *m/z* (M + 1): 478; IR (KBr): 3354, 2955, 2932, 2871, 1644, 1620, 1584, 1533, 1503, 1446, 1368, 1231, 1129, 1055, 1004, 735; ^{1}H NMR (500 MHz, CDCl$_3$): δ 8.96 (1H, s, H-4); 8.25–8.26 (1H, d, *J* = 6.5 Hz, H-8); 7.61–7.65 (1H, m, H-5); 7.46–7.47 (1H, d, *J* = 8.5 Hz, H-6); 7.35–7.38 (1H, m, H-7); 6.80 (2H, s, Ph*H*); 3.91–3.98 (11H, m, OCH_3, NCH_2CH$_2$CH$_2$CH$_3$); 3.85–3.87 (2H, m, NHCH$_2$CH_2OH); 3.66–3.69 (2H, m, NHCH_2CH$_2$OH); 1.44–1.51 (2H, m, NCH$_2$CH_2CH$_2$CH$_3$); 0.94–1.02 (2H, m, NCH$_2$CH$_2$CH_2CH$_3$); 0.71–0.74 (3H, s, NCH$_2$CH$_2$CH$_2$CH_3); Anal. Calcd for $C_{27}H_{31}N_3O_5$: C, 67.91; H, 6.54; N, 8.80. Found: C, 67.74; H, 6.51; N, 8.71.

*4.1.9. General procedure for the preparation of 1-(3,4,5-trimethoxy)phenyl-3-hydroxymethyl-9-substituted-β-carboline (**9a–d**)*

A fine suspension of compounds **6a–d** (10 mmol) in dry THF (100 ml) was treated with $LiBH_4$ (30 mmol), and the mixture was stirred at room temperature for 9 h. The reaction was cooled, treated with 10% aq. HCl (20 ml), and stirred for 4 h. The reaction mixture was neutralized with 10% aq. NaOH solution and extracted with ethyl acetate. The organic phase was washed with water and brine, then dried over anhydrous sodium sulfate, filtered and evaporated. The residue obtained was purified by silica column chromatography with ethyl acetate as the eluent. Upon recrystallization, white crystals were obtained.

*4.1.9.1. 1-(3,4,5-Trimethoxy)phenyl-3-hydroxymethyl-9-methyl-β-carboline (**9a**).* White crystals were obtained (3.0 g, 79%), mp 151–152 °C. FAB-MS *m/z* (M + 1): 379; IR (KBr): 3275, 3060, 2998, 2930, 2833, 1622, 1581, 1504, 1462, 1413, 1370, 1234, 1126, 1063, 1003, 836, 738; ^{1}H NMR (500 MHz, CDCl$_3$): δ 8.30–8.32 (1H, d, *J* = 10.0 Hz, H-4); 8.19 (1H, s, H-6); 7.60–7.61 (2H, m, H-5, H-8); 7.27–7.31 (1H, m, H-7); 6.86 (2H, s, Ph*H*); 4.73–4.75 (2H, d, *J* = 7.0 Hz, CH_2OH); 3.82 (6H, s, OCH_3); 3.76 (3H, s,

OCH_3); 3.50 (3H, s, NCH_3); Anal. Calcd for $C_{22}H_{22}N_2O_4$: C, 69.83; H, 5.86; N, 7.40. Found: C, 69.70; H, 5.92; N, 7.35.

*4.1.9.2. 1-(3,4,5-Trimethoxy)phenyl-3-hydroxymethyl-9-ethyl-β-carboline (**9b**).* White crystals were obtained (2.9 g, 74%), mp 154–155 °C. FAB-MS *m/z* (M + 1): 393; IR (KBr): 3328, 3068, 2968, 2936, 2835, 1621, 1582, 1504, 1450, 1410, 1370, 1231, 1125, 1052, 1013, 832, 742; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.30–8.32 (1H, d, J = 10.5 Hz, H-4); 8.20 (1H, s, H-6); 7.57–7.65 (2H, m, H-5, H-8); 7.26–7.30 (1H, m, H-7); 6.86 (2H, s, Ph*H*); 4.73–4.74 (2H, d, J = 7.0 Hz, CH_2OH); 4.02–4.07 (2H, s, NCH_2CH$_3$); 3.81 (6H, s, OCH_3); 3.77 (3H, s, OCH_3); 0.93–0.97 (3H, m, NCH_2CH$_3$); Anal. Calcd for $C_{23}H_{24}N_2O_4$: C, 70.39; H, 6.16; N, 7.14. Found: C, 70.28; H, 6.19; N, 7.09.

*4.1.9.3. 1-(3,4,5-Trimethoxy)phenyl-3-hydroxymethyl-9-n-butyl-β-carboline (**9c**).* White crystals were obtained (3.2 g, 76%), mp 127–128 °C. FAB-MS *m/z* (M + 1): 421; IR (KBr): 3338, 3181, 3061, 2986, 2832, 1621, 1583, 1505, 1465, 1409, 1366, 1297, 1235, 1179, 1126, 1051, 998, 833, 739; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.30–8.31 (1H, d, J = 10.5 Hz, H-4); 8.20 (1H, s, H-6); 7.57–7.65 (2H, m, H-5, H-8); 7.25–7.29 (1H, m, H-7); 6.84 (2H, s, Ph*H*); 4.72–4.74 (2H, d, J = 7.0 Hz, CH_2OH); 3.93–3.97 (2H, m, NCH_2CH$_2$CH$_2$CH$_3$); 3.80 (6H, s, OCH_3); 3.75 (3H, s, OCH_3); 1.31–1.39 (2H, m, NCH$_2$CH_2CH$_2$CH$_3$); 0.86–0.92 (2H, m, NCH$_2$CH$_2$CH_2CH$_3$); 0.62–0.66 (3H, m, NCH$_2$CH$_2$CH$_2$CH_3); Anal. Calcd for $C_{25}H_{28}N_2O_4$: C, 71.41; H, 6.71; N, 6.66. Found: C, 71.19; H, 6.81; N, 6.58.

*4.1.9.4. 1-(3,4,5-Trimethoxy)phenyl-3-hydroxymethyl-9-phenylpropyl-β-carboline (**9d**).* White crystals were obtained (3.5 g, 72%), mp 124–125 °C. FAB-MS *m/z* (M + 1): 483; IR (KBr): 3324, 3062, 2938, 2836, 1621, 1582, 1503, 1459, 1410, 1364, 1235, 1177, 1126, 1052, 838, 746; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.30–8.32 (1H, d, J = 10.5 Hz, H-4); 8.21 (1H, s, H-6); 7.56–7.63 (2H, m, H-5, H-8); 6.98–7.30 (6H, m, H-7, Ph*H*); 6.87 (2H, s, Ph*H*); 4.73–4.74 (2H, d, J = 7.0 Hz, CH_2OH); 4.03–4.05 (2H, m, NCH_2CH$_2$CH$_2$Ph); 3.79 (9H, s, OCH_3); 2.12–2.16 (2H, m, NCH$_2$CH$_2$CH_2Ph); 1.62–1.70 (2H, m, NCH$_2$CH_2CH$_2$Ph); Anal. Calcd for $C_{30}H_{30}N_2O_4$: C, 74.67; H, 6.27; N, 5.81. Found: C, 74.76; H, 6.35; N, 5.76.

*4.1.10. General procedure for the preparation of 1-(3,4,5-trimethoxy)phenyl-9-substituted-β-carboline-3-carboxaldehyde (**10a–d**)*

To a solution of **9a–d** (5 mmol) in CH_3CN (300 ml) was added activated MnO_2 (20 mmol). The suspension was refluxed for 2 h and then cooled and filtered through Celite. The filtrate was passed through silica gel and washed with ethyl acetate, and the solvent was removed under reduced pressure. The residue was crystallized from acetone or acetone-petroleum ether to afford white crystals.

*4.1.10.1. 1-(3,4,5-Trimethoxy)phenyl-9-methyl-β-carboline-3-carboxaldehyde (**10a**).* White crystals were obtained (1.4 g, 74%), mp 200–201 °C. FAB-MS *m/z* (M + 1): 377; IR (KBr): 3057, 2986, 2939, 2815, 1684, 1620, 1585, 1501, 1463, 1367, 1238, 1123, 997, 837, 740; ^{1}H NMR (500 MHz, $CDCl_3$): δ 10.27 (1H, s, C*H*O); 8.76 (1H, s, H-4); 8.25–8.27 (1H, d, J = 9.5 Hz, H-6); 7.66–7.70 (1H, m, H-5); 7.49–7.51 (1H, d, J = 9.5 Hz, H-8); 7.40–7.44 (1H, m, H-7); 6.84 (2H, s, Ph*H*); 3.94 (3H, s, OCH_3); 3.92 (6H, s, OCH_3); 3.59 (3H, s, NCH_3); Anal. Calcd for $C_{22}H_{20}N_2O_4$: C, 70.20; H, 5.36; N, 7.44. Found: C, 69.96; H, 5.46; N, 7.35.

*4.1.10.2. 1-(3,4,5-Trimethoxy)phenyl-9-ethyl-β-carboline-3-carboxaldehyde (**10b**).* White crystals were obtained (1.6 g, 82%), mp 209–210 °C. FAB-MS *m/z* (M + 1): 391; IR (KBr): 3065, 3001, 2935, 2829, 1687, 1621, 1580, 1503, 1457, 1404, 1325, 1233, 1127, 1008, 865, 743; ^{1}H NMR (500 MHz, $CDCl_3$): δ 10.27 (1H, s, C*H*O); 8.77 (1H, s, H-4); 8.26–8.28 (1H, d, J = 9.5 Hz, H-6); 7.67–7.69 (1H, m, H-5); 7.50–7.53 (1H, d, J = 10.5 Hz, H-8); 7.39–7.43 (1H, m, H-7); 6.84 (2H, s, Ph*H*); 4.07–4.12 (2H, m, NCH_2CH$_3$); 3.94 (3H, s, OCH_3); 3.91 (6H, s, OCH_3); 3.59 (3H, s, NCH_3); 1.12–1.15 (3H, m, NCH$_2$CH_3); Anal. Calcd for $C_{23}H_{22}N_2O_4$: C, 70.75; H, 5.68; N, 7.17. Found: C, 70.51; H, 5.79; N, 7.10.

*4.1.10.3. 1-(3,4,5-Trimethoxy)phenyl-9-n-butyl-β-carboline-3-carboxaldehyde (**10c**).* White crystals were obtained (1.5 g, 72%), mp 150–151 °C. FAB-MS *m/z* (M + 1): 419; IR (KBr): 3059, 3002, 2958, 2809, 1694, 1618, 1582, 1505, 1464, 1410, 1363, 1244, 1203, 1124, 1001, 969, 851, 754; ^{1}H NMR (500 MHz, $CDCl_3$): δ 10.26 (1H, s, C*H*O); 8.76 (1H, s, H-4); 8.25–8.27 (1H, d, J = 10.0 Hz, H-6); 7.64–7.68 (1H, m, H-5); 7.49–7.51 (1H, d, J = 10.5 Hz, H-8); 7.38–7.42 (1H, m, H-7); 6.84 (2H, s, Ph*H*); 3.98–4.02 (2H, m, NCH_2CH$_2$CH$_2$CH$_3$); 3.93 (3H, s, OCH_3); 3.91 (6H, s, OCH_3); 1.48–1.56 (2H, m, NCH$_2$CH_2CH$_2$CH$_3$); 0.96–1.06 (2H, m, NCH$_2$CH$_2$CH_2CH$_3$); 0.73–0.76 (3H, m, NCH$_2$CH$_2$CH$_2$CH_3); Anal. Calcd for $C_{25}H_{26}N_2O_4$: C, 71.75; H, 6.26; N, 6.69. Found: C, 71.62; H, 6.29; N, 6.63.

*4.1.10.4. 1-(3,4,5-Trimethoxy)phenyl-9-phenylpropyl-β-carboline-3-carboxaldehyde (**10d**).* White crystals were obtained (1.8 g, 75%), mp 174–175 °C. FAB-MS *m/z* (M + 1): 481; IR (KBr): 3065, 2998, 2957, 2934, 2828, 1689, 1621, 1581, 1504, 1456, 1405, 1366, 1236, 1127, 1006, 746; ^{1}H NMR (500 MHz, $CDCl_3$): δ 10.30 (1H, s, C*H*O); 8.76 (1H, s, H-4); 8.24–8.26 (1H, d, J = 10.0 Hz, H-6); 7.62–7.66 (1H, m, H-5); 7.38–7.42 (2H, m, H-7, H-8); 6.97–7.26 (5H, m, Ph*H*); 6.85 (2H, s, Ph*H*); 4.05–4.09 (2H, m, NCH_2CH$_2$CH$_2$Ph); 3.97 (3H, s, OCH_3); 3.89 (6H, s, OCH_3); 2.25–2.29 (2H, m, NCH$_2$CH$_2$CH_2Ph); 1.80–1.88 (2H, m, NCH$_2$CH_2CH$_2$Ph); Anal. Calcd for $C_{30}H_{28}N_2O_4$: C, 74.98; H, 5.87; N, 5.83. Found: C, 74.82; H, 5.90; N, 5.79.

4.2. Cytotoxicity assays in vitro

Cytotoxicity assays *in vitro* were carried out using 96-microtitre plate cultures and MTT staining according to the

procedures described by Al-Allaf and Rashan [35] with a slight modification. Cells were grown in RPMI-1640 medium containing 10% (v/v) fetal calf serum and 50 μg/ml penicillin and 50 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 cervical carcinoma (Hela), liver carcinoma (HepG2 and Bel-7402), gastric carcinoma (BGC-823) and breast carcinoma (MCF-7). In all of these experiments, three replicate wells were used to determine each point.

Acknowledgements

This work was supported by grants from the National New Drug Funds for Doctors (96-901-06-36) of the Ministry of Science and Technology, the Guangzhou Bureau of Science and Technology (97-Z-12-01), and Xinjiang Huashidan Pharmaceutical Co. Ltd.

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