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# Synthesis and cytotoxic evaluation of $N^2$-benzylated quaternary β-carboline amino acid ester conjugates

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ABSTRACT

The β-carboline alkaloids have been characterized as a class of potential antitumor agents. To further enhance the cytotoxic potency and improve water solubility of β-carboline, a series of new β-carboline amino acid ester, β-carboline amino acid and $N^2$-benzylated quaternary β-carboline amino acid ester conjugates were designed and synthesized, and the cytotoxic activities of these compounds were evaluated using a panel of human tumor cell lines. The $N^2$-benzylated quaternary β-carboline amino acid ester conjugates represented the most interesting cytotoxic activities. Particularly, compounds **8b** and **8g** were found to be the most potent compounds with $IC_{50}$ values lower than 20 μM against all human tumor cell lines investigated. These results confirmed that the $N^2$-benzyl substituent on the β-carboline ring played an important role in the modulation of the cytotoxic potencies.



## 1. Introduction

The β-carboline core is the common structural unit of many naturally occurring and synthetic indole alkoloids associated with a broad spectrum of biochemical and pharmacological properties [1]. Previously, considerable attention focused on the effects of β-carboline alkoloids on the central nervous system (CNS) including their affinity with benzodiazepine (BZ) [2], 5-hydroxytryptamine (5-HT) [3], dopamine (DA) [4], and imidazoline receptors [5]. Recent interests in β-carboline alkoloids have been stimulated by their potential antitumor activities. Several groups have investigated the syntheses, cytotoxic activities of β-carbolines bearing various substituents at postion-1, 3, 7 and 9 of β-carboline nucleus. Ishida et al. [6] reported that harmine and its β-carboline analogues displayed significant cytotoxic activities against a panel of tumor cell lines. Xiao et al. [7] described that β-carbolines bearing a flexible alkylamine side chain at position-3 exhibited potent cytotoxic activities. Shen et al. [8] observed that 1-substituted 1,2,3,4-tetrahydro- and 3,4-dihydro-β-carboline demonstrated remarkable cytotoxic potencies. Moreover, some amino acid functionalized 1,2,3,4-tetrahydro-β-carbolines were reported to inhibit topoisomerase II resulting in potent antitumor effects [9], and β-carboline amino acid ester conjugates were found to display prominent cytotoxic potencies [10].

Our previous reports also described the syntheses of numerous β-carboline derivatives bearing various substituents at position-1, 2, 3, 7 and 9 of β-carboline nucleus and evaluated their antitumor activities *in vitro* [11–16] and *in vivo* [11,13]. The SARs analysis revealed that (1) the common β-carboline moiety was very important for their potent antitumor activities; (2) the introduction of appropriate substituents into position C-1, N-2, C-3 and N-9 of β-carboline ring enhanced the antitumor activities; and the *n*-butyl, benzyl and phenylpropyl substituents at position-9 and the benzyl group at position-2 played a vital role in facilitating their antitumor potencies.

It is well known that amino acids are attractive substrates because there are not only the fundamental building blocks of biological systems and many natural products, but also they are commercially available and possess structurally diverse side chain [9]. So far, amino acids have been introduced into many leading compounds aiming to develop potential bioactive agents with low toxicity [10,17–20]. In addition, amino acids, as prodrugs, can significantly improve oral bioavailability of the parent drugs [21], such as melphalan [22] and gabapentin [23], which is ascribed to their advantages of being able to be efficiently delivered by the human peptide transporter.

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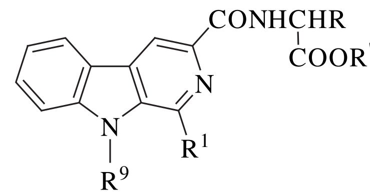
In our continuing search for novel and effective antitumor agents, we designed and synthesized a series of novel β-carboline amino acid ester conjugates. The design of substituents at position-9 of β-carboline ring was based on the previous SARs analysis [1], and the choice of L-amino acid was limited to L-alanine, L-valine, L-methionine, L-phenylalanine and L-tyrosine. The focus of this investigation was to probe the optimal structural requirement of these compounds with regard to antitumor activities, and further develop new antitumor β-carbolines with improved water solubility and bioavailability. To the best of our knowledge, all β-carboline amino acid ester conjugates are novel. We report herein the preparation of novel β-carboline derivatives and their cytotoxic activities.

## 2. Chemistry

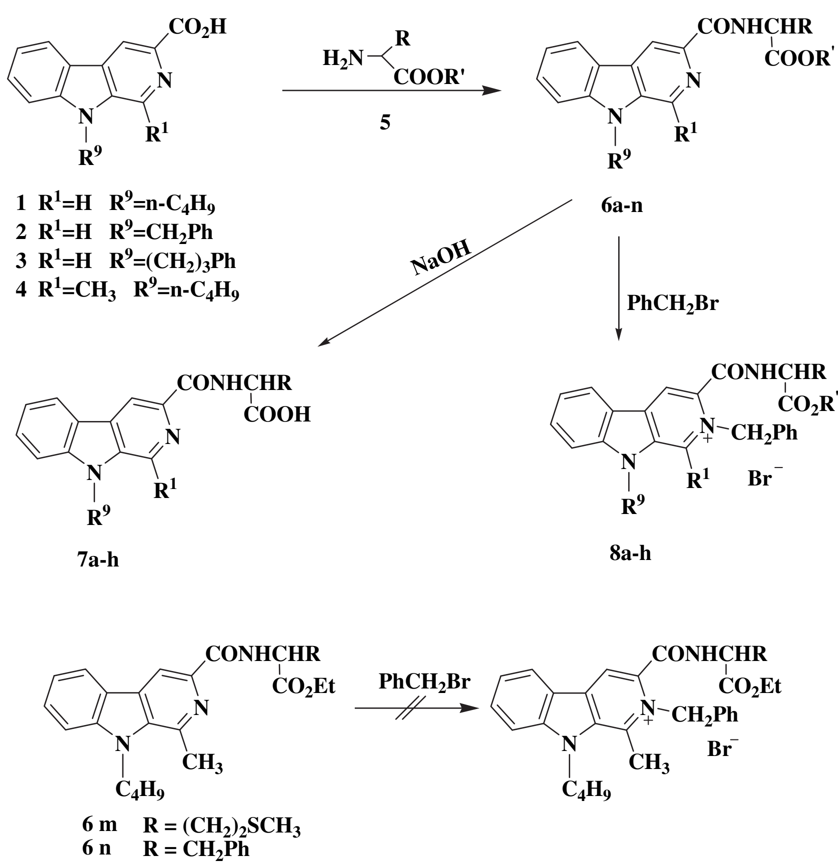
The synthetic routes of β-carboline amino acid conjugates are outlined in Scheme 1. 9-Substituted-β-carboline-3-carboxylic acids **1**–**4** were prepared from the L-tryptophan *via* five steps including the Pictet–Spengler condensation, esterification, aromatization, *N*-alkylation or *N*-benzylation and hydrolyzation as previously described [11,12]. Esterification of various L-amino acid with methanol or ethanol in the presence of $SOCl_2$ at −5 °C gave the corresponding L-amino acid esters **5** in good yield.

The conversion of compounds **1**–**4** to β-carboline amino acid ester conjugates **6a**–**n** (Table 1) was carried out *via* different amidating approach either by activation of β-carboline-3-carboxylic acid **1**–**4** with *N*,*N*'-dicyclohexxylcarbodiimide (DCC) in anhydrous THF [10], benzotriazole-1-yl-oxytri-pyrrolidino phosphonium hexafluorophosphate (Py-BOP) in anhydrous $CH_2Cl_2$ [9], and *N*, *N*'-carbonyldiimidazole (CDI) in anhydrous DMF. And the CDI was proven to be the most efficient and convenient reagent with mild reaction conditions, and compounds **1**–**4** were coupled to amino acid esters **5** using CDI in anhydrous DMF to provide the target compounds **6a**–**n** in 82–96% yield. Refluxing of the corresponding β-carboline amino acid ester **6** with aqueous sodium hydroxide for

**Table 1**
Chemical structure of compounds **6a**–**n**




| Compounds | $R^1$ | $R^9$ | R | R′ |
|---|---|---|---|---|
| **6a** | H | $n$-$C_4H_9$ | $CH_3$ | $CH_3$ |
| **6b** | H | $n$-$C_4H_9$ | $CH(CH_3)_2$ | $CH_3$ |
| **6c** | H | $n$-$C_4H_9$ | $CH_2CH_2SCH_3$ | $C_2H_5$ |
| **6d** | H | $n$-$C_4H_9$ | $CH_2Ph$ | $CH_3$ |
| **6e** | H | $n$-$C_4H_9$ | $CH_2Ph$ | $C_2H_5$ |
| **6f** | H | $n$-$C_4H_9$ | $CH_2Ph(p\text{-}OH)$ | $CH_3$ |
| **6g** | H | $CH_2Ph$ | $CH_2CH_2SCH_3$ | $C_2H_5$ |
| **6h** | H | $CH_2Ph$ | $CH_2Ph$ | $C_2H_5$ |
| **6i** | H | $(CH_2)_3Ph$ | $CH_3$ | $CH_3$ |
| **6j** | H | $(CH_2)_3Ph$ | $CH(CH_3)_2$ | $C_2H_5$ |
| **6k** | H | $(CH_2)_3Ph$ | $CH_2CH_2SCH_3$ | $C_2H_5$ |
| **6l** | H | $(CH_2)_3Ph$ | $CH_2Ph$ | $C_2H_5$ |
| **6m** | $CH_3$ | $n$-$C_4H_9$ | $CH_2CH_2SCH_3$ | $C_2H_5$ |
| **6n** | $CH_3$ | $n$-$C_4H_9$ | $CH_2Ph$ | $C_2H_5$ |




**Scheme 1.** Synthesis of β-carboline amino acid ester derivatives.

1h almost quantitatively generated β-carboline amino acid conjugates **7a–h** (Table 2), while the amide bonds of β-carboline amino acid ester conjugates were not affected.

The *N*2-benzylated β-carbolinium bromate derivatives **8a–h** (Table 3) was prepared from the corresponding β-carboline amino acid ester conjugates **6** by the addition of benzyl bromide in refluxing ethyl acetate [15]. Compared with the *N*2-benzylated reaction of other β-carbolines [15] with benzyl bromide, compounds **8a–h** were obtained with longer reaction time and lower yield (32–62%), which might be attrituted to the inaccessibility of the sterically hindered amino acid side chains. Unfortunately, the same synthetic procedure was used for the preparation of *N*2-benzylated compounds **6m** and **6n** but failed to afford the expected β-carbolinium bromates. The chemical structures of all the synthesized novel compounds were characterized by elemental analysis, MS, IR, $^{1}$H NMR, and $^{13}$C NMR spectra.

## 3. Results and discussion

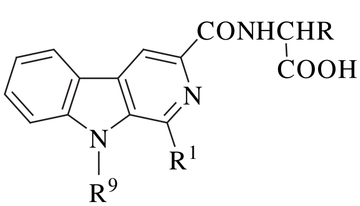
All compounds were evaluated for their cytotoxic activity *in vitro* against a panel of human tumor cell lines using cisplatin and paclitaxel as the reference drug. In order to enhance the solubility in aqueous solution, compounds **6a–n** were prepared in the form of hydrochloride and compounds **7a–h** were converted into their water-soluble sodium salts by the usual methods before use. The $IC_{50}$ results were summarized in Table 4.

Our efforts to improve the water solubility and cytotoxic potency of β-carboline derivatives resulted in the compounds **6a–n**, **7a–h** and **8a–h**. As predicted, compounds **7a–h** displayed improved water solubility with ClogP values ranged from 3.88 to 6.04 (Table 4). Interestingly, *N*2-benzylated quaternary β-carboline-3-amino acid ester conjugates **8a–h** exhibited excellent water solubility with ClogP values ranged from 1.38 to 3.54. However, compounds **6a–n** were almost insoluble in aqueous solution.

Unfortunately, as shown in Table 4, the β-carboline amino acid ester conjugates **6a–n** were almost inactive to all tumor cell lines investigated at the concentration of 200 μM. This was in disagreement with earlier findings [10]. The poor water solubility of these compounds might be significantly affected their potencies *in vitro*. Whereas, their corresponding hydrolyzed congener **7a–h**, bearing a free carboxyl group at the side chain of amino acid residue, exhibited moderate to weak cytotoxic activities against all tested human tumor cell lines. Noticeably, most of the *N*2-benzylated β-carboline-3-amino acid ester conjugates **8a–h** displayed a broad spectrum of cytotoxic activities against all human tumor cell lines with $IC_{50}$ values of lower than 50 μM.

Of all β-carboline-3-amino acid conjugates **7a–h**, compounds **7f**, **7g** and **7h** bearing a phenylpropyl substituent at position-9 of

**Table 2**
Chemical structure of compounds **7a–h**

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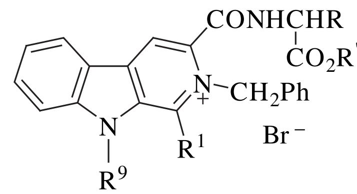
| Compounds | $R^1$ | $R^9$ | R |
|---|---|---|---|
| **7a** | H | $n$-$C_4H_9$ | $CH_2CH_2SCH_3$ |
| **7b** | H | $n$-$C_4H_9$ | $CH_2Ph$ |
| **7c** | H | $n$-$C_4H_9$ | $CH_2Ph$(p-OH) |
| **7d** | H | $CH_2Ph$ | $CH_2CH_2SCH_3$ |
| **7e** | H | $(CH_2)_3Ph$ | $CH_3$ |
| **7f** | H | $(CH_2)_3Ph$ | $CH(CH_3)_2$ |
| **7g** | H | $(CH_2)_3Ph$ | $CH_2CH_2SCH_3$ |
| **7h** | H | $(CH_2)_3Ph$ | $CH_2Ph$ |

**Table 3**
Chemical structure of compounds **8a–h**

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| Compounds | $R^1$ | $R^9$ | R | R′ |
|---|---|---|---|---|
| **8a** | H | $n$-$C_4H_9$ | $CH(CH_3)_2$ | $C_2H_5$ |
| **8b** | H | $n$-$C_4H_9$ | $CH_2CH_2SCH_3$ | $C_2H_5$ |
| **8c** | H | $n$-$C_4H_9$ | $CH_2Ph$ | $C_2H_5$ |
| **8d** | H | $CH_2Ph$ | $CH(CH_3)_2$ | $C_2H_5$ |
| **8e** | H | $CH_2Ph$ | $CH_2Ph$ | $C_2H_5$ |
| **8f** | H | $(CH_2)_3Ph$ | $CH(CH_3)_2$ | $C_2H_5$ |
| **8g** | H | $(CH_2)_3Ph$ | $CH_2CH_2SCH_3$ | $C_2H_5$ |
| **8h** | H | $(CH_2)_3Ph$ | $CH_2Ph$ | $C_2H_5$ |

**Table 4**
Cytotoxicity of β-carboline derivatives *in vitro*[c] ($IC_{50}$,[a] μM).

| Compounds | 769-P[b] | BGC[b] | KB[b] | 786-0[b] | A375[b] | ClogP[d] |
|---|---|---|---|---|---|---|
| **6a** | 27.2 | >200 | >200 | >200 | >200 | 4.40 |
| **6b** | >200 | >200 | >200 | >200 | >200 | 5.32 |
| **6c** | >200 | >200 | >200 | >200 | >200 | 5.07 |
| **6d** | >200 | >200 | >200 | >200 | >200 | 5.81 |
| **6e** | 32.5 | >200 | 113 | >200 | >200 | 6.34 |
| **6f** | >200 | >200 | >200 | >200 | >200 | 5.15 |
| **6g** | >200 | >200 | >200 | >200 | >200 | 5.25 |
| **6h** | >200 | >200 | >200 | >200 | >200 | 6.52 |
| **6i** | >200 | 148 | >200 | >200 | >200 | 5.28 |
| **6j** | >200 | >200 | >200 | >200 | >200 | 6.74 |
| **6k** | >200 | >200 | 176 | >200 | >200 | 5.96 |
| **6l** | 187 | >200 | 89.2 | >200 | >200 | 7.23 |
| **6m** | >200 | >200 | >200 | >200 | >200 | 5.57 |
| **6n** | >200 | >200 | >200 | >200 | >200 | 6.84 |
| **7a** | 105 | >200 | >200 | >200 | >200 | 3.88 |
| **7b** | 114 | >200 | 155 | 191 | 160 | 5.15 |
| **7c** | >200 | >200 | >200 | >200 | 121 | 4.48 |
| **7d** | 145 | >200 | 135 | >200 | 156 | 4.06 |
| **7e** | 120 | 162 | 179 | 78.3 | 96.3 | 4.62 |
| **7f** | 78.3 | 139 | 106 | 84.6 | 58.0 | 5.55 |
| **7g** | 116 | 136 | 96.6 | 70.0 | 58.9 | 4.77 |
| **7h** | 8.0 | 76.1 | 38.8 | 52.7 | 33.8 | 6.04 |
| **8a** | 13.7 | 44.2 | 28.4 | 14.4 | 22.5 | 2.16 |
| **8b** | 7.5 | 13.4 | 10.8 | 1.7 | 8.2 | 1.38 |
| **8c** | 19.2 | 21.4 | 11.3 | 15.0 | 41.7 | 2.65 |
| **8d** | 12.5 | 21.9 | 25.3 | 10.8 | 15.8 | 2.34 |
| **8e** | 13.5 | 92.5 | 51.4 | 69.4 | 25.8 | 2.84 |
| **8f** | 14.0 | 17.8 | 12.5 | 4.0 | 10.4 | 3.05 |
| **8g** | 7.6 | 12.0 | 9.4 | 10.1 | 7.3 | 2.28 |
| **8h** | 22.9 | 23.7 | 18.2 | 23.4 | 21.3 | 3.54 |
| **Cisplatin** | 19.2 | 13.4 | 4.6 | 4.9 | 9.4 | – |
| **Paclitaxel** | 7.1 | 1.5 | 0.08 | <0.08 | 0.81 | – |

[a] Cytotoxicity as $IC_{50}$ for each cell line is the concentration of compound, which reduced by 50% the optical density of treated cells with respect to untreated using the MTT assay.
[b] Cell lines include renal carcinoma (769-P), gastric carcinoma (BGC-823), epidermoid carcinoma of the nasopharynx (KB), renal carcinoma (786-0), melanoma (A375).
[c] The data represent the mean values of three independent determinations.
[d] ClogP represent the calculated $n$-octanol/water partition coefficient (log Pow), and the values produced by Chemdraw software.

β-carboline core displayed moderate cytotoxic activities. However, the others only had marginal or no cytotoxic effects in any cell lines. Noticeably, compound **7h** showed the best activity with, for example, 50% growth inhibition, $IC_{50}$ 8.0 μM against 769-P renal carcinoma cell lines. Our previous reports [15] and the above-mentioned results further confirmed that the phenylpropyl substituent represented the optimal structure at postion-9 of β-carboline core for this class of compounds to exhibit potent cytotoxic activities.

As predicted, the incorporating a benzyl substituent into position-2 of β-carboline core led to the $N^2$-benzylated quaternary β-carboline-3-amino acid ester conjugates **8a**–**h**, which represented the most interesting cytotoxic activities. Compounds **8b**, **8f** and **8g** exhibited significant cytotoxic activities against all human tumor cell lines with $IC_{50}$ value of lower than 20 μM. These results further confirmed that the $N^2$-benzylated substituent on the β-carboline ring played a very vital role in the modulation of the cytotoxic activities. Particularly, compounds **8b** and **8g,** having a common methionine residue at position-3 of β-carboline nucleus but bearing an *n*-butyl and phenylpropyl substituent at position-9 of β-carboline core, respectively, demonstrated the most potent cytotoxic activities with $IC_{50}$ value of 1.7 μM and 4.0 μM against 786-0 renal carcinoma cell lines, respectively. This observation indicated that methionine side chain might be more favorable for the cytotoxic potency of $N^2$-benzylated quaternary β-carboline amino acid ester conjugates.

## 4. Conclusions

A series of new $N^2$-benzylated quaternary β-carboline amino acid ester conjugates described in this paper were proved to be significantly cytotoxic activities. Current investigation corroborated our previous observations that (1) the cytotoxic potencies of β-carbolines were substituent-dependant; (2) introducing appropriate substituents into position-9 of β-carboline nucleus enhanced their cytotoxic activities; (3) the $N^2$-benzyl substituent on the β-carboline core played a very important role in the modulation of the cytotoxic potencies.

Some important molecular mechanisms of action of β-carbolines have recently been reviewed [1]. However, the underlying mechanism and the cellular target molecules responsible for such activity has not been completely understood. Some β-carbolines were reported to have DNA intercalating activity [7,24] and inhibitory activity of topoisomerase [9], other β-carbolines were identified as inhibitors of CDK [25] and MAPKAP-K2 [26]. Our previous investigations reported that the ability of β-carbolines to act as DNA intercalating agents and Topoisomerase I inhibitors was related to their potent antitumor activities [27], and β-carbolines can pass through cell membrane and penetrate into cell nucleus quickly resulting in intercalating into DNA in cells [28]. Moreover, our group also found that some β-carbolines can induce apoptosis in HepG2 cells and other β-carbolines can inhibit the expression of *Bcl-2* gene and upregulate the expression of death receptor *Fas* [29]. Unfortunately, the weak effects of β-carbolines on the above-mentioned cellular molecules indicated that they are unlikely to be the targets for the potent inhibition of tumor cell growth. There is no doubt that further investigation is urgently required to completely elucidate the mechanisms of action of β-carbolines.

In order to confirm the utility of the $N^2$-benzylated β-carboline amino acid ester conjugates as an interesting antitumor agent, compounds **8b**, **8f** and **8g** are now selected and submitted to further acute toxicity and antitumor activity studies in animal models, and the relative possible results will be reported in due course. Moreover, to acquire more information about the structural requirements for enhancing cytotoxic potencies and improving water solubility and bioavailability, the synthesis of more $N^2$-benzylated quaternary β-carboline amino acid ester conjugates is needed.

## 5. Experimental

### *5.1. Reagents and general procedures*

All reagents were purchased from commercial suppliers and were dried and purified when necessary. Melting points were determined with a Kofler micromelting point apparatus without correction. ESI-MS spectra were obtained from VG ZAB-HS spectrometer. $^1$H NMR and $^{13}$C NMR spectra were recorded on a Mercury-Plus 300 spectrometer at 300 MHz and 75 MHz respectively, using TMS as internal standard and $CDCl_3$ or DMSO-$d_6$ as solvent and chemical shifts ($\delta$) were expressed in ppm. 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.

### *5.2. Gerneral procedure for the preparation of amino acid methyl/ethyl esters **5***

To the solution of L-amino acid (10 mmol) and anhydrous methanol/ethanol (100 mL), $SOCl_2$ (1.0 mL, 12 mmol) was added slowly at −5 °C for about 10–30 min until clear. And then the reaction mixture was stirred for about 5 h at room temperature. After the reaction was completed, the superfluous $SOCl_2$ and methanol/ethanol were removed on the rotary evaporator to give the L-amino acid methyl/ethyl ester hydrochloride in 96–98% yield as white solid.

To the mixture of the above-mentioned amino acid methyl/ethyl ester hydrochlorides and anhydrous methanol/ethanol (100 mL), anhydrous baryta (20 mmol) was added, and the reaction mixture was stirred at room temperature overnight. After filtration and evaporation, the amino acid esters were obtained as oil or solid, which could be used directly for the next step without further purification.

### *5.3. Gerneral procedure for the preparation of compounds 6a–n*

A mixture of β-carboline-3-carboxylic acid (1 mmol), CDI (0.18 g, 1.1 mmol) and anhydrous DMF (15 mL) was stirred at room temperature until clear, and then L-amino acid ester (1.2 mmol) was added and stirred at room temperature for 3–8 h. After completion of the reaction as indicated by TLC, the resulting solution was poured into $H_2O$ (100 mL), and extracted with ethyl acetate (3 × 50 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/petroleum ether as the eluent to give compounds **6a**–**n** as white crystals.

#### *5.3.1. N-(9-Butyl-β-carboline-3-carbonyl)-L-alanine methyl ester (**6a**)*

Yield 91%, mp 110–112 °C; ESI-MS *m/z* 354 $[M + H]^+$; IR (KBr): 3402 (N − H), 3049, 2962, 2931, 2853, 1739 (C=O), 1666 (C=O), 1624, 1588, 1496, 1211, 748 cm$^{-1}$; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$: 8.89 (1H, s, ArH), 8.78 (1H, s, ArH), 8.59 (1H, d, $J = 7.8$ Hz, CONH), 8.20 (1H, d, $J = 7.8$ Hz), 7.62 (1H, t, $J = 7.2$ Hz, ArH), 7.49 (1H, d, $J = 8.4$ Hz, ArH), 7.34 (1H, t, $J = 7.5$ Hz, ArH), 4.90 (1H, m, CH), 4.42 (2H, t, $J = 7.2$ Hz, $NCH_2$), 3.80 (3H, s, $OCH_3$), 1.91 (2H, m, $CH_2$), 1.61 (3H, d, $J = 6.9$ Hz, $CHC\boldsymbol{H}_3$), 1.40 (2H, m, $CH_2$), 0.96 (3H, t, $J = 7.2$ Hz, $CH_3$); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 173.59 (C=O), 165.02 (C=O), 141.85, 139.76, 137.99, 131.74, 129.39, 128.44, 123.00, 121.34, 120.82, 114.70, 111.18, 52.80, 48.57, 43.38, 31.67, 20.53, 18.31, 14.45. Anal. Calc. for $C_{20}H_{23}N_3O_3$: C, 67.97; H, 6.56; N, 11.89. Found: C, 67.85; H, 6.74; N, 11.80.

### 5.3.2. *N-(9-Butyl-β-carboline-3-carbonyl)-L-valine methyl ester (**6b**)*

Yield 83%, mp 99–101 °C; ESI-MS *m/z* 382 $[M + H]^+$; IR (KBr): 3384 (N − H), 3035, 2968, 2931, 2863, 1735 (C=O), 1669(C=O), 1621, 1586, 1497, 1201, 749 $cm^{-1}$; $^1H$ NMR (300 M Hz, $CDCl_3$) δ: 8.87 (1H, s, ArH), 8.75 (1H, s, ArH), 8.59 (1H, d, *J* = 7.8 Hz, CONH), 8.19 (1H, d, *J* = 7.8 Hz), 7.63 (1H, t, *J* = 7.2 Hz, ArH), 7.51 (1H, d, *J* = 8.4 Hz, ArH), 7.36 (1H, t, *J* = 7.5 Hz, ArH), 4.85 (1H, m, CH), 4.40 (2H, t, *J* = 7.2 Hz, $NCH_2$), 3.78 (3H, s, $OCH_3$), 3.11 (1H, m, CH), 1.41 (2H, m, $CH_2$), 1.21 (6H, s, CH (C***H***$_3$)$_2$), 0.96 (3H, t, *J* = 7.2 Hz, $CH_3$); $^{13}C$ NMR (75 MHz, $CDCl_3$) δ: 171.61 (C=O), 162.57 (C=O), 143.43, 136.96, 134.24, 131.36, 130.83, 129.09, 123.66, 122.08, 120.77, 116.78, 111.91, 61.42, 59.42, 44.00, 31.74, 30.72, 20.43, 19.84, 19.58, 14.47. Anal. Calc. for $C_{22}H_{27}N_3O_3$: C, 69.27; H, 7.13; N, 11.02. Found: C, 69.17; H, 7.16; N, 10.95.

### 5.3.3. *N-(9-Butyl-β-carboline-3-carbonyl)-L-methionine ethyl ester (**6c**)*

Yield 95%, mp 100–102 °C; ESI-MS *m/z* 428 $[M + H]^+$; IR (KBr): 3372 (N − H), 3050, 2956, 2927, 2858, 1730 (C=O), 1674 (C=O), 1625, 1518, 1495, 1211, 745 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ: 8.91 (1H, s, ArH), 8.80 (1H, s, ArH), 8.70 (1H, d, *J* = 8.7 Hz, CONH), 8.21 (1H, d, *J* = 7.8 Hz, ArH), 7.64 (1H, t, *J* = 7.2 Hz, ArH), 7.51 (1H, d, *J* = 8.4 Hz, ArH), 7.35 (1H, t, *J* = 7.5 Hz, ArH), 5.00 (1H, m, CH), 4.43 (2H, t, *J* = 7.2 Hz, $NCH_2$), 4.27 (2H, q, $OCH_2$), 2.67 (2H, m, $SCH_2$), 2.41–2.17 (2H, m, CHC***H***$_2$), 2.14 (3H, s, $SCH_3$), 1.92 (2H, m, $CH_2$), 1.42 (2H, m, $CH_2$), 1.33 (3H, t, *J* = 7.2 Hz, $CH_3$), 0.97 (3H, t, *J* = 7.2 Hz, $CH_3$); $^{13}C$ NMR (75 MHz, $CDCl_3$) δ: 172.36 (C=O), 165.51 (C=O), 141.88, 139.72, 138.03, 131.83, 129.45, 128.45, 123.07, 121.34, 120.88, 114.81, 111.27, 61.56, 52.05, 43.42, 31.71, 31.44, 30.63, 20.56, 15.47, 14.94, 14.52. Anal. Calc. for $C_{23}H_{29}N_3O_3S$: C, 64.61; H, 6.84; N, 9.83. Found: C, 64.50; H, 6.95; N, 9.76.

### 5.3.4. *N-(9-Butyl-β-carboline-3-carbonyl)-L-phenylalanine methyl ester (**6d**)*

Yield 95%, mp 102–104 °C; ESI-MS *m/z* 431 $[M + H]^+$; IR (KBr): 3368 (N − H), 3031, 2955, 2882, 1737 (C=O), 1668 (C=O), 1624, 1588, 1511, 1491, 1203, 748, 700 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ: 8.86 (1H, s, ArH), 8.71 (1H, s, ArH), 8.60 (1H, d, *J* = 8.4 Hz, CONH), 8.16 (1H, d, *J* = 7.8 Hz, ArH), 7.59 (1H, t, *J* = 7.5 Hz, ArH), 7.45 (1H, d, *J* = 8.4 Hz, ArH), 7.30–7.22 (6H, m, 6ArH), 5.17 (1H, m, CH), 4.35 (2H, t, *J* = 7.2 Hz, $NCH_2$), 3.74 (3H, s, $OCH_3$), 3.30 (2H, m, CHC***H***$_2$), 1.88 (2H, m, $CH_2$), 1.38 (2H, m, $CH_2$), 0.94 (3H, t, *J* = 7.2 Hz, $CH_3$); $^{13}C$ NMR (75 MHz, $CDCl_3$) δ: 172.02 (C=O), 162.53 (C=O), 143.35, 137.92, 136.97, 134.25, 131.37, 130.78, 129.85, 129.35, 128.90, 127.21, 123.53, 122.10, 120.73, 116.29, 111.88, 55.25, 48.90, 43.95, 37.53, 31.76, 20.34, 14.48. Anal. Calc. for $C_{26}H_{27}N_3O_3$: C, 72.71; H, 6.34; N, 9.78. Found: C, 72.63; H, 6.40; N, 9.68.

### 5.3.5. *N-(9-Butyl-β-carboline-3-carbonyl)-L-phenylalanine ethyl ester (**6e**)*

Yield 92%, mp 107–108 °C; ESI-MS *m/z* 444 $[M + H]^+$; IR (KBr): 3370 (N − H), 3063, 3028, 2959, 2928, 2872, 1735 (C=O), 1665 (C=O), 1624, 1586, 1516, 1494, 1199, 750, 702 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ: 8.88 (1H, s, ArH), 8.76 (1H, s, ArH), 8.61 (1H, d, *J* = 8.1 Hz, CONH), 8.20 (1H, d, *J* = 7.8 Hz, ArH), 7.62 (1H, t, *J* = 7.8 Hz, ArH), 7.49 (1H, d, *J* = 8.1 Hz, ArH), 7.36–7.20 (6H, m, 6ArH), 5.13 (1H, q, CH), 4.42 (2H, t, *J* = 7.5 Hz, $NCH_2$), 4.19 (2H, q, $OCH_2$), 3.29 (2H, d, *J* = 6.3 Hz, CHC***H***$_2$Ph), 1.92 (2H, m, $CH_2$), 1.42 (2H, m, $CH_2$), 1.25 (3H, t, *J* = 7.2 Hz, $CH_3$), 0.98 (3H, t, *J* = 7.2 Hz, $CH_3$); $^{13}C$ NMR (75 MHz, $CDCl_3$) δ: 171.62 (C=O), 162.41 (C=O), 143.42, 137.83, 136.91, 134.17, 131.42, 130.75, 129.80, 129.26, 128.91, 127.24, 123.48, 122.13, 120.69, 116.37, 111.93, 61.63, 55.30, 44.01, 37.22, 31.73, 20.42, 14.85, 14.47. Anal. Calc. for $C_{27}H_{29}N_3O_3$: C, 73.11; H, 6.59; N, 9.47. Found: C, 73.28; H, 6.70; N, 9.40.

### 5.3.6. *N-(9-Butyl-β-carboline-3-carbonyl)-L-tyrosine methyl ester (**6f**)*

Yield 88%, mp 145–147 °C; ESI-MS *m/z* 447 $[M + H]^+$; IR (KBr): 3373 (N − H), 3240, 3059, 3030, 2958, 2930, 2872, 1723 (C=O), 1622 (C=O), 1589, 1515, 1216, 828, 750 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ: 8.86 (1H, s, ArH), 8.74 (1H, s, ArH), 8.65 (1H, d, *J* = 8.1 Hz, CONH), 8.17 (1H, d, *J* = 7.5 Hz, ArH), 7.61 (1H, t, *J* = 7.8 Hz, ArH), 7.48 (1H, d, *J* = 8.1 Hz, ArH), 7.32 (1H, t, *J* = 7.5 Hz, ArH), 7.07 (2H, d, *J* = 7.8 Hz, 2ArH), 6.74 (1H, d, *J* = 7.8 Hz, 2ArH), 6.17 (1H, s, OH), 5.11 (1H, q, CH), 4.39 (2H, t, *J* = 7.2 Hz, $NCH_2$), 3.74 (1H, s, $OCH_3$), 3.20 (2H, m, CHC***H***$_2$), 1.89 (2H, m, $CH_2$), 1.39 (2H, m, $CH_2$), 0.96 (3H, t, *J* = 7.2 Hz, $CH_3$); $^{13}C$ NMR (75 MHz, $CDCl_3$) δ: 172.30 (C=O), 162.73 (C=O), 156.73, 143.16, 137.04, 134.89, 131.14, 130.71, 130.37, 129.61, 127.67, 123.40, 121.96, 120.74, 116.03, 115.84, 111.82, 55.34, 52.91, 43.90, 36.49, 31.72, 20.45, 14.49. Anal. Calc. for $C_{26}H_{27}N_3O_4$: C, 70.09; H, 6.11; N, 9.43. Found: C, 69.94; H, 6.20; N, 9.37.

### 5.3.7. *N-(9-Benzyl-β-carboline-3-carbonyl)-L-methionine ethyl ester (**6g**)*

Yield 87%, mp 105–107 °C; ESI-MS *m/z* 462 $[M + H]^+$; IR (KBr): 3312 (N − H), 3058, 2971, 2918, 1741 (C=O), 1654 (C=O), 1586, 1521, 1458, 1208, 746, 696 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ: 8.92 (1H, s, ArH), 8.73 (1H, s, ArH), 8.63 (1H, d, *J* = 8.1 Hz, CONH), 8.23 (1H, d, *J* = 7.8 Hz, ArH), 7.61 (1H, t, *J* = 7.5 Hz, ArH), 7.49 (1H, d, *J* = 8.1 Hz, ArH), 7.38 (1H, t, *J* = 7.5 Hz, ArH), 7.29–7.09 (5H, m, 5ArH), 5.63 (2H, s, $NCH_2Ph$), 4.99 (1H, m, CH), 4.26 (2H, q, $OCH_2$), 2.63 (2H, m, $SCH_2$), 2.32–2.16 (2H, m, CHC***H***$_2$), 2.13 (3H, s, $SCH_3$), 1.32 (3H, t, *J* = 7.2 Hz, $CH_3$); $^{13}C$ NMR (75 MHz, $CDCl_3$) δ: 172.12 (C=O), 163.63 (C=O), 143.02, 137.57, 137.32, 136.55, 130.96, 130.46, 130.19, 129.36, 128.30, 127.49, 123.50, 122.04, 121.18, 116.16, 111.99, 61.65, 52.54, 47.26, 30.98, 30.66, 15.39, 14.95. Anal. Calc. for $C_{26}H_{27}N_3O_3S$: C, 67.65; H, 5.90; N, 9.10. Found: C, 67.47; H, 6.02; N, 9.05.

### 5.3.8. *N-(9-Benzyl-β-carboline-3-carbonyl)-L-phenylalanine ethyl ester (**6h**)*

Yield 96%, mp 147–149 °C; ESI-MS *m/z* 478 $[M + H]^+$; IR (KBr): 3383 (N − H), 3060, 3031, 2979, 2931, 2867, 1734 (C=O), 1665 (C=O), 1619, 1584, 1513, 1459, 1205, 734, 697 $cm^{-1}$;$^1H$ NMR (300 MHz, $CDCl_3$) δ: 8.91 (1H, s, ArH), 8.70 (1H, s, ArH), 8.58 (1H, d, *J* = 8.1 Hz, CONH), 8.23 (1H, d, *J* = 7.8 Hz, ArH), 7.60 (1H, t, *J* = 7.8 Hz, ArH), 7.48 (1H, d, *J* = 8.1 Hz, ArH), 7.36 (1H, t, *J* = 7.5 Hz, ArH), 7.29–7.13 (10H, m, 10ArH), 5.62 (2H, s, $NCH_2Ph$), 5.12 (1H, m, CH), 4.19 (2H, q, $OCH_2$), 3.28 (2H, d, *J* = 6.3 Hz, CHC***H***$_2$), 1.24 (3H, t, *J* = 7.2 Hz, $CH_3$); $^{13}C$ NMR (75 MHz, $CDCl_3$) δ: 171.94 (C=O), 165.03 (C=O), 142.01, 140.00, 138.14, 137.69, 132.08, 129.79, 129.62, 129.35, 128.94, 128.82, 128.21, 127.51, 127.24, 123.12, 121.54, 121.20, 114.78, 111.47, 61.57, 54.27, 47.00, 37.65, 14.87. Anal. Calc. for $C_{30}H_{27}N_3O_3$: C, 75.45; H, 5.70; N, 8.80. Found: C, 75.27; H, 5.82; N, 8.70.

### 5.3.9. *N-(9-Phenylpropyl-β-carboline-3-carbonyl)-L-alanine methyl ester (**6i**)*

Yield 93%, mp 141–143 °C; ESI-MS *m/z* 416 $[M + H]^+$; IR (KBr): 3315 (N − H), 3054, 2944, 2853, 1748 (C=O), 1652 (C=O), 1588, 1518, 1459, 1215, 749, 699 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ: 8.91 (1H, s, ArH), 8.69 (1H, s, ArH), 8.63 (H, d, *J* = 7.2 Hz, CONH), 8.22 (1H, d, *J* = 7.8 Hz, ArH), 7.62 (1H, t, *J* = 7.8 Hz, ArH), 7.42–7.15 (7H, m, 7ArH), 4.89 (1H, m, CH), 4.44 (2H, t, *J* = 7.2 Hz, $NCH_2$), 3.81 (3H, s, $OCH_3$), 2.74 (2H, t, *J* = 7.5 Hz, $CH_2Ph$), 2.29 (2H, m, $CH_2$), 1.62 (3H, d, *J* = 7.2 Hz, CHC***H***$_3$); $^{13}C$ NMR (75 MHz, $CDCl_3$) δ: 173.60 (C=O), 165.01 (C═O), 141.83, 141.67, 139.77, 137.91, 131.69, 129.50, 128.99, 128.75, 128.54, 126.56, 123.14, 121.37, 120.95, 114.80, 111.18, 52.84, 48.56, 43.35, 33.17, 31.19, 18.25. Anal. Calc. for $C_{25}H_{25}N_3O_3$: C, 72.27; H, 6.06; N, 10.11. Found: C, 72.10; H, 6.11; N, 10.02.

### 5.3.10. *N-(9-Phenylpropyl-β-carboline-3-carbonyl)-L-valine ethyl ester (**6j**)*

Yield 82%, mp 123–125 °C; ESI-MS *m/z* 459 $[M + H]^+$; IR (KBr): 3366 (N − H), 3061, 3027, 2960, 1730 (C═O), 1669 (C═O), 1588, 1518, 1463, 1193, 746, 699 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$: 8.90 (1H, s, ArH), 8.71 (1H, s, ArH), 8.63 (1H, d, $J = 8.4$ Hz, CONH), 8.20 (1H, d, $J = 7.8$ Hz, ArH), 7.60 (1H, t, $J = 7.8$ Hz, ArH), 7.41–7.15 (7H, m, 7ArH), 4.81 (1H, m, CH), 4.43 (2H, t, $J = 6.9$ Hz, $NCH_2$), 4.26 (2H, q, $OCH_2$), 2.74 (2H, t, $J = 7.2$ Hz, $CH_2Ph$), 2.40–2.26 (3H, m, $CH_2$, C***H*** $(CH_3)_2$), 1.33 (3H, t, $J = 6.9$ Hz, $CH_3$), 1.07 (6H, t, $J = 6.9$ Hz, $2CH_3$); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$: 171.99 (C═O), 165.05 (C═O), 141.87, 141.65, 139.49, 137.98, 131.91, 129.50, 128.94, 128.73, 128.61, 126.52, 123.13, 121.37, 120.96, 114.73, 111.15, 61.53, 57.93, 43.45, 33.23, 31.43, 31.20, 19.82, 18.85, 14.97. Anal. Calc. for $C_{28}H_{31}N_3O_3$: C, 73.50; H, 6.83; N, 9.18. Found: C, 73.28; H, 6.99; N, 9.20.

### 5.3.11. *N-(9-Phenylpropyl-β-carboline-3-carbonyl)-L-methionine ethyl ester (**6k**)*

Yield 84%, mp 113–115 °C; ESI-MS *m/z* 491 $[M + H]^+$; IR (KBr): 3323 (N–H), 3055, 2974, 2918, 2855, 1743 (C═O), 1653 (C═O), 1588, 1517, 1500, 1214, 749, 699 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$: 8.91 (1H, s, ArH), 8.74–870 (2H, ArH, CONH), 8.21 (1H, d, $J = 7.8$ Hz, ArH), 7.62 (1H, t, $J = 7.5$ Hz, ArH), 7.43–7.15 (7H, m, 7ArH), 4.98 (1H, m, CH), 4.45 (2H, t, $J = 7.2$ Hz, $NCH_2$), 4.27 (2H, q, $OCH_2$), 2.75 (2H, t, $CH_2Ph$), 2.67 (2H, m, CHC***H***$_2$), 2.40–2.18 (4H, m, $SCH_2$, $CH_2$), 2.14 (3H, s, $SCH_3$), 1.34 (3H, t, $J = 7.2$ Hz, $CH_3$); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$: 172.19 (C═O), 165.52 (C═O), 141.67, 140.52, 139.59, 137.88, 130.28, 129.14, 128.95, 128.80, 128.43, 126.56, 122.47, 121.77, 120.72, 114.78, 109.95, 61.87, 52.08, 43.30, 33.51, 32.88, 30.64, 30.58, 15.92, 14.63. Anal. Calc. for $C_{28}H_{31}N_3O_3S$: C, 68.68; H, 6.38; N, 8.58. Found: C, 68.81; H, 6.40; N, 8.47.

### 5.3.12. *N-(9-Phenylpropyl-β-carboline-3-carbonyl)-L-phenylalanine ethyl ester (**6l**)*

Yield 89%, mp 109–111 °C; ESI-MS *m/z* 507 $[M + H]^+$; IR (KBr): 3381 (N − H), 3034, 2935, 2862, 1741 (C═O), 1661 (C═O), 1590, 1509, 1459, 1197, 746, 700 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$: 8.88 (1H, s, ArH), 8.66 (1H, s, ArH), 8.61 (1H, d, $J = 8.1$ Hz, CONH), 8.19 (1H, d, $J = 7.8$ Hz, ArH), 7.61 (1H, t, $J = 7.8$ Hz, ArH), 7.41–7.15 (12H, m, 12ArH), 5.14 (1H, m, CH), 4.41 (2H, t, $J = 7.2$ Hz, $NCH_2$), 4.20 (2H, q, $OCH_2$), 3.29 (2H, d, $J = 6.0$ Hz, CHC***H***$_2$), 2.73 (2H, t, $J = 7.5$ Hz, $CH_2Ph$), 2.28 (2H, m, $CH_2$), 1.25 (3H, t, $J = 6.9$ Hz, $CH_3$); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$: 171.86 (C═O), 165.29 (C═O), 141.61, 140.56, 139.65, 137.82, 136.60, 130.34, 129.65, 129.04, 128.91, 128.80, 128.69, 128.45, 127.14, 126.56, 122.43, 121.76, 120.68, 114.66, 109.94, 61.69, 53.96, 43.26, 38.97, 33.51, 30.65, 14.59. Anal. Calc. for $C_{32}H_{31}N_3O_3$: C, 76.02; H, 6.18; N, 8.31. Found: C, 75.90; H, 6.28; N, 8.28.

### 5.3.13. *N-(1-Methyl-9-butyl-β-carboline-3-carbonyl)-L-methionine ethyl ester (**6m**)*

Yield 91%, mp 104–105 °C; ESI-MS *m/z* 442 $[M + H]^+$; IR (KBr): 3363 (N − H), 3054, 2957, 2915, 2856, 1727 (C═O), 1675 (C═O), 1620, 1560, 1514, 1211, 745 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$: 8.75–8.73 (2H, ArH, CONH), 8.17 (1H, d, $J = 8.1$ Hz, ArH), 7.60 (1H, t, $J = 8.1$ Hz, ArH), 7.48 (1H, d, $J = 8.4$ Hz, ArH), 7.32 (1H, t, $J = 7.8$ Hz, ArH), 4.98 (1H, m, CH), 4.58 (2H, t, $J = 7.8$ Hz, $NCH_2$), 4.27 (2H, q, $OCH_2$), 3.08 (3H, s, $ArCH_3$), 2.65 (2H, m, $SCH_2$), 2.36–2.18 (2H, m, CHC***H***$_2$), 2.14 (3H, s, $SCH_3$), 1.86 (2H, m, $CH_2$), 1.47 (2H, m, $CH_2$), 1.34 (3H, t, $J = 7.2$ Hz, $CH_3$), 1.01(3H, t, $J = 7.2$ Hz, $CH_3$); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$: 172.00 (C═O), 161.59 (C═O), 143.86, 141.08, 134.66, 132.31, 131.64, 131.03, 123.06, 122.40, 120.19, 115.76, 111.80, 61.66, 53.18, 45.38, 33.35, 30.81, 30.54, 20.25, 19.42, 15.34, 14.98, 14.42. Anal. Calc. for $C_{24}H_{31}N_3O_3S$: C, 65.28; H, 7.08; N, 9.52. Found: C, 65.38; H, 7.04; N, 9.43.

### 5.3.14. *N-(1-Methyl-9-butyl-β-carboline-3-carbonyl)-L-phenylalanine ethyl ester (**6n**)*

Yield 92%, mp 110–111 °C; ESI-MS *m/z* 459 $[M + H]^+$; IR (KBr): 3379 (N − H), 3060, 2952, 2910, 2870, 1740 (C═O), 1655 (C═O), 1621, 1581, 1515, 1192, 750 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$: 8.64 (s, 1H, ArH), 8.58 (1H, d, $J = 8.1$ Hz, CONH), 8.06 (1H, d, $J = 7.8$ Hz, ArH), 7.49 (1H, t, $J = 7.8$ Hz, ArH), 7.36 (1H, d, $J = 8.4$ Hz, ArH), 7.23–7.14 (6H, m, 6ArH), 5.02 (1H, q, CH), 4.44 (2H, t, $J = 7.8$ Hz, $NCH_2$), 4.10 (2H, q, $OCH_2$), 3.19 (2H, m, CHC***H***$_2$), 2.93 (3H, s, $ArCH_3$), 1.72 (2H, m, $CH_2$), 1.36 (2H, m, $CH_2$), 1.15 (3H, t, $J = 7.2$ Hz, $CH_3$), 0.90 (3H, t, $J = 7.2$ Hz, $CH_3$).; $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$: 171.94 (C═O), 164.86 (C═O), 142.10, 140.65, 138.51, 137.48, 136.38, 129.85, 129.24, 129.16, 128.98, 127.35, 122.45, 121.41, 120.87, 112.93, 111.26, 61.57, 54.01, 44.82, 37.75, 33.38, 24.09, 20.34, 14.86, 14.47. Anal. Calc. for $C_{28}H_{31}N_3O_3$: C, 73.50; H, 6.83; N, 9.18. Found: C, 73.41; H, 6.99; N, 9.10.

## 5.4. *General procedure for the preparation of compounds **7a**–**h***

A mixture of the corresponding β-carboline-3-carbonyl-L-amino acid ester **6** (1 mmol), NaOH (5 mmol), ethanol (10 mL) and $H_2O$ (10 mL) was stirred for 1 h at refluxing, and then the ethanol was removed on the rotary evaporator. The mixture was neutralized to pH 5 with 5M hydrochloride and cooled. The precipitate was collected, washed well with $H_2O$ and dried to give compounds **7a**–**h** as yellow solid. The solid was further crystallized from ethanol.

### 5.4.1. *N-(9-Butyl-β-carboline-3-carbonyl)-L-methionine (**7a**)*

Yield 95%, mp 195–196 °C; ESI-MS *m/z* 420 $[M - H]^-$; IR (KBr): 33 375 (N − H), 3030, 2966, 2937, 2868, 1733 (C═O), 1670 (C═O), 1624, 1510, 1496, 1201, 745 $cm^{-1}$; $^1H$ NMR (300 MHz, DMSO-$d_6$) $\delta$: 9.07 (1H, s, ArH), 8.84 (1H, s, ArH), 8.79 (1H, d, $J = 8.4$ Hz, CONH), 8.41 (1H, d, $J = 7.8$ Hz, ArH), 7.77 (1H, d, $J = 8.4$ Hz, ArH), 7.65 (1H, t, $J = 8.4$ Hz, ArH), 7.33 (1H, t, $J = 7.5$ Hz, ArH), 4.67 (1H, q, CH), 4.58 (2H, t, $J = 6.6$ Hz, $NCH_2$), 2.55 (2H, m, CHC***H***$_2$), 2.16 (2H, q, $SCH_2$), 2.05 (3H, s, $SCH_3$), 1.82 (2H, m, $CH_2$), 1.27 (2H, m, $CH_2$), 0.87 (3H, t, $J = 7.2$ Hz, $CH_3$); $^{13}C$ NMR (75 MHz, DMSO-$d_6$) $\delta$: 173.87 (C═O), 165.32 (C═O), 141.86, 139.80, 137.99, 131.79, 129.42, 128.45, 123.05, 121.34, 120.86, 114.70, 111.23, 51.95, 43.41, 31.70, 30.69, 20.56, 15.48, 14.50. Anal. Calc. for $C_{21}H_{25}N_3O_3S$: C, 63.13; H, 6.31; N, 10.52. Found: C, 63.00; H, 6.47; N, 10.45.

### 5.4.2. *N-(9-Butyl-β-carboline-3-carbonyl)-L-phenylalanine (**7b**)*

Yield 94%, mp 190–192 °C; ESI-MS *m/z* 436 $[M - H]^-$; IR (KBr): 3346, 3030, 2927, 2853, 1734 (C═O), 1636 (C═O), 1590, 1521, 1500, 1201, 742, 698 $cm^{-1}$; $^1H$ NMR (300 MHz, DMSO-$d_6$) $\delta$: 9.03 (1H, s, ArH), 8.80 (1H, s, ArH), 8.63 (1H, d, $J = 8.1$ Hz, CONH), 8.39 (1H, d, $J = 7.5$ Hz, ArH), 7.75 (1H, d, $J = 8.4$ Hz, ArH), 7.63 (1H, t, $J = 7.5$ Hz, ArH), 7.31 (1H, t, $J = 7.5$ Hz, ArH), 7.26–7.14 (5H, m, 5ArH), 4.82 (1H, q, CH), 4.55 (2H, t, $J = 6.9$ Hz, $NCH_2$), 3.25 (2H, m, CHC***H***$_2$), 1.80 (2H, m, $CH_2$), 1.26 (2H, m, $CH_2$), 0.86 (3H, t, $J = 7.2$ Hz, $CH_3$); $^{13}C$ NMR (75 MHz, DMSO-$d_6$) $\delta$: 173.24 (C═O), 162.87 (C═O), 143.27, 138.01, 136.78, 135.37, 131.43, 130.82, 129.76, 128.98, 128.93, 127.03, 122.76, 122.59, 120.45, 115.28, 111.86, 55.35, 44.05, 37.50, 31.65, 20.22, 14.52. Anal. Calc. for $C_{25}H_{25}N_3O_3$: C, 72.27; H, 6.06; N, 10.11. Found: C, 72.05; H, 6.24; N, 10.10.

### 5.4.3. *N-(9-Butyl-β-carboline-3-carbonyl)-L-tyrosine (**7c**)*

Yield 94%, mp 183–185 °C; ESI-MS *m/z* 430 $[M - H]^-$; IR (KBr): 3373 (N − H), 3240, 3059, 3030, 2958, 2930, 2872, 1723 (C═O), 1622 (C=O), 1589, 1515, 1216, 828, 750 $cm^{-1}$; $^{1}H$ NMR (300 MHz, DMSO-$d_6$) $\delta$: 9.06 (1H, s, ArH), 8.84 (1H, s, ArH), 8.61 (1H, d, $J = 8.1$ Hz, CONH), 8.41 (1H, d, $J = 7.8$ Hz, ArH), 7.77 (1H, d, $J = 8.1$ Hz, ArH), 7.65 (1H, t, $J = 7.8$ Hz, ArH), 7.33 (1H, t, $J = 7.5$ Hz, ArH), 7.01 (2H, d, $J = 7.8$ Hz, 2ArH), 6.62 (1H, d, $J = 7.8$ Hz, 2ArH), 4.73 (1H, q, CH), 4.57 (2H, t, $J = 6.9$ Hz, $NCH_2$), 3.11 (2H, m, CHC***H***$_2$), 1.81 (2H, m, $CH_2$), 1.28 (2H, m, $CH_2$), 0.87 (3H, t, $J = 7.2$ Hz, $CH_3$); $^{13}C$ NMR (75 MHz, DMSO-$d_6$) $\delta$: 173.46 (C=O), 164.37 (C=O), 156.66, 142.12, 138.67, 137.73, 131.34, 130.78, 129.78, 128.83, 127.81, 123.05, 121.16, 121.09, 115.82, 114.76, 111.28, 54.44, 43.51, 36.83, 31.68, 20.53, 14.46. Anal. Calc. for $C_{25}H_{25}N_3O_4$: C, 69.59; H, 5.84; N, 9.74. Found: C, 69.50; H, 6.03; N, 9.63.

#### 5.4.4. *N-(9-Benzyl-β-carboline-3-carbonyl)-L-methionine (**7d**)*

Yield 96%, mp 233–234 °C; ESI-MS *m/z* 454 [M − H]$^-$; IR (KBr): 3428, 3037, 2925, 1732 (C=O), 1634 (C=O), 1530, 1501, 1459, 1181, 748 $cm^{-1}$; $^{1}H$ NMR (300 MHz, DMSO-$d_6$) $\delta$: 9.10 (1H, s, ArH), 8.85 (1H, s, ArH), 8.79 (1H, d, $J = 8.1$ Hz, CONH), 8.43 (1H, d, $J = 7.5$ Hz, ArH), 7.78 (1H, d, $J = 7.5$ Hz, ArH), 7.62 (1H, t, $J = 7.5$ Hz, ArH), 7.33 (1H, t, $J = 7.5$ Hz, ArH), 7.27–7.19 (5H, m, 5ArH), 5.84 (2H, s, $NCH_2Ph$), 4.65 (1H, q, CH), 2.52 (2H, m, CHC***H***$_2$), 2.14 (2H, q, $SCH_2$), 2.04 (3H, s, $SCH_3$); $^{13}C$ NMR (75 MHz, DMSO-$d_6$) $\delta$: 173.83 (C=O), 165.27 (C=O), 142.00, 140.24, 138.12, 137.70, 132.03, 129.63, 129.37, 128.85, 128.23, 127.53, 123.16, 121.57, 121.21, 114.79, 111.50, 51.97, 46.99, 31.68, 30.70, 15.47. Anal. Calc. for $C_{24}H_{23}N_3O_3S$: C, 66.49; H, 5.35; N, 9.69. Found: C, 66.25; H, 5.55; N, 9.71.

#### 5.4.5. *N-(9-Phenylpropyl-β-carboline-3-carbonyl)-L-alanine (**7e**)*

Yield 94%, mp 178–179 °C; ESI-MS *m/z* 423 [M − H]$^-$; IR (KBr): 3383 (N − H), 3028, 2932, 1733 (C=O), 1627 (C=O), 1528, 1505,1457, 1213, 745 $cm^{-1}$; $^{1}H$ NMR (300 MHz, DMSO-$d_6$) $\delta$: 12.80 (1H, s, COOH), 9.02 (1H, s, ArH), 8.84 (1H, s, ArH), 8.75 (1H, d, $J = 7.5$ Hz, CONH), 8.42 (1H, d, $J = 7.8$ Hz, ArH), 7.72 (1H, d, $J = 8.1$ Hz, ArH), 7.64 (1H, t, $J = 8.1$ Hz, 0.9 Hz, ArH), 7.32 (1H, t, $J = 7.5$ Hz, ArH), 7.26–7.14 (4H, m, 4ArH), 4.61 (2H, t, $J = 6.9$ Hz, $NCH_2$), 4.54 (1H, m, CH), 2.65 (2H, t, $J = 7.8$ Hz, $CH_2Ph$), 2.14 (2H, m, $CH_2$), 1.46 (3H, d, $J = 7.2$ Hz, CHC***H***$_3$); $^{13}C$ NMR (75 MHz, DMSO-$d_6$) $\delta$: 176.63 (C=O), 164.12 (C=O), 141.66, 141.45, 140.83, 137.61, 131.53, 129.26, 128.81, 128.57, 128.46, 126.41, 122.76, 121.33, 120.74, 114.06, 110.86, 50.98, 43.20, 33.10, 31.05, 20.52. Anal. Calc. for $C_{24}H_{23}N_3O_3$: C, 71.80; H, 5.77; N, 10.47. Found: C, 71.73; H, 5.89; N, 10.40.

#### 5.4.6. *N-(9-Phenylpropyl-β-carboline-3-carbonyl)-L-valine (**7f**)*

Yield 96%, mp 93–95 °C; ESI-MS *m/z* 429 [M − H]$^-$; IR (KBr): 3379 (N − H), 3029, 2962, 2883, 1730 (C=O), 1628 (C=O), 1523, 1510, 1460, 1208, 745 $cm^{-1}$; $^{1}H$ NMR (300 MHz, DMSO-$d_6$) $\delta$: 9.02 (1H, s, ArH), 8.81 (2H, ArH, CONH), 8.23 (1H, d, $J = 7.5$ Hz, ArH), 7.64 (1H, t, $J = 7.8$ Hz, ArH), 7.41–7.13 (7H, m, 7ArH), 4.72 (1H, m, CH), 4.46 (2H, t, $J = 6.9$ Hz, $NCH_2$), 2.76 (2H, t, $J = 7.2$ Hz, $CH_2Ph$), 2.31–2.26 (3H, m, $CH_2$, C***H***$(CH_3)_2$), 1.14 (6H, t, $J = 6.3$ Hz, $2CH_3$); $^{13}C$ NMR (75 MHz, DMSO-$d_6$) $\delta$: 173.41 (C=O), 164.49 (C=O), 142.19, 141.66, 138.69, 137.80, 131.45, 129.88, 129.05, 128.95, 128.74, 126.53, 123.24, 121.29, 121.20, 114.95, 111.32, 57.94, 43.56, 33.20, 31.35, 31.20, 20.01, 18.73. Anal. Calc. for $C_{26}H_{27}N_3O_3$: C, 72.71; H, 6.34; N, 9.78. Found: C, 72.60; H, 6.50; N, 9.74.

#### 5.4.7. *N-(9-Phenylpropyl-β-carboline-3-carbonyl)-L-methionine (**7g**)*

Yield 96%, mp 192–194 °C; ESI-MS *m/z* 483 [M − H]$^-$; IR (KBr): 3430, 3376 (N − H), 3027, 2926, 1732 (C=O), 1635 (C=O), 1534, 1502, 1460, 1170, 747 $cm^{-1}$; $^{1}H$ NMR (300 MHz, DMSO-$d_6$) $\delta$: 12.88 (1H, s, COOH), 9.02 (1H, d, $J = 0.9$ Hz, ArH), 8.84 (H, d, $J = 0.6$ Hz, ArH), 8.81 (1H, d, $J = 8.1$ Hz, CONH), 8.42 (1H, d, $J = 7.8$ Hz, ArH), 7.73 (1H, d, $J = 8.1$ Hz, ArH), 7.64 (1H, dt, $J = 7.2$ Hz, 0.9 Hz, ArH), 7.33 (1H, dt, $J = 7.8$ Hz, 0.6 Hz, ArH), 7.26–7.14 (4H, m, 4ArH), 4.67 (1H, m, CH), 4.61 (2H, t, $J = 7.2$ Hz, $NCH_2$), 2.65 (2H, t, $J = 7.8$ Hz, $CH_2Ph$), 2.53 (2H, m, CHC***H***$_2$), 2.20–2.11 (4H, m, $SCH_2$, $CH_2$), 2.05 (3H, s, $SCH_3$); $^{13}C$ NMR (75 MHz, DMSO-$d_6$) $\delta$: 173.84 (C=O), 165.31 (C=O), 141.83, 141.64, 139.89, 137.91, 131.69, 129.45, 128.95, 128.73, 128.55, 126.53, 123.08, 121.39, 120.91, 114.73, 111.13, 51.97, 43.37, 33.20, 31.74, 31.18, 30.70, 15.49. Anal. Calc. for $C_{26}H_{27}N_3O_3S$: C, 67.65; H, 5.90; N, 9.10. Found: C, 67.77; H, 5.88; N, 9.11.

#### 5.4.8. *N-(9-Phenylpropyl-β-carboline-3-carbonyl)-L-phenylalanine (**7h**)*

Yield 97%, mp 179–180 °C; ESI-MS *m/z* 477 [M − H]$^-$; IR (KBr): 3376 (N − H), 3027, 2929, 2860, 1735 (C=O), 1626 (C=O), 1591, 1526, 1500, 1210, 746, 699 $cm^{-1}$; $^{1}H$ NMR (300 MHz, DMSO-$d_6$) $\delta$: 9.02 (1H, s, ArH), 8.86 (1H, s, ArH), 8.73 (1H, d, $J = 8.1$ Hz, CONH), 8.40 (1H, d, $J = 7.8$ Hz, ArH), 7.72 (1H, d, $J = 8.1$ Hz, ArH), 7.65 (1H, t, $J = 7.5$ Hz, ArH), 7.33 (1H, t, $J = 7.5$ Hz, ArH), 7.25–7.14 (10H, m, 10ArH), 4.90 (1H, m, CH), 4.60 (2H, t, $J = 6.9$ Hz, $NCH_2$), 3.24 (2H, m, CHC***H***$_2$), 2.65 (2H, t, $J = 7.8$ Hz, $CH_2Ph$), 2.13 (2H, m, $CH_2$); $^{13}C$ NMR (75 MHz, DMSO-$d_6$) $\delta$: 173.29 (C=O), 164.36 (C=O), 142.13, 141.59, 138.63, 138.00, 137.68, 131.20, 129.83, 129.03, 128.89, 128.70, 127.16, 126.49, 123.11, 121.24, 121.16, 114.91, 111.24, 54.26, 43.49, 37.52, 33.15, 31.12. Anal. Calc. for $C_{30}H_{27}N_3O_3$: C, 75.45; H, 5.70; N, 8.80. Found: C, 75.31; H, 5.85; N, 8.72.

### 5.5. *General procedure for the preparation of compounds 8a–h*

A mixture of *N*-(β-carboline-3-carbonyl)-L-amino acid esters (2 mmol) and benzyl bromide (20 mmol) in ethyl acetate (10 mL) was refluxed for 24–36 h. The reaction mixture was monitored by TLC and then cooled at 0 °C. The yellow solid was filtered under reduced pressure and washed well with ethyl acetate, and then recrystallized from ethanol, dried in vacuum to give yellow crystals **8a**–**h**.

#### 5.5.1. *2-Benzyl-9-butyl-3-(carbonyl-valine ethyl ester)-β-carbolinium bromate (**8a**)*

Yield 39%, mp 174–175 °C; ESI-MS *m/z* 487 [M − Br]$^+$; IR (KBr): 3429 (N − H), 3173, 2963, 2934, 2874, 1739 ($CO_2Et$), 1668 (CONH), 1635, 1536, 1513, 1461, 756, 714 $cm^{-1}$; $^{1}H$ NMR (300 MHz, DMSO-$d_6$) $\delta$: 10.00 (1H, s, ArH), 9.53 (1H, d, $J = 7.5$ Hz, CONH), 8.94 (1H, s, ArH), 8.64 (1H, d, $J = 7.8$ Hz, ArH), 8.03 (1H, d, $J = 8.4$ Hz, ArH), 7.91 (1H, t, $J = 8.1$ Hz, ArH), 7.54 (1H, t, $J = 7.5$ Hz, ArH), 7.56–7.29 (5H, m, 5ArH), 6.13 (2H, q, $N^+CH_2Ph$), 4.69 (2H, t, $J = 6.9$ Hz, $NCH_2$), 4.31 (1H, t, $J = 7.2$ Hz, CH), 4.16 (2H, m, $OCH_2$), 2.11 (1H, m, ***CH***Me$_2$), 1.84 (2H, m, $CH_2$), 1.27 (2H, m, $CH_2$), 1.22 (3H, t, $J = 7.2$ Hz, $CH_3$), 0.91–0.83 (9H, m, $CH_3$, $CH(CH_3)_2$); $^{13}C$ NMR (75 MHz, DMSO-$d_6$) $\delta$: 171.04 (C=O), 162.74 (C=O), 145.31, 136.82, 135.89, 135.33, 133.46, 132.07, 131.55, 129.48, 128.49, 124.88, 123.12, 119.96, 119.62, 112.53, 61.79, 61.65, 59.42, 44.52, 31.64, 30.52, 20.45, 19.11, 14.97, 14.49. Anal. Calc. for $C_{30}H_{36}BrN_3O_3$: C, 63.60; H, 6.40; N, 7.42. Found: C, 63.65; H, 6.51; N, 7.37.

#### 5.5.2. *2-Benzyl-9-butyl-3-(carbonyl-methionine ethyl ester)-β-carbolinium bromate (**8b**)*

Yield 46%, mp 138–140 °C; ESI-MS *m/z* 519 [M − Br]$^+$; IR (KBr): 3428 (N − H), 3160, 2593, 2932, 2870, 1739 ($CO_2Et$), 1671 (CONH), 1633, 1577, 1513, 1458, 1215, 754, 714 $cm^{-1}$; $^{1}H$ NMR (300 MHz, DMSO-$d_6$) $\delta$: 9.96 (1H, s, ArH), 9.44 (1H, d, $J = 7.5$ Hz, CONH), 9.17 (1H, s, ArH), 8.42 (1H, s, ArH), 8.05 (1H, d, $J = 8.1$ Hz, ArH), 7.57 (1H, t, $J = 7.8$ Hz, ArH), 7.32–7.05 (6H, m, 6ArH), 6.12 (2H, q, $N^+CH_2Ph$), 4.64 (1H, q, CH), 4.24 (2H, t, $J = 6.9$ Hz, $NCH_2$), 4.02 (2H, q, $OCH_2$), 2.10 (2H, m, $CH_2$), 1.55 (4H, m, $2CH_2$), 1.06 (3H, t, $J = 7.2$ Hz, $CH_3$), 0.62 (3H, t, $J = 6.9$ Hz, $CH_3$); $^{13}C$ NMR (75 MHz, DMSO-$d_6$) $\delta$: 171.27 (C=O), 162.57 (C=O), 145.28, 136.32, 135.88, 135.42, 133.47, 132.03, 131.93, 129.04, 128.59, 127.49, 124.78, 123.18, 119.96, 119.63, 112.59, 61.96, 61.68, 52.93, 44.61, 35.70, 31.69, 31.01, 28.03, 20.45, 14.89, 14.51. Anal. Calc. for $C_{30}H_{36}BrN_3O_3S$: C, 60.19; H, 6.06; N, 7.02. Found: C, 60.00; H, 6.27; N, 6.95.

### 5.5.3. *2-Benzyl-9-butyl-3-(carbonyl-phenylalanine ethyl ester)-β-carbolinium bromate (**8c**)*

Yield 62%, mp 155–157 °C; ESI-MS *m/z* 535 [M − Br]$^+$; IR (KBr) 3427(N − H), 3169, 3060, 2960, 1741 ($CO_2Et$), 1670 (CONH), 1634, 1513, 1458, 1216, 753, 714 $cm^{-1}$; $^1H$ NMR (300 MHz, DMSO-$d_6$) δ: 10.07 (1H, s, ArH), 9.82 (1H, d, $J = 7.2$ Hz, CONH), 8.69 (1H, s, ArH), 8.53 (1H, d, $J = 7.8$ Hz, ArH), 8.03 (1H, d, $J = 8.7$ Hz, ArH), 7.91 (1H, t, $J = 7.2$ Hz, ArH), 7.55 (1H, t, $J = 7.2$ Hz, ArH), 7.31–7.27 (8H, m, 8ArH), 6.00 (2H, q, $N^+CH_2Ph$), 4.71 (3H, m, CH, $NCH_2$), 4.10 (2H, q, $OCH_2$), 3.17–2.94 (2H, m, $CH_{\mathbf{2}}Ph$), 1.80 (2H, m, $CH_2$), 1.25 (2H, m, $CH_2$), 1.13 (3H, t, $J = 7.2$ Hz, $CH_3$), 0.85 (3H, t, $J = 7.2$ Hz, $CH_3$); $^{13}C$ NMR (75 MHz, DMSO-$d_6$) δ: 171.05 (C=O), 162.28 (C=O), 145.23, 137.32, 136.39, 135.35, 133.44, 131.80, 129.86, 129.43, 129.09, 128.73, 127.51, 124.54, 123.21, 119.86, 119.40, 112.59, 61.84, 61.48, 55.31, 44.63, 37.33, 31.72, 20.45, 14.82, 14.53. Anal. Calc. for $C_{34}H_{36}BrN_3O_3$: C, 66.45; H, 5.90; N, 6.84. Found: C, 66.46; H, 6.03; N, 6.70.

### 5.5.4. *2,9-Dibenzyl-3-(carbonyl-valine ethyl ester)-β-carbolinium bromate (**8d**)*

Yield 36%, mp 195–196 °C; ESI-MS *m/z* 521 [M − Br]$^+$; IR (KBr) 3429 (N − H), 3151, 3030, 2966, 2934, 1737 ($CO_2Et$), 1667 (CONH), 1632, 1536, 1512, 1202, 756, 712 $cm^{-1}$; $^1H$ NMR (300 MHz, DMSO-$d_6$) δ: 10.07 (1H, s, ArH), 9.56 (1H, d, $J = 6.3$ Hz, CONH), 8.98 (1H, s, ArH), 8.66 (1H, d, $J = 7.8$ Hz, ArH), 8.01 (1H, d, $J = 8.7$ Hz, ArH), 7.89 (1H, t, $J = 7.8$ Hz, ArH), 7.54 (1H, t, $J = 7.5$ Hz, ArH), 7.33–7.26 (10H, m, 10ArH), 6.10 (2H, q, $N^+CH_2Ph$), 5.96 (2H, s, $NCH_2Ph$), 4.34 (1H, t, $J = 6.3$ Hz, CH), 4.17 (2H, q, $OCH_2$), 2.14 (1H, m, ***CH***$Me_2$), 1.22 (3H, t, $J = 6.6$ Hz, $CH_3$), 0.92 (6H, d, $J = 6.0$ Hz, $CH(\boldsymbol{CH_3})_2$); $^{13}C$ NMR (75 MHz, DMSO-$d_6$) δ: 171.03 (C=O), 162.70 (C=O), 145.29, 137.49, 136.52, 136.16, 135.18, 133.67, 132.65, 131.35, 129.52, 129.47, 128.66, 127.71, 125.01, 123.40, 120.21, 119.66, 112.77, 61.85, 61.70, 59.45, 47.83, 30.53, 19.71, 19.07, 14.98. Anal. Calc. for $C_{33}H_{34}BrN_3O_3$: C, 66.00; H, 5.71; N, 7.00. Found: C, 65.71; H, 5.93; N, 7.08.

### 5.5.5. *2,9-Dibenzyl-3-(carbonyl-phenylalanine ethyl ester)-β-carbolinium bromate (**8e**)*

Yield 43%, mp 189–190 °C; ESI-MS *m/z* 569 [M − Br]$^+$; IR (KBr) 3427 (N − H), 3156, 3057, 2977, 1738 ($CO_2Et$), 1671 (CONH), 1632, 1541, 1514, 1456, 1220, 743, 700 $cm^{-1}$; $^1H$ NMR (300 MHz, DMSO-$d_6$) δ: 10.01 (1H, s, ArH), 9.83 (1H, d, $J = 7.5$ Hz, CONH), 8.72 (1H, s, ArH), 8.56 (1H, d, $J = 8.1$ Hz, ArH), 7.99 (1H, d, $J = 8.4$ Hz, ArH), 7.89 (1H, t, $J = 7.2$ Hz, ArH), 7.56 (1H, t, $J = 7.5$ Hz, ArH), 7.33–7.29 (5H, m, 5ArH), 7.25–7.22 (5H, m, 5ArH), 5.94 (4H, m, $N^+CH_2Ph$, $NCH_2Ph$), 4.75 (1H, m, CH), 4.11 (2H, q, $OCH_2$), 3.19–2.94 (2H, m, $CH_2Ph$), 1.13 (3H, t, $J = 7.2$ Hz, $CH_3$); $^{13}C$ NMR (75 MHz, DMSO-$d_6$) δ: 171.03 (C=O), 162.20 (C=O), 145.28, 137.22, 137.10, 136.40, 136.14, 135.11, 133.76, 132.51, 131.48, 129.84, 129.51, 129.13, 128.70, 127.70, 127.56, 124.66, 123.58, 120.10, 119.35, 112.81, 61.92, 61.80, 55.25, 47.86, 37.37,14.78. Anal. Calc. for $C_{37}H_{34}BrN_3O_3$: C, 68.52; H, 5.28; N, 6.48. Found: C, 68.28; H, 5.48; N, 6.22.

### 5.5.6. *2-Benzyl-3-(carbonyl-valine ethyl ester)-9-phenylpropyl-β-carbolinium bromate (**8f**)*

Yield 40%, mp 148–150 °C; ESI-MS *m/z* 549 [M − Br]$^+$; IR (KBr) 3428 (N − H), 3166, 2970, 1738 ($CO_2Et$), 1668 (CONH), 1634, 1514, 1460, 1198, 749 $cm^{-1}$; $^1H$ NMR (300 MHz, DMSO-$d_6$) δ: 10.04 (1H, s, ArH), 9.52 (1H, d, $J = 7.2$ Hz, NH), 8.92 (1H, s, ArH), 8.63 (1H, d, $J = 8.1$ Hz, ArH), 8.00 (1H, d, $J = 7.5$ Hz, ArH), 7.92 (1H, t, $J = 6.9$ Hz, ArH), 7.54 (1H, t, $J = 7.5$ Hz, ArH), 7.47–7.08 (10H, m, 10ArH), 6.15 (2H, q, $N^+CH_2Ph$), 4.77 (2H, t, $J = 6.0$ Hz, $NCH_2$), 4.32 (1H, t, $J = 6.9$ Hz, CH), 4.17 (2H, q, $OCH_2$), 2.64 (2H, t, $J = 7.5$ Hz, $CH_2Ph$), 2.20 (2H, m, $CH_2$), 2.10 (1H, m, ***CH***$Me_2$), 1.23 (3H, t, $J = 6.9$ Hz, $CH_3$), 0.91 (6H, d, $J = 6.3$ Hz, $CH(\boldsymbol{CH_3})_2$); $^{13}C$ NMR (75 MHz, DMSO-$d_6$) δ: 171.04 (C=O), 162.75 (C=O), 145.28, 141.42, 136.79, 135.88, 135.35, 133.42, 132.12, 131.65, 131.27, 129.46, 129.26, 128.88, 128.59, 126.53, 124.86, 123.11, 120.02, 119.58, 112.45, 61.65, 59.43, 44.60, 33.08, 31.02, 30.50, 19.69, 19.13, 14.97. Anal. Calc. for $C_{35}H_{38}BrN_3O_3$: C, 66.87; H, 6.09; N, 6.68. Found: C, 66.78; H, 6.23; N, 6.60.

### 5.5.7. *2-Benzyl-3-(carbonyl-methionine ethyl ester)-9-phenylpropyl-β-carbolinium bromate (**8g**)*

Yield 32%, mp 134–136 °C; ESI-MS *m/z* 581 [M − Br]$^+$; IR (KBr) 3428, 3161, 3027, 2980, 2940, 1739 (C=O), 1669 (C=O), 1632, 1578, 1514, 1456, 1340, 1213, 749, 707 $cm^{-1}$; $^1H$ NMR (300 MHz, DMSO-$d_6$) δ: 10.17 (1H, d, $J = 4.2$ Hz, ArH), 9.75 (1H, dd, $J = 28.8$, 7.2 Hz), 8.97 (1H, d, $J = 21.0$ Hz, ArH), 8.60 (1H, t, $J = 7.8$ Hz, ArH), 8.01 (1H, d, $J = 8.1$ Hz, ArH), 7.91 (1H, t, $J = 7.8$ Hz, ArH), 7.54 (1H, t, $J = 7.8$ Hz, ArH), 7.35–7.06 (10H, m, 10ArH), 6.20 (2H, m, $N^+CH_2Ph$), 4.79 (2H, t, $J = 6.6$ Hz, $NCH_2$), 4.57 (1H, q, CH), 4.16 (2H, q, $OCH_2$), 2.66 (2H, t, $J = 7.8$ Hz, $CH_2Ph$), 2.40–2.20 (4H, m, $2CH_2$), 1.98 (2H, m, $CH_2$), 1.21 (3H, t, $J = 7.2$ Hz, $CH_3$); $^{13}C$ NMR (75 MHz, DMSO-$d_6$) δ: 172.89 (C=O), 162.37 (C=O), 145.13, 141.47, 135.78, 135.51, 135.38, 133.33, 132.28, 131.93, 129.48, 129.27, 128.90, 128.78, 128.59, 126.43, 124.72, 123.11, 120.04, 119.69, 112.40, 61.61, 61.47, 53.00, 49.74, 44.72, 35.64, 33.02, 31.11, 28.39, 14.70. Anal. Calc. for $C_{35}H_{38}BrN_3O_3S$: C, 63.63; H, 5.80; N, 6.36. Found: C, 63.42; H, 5.90; N, 6.40.

### 5.5.8. *2-Benzyl-3-(carbonyl-phenylalanine ethyl ester)-9-phenylpropyl-β-carbolinium bromate (**8h**)*

Yield 40%, mp 188–189 °C; ESI-MS *m/z* 597 [M − Br]$^+$; IR (KBr) 3410, 3181, 3027, 2983, 2939, 2851, 1736 (C=O), 1670 (C=O), 1633, 1541, 1515, 1457, 1341, 1244, 748, 704 $cm^{-1}$; $^1H$ NMR (300 MHz, DMSO-$d_6$) δ: 9.98 (1H, s, ArH), 9.80 (1H, d, $J = 7.5$ Hz, CONH), 8.65 (1H, s, ArH), 8.52 (1H, d, $J = 8.1$ Hz, ArH), 8.01 (1H, d, $J = 8.1$ Hz, ArH), 7.92 (1H, t, $J = 7.5$ Hz, ArH), 7.56 (1H, t, $J = 7.5$ Hz, ArH), 7.33–7.04 (15H, m, 15ArH), 6.00 (2H, m, $N^+CH_2Ph$), 4.75 (3H, m, CH, $NCH_2$), 4.11 (2H, q, $OCH_2$), 3.05 (2H, m, $CHC\boldsymbol{H_2}$), 2.63 (2H, t, $J = 7.5$ Hz, $CH_2Ph$), 2.19 (2H, m, $CH_2$), 1.13 (3H, t, $J = 6.6$ Hz, $CH_3$); $^{13}C$ NMR (75 MHz, DMSO-$d_6$) δ: 171.06 (C=O), 162.31 (C=O), 145.22, 141.46, 137.37, 136.39, 135.86, 135.37, 133.42, 131.92, 131.82, 129.86, 129.44, 129.08, 128.80, 128.59, 127.50, 126.47, 124.51, 123.24, 119.97, 119.38, 112.51, 61.86, 61.47, 55.32, 44.71, 37.32, 33.03, 31.05, 14.81. Anal. Calc. for $C_{39}H_{38}BrN_3O_3$: C, 69.23; H, 5.66; N, 6.21. Found: C, 69.22; H, 5.65; N, 6.03.

## 5.6. *Cytotoxicity assays in vitro*

Cytotoxicity assays *in vitro* were carried out using 96 microtitre plate cultures and MTT staining according to the procedures described in our previous report [11]. Briefly, 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 renal carcinoma (769-P), gastric carcinoma (BGC-823), epidermoid carcinoma of the nasopharynx (KB), renal carcinoma (786-0), melanoma (A375). In all of these experiments, three replicate wells were used to determine each point.

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