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# A new class of anti-thrombosis hexahydropyrazino-[1′,2′:1,6]pyrido-[3,4-*b*]-indole-1,4-dions: Design, synthesis, log *K* determination, and QSAR analysis

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**Abstract**—Based on the fact that the cyclization of *N*-[(3*S*)]-1,2,3,4-tetrahydro-β-carboline-3-carboxyl]-L-lysine in both of acetic acid aqueous (5%) and rat plasma gave the same product and the hypothesis that the cyclization product is antithrombotic active, we report the synthesis, in vitro anti-aggregation, and in vivo anti-thrombosis activity of 20 hexahydropyrazino[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4-dions (**5a**–**t**) as potential anti-thrombosis agents in this study. Two intermediates (tetrahydro-β-carboline-3-carboxy-L-amino acid benzylesters, 2-aminoacyltetrahydro-β-carboline-3-carboxylic acid benzylesters) were prepared and used for the cyclization to form **5a**–**t**. Coupling hydrochloric acid salt of tetrahydro-β-carboline-3-carboxylic acid esters and Boc-amino acids in the reported literature usually generates very low yield products accompanied by racemization. However, in our case, the free base of tetrahydro-β-carboline-3-carboxylic acid benzylester produced the desired products in high yields and without racemization. The anti-thrombosis results from both in vitro and in vivo studies revealed that **5a**–**t** may be a new class of anti-thrombosis agents with potent effective concentration at 0.5 μmol/kg with oral administration. Moreover, a QSAR analysis was performed on these 20 compounds by using molecular descriptors generated by e-dragon server. Although the activities of these compounds are weakly correlated with the log *P* values, the current QSAR analysis revealed that the anti-thrombotic activity of these compounds can be explained by their steric and electrostatic effects.


## 1. Introduction

Intravascular thrombosis is one of the most frequent pathological events and a major cause of morbidity and mortality worldwide. Critical steps in the development of acute coronary syndromes are the disruption, rupture, erosion of atherosclerotic plaque with the formation of either partial or complete occlusive thrombus.[1–3] Factors contribute to thrombosis including vascular damage, stimulation of platelets, and activation of the coagulation cascade. Platelets adhere to exposed subendothelium surfaces of injured vessels with various pathological conditions, including cardiovascular and cerebrovascular thromboembotic disorders, such as unstable angina, myocardial infarction, transient ischemic attack, stroke, and atherosclerosis.[3–7]

Despite the well-established application of antithrombotic drugs, such as anticoagulants, antiplatelet drugs, and thrombolytic drugs, there is still an urgent need for more potent and safer compounds for the prevention and treatment of ischemic symptoms. Many antithrombotic drug candidates fail to exert their therapeutic potential due to their poor bioavailability. To improve the pharmacological and the pharmacokinetic properties, we have developed a series of small-molecule antithrombotic and thrombolytic agents.[8–14] Recently, a promising strategy for overcoming this poor bioavailability problem was to target the intestinal peptide transport system. In this context, we have synthesized

*Keywords*: Anti-thrombotic agent; Hexahydropyrazino[1′,2′:1,6]-pyrido[3,4-*b*]indole-1,4-dions; Intramolecular cyclization; Tetrahydro-β-carboline-3-carboxylic acid.
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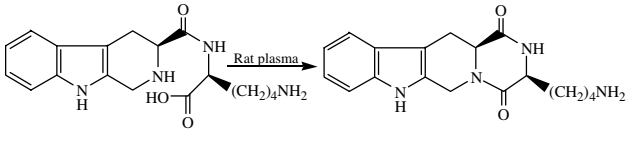
and evaluated a series of novel dipeptide analogues as a new strategy to improve pharmacological and pharmacokinetic properties. The main feature of our approach is to design drug candidates in the form of dipeptide analogues that can be readily absorbed across the intestinal brush border membrane via the peptide transport system. More specifically, 3-(*S*)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid isolated from A. Chinese G. Don was found to possess moderate anti-aggregation activity, but with poor bioavailability. To improve its biological property, we focused on improving the absorption of 3-(*S*)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid by converting it into a dipeptide analogue for improved peptide transport.[9]

In the investigation we found that these dipeptide analogues exhibited a strong tendency of intramolecular cyclization. In order to confirm their cyclization and the possible use *N*-[(3*S*)]-1,2,3,4-tetrahydro-β-carboline-3-carboxyl]-L-lysine was selected as a representative compound, incubated in acetic acid aqueous (5%) and in rat plasma. Then 3.5 mg of *N*-[(3*S*)]-1,2,3,4-tetrahydro-β-carboline-3-carboxyl]-L-lysine was incubated in 15 ml of acetic acid aqueous (5%) at 37 °C for 30 min and then exposed to a gentle nitrogen-flow to evaporate to dryness. The obtained residue was dissolved in 100 μl of aqueous methanol (50%, v/v) to prepare sample solution of HPLC-MS analysis. After 20 μl of the sample solution was loaded, the column was eluted with aqueous methanol (50%, v/v) as the mobile phase. The flow rate was 1 ml/min. The sample was monitored with UV detector at 254.8 nm. The MS analysis was carried out on HP ES-5989x instrument (cone, 30 V; source temperature, 120 °C; desolvation temperature, 350 °C; desolvation gas, 350 L/h; cone gas, 50 L/h) and a molecule ion with 327 amu was found, which was deduced as 3-(4-aminobutyl)-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4-dione. Then its incubation procedure was further used with 15 ml of rat plasma instead of acetic acid aqueous, a molecule ion with 327 amu was again found and the same product was deduced. The cyclization may be presented by Scheme 1. Based on these observations the cyclized product was supposed as antithrombotic active and was consequently synthesized for bioassay. The data indicated that 3-(4-aminobutyl)-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido [3,4-*b*]indole-1,4-dione was in vitro and in vivo anti-thrombotic active.

Thus in this study 20 hexahydropyrazino[1′,2′:1,6]pyrido[3,4-*b*]-indole-1,4-diones were prepared via the intramolecular cyclization of *N*-[(3*S*)-1,2,3,4-tetrahydro-β-carboline-3-carboxyl]-L-amino acid benzylesters and (3*S*)-*N*-(L-aminoacyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid benzylesters, and their in vitro and in vivo anti-thrombotic activities were evaluated. Considering the significant correlation between log *P* and retention time in HPLC (alternatively represented as log *K*),[15–17] moreover, the retention time of these newly characterized anti-thrombotic agents was measured to replace the time-consuming log *P* determination. Finally, an optimal QSAR model was constructed by analyzing the determined log *K* values and other molecular descriptors generated from e-dragon server.




**Scheme 1.** When *N*-[(3*S*)]-1,2,3,4-tetrahydro-β-carboline-3-carboxyl]-L-lysine was incubated in 15 ml of rat plasma or acetic acid aqueous (5%) at 37 °C for 30 min, a cyclization product with 326 amu was monitored by HPLC-MS analysis, which may be deduced as 3-(4-aminobutyl)-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido[3,4-b]-indole-1,4-dione.

## 2. Results and discussion

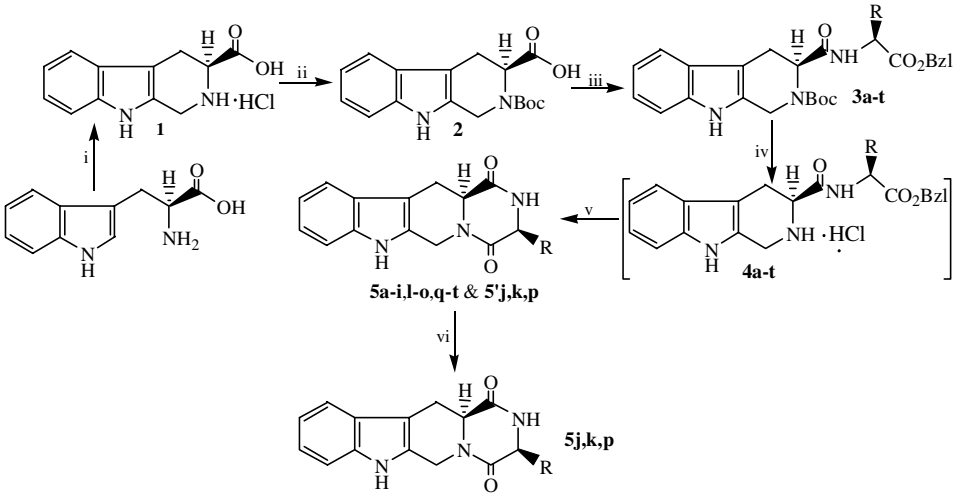
### 2.1. Coupling, cyclization, and stereochemistry

In general, 3-aminoacyl-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4-diones can be synthesized from the intramolecular cyclization of *N*-[(3*S*)-1,2,3,4-tetrahydro-β-carboline-3-carboxyl]amino acids (Scheme 2) or (3*S*)-*N*-(L-aminoacyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acids (Scheme 3). For these two cyclization models, (3*S*)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid is the key intermediate. The results of the preparation reactions and the stereochemistry were described as follows.

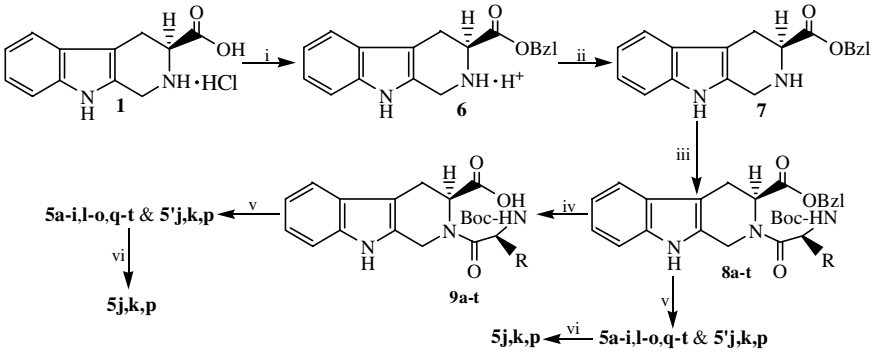
**2.1.1. Cyclization of *N*-[(3*S*)-1,2,3,4-tetrahydro-β-carboline-3-carboxyl]-L-amino acid benzylesters (3a–t).** (3*S*)-1,2,3,4-Tetrahydro-β-carboline-3-carboxylic acid **1** was first transformed into *N*-Boc-tetrahydro-β-carboline-carboxylic acid **2** via the acylation with Boc-$N_3$ (76%), which was then conjugated with amino acid benzylesters to give **3a–t** (85–96% yield). Upon removal of their *N*-Boc group **4a-t** were obtained. Interestingly, without the additional coupling agents, in polar solvent the intramolecular cyclization occurred forming a diketopiperazine ring to give **5a–l**; **5′j,k**; **5m–o**; **5′p**; **5q–t** (87–92%).

**2.1.2. Coupling phosphoric salt of 3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid benzylester (6).** After benzyl esterification of **1** in polyphosphoric acid/benzyl alcohol system the phosphoric salt of 3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid benzylester (**6**) was obtained in 89% yield. A general coupling of phosphoric salt **6** and Boc-amino acids provided *N*-(Boc-aminoacyl) tetrahydro-β-carbolinecarboxylic acid benzylesters **8a–t** in low yield (7–60%).

**2.1.3. Coupling free base 3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid benzylester (7).** Treating phosphoric salt **6** with $Et_3N$ gave the free base 3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid benzylester (**7**), which was then coupled with Boc-amino acids. In contrast to phosphoric salt **6**, the coupling yields of free base **7** were quite enormous (86–95%). With respect to the difference between the yields of coupling phosphoric salt **6** and free base **7** it could be considered that under the general coupling condition *N*-methyl morpholine is unable to



**Scheme 2.** Preparing 3-aminoacyl-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4-dione via the intramolecular cyclization of *N*-[(3*S*)-1,2,3,4-tetrahydro-β-carboline-3-carboxyl]-L-amino acid benzylester. (i) Formaldehyde and $H_2SO_4$; (ii) Triethylamine and Boc-$N_3$; (iii) H-AA-OBzl/DCC/NMM; (iv) Hydrogen chloride in ethyl acetate (4 mol/L); (v) Methanol, ethanol or acetone, and NMM; (vi) Pd/C, $H_2$. In **3a**,**4a**, and **5a**, R = $CH_3$; **3b**, **4b**, and **5b**, R = $CH_2C_6H_5$; **3c**,**4c**, and **5c**, R = $CH(CH_3)_2$; **3d**, **4d**, and **5d**, R = $CH_2OH$; **3e**, **4e**, and **5e**, R = $CH(OH)CH_3$; **3f**, **4f**, and **5f**, R = $CH_2C_6H_4$-OH-p; **3g**, **4g**, and **5g**, R = tetrahydropyrrol-2-yl; **3h**, **4h**, and **5h**, R = $CH_2SH$; **3i**, **4i**, and **5i**, R = $CH_2CH_2SCH_3$; **3j**, **4j**, and **5j′**, R = $CH_2CH_2COOCH_2C_6H_5$; **5j**, R = $CH_2CH_2COOH$; **3k**, **4k**, and **5′k**, R = $CH_2COOCH_2C_6H_5$; **5k**, R = $CH_2COOH$; **3l**, **4l** and **5l**, R = 1,3-imidazol-5-methylene; **3m**, **4m**, and **5m**, R = indol-3-methylene; **3n**, **4n**, and **5n**, R = $CH_2(CH_2)_2NHC(NH_2)$=NH, **3o**, **4o** and **5o**, R = H; **3p**, **4p**, and **5′p**, R = $CH_2(CH_2)_3NHZ$; **5p**, R = $CH_2(CH_2)_3NH_2$; **3q**, **4q**, and **5q**, R = $CH_2CH_2CONH_2$; **3r**, **4r**, and **5r**, R = $CH_2CONH_2$; **3s**, **4s** and **5s**, R = $CH_2CH(CH_3)_2$; **3t**, **4t**, and **5t**, R = $CH(CH_3)CH_2CH_3$.



**Scheme 3.** Preparing 3-substituted-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido[3,4-*b*]-indole-1,4-diones via the intramolecular amination of *N*-aminoacylcarbolinecarboxylic benzylesters **8a**–**t**, and the intramolecular acylation of *N*-aminoacylcarbolinecarboxylic acids **9a**–**t**. (i) polyphosphoric acid, benzyl alcohol/80 °C; (ii) Triethylamine; (iii) Boc-AA-OH, DCC, and NMM; (iv) Aqueous NaOH (2 N) and hydrochloric acid; (v) Hydrogen chloride in ethyl acetate (4 mol/L); (vi) Pd/C, $H_2$. In **5a**, **8a**, and **9a**, $R_1$ = $CH_3$; **5b**, **8b**, and **9b**, R = $CH_2C_6H_5$; **5c**, **8c**, and **9c**, R = $CH(CH_3)_2$; **5d**, **8d**, and **9d**, R = $CH_2OH$; **5e**, **8e**, and **9e**, R = $CH(OH)CH_3$; **5f**, **8f**, and **9f**, R = $CH_2C_6H_4$-OH-p; **5g**, **8g**, and **9g**, R = tetrahydropyrrol-2-yl; **5h**, **8h**, and **9h**, R = $CH_2SH$; **5i**, **8i** and **9i**, R = $CH_2CH_2SCH_3$; **5j′** and **8j**, R = $CH_2CH_2CO_2CH_2C_6H_5$; **5j** and **9j**, R = $CH_2CH_2CO_2H$; **5′k** and **8k**, R = $CH_2CO_2CH_2C_6H_5$; **5k** and **9k**, R = $CH_2CO_2H$; **5l**, **8l**, and **9l**, R = 1,3-imidazol-5-methylene; **5m**, **8m**, and **9m**, R = indol-3-yl-methylene; **5n**, **8n**, and **9n**, R = $CH_2(CH_2)_2NHC(NH_2)$=NH; **5o**, **8o**, and **9o**, R = H; **5′p**, **8p**, and **9p**, R = $CH_2(CH_3)_3NHZ$; **5q**, **8q**, and **9q**, R = $CH_2CH_2CONH_2$; **5r**, **8r**, and **9r**, R = $CH_2CONH_2$; **5s**, **8s**, and **9s**, R = $CH_2CH(CH_3)_2$; **5t**, **8t**, and **9t**, R = $CH(CH_3)CH_2CH_3$.

neutralize phosphoric acid and convert **6** into free base **7** to undergo the coupling.

**2.1.4. Cyclization of (3*S*)-*N*-(Boc-L-aminoacyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid benzylesters (8a–t).** Upon removal of Boc group an intramolecular cyclization may simultaneously occur for **8a**–**t**. This two-step-one-pot reaction gave 3-substituted-2,3,6,7,12,12a-hexahydropyrazino-[1′,2′:1,6]pyrido-[3,4-*b*]indole-1,4-diones in good yield (87–92%).

**2.1.5. Cyclization of (3*S*)-*N*-(Boc-L-aminoacyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acids (9a–t).** In aqueous NaOH, **8a**–**t** may be converted into **9a**–**t** in 90–94% yield. After removing Boc group **9a**–**t** also simultaneously underwent intramolecular cyclization to form **5a**–**t** in 86–91% yields (Scheme 3).

**2.1.6. Removing the benzyloxyl group of 5′j,k and the benzyloxycarbonyl of 5′p by platinum catalyzed hydrogenolysis.** The platinum catalyzed hydrogenolysis of **5j′**,**k**,**p** resulted in a smooth removal of both benzyloxyl group and benzyloxycarbonyl group, and **5′j**,**k**,**p** were converted into **5j**,**k**,**p** in 95–95% yields (Scheme 3).

**2.1.7. Optical purity of 5a–t and 8a–t.** In a solid phase synthesis the coupling of hydrochloric salt of 3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid benzyl-

ester was reported inevitably leading to racemization and giving a product consisted of four diastereoisomers. Thus the reported bioassays in the literature were generally related to diastereomeric mixtures.[18,19] To determine if any racemization of the preexisting stereogenic center had occurred during the course of the coupling, upon removal of the solvent and volatile side products, the coupling products (**5a**–**t** and **8a**–**t**) were then directly analyzed by HPLC, which was carried out on Agilent 1100 series, the column was a reversed-phase $C_{18}$ column (Agilent Zorbax Extend-C18, 4.6 × 15 mm, 5um). After 20 μl of the sample (10 μM) was loaded, the column was eluted with aqueous methanol (50%) as the mobile phase for 40 min. The flow rate was 1 ml/min, **5a**–**t** and **8a**–**t** in the sample were monitored with UV detector at 254.8 nm and the peak area was recorded. Based on the relative proportion of the peak areas it was found that the diastereopurities of **5a**–**t** and **8a**–**t** were higher than 98%.

## 2.2. In vitro anti-platelet aggregation activities of 5a–t

The in vitro anti-platelet aggregation activities of **5a**–**t** were tested by use of a general procedure. After collection, the pig blood was centrifuged at 100 g for 10 min and the platelet rich plasma (PRP) was removed. The remaining blood was centrifuged for a further 10 min at 1,500 g to prepare platelet poor plasma (PPP). The final platelet count of the citrated plasma samples was adjusted to $2 \times 10^8$ platelets/ml with autologous PPP. To a testing tube of the optical aggregometry 0.5 ml of the adjusted plasma sample and 5 μl of NS or 5 μl of the solution of **5a**–**t** (in a series of concentrations of a range from 1 μmol/L to 0.01 μmol/L) were added. After adjustment of the baseline, 5 μl of the solution of platelet-activating factor in NS (PAF, final concentration $10^{-7}$ mol/L), or 5 μl of the solution of adenosine diphosphate in NS (ADP, final concentration $10^{-5}$ mol/L) was added and aggregation was recorded at 37 °C for 5 min. The effects of **5a**–**t** (final concentration 1–0.01 μmol/L) on PAF or ADP induced platelet aggregation were observed. The maximal rate of platelet aggregation (Am%) was represented by the peak height of aggregation curve. The inhibition rate was calculated by %Inhibition = (Am% of NS, 50.16 ± 3.65%) − (Am% of **5a**–**t**) ÷ (Am% of NS, 50.16 ± 3.65%) and the $IC_{50}$ values were determined. The range of the $IC_{50}$ values of **5a**–**t** for ADP-induced aggregation of platelet in PRP plasma of pig is 0.112 ± 0.013 μmol/L to 2.045 ± 0.095 μmol/L, for PAF-induced aggregation of platelet in PRP plasma of pig is 0.294 ± 0.035 μmol/L to 1.134 ± 0.102 μmol/L. The results clearly indicate that the platelet aggregations induced by the two aggregators are sensitive to **5a**–**t** (Fig. 1).

## 2.3. In vivo anti-thrombotic activities of intravenous 5a–t

The in vivo anti-thrombosis activities of **5a**–**t** were tested on Wistar rats using standard derivative circulation via polyethylene tube. The anti-thrombosis potencies of **5a**–**t** were represented by reduced weights of thrombus and the results are listed in Table 1. Statistical analysis of the data is carried out using one way ANOVA test with $p < 0.05$ as significant cut-off. The in vivo activities listed in Table 1 demonstrated that at 0.5 μmol/kg, **5a**–**t** were able to prevent rats from developing thrombus.

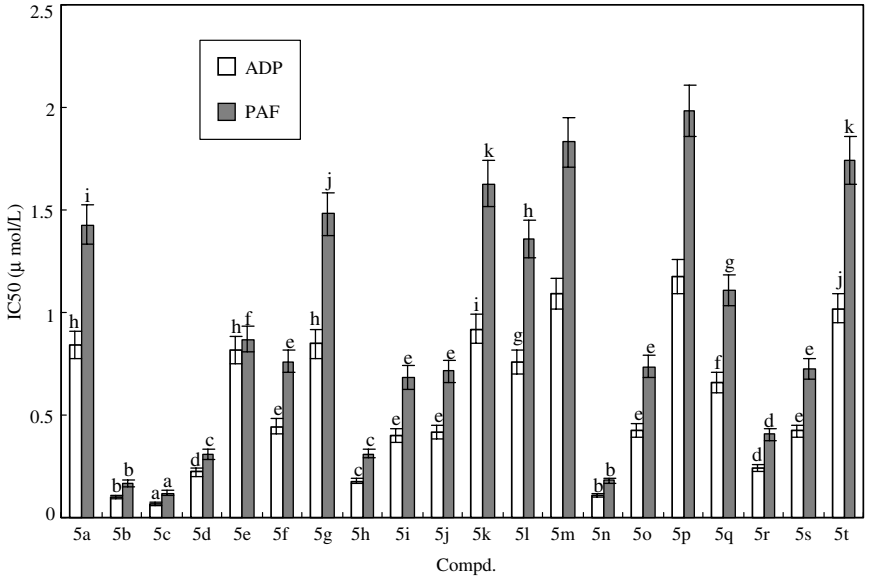



**Figure 1.** In vitro anti-platelet aggregation activities of **5a**–**t**. $n = 6$; for ADP induced aggregation, a = **5c**. Compare to **5a**,**b**,**d**–**t** $p < 0.001$; b = **5b**,**n**. Compare to **5a**,**d**–**m**, **o**–**t** $p < 0.001$; c = **5h**. Compare to **5a**,**d**–**g**,**i**–**m**, **o**–**t** $p < 0.001$; d = **5d**,**r**. Compare to **5a**,**e**–**g**,**i**–**m**, **o**–**q**,**s**,**t** $p < 0.001$; e = **5f**,**i**,**j**,**o**,**s**. Compare to **5a**,**e**,**g**,**k**–**m**, **p**,**q**,**t** $p < 0.001$; f = **5q**. Compare to **5l** $p < 0.05$, **5a**,**e**,**g**,**k**,**m**,**p**,**t** $p < 0.001$; g = **5l**. Compare to **5k**,**m**,**p**,**t** $p < 0.001$; h = **5a**,**e**,**g**. Compare to **5m**,**p**,**t** $p < 0.001$; i = **5k**. Compare to **5p** $p < 0.001$; j = **5t**. Compare to **5m**, **p** $p < 0.01$. for PAF induced aggregation, a = **5c**. Compare to **5a**,**b**,**d**–**t** $p < 0.001$; b = **5b**,**n**. Compare to **5a**,**d**–**m**, **o**–**t** $p < 0.001$; c = **5d**,**h**. Compare to **5a**,**e**–**g**,**i**–**m**, **o**–**t** $p < 0.001$; d = **5r**. Compare to **5a**,**e**–**g**,**i**–**m**,**o**–**q**,**s**,**t** $p < 0.001$; e = **5f**,**i**,**j**,**o**,**s**. Compare to **5e** $p < 0.05$, to **5a**,**g**,**k**–**m**, **p**,**q**,**t** $p < 0.001$; f = **5e**. Compare to **5a**,**g**,**k**–**m**,**p**,**q**,**t** $p < 0.001$; g = **5q**. Compare to **5a**,**g**,**k**–**m**,**p**,**t** $p < 0.001$; h = **5l**. Compare to **5k**,**m**,**p**,**t** $p < 0.001$; i = **5a**. Compare to **5g** $p < 0.05$, to **5k**,**m**,**p**,**t** $p < 0.001$; j = **5g**. Compare to **5k**,**m**,**p**,**t** $p < 0.001$; k = **5k**,**t**. Compare to **5m** $p < 0.05$, to **5p** $p < 0.001$.

**Table 1.** Effect of intravenous **5a–t** on the thrombus weight ($\overline{X} \pm$ SD mg)

| Compound | Wet thrombus | Dry thrombus |
|---|---|---|
| NS | 25.75 ± 2.21 | 5.43 ± 0.62 |
| Aspirin | 7.56 ± 1.31[a] | 2.40 ± 0.5[a] |
| **5a** | 12.05 ± 1.08[a] | 3.83 ± 0.49[a] |
| **5b** | 8.58 ± 1.24[b] | 2.72 ± 0.44[b] |
| **5c** | 8.19 ± 1.76[b] | 2.59 ± 0.36[b] |
| **5d** | 9.44 ± 1.17[c] | 2.98 ± 0.46[c] |
| **5e** | 11.49 ± 1.27[a] | 3.65 ± 0.49 |
| **5f** | 10.49 ± 1.75[d] | 3.30 ± 0.39[d] |
| **5g** | 11.28 ± 1.07[a] | 3.58 ± 0.42[a] |
| **5h** | 9.06 ± 1.03[c] | 2.88 ± 0.39[c] |
| **5i** | 10.41 ± 0.93[d] | 3.28 ± 0.54[d] |
| **5j** | 10.42 ± 1.82[d] | 3.31 ± 0.32 |
| **5k** | 12.26 ± 1.83[a] | 3.89 ± 0.54[a] |
| **5l** | 11.43 ± 0.57[a] | 3.63 ± 0.27[a] |
| **5m** | 12.42 ± 1.65[a] | 3.94 ± 0.54[a] |
| **5n** | 8.62 ± 1.82[b] | 2.74 ± 0.33[b] |
| **5o** | 10.68 ± 1.55[d] | 3.39 ± 0.41[d] |
| **5p** | 12.78 ± 1.57[a] | 4.06 ± 0.31[a] |
| **5q** | 11.10 ± 1.29[a] | 3.52 ± 0.53[a] |
| **5r** | 9.54 ± 1.61[c] | 3.03 ± 0.51[c] |
| **5s** | 10.40 ± 1.88[d] | 3.30 ± 0.39[d] |
| **5t** | 12.45 ± 0.23[a] | 3.95 ± 0.31[a] |

$n = 11$; Aspirin: dosage (iv) = $1.8 \times 10^5$μmol/kg; **5a–t**: dosage (iv)=0.5 μmol/kg.
[a] Compare to NS, $p < 0.001$.
[b] Compare to **5a**,**m**,**t**,**p** and NS $p < 0.001$, to **5f**,**i**,**j**,**o**,**s** $p < 0.05$.
[c] Compare to **5e**,**g**,**l**,**q** $p < 0.05$, to NS $p < 0.001$.
[d] Compare to **5a**,**t**,**p** $p < 0.05$, to NS $p < 0.001$.

The inhibiting potency was significantly greater than that of NS and equal to Aspirin (at $1.8 \times 10^5$μmol/kg). These data again explored that the substituents at the 3-position of 2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4-dione affected the anti-thrombotic activity. Comparing to **5o** (3-position without substituent), compounds with 3-phenyl (**5b**), isopropyl (**5c**), hydroxymethyl (**5d**), and mercaptomethyl (**5h**) had higher anti-thrombosis activity. Compounds with 3-carboxyl (**5k**), heterocyclic ring (**5m**), and long aliphatic chain (**5t**) had lower anti-thrombotic activity. Compounds with 3-hydroxylphenyl (**8f**) and methylmercapto (**5i**) had equal anti-thrombotic activity. It is possible that the anti-thrombosis activities relate to the stabilities of the compounds in vivo, which is also consistent with their chemical stabilities.

### 2.4. Dose-dependent in vivo anti-thrombotic activities of intravenous 5b,c,n

Compounds with significant activity (**5b**,**c**,**n**) were administered using three dosages to conduct detailed pharmacological activity profile study (Table 2). Statistical analysis was carried out using one way ANOVA test with $p < 0.05$ as significant cut-off. The data indicated that the in vivo anti-thrombotic activities of intravenous **5b,c,n** were dose-dependent.

### 2.5. In vivo anti-thrombotic activities of oral 5k,o,p

To expand the administration route for all compounds **5k**,**o**,**p**, the moderately bioactive compounds, with neutral, acidic, and alkaline side chain, were administered orally. Results from oral and intravenous are listed together in Table 3. The statistical analysis was carried out using one way ANOVA test with $p < 0.05$ as significant cut-off. The data of Table 3 indicated that at the dose of 0.5 μmol/kg the oral administration of **5k**,**o**,**p** effectively reduced rat thrombus and the potency was equal to that of intravenous administration. These observations indicate that this class of compounds are promising as oral antithrombotic agents.

### 2.6. The retention time of 5a–t

The HPLC analysis was carried out to determine the retention time ($t_R$) of **5a–t**, which were further represented as the log *K* values (Table 4). More details about the measurement of retention time can be found in the Experimental section. The log *P* value, defined as the logarithm of the partition coefficient between *n*-octanol and water, is an important parameter to reflect a compound's drugability. Meanwhile, it has been widely used as an important molecular descriptor in QSAR analysis such as the classical Hansch approach.[20] Since it has been well established that the log *K* values are highly correlated with the corresponding log *P* values, therefore the determination of log *K* was carried out in this study to replace the time-consuming log *P* determination.

### 2.7. QSAR analysis of 5a–t

In the present study, the in vivo anti-thrombotic activities of intravenous **5a–t**, represented as the weights of

**Table 2.** Effect of intravenous **5b**,**c**,**n** at different dosages on the thrombus weight

| Compound | Wet thrombus ($\overline{X} \pm$ SD mg) | | | Dry thrombus ($\overline{X} \pm$ SD mg) | | |
|---|---|---|---|---|---|---|
| | 0.5 μmol/kg | 5 nmol/kg | 0.5 nmol/kg | 0.5 μmol/kg | 5 nmol/kg | 0.5 nmol/kg |
| NS | | 25.75 ± 2.21 | | | 5.43 ± 0.62 | |
| **5b** | 8.58 ± 1.24[a] | 13.30 ± 2.19[c] | 15.30 ± 2.20[d] | 1.33 ± 0.30[a] | 2.08 ± 0.33[c] | 2.39 ± 0.40[d] |
| **5c** | 8.19 ± 2.76[a] | 14.79 ± 1.33[b] | 16.88 ± 1.56[d] | 1.20 ± 0.26[a] | 2.31 ± 0.43[b] | 2.64 ± 0.36[d] |
| **5n** | 8.62 ± 1.82[a] | 14.52 ± 1.10[c] | 15.71 ± 0.88[d] | 1.22 ± 0.32[a] | 2.27 ± 0.27[c] | 2.45 ± 0.39[d] |

$n = 11$.
[a] Compare to 5 nmol/kg and NS groups, $p < 0.001$.
[b] Compare to 0.5 nmol/kg group $p < 0.01$, to NS group $p < 0.01$.
[c] Compare to 0.5 nmol/kg group $p < 0.05$, to NS group, $p < 0.001$.
[d] Compare to NS group, $p < 0.001$.

**Table 3.** Effect of oral **5k**,**o**,**p** on the thrombus weight

| Compound | Wet thrombus ($\overline{X} \pm$ SD mg) | | Wet thrombus ($\overline{X} \pm$ SD mg) | |
|---|---|---|---|---|
| | Oral | Intravenous | Oral | Intravenous |
| NS | 26.03 ± 2.17 | 25.75 ± 2.21 | 5.50 ± 0.32 | 5.43 ± 0.62 |
| **5k** | 12.42 ± 2.83[a] | 12.26 ± 1.83[a] | 3.94 ± 0.47[a] | 3.89 ± 0.54[a] |
| **5o** | 11.57 ± 2.00[a] | 10.68 ± 1.55[a] | 3.67 ± 0.39[a] | 3.39 ± 0.41[a] |
| **5p** | 11.75 ± 2.97[a] | 12.78 ± 1.57[a] | 3.73 ± 0.34[a] | 4.06 ± 0.31[a] |

$n = 11$, dosage = 0.5 μmol/kg.
[a] Compare to NS group, $p < 0.001$.

**Table 4.** log $K$ values of compounds **5a**–**t**

| Compound | log $K$ |
|---|---|
| **5a** | 0.47 |
| **5b** | 1.05 |
| **5c** | 0.97 |
| **5d** | −0.16 |
| **5e** | −0.14 |
| **5f** | 0.01 |
| **5g** | 0.94 |
| **5h** | 0.71 |
| **5i** | 0.97 |
| **5j** | 0.21 |
| **5k** | 0.15 |
| **5l** | 0.41 |
| **5m** | 0.66 |
| **5n** | −0.58 |
| **5o** | 0.19 |
| **5p** | −0.26 |
| **5q** | 0.28 |
| **5r** | 0.21 |
| **5s** | 1.32 |
| **5t** | 1.26 |

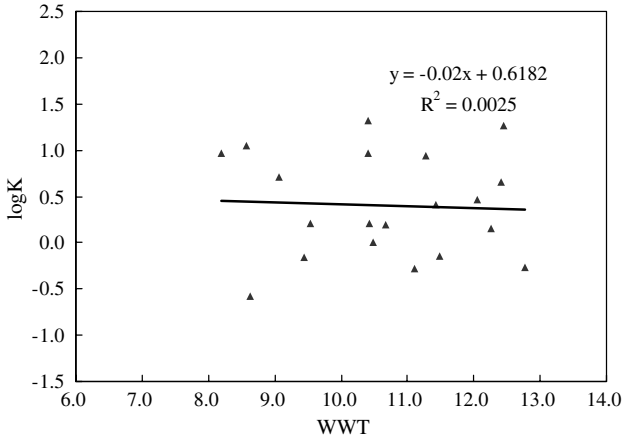



**Figure 2.** The correlation between the WWT values and the measured log $K$ values for **5a**–**t**.

wet thrombus (WWT), are regarded as the anti-thrombotic activities in the QSAR analysis. The detailed procedures to measure the WWT value can be found in the Experimental section. Since only very weak correlation between the log $K$ values and the activities (i.e., WWT) was observed in **5a**–**t** (cf. Fig. 2), the classical Hansch method is not possible to obtain a suitable QSAR model for **5a**–**t**. Therefore, the QSAR analysis based on other molecular descriptors was carried out. By selecting the molecular descriptors generated from e-dragon webserver,[21] the multiple linear regression method (MLR) was employed to derive the QSAR equation of **5a**–**t**. The resubstitution and Leave-One-Out (LOO) tests were carried out to validate the established equations. For **5a**–**t** Eq. 1 could give the highest predictive accuracy.

$$\begin{aligned} WWT &= 28.79(\pm 5.52) - 124.97(\pm 31.21) \times \mathrm{Gm} + 0.53(\pm 0.12) \times \mathrm{RDF060m} \\ &\quad -1.61(\pm 0.34) \times \mathrm{RDF130m} + 299.96(\pm 60.86) \times \mathrm{JGI10} \\ N &= 20, R^2 = 0.74, S = 0.68, \bar{e} = 0.50, R^2_{\mathrm{LOO}} = 0.64, S_{\mathrm{LOO}} = 0.83, \bar{e}_{\mathrm{LOO}} = 0.66, \\ F &= 11.27, p = 0.0003 \end{aligned} \quad (1)$$

The squared correlation coefficients in the resubstitution test ($R^2$) and LOO test ($R^2_{\mathrm{LOO}}$) are 0.74 and 0.64, respectively. Generally, $R^2$ measures goodness-of-fit, whereas $R^2_{\mathrm{LOO}}$ measures goodness-of-prediction. In addition, the resubstitution analysis gave an absolute average error ($\bar{e}$) of 0.50, whereas the absolute average error for the LOO test ($\bar{e}_{\mathrm{LOO}}$) is 0.66. The detailed description of the other statistical measurements in Eq. 1 is available in the Experimental section. Grouped into Weighted Holistic Invariant Molecular (WHIM) descriptors, Gm encodes information about the overall symmetry of a molecule weighted by atomic masses.[22] The RDF060m and RDF130m are classified into the RDF molecular descriptors, which were obtained by radial distribution functions centered on different interatomic distances (from 0.5 Å to 15.5 Å).[23] The RDF molecular descriptors can be interpreted as the probability distribution of finding an atom in a spherical volume of certain radius. For RDF060m and RDF130m, the atomic mass weights are used and the corresponding sphere radius is of 6.0 and 13.0 Å, respectively. The JGI10 belongs to the descriptors of charge indices, which evaluate the global charge transferred between pairs of atoms inside the molecule.[24] A pair-wise correlation analysis between the above four variables (Gm, RDF060m, RDF130m, and JGI10) and activity (i.e., WWT) was carried out and is summarized in Table 5. Generally, the selected four molecular descriptors share medium correlation with the in vivo anti-thrombotic activity, and the correlation between Gm and WWT is the most significant (the correlation coefficient is equal to 0.308). Additionally, all the pair-wise correlation coefficients between different molecular descriptors are in the range from −0.308 to 0.325 (Table 5), implying that there is no significant correlation existing between any two molecular descriptors.

Considering the standard deviation in measuring the WWT value for each compound (*cf.* Table 1), the

**Table 5.** Correlation matrix of different parameters, including four molecular descriptors and the in vivo anti-thrombotic activity (i.e., WWT)

| | WWT | Gm | RDF060m | RDF130m | JGI10 |
|---|---|---|---|---|---|
| WWT | 1.000 | −0.308 | 0.302 | −0.267 | 0.265 |
| Gm | | 1.000 | −0.173 | −0.202 | −0.308 |
| RDF060m | | | 1.00 | 0.325 | 0.298 |
| RDF130m | | | | 1.000 | −0.144 |
| JGI10 | | | | | 1.000 |

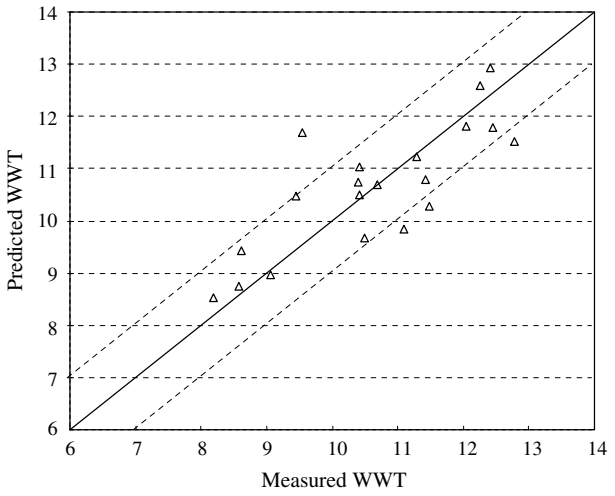



**Figure 3.** Comparison of the predicted anti-thrombotic activities of compounds **5a**–**t** in the LOO test with the measured activities (i.e., the weight of wet thrombus). The data points situated at the zone between the two diagonal-dash lines correspond to the compounds, in which the absolute values of the prediction errors are less than 1.0.

current predictive accuracy of **5a**–**t** is relatively high (Fig. 3). Therefore, Eq. 1 can be practically used to forecast the anti-thrombotic activities of new derivatives. In Eq. 1, three descriptors (Gm, RDF060m, RDF130m) are significantly related to a molecule's steric property, while the remained descriptor JGI10 is more correlated with the electrostatic character of a molecule. Therefore, the overall anti-thrombotic activities of **5a**–**t** can be reasonably explained by their electrostatic and steric effects, although they are weakly correlated with the log *K* values.

## 3. Conclusion

In conclusion, the dipeptide analogues of tetrahydro-β-carboline-3-carboxylamino acids and 2-aminoacyltetrahydro-β-carboline-3-carboxylic acids were easy to cyclize to 3-aminoacyl-hexahydropyrazino[1′,2′:1,6]pyrido-[3,4-*b*]indole-1,4-diones **5a**–**t** via either 2-Boc-β-carboline-3-carboxylamino acid benzylesters (**3a**–**t**) or 2-(Boc-aminoacyl)-tetrahydro-β-carboline-3-carboxylic acid benzylesters (**8a**–**t**) in satisfactory yield. In the preparation of **8a**–**t** the comparisons of low yields/high racemization and high yields/low racemization were observed in the couplings of the phosphoric salts and the free base of tetrahydro-β-carboline-3-carboxylic acid benzylester with Boc-amino acids, respectively. This result demonstrated that the 2-position binding acid should be responsible for the low yields/high racemization occurring in the coupling of some tetrahydro-β-carboline-3-carboxylic acid benzylester with Boc-amino acids. The good in vitro anti-platelet and in vivo anti-thrombotic activities of **5a**–**t** confirmed that the intramolecular cyclization of the dipeptide analogues, tetrahydro-β-carboline-3-carboxylamino acids and 2-aminoacyltetrahydro-β-carboline-3-carboxylid acids, indeed resulted in antithrombotic active products. The data from three doses of the most potential **5b,c,n** suggested that the in vivo anti-thrombolysis action may be dose-dependent. The substantially equal potency of intravenous and oral administration of **5k**,**o**,**p** (the moderately bioactive and with neutral, acidic, and alkaline side chain compounds) implies that the cyclization products of the mentioned dipeptides are promising as oral anti-thrombotic agents.

## 4. Experimental

*General.* The protected amino acids with L-configuration were purchased from Sigma Chemical Co. All coupling and deprotective reactions were carried out under anhydrous conditions. Chromatography was performed on Qingdao silica gel H. The purities of the intermediates and products were confirmed by TLC (Merck silica gel plates of type 60 $F_{254}$, 0.25 mm layer thickness) and HPLC (Waters, $C_{18}$ column 4.6 × 150 mm). The amino acid analysis was determined with a Hitachi 835-50 instrument. FAB-MS was determined by VG-ZAB-MS high resolution GC/MS/DS and HP ES-5989x. Optical rotations were determined with a Schmidt + Haensch Polartromic D instrument. Statistical analysis of all biological data was carried out using one way ANOVA test with $p < 0.05$ as significant cut-off.

### 4.1. Preparation of 3*S*-1,2,3,4-Tetrahydro-*β*-carboline-3-carboxylic acid and derivatives

**4.1.1. 3*S*-1,2,3,4-Tetrahydro-β-carboline-3-carboxylic acid (1).** To a mixture of 5.0 g (24.5 mmol) of L-tryptophane, 25 ml of $H_2SO_4$ (1 mol/L), and 80 ml of water, 8 ml of formaldehyde (36–38%) was added. The reaction mixture was stirred at room temperature for 2 h and adjusted to pH 6–7 with concentrated ammonia liquor. The mixture was kept at 0 °C for 12 h and the formed precipitates were collected by filtration. After recrystallization (ethanol/aqueous ammonia) 3.97 g (75%) of the title compound was obtained as a colorless powder. Mp 280–282 °C; ESI/MS (*m*/*z*) 217 $[M+H]^+$; IR (KBr): 3450, 3200, 3000, 2950, 2850, 1700, 1601, 1452, 1070, 900 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 10.99 (s, 1H),9.89 (s, 1H), 7.30 (t, $J$ = 7.5 Hz, 1H), 7.22 (t, $J$ = 8.0 Hz, 1H), 7.01 (d, $J$ = 8.0 Hz, 1H), 6.81 (d, $J$ = 7.5 Hz, 1H), 4.01 (t, $J$ = 4.8 Hz, 1H), 3.75 (dd, $J$ = 10.5 Hz, $J$ = 5.0 Hz, 1H), 3.64 (dd, $J$ = 10.5 Hz, $J$ = 2.4 Hz, 1H), 2.91 (d, $J$ = 10.5 Hz, 2H), 2.86 (s, 1H). Anal. Calcd for $C_{12}H_{12}N_2O_2$ C 66.65, H, 5.59, N 12.96. Found C 66.45, H 5.72, N 12.79.

**4.1.2. *N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (2).** The suspension of 1.1 g (5.0 mmol) of compound **1** in 15 ml of DMF and 1.4 ml of triethylamine

was vigorously stirred at room temperature, to which 1.1 g (7.7 mmol) of Boc-$N_3$ was added for 30 min. The reaction mixture was stirred at room temperature for 24 h and at 40 °C for 80 h. To the reaction mixture, 5 ml of citrate in water (20%) was added and the solution was extracted with ethyl acetate (30 ml × 3). The separated ethyl acetate layer was dried with anhydrous $MgSO_4$. After removal of $MgSO_4$ by filtration, the filtrate was evaporated to dryness. The residue obtained was crystallized in $CHCl_3$ to give 1.20 g (76%) of the title compound. Mp 165–170 °C; ESI/MS (*m/z*) 317 $[M+H]^+$; IR (KBr): 3452, 3205, 3001, 2952, 2848, 1705, 1645, 1600, 1450, 1072, 901 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 10.87 (s, 1H), 9.86 (s, 1H), 7.32 (t, $J$ = 7.6 Hz, 1H), 7.21 (t, $J$ = 7.9 Hz, 1H), 7.00 (d, $J$ = 7.9 Hz, 1H), 6.84 (t, $J$ = 7.6 Hz, 1H), 4.84 (t, $J$ = 5.0 Hz, 1H), 4.20 (dd, $J$ = 10.2 Hz, $J$ = 4.8 Hz, 1H), 3.98 (dd, $J$ = 10.2 Hz, $J$ = 3.2 Hz, 1H), 2.93 (d, $J$ = 10.2 Hz, 2H), 1.46 (s, 9H). Anal. Calcd for $C_{17}H_{20}N_2O_4$ C 64.54, H, 6.37, N 8.86. Found C 64.41, H 6.25, N 8.74.

**4.1.3. Phosphoric salt of 3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid benzylester (6).** At 70 °C the mixture of 60 g polyphosphoric acid and 600 ml of benzyl alcohol was stirred for 20 min to form a clean solution. The temperature of the solution was raised to 80 °C, 3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (30 g) was then added. The reaction mixture was stirred for another 24h and TLC ($CHCl_3$/MeOH, 10/1) indicated the complete disappearance of 3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid. The reaction mixture was cooled to room temperature, diluted with 500 ml of ether and 2100 ml of water, and stirred at room temperature for 48h. The formed precipitates were collected by filtration, washed with water and ether, dried over anhydrous $CaCl_2$ to provide 35 g (85%) of phosphoric salt of 3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid benzylester as a colorless powder. Mp 192–194 °C; ESI/MS (*m/z*) 307 $[M+H]^+$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 8.79 (s, 1H), 8.22 (s, 1H), 7.48 (d, $J$ = 7.5 Hz, 1H), 7.40 (d, $J$ = 7.2 Hz, 2H), 7.33 (t, $J$ = 8.0 Hz, 1H), 7.20 (t, $J$ = 7.6 Hz, 2H), 7.08 (t, $J$ = 8.0 Hz, 1H), 7.01 (t, $J$ = 7.5Hz, 1H), 6.99 (t, $J$ = 7.5Hz, 1H), 4.22 (d, $J$ = 4.8 Hz, 1H), 3.69 (dd, $J$ = 10.5 Hz, $J$ = 5.0 Hz, 1H), 3.56 (s, 2H), 3.14 (dd, $J$ = 10.5 Hz, $J$ = 2.4 Hz, 1H), 2.83 (ddd, $J$ = 10.5 Hz, $J$ = 5.0 Hz, $J$ = 2.4 Hz, 1H).

**4.1.4. 3*S*-1,2,3,4-Tetrahydro-β-carboline-3-carboxylic acid benzylester (7).** At 0 °C to the stirring suspension of 402 mg (1.0 mmol) of **6** in 30 ml of ethyl acetate, 303 mg (3.0 mmol) of triethylamine was added. The mixture was stirred at room temperature until a clean solution was formed. The solution was then washed with aqueous $NaHCO_3$ (5%, 50 ml × 6) and saturated aqueous NaCl (50 ml × 3), and dried over anhydrous $Na_2SO_4$. After filtration the filtrate was evaporated to provide 272 mg (89%) of **8** as a pale yellow powder. Mp 133–135 °C; ESI/MS (*m/z*) 307 $[M+H]^+$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 8.74 (s, 1H) 7.45 (d, $J$ = 7.4 Hz, 1H), 7.37 (d, $J$ = 7.2 Hz, 2H), 7.30 (t, $J$ = 8.1 Hz, 1H), 7.17 (t, $J$ = 7.4 Hz, 2H), 7.03 (t, $J$ = 8.1 Hz, 1H), 7.00 (t, $J$ = 7.3 Hz, 1H), 6.95 (t, $J$ = 7.3Hz, 1H), 4.24 (d, $J$ = 4.7 Hz, 1H), 3.67 (dd, $J$ = 10.1 Hz, $J$ = 5.2 Hz, 1H), 3.55 (s, 2H), 3.15 (dd, $J$ = 10.1 Hz, $J$ = 2.4 Hz, 1H), 2.84 (ddd, $J$ = 10.1 Hz, $J$ = 5.2 Hz, $J$ = 2.4 Hz, 1H), 2.81 (s, 1H).

### 4.2. General procedure for the preparation of *N*-(*N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxyl)-L-amino acid benzylesters (3a–t)

At 0 °C to the solution of 2.0 g (6.33 mmol) *N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid in 30 ml of anhydrous THF, 1.2 g (8.9 mmol) of HOBt was added, after 10 min, 1.75 g (8.5 mmol) of DCC was then added. The suspension of 6.96 mmol of HCl · L-AA-OBzl in 3 ml of anhydrous THF was adjusted to pH 8–9 with *N*-methyl morpholine and stirred at room temperature for another 20 min. This suspension then was added to the solution of *N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid and the reaction mixture was stirred at 0 °C for 2h and at room temperature for 16 h. On evaporation the residue was dissolved in 30 ml of ethyl acetate. The solution was washed successively with 5% sodium bicarbonate, 5% citric acid, and saturated sodium chloride and the organic phase was separated and dried over anhydrous sodium sulfate. After filtration and evaporation under reduced pressure the title compound was obtained as powder.

**4.2.1. *N*-[(3*S*)-*N*-Boc-1,2,3,4-tetrahydro-β-carboline-3-carboxyl]-L-alaninebenzylester (3a).** Yield: 95%. Mp 110–112 °C; ESI/MS (*m/z*) 478 $[M+H]^+$. IR (KBr): 3447, 3342, 3001, 2945, 2842, 1761, 1733, 1602, 1455, 1391, 1373, 1062, 899 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 9.93 (s, 1H), 8.04 (s, 1H), 7.30 (t, $J$ = 7.3 Hz, 1H), 7.24 (t, $J$ = 7.3 Hz, 1H), 7.22 (t, $J$ = 7.2 Hz, 2H), 7.14 (d, $J$ = 7.2 Hz, 2H), 7.11 (t, $J$ = 7.2 Hz, 1H), 6.95 (d, $J$ = 7.3 Hz, 1H), 6.83 (d, $J$ = 7.3 Hz, 1H), 5.35 (s, 2H), 4.86 (d, $J$ = 5.4 Hz, 1H), 4.56 (m, $J$ = 5.2 Hz, 1H), 4.22 (dd, $J$ = 10.1 Hz, $J$ = 4.5 Hz, 1H), 4.15 (dd, $J$ = 10.0 Hz, $J$ = 3.7 Hz, 1H), 2.92 (d, $J$ = 10.0 Hz, 2H), 1.57 (d, $J$ = 5.0 Hz, 3H), 1.46 (s, 9H). $[\alpha]_D^{20}$ − 100° (*c* 0.38, $CHCl_3$/$CH_3OH$, 1:1, v/v). Anal. Calcd for $C_{27}H_{31}N_3O_5$ C 67.91, H, 6.54, N 8.80. Found C 67.72, H 6.40, N 8.90.

**4.2.2. *N*-[(3*S*)-*N*-Boc-1,2,3,4-tetrahydro-β-carboline-3-carboxyl]-L-phenylalanine benzyl-ester (3b).** Yield: 96%. Mp 144–146 °C; ESI/MS (*m/z*) 554 $[M+H]^+$. IR (KBr): 3442, 3350, 3202, 3009, 2944, 2842, 1758, 1734, 1642, 1601, 1455, 1390, 1371, 1065, 902 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 9.95 (s, 1H), 7.99 (s, 1H), 7.28 (t, $J$ = 7.3 Hz, 1H), 7.25 (t, $J$ = 7.6 Hz, 2H), 7.22 (t, $J$ = 7.3 Hz, 2H),7.17 (t, $J$ = 7.3 Hz, 1H), 7.15 (d, $J$ = 7.4 Hz, 2H), 7.13 (d, $J$ = 7.3 Hz, 2H), 7.11 (t, $J$ = 7.3 Hz, 1H), 7.03 (t, $J$ = 7.3 Hz, 1H), 6.98 (d, $J$ = 7.6 Hz, 1H), 6.82 (d, $J$ = 7.2 Hz, 1H), 5.34 (s, 2H), 4.95 (d, $J$ = 5.2 Hz, 1H), 4.84 (t, $J$ = 5.2 Hz, 1H), 4.25 (dd, $J$ = 10.0 Hz, $J$ = 4.1 Hz, 1H), 4.15 (dd, $J$ = 10.0 Hz, $J$ = 3.5 Hz, 1H), 3.19 (d, $J$ = 5.2 Hz, 2H), 2.92 (d, $J$ = 10.0 Hz, 2H), 1.49 (s, 9H). $[\alpha]_D^{20}$ − 52° (*c* 0.38, $CHCl_3$/$CH_3OH$, 1:1, v/v). Anal. Calcd for

$C_{33}H_{35}N_3O_5$ C 71.59, H 6.37, N 7.59. Found C 71.74, H 6.22, N 7.77.

**4.2.3. *N*-(*N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3- carboxyl)-L-valine benzylester (3c).** Yield: 94%. Mp 169–171 °C; ESI/MS (*m*/*z*) 506 $[M+H]^+$. IR (KBr): 3441, 3336, 3205, 3004, 2953, 2843, 1757, 1732, 1645, 1600, 1453, 1390, 1372, 1064, 900 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 10.00 (s, 1H), 7.98 (s, 1H), 7.27 (t, *J* = 7.4 Hz, 1H), 7.23 (t, *J* = 7.0 Hz, 2H), 7.20 (t, *J* = 7.4 Hz, 1H), 7.02 (d, *J* = 7.4 Hz, 1H), 7.13 (d, *J* = 7.0 Hz, 2H), 7.10 (t, *J* = 7.0 Hz, 1H), 6.87 (d, *J* = 7.4 Hz, 1H), 5.34 (s, 2H), 4.86 (t, *J* = 5.2 Hz, 1H), 4.40 (d, *J* = 5.1 Hz, 1H), 4.24 (dd, *J* = 10.0 Hz, *J* = 4.3 Hz, 1H), 4.05 (dd, *J* = 10.0 Hz, *J* = 3.5 Hz, 1H), 3.12 (m, *J* = 5.2 Hz, 1H), 2.93 (d, *J* = 6.4 Hz, 2H), 1.49 (s, 9H), 1.03 (d, *J* = 5.1 Hz, 6H). Anal. Calcd for $C_{29}H_{35}N_3O_5$ C 68.89, H 6.98, N 8.31. Found C 68.74, H 7.08, N 8.48.

**4.2.4. *N*-[(3*S*)-*N*-Boc-1,2,3,4-tetrahydro-β-carboline-3-carboxyl]-L-serine benzylester (3d).** Yield: 93%. Mp 125–127 °C; ESI/MS (*m*/*z*) 494 $[M+H]^+$. IR (KBr): 3445, 3338, 3207, 3005, 2956, 2843, 1762, 1733, 1642, 1601, 1457, 1390, 1371, 1062, 903 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 9.97 (s, 1H), 7.99 (s, 1H), 7.27 (t, *J* = 7.2 Hz, 1H), 7.23 (t, *J* = 7.3 Hz, 1H), 7.21 (t, *J* = 7.0 Hz, 2H), 7.13 (d, *J* = 7.0 Hz, 2H), 7.10 (t, *J* = 7.0 Hz, 1H), 6.99 (d, *J* = 7.3 Hz, 1H), 6.85 (t, *J* = 7.2 Hz, 1H), 5.33 (s, 2H), 4.89 (d, *J* = 5.2 Hz, 1H), 4.50 (t, *J* = 5.0 Hz, 1H), 4.17 (d, *J* = 5.0Hz, 2H), 4.15 (d, *J* = 5.2 Hz, 2H), 2.94 (d, *J* = 5.3 Hz, 1H), 2.90 (d, *J* = 5.3 Hz, 1H), 2.25 (s, 1H), 1.47 (s, 9H). $[\alpha]_D^{20}$ − 51° (*c* 0.35, $CHCl_3$/$CH_3OH$, 1:1, v/v). Anal. Calcd for $C_{27}H_{31}N_3O_6$ C 65.71, H 6.33, N 8.51. Found C 65.58, H 6.39, N 8.70.

**4.2.5. *N*-(*N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxyl)-L-threonine benzylester (3e).** 90%. Mp 145–147 °C; ESI/MS (*m*/*z*) 508 $[M+H]^+$; IR (KBr): 3440, 3334, 3201, 3002, 2951, 2845, 1760, 1731, 1640, 1600, 1452, 1395, 1375, 1060, 900 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 9.95 (s, 1H), 7.84 (s, 1H), 7.30 (t, *J* = 7.2 Hz, 1H), 7.24 (t, *J* = 7.2 Hz, 1H), 7.19 (t, *J* = 7.0 Hz, 2H), 7.10 (d, *J* = 7.0 Hz, 2H), 7.07 (t, *J* = 7.0 Hz, 1H), 6.97 (d, *J* = 7.2 Hz, 1H), 6.85 (t, *J* = 7.2 Hz, 1H), 5.33 (s, 2H), 4.85 (t, *J* = 5.5 Hz, 1H), 4.63 (m, *J* = 5.5 Hz, 1H), 4.45 (t, *J* = 5.5 Hz, 1H), 3.95 (m, *J* = 5.3 Hz, 2H), 2.94 (d, *J* = 5.6Hz, 2H), 2.20 (s, 1H), 1.49 (s, 9H), 1.22 (d, *J* = 5.5 Hz, 3H). Anal. Calcd for $C_{28}H_{33}N_3O_6$ C 66.26, H 6.55, N 8.28. Found C 66.11, H 6.38, N 8.44.

**4.2.6. *N*-(*N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxyl)-L-tyrosine benzylester (3f).** Yield: 90%. Mp 155–157 °C; ESI/MS (*m*/*z*) 570 $[M+H]^+$; IR (KBr): 3444, 3200, 3006, 2950, 2842, 1730, 1647, 1600, 1456, 1395, 1370, 1060, 900 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 9.95 (s, 1H), 8.04 (s, 1H), 7.37 (t, *J* = 7.4 Hz, 1H), 7.23 (t, *J* = 7.6 Hz, 1H), 7.16 (d, *J* = 7.4 Hz, 2H), 7.14 (t, *J* = 7.1 Hz, 2H), 7.12 (d, *J* = 7.1 Hz, 2H), 7.09 (t, *J* = 7.1 Hz, 1H), 7.05 (d, *J* = 7.4 Hz, 1H), 6.93 (d, *J* = 7.6 Hz, 1H), 6.90 (d, *J* = 7.4 Hz, 2H), 5.40 (s, 2H), 4.99 (s, 1H), 4.91 (d, *J* = 5.5 Hz, 1H), 4.82 (t, *J* = 5.7 Hz, 1H), 4.26 (m, *J* = 5.3 Hz, 2H), 3.17 (d, *J* = 5.3 Hz, 2H), 2.95 (d, *J* = 5.1 Hz, 2H), 1.47 (s, 9H). Anal. Calcd for $C_{33}H_{35}N_3O_6$ C 69.58, H 6.19, N 7.38. Found C 69.41, H 6.10, N 7.53.

**4.2.7. *N*-(*N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxyl)-L-proline benzylester (3g).** Yield: 93%. Mp 133–135 °C; ESI/MS (*m*/*z*) 504 $[M+H]^+$; IR (KBr): 3439, 3206, 3005, 2952, 2843, 1730, 1641, 1600, 1452, 1395, 1373, 1060, 905 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 10.04 (s, 1H), 7.33 (t, *J* = 7.3 Hz, 1H), 7.22 (t, *J* = 7.6 Hz, 1H), 7.20 (t, *J* = 7.2 Hz, 2H), 7.13 (d, *J* = 7.2 Hz, 2H), 7.09 (t, *J* = 7.2 Hz, 1H), 7.05 (d, *J* = 7.6 Hz, 1H), 6.93 (d, *J* = 7.3 Hz, 1H), 5.30 (s, 2H), 4.86 (t, *J* = 5.3 Hz, 1H), 4.33 (t, *J* = 5.5 Hz, 1H), 4.24 (d, *J* = 5.2 Hz, 2H), 3.49 (t, *J* = 5.5 Hz, 2H), 2.93 (d, *J* = 5.5 Hz, 2H), 2.27 (d, *J* = 5.5Hz, 2H), 1.96 (t, *J* = 4.8 Hz, 2H), 1.45 (s, 9H). Anal. Calcd for $C_{29}H_{33}N_3O_5$ C 69.17, H 6.60, N 8.34. Found C 69.02, H 6.50, N 8.51.

**4.2.8. *N*-(*N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxyl)-L-cysteine benzylester (3h).** Yield: 90%. Mp 151–153 °C; ESI/MS (*m*/*z*) 510 $[M+H]^+$; IR (KBr): 3441, 3205, 3003, 2940, 2842, 1735, 1640, 1603, 1450, 1392, 1375, 1062, 899 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 9.96 (s, 1H), 7.95 (s, 1H), 7.30 (t, *J* = 7.4 Hz, 1H), 7.24 (t, *J* = 7.6 Hz, 1H), 7.22 (t, *J* = 7.5 Hz, 2H), 7.15 (d, *J* = 7.5 Hz, 2H), 7.11 (t, *J* = 7.5 Hz, 1H), 7.07 (d, *J* = 7.6 Hz, 1H), 6.86 (d, *J* = 7.4 Hz, 1H), 5.32 (s, 2H), 4.95 (t, *J* = 5.4 Hz, 1H), 4.74 (t, *J* = 5.6 Hz, 1H), 4.23 (d, *J* = 5.4 Hz, 2H), 3.14 (d, *J* = 5.4 Hz, 2H), 3.02 (d, *J* = 5.5 Hz, 2H), 1.47 (s, 9H), 1.65 (s, 1H). Anal. Calcd for $C_{27}H_{31}N_3O_5S$ C 63.63, H 6.13, N 8.25. Found C 63.45, H 6.02, N 8.10.

**4.2.9. *N*-(*N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxyl)-L-methionine benzylester (3i).** Yield: 91%.Mp 167–169 °C; ESI/MS (*m*/*z*) 538 $[M+H]^+$; IR (KBr): 3445, 3200, 3007, 2956, 2849, 1730, 1645, 1600, 1452, 1395, 1370, 1064, 905 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 10.07 (s, 1H), 7.94 (s, 1H), 7.33 (t, *J* = 7.4 Hz, 1H), 7.23 (t, *J* = 7.6 Hz, 1H), 7.20 (t, *J* = 7.5 Hz, 2H), 7.17 (d, *J* = 7.5 Hz, 2H), 7.12 (t, *J* = 7.5 Hz, 1H), 6.95 (d, *J* = 7.4 Hz, 1H), 6.83 (d, *J* = 7.4 Hz, 1H), 5.32 (s, 2H), 4.87 (t, *J* = 5.1 Hz, 1H), 4.46 (t, *J* = 5.4 Hz, 1H), 4.26 (d, *J* = 5.2 Hz, 2H), 2.95 (d, *J* = 5.1 Hz, 2H), 2.44 (t, *J* = 5.3 Hz, 2H), 2.25 (d, *J* = 5.4 Hz, 2H), 2.13 (s, 3H), 1.49 (s, 9H). Anal. Calcd for $C_{29}H_{35}N_3O_5S$ C 64.78, H 6.56, N 7.82. Found C 64.62, H 6.45, N 7.99.

**4.2.10. *N*-(*N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxyl)-L-glutamic acid dibenzyl-ester (3j).** Yield: 90%. Mp 144–146 °C; ESI/MS (*m*/*z*) 626 $[M+H]^+$; IR (KBr): 3445, 3204, 3007, 2948, 2826, 1735, 1640, 1600, 1452, 1391, 1375, 1062, 900 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 9.92 (s, 1H), 8.03 (s, 1H), 7.36 (t, *J* = 7.5 Hz, 1H), 7.27 (t, *J* = 7.5 Hz, 1H), 7.24 (t, *J* = 7.3 Hz, 2H), 7.22 (t, *J* = 7.4 Hz, 2H), 7.21 (d, *J* = 7.3 Hz, 2H), 7.19 (d, *J* = 7.4 Hz, 2H), 7.16 (t,

$J$ = 7.3 Hz, 1H), 7.14 (t, $J$ = 7.4 Hz, 1H), 7.04 (d, $J$ = 7.5 Hz, 1H), 6.86 (d, $J$ = 7.5 Hz, 1H), 5.36 (s, 2H), 5.33 (s, 2H), 4.93 (d, $J$ = 5.3 Hz, 1H), 4.46 (t, $J$ = 5.5 Hz, 1H), 4.25 (d, $J$ = 5.4 Hz, 2H), 2.95 (d, $J$ = 5.2 Hz, 2H), 2.25 (t, $J$ = 5.5 Hz, 2H), 2.22 (t, $J$ = 5.6 Hz, 2H), 1.48 (s, 9H). Anal. Calcd for $C_{36}H_{39}N_3O_7$ C 69.10, H 6.28, N 6.72. Found C 69.27, H 6.18, N 6.89.

**4.2.11. *N*-(*N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxyl)-L-aspartic acid dibenzyl-ester (3k).** Yield: 92%. Mp 132–134 °C; ESI/MS (*m*/*z*) 612 $[M+H]^+$; IR (KBr): 3445, 3340, 3214, 3002, 2952, 2843, 1758, 1730, 1646, 1602, 1455, 1388, 1369, 1064, 901 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 10.01 (s, 1H), 8.02 (s, 1H), 7.30 (t, $J$ = 7.2 Hz, 1H), 7.24 (t, $J$ = 7.2 Hz, 1H), 7.22 (t, $J$ = 7.0 Hz, 2H), 7.20 (t, $J$ = 7.0 Hz, 2H), 7.15 (d, $J$ = 7.0 Hz, 2H), 7.13 (d, $J$ = 7.0 Hz, 2H), 7.11 (t, $J$ = 7.0 Hz, 1H), 7.10 (t, $J$ = 7.0 Hz, 1H), 7.02 (d, $J$ = 7.2 Hz, 1H), 6.98 (d, $J$ = 7.2 Hz, 1H), 5.36 (s, 2H), 5.34 (s, 2H), 4.94 (d, $J$ = 5.2 Hz, 1H), 4.79 (t, $J$ = 5.2 Hz, 1H), 4.27 (d, $J$ = 5.2 Hz, 2H), 2.94 (d, $J$ = 5.0 Hz, 2H), 2.87 (d, $J$ = 5.2 Hz, 2H), 1.47 (s, 9H). Anal. Calcd for $C_{35}H_{37}N_3O_7$ C 68.72, H 6.10, N 6.87. Found C 68.54, H 6.19, N 7.00.

**4.2.12. *N*-(*N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxyl)-L-histidine benzylester (3l).** Yield: 91%. Mp 153–155 °C; ESI/MS (*m*/*z*) 544 $[M+H]^+$; IR (KBr): 3445, 3202, 3007, 2943, 2832, 1735, 1640, 1600, 1452, 1395, 1371, 1060, 905 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 12.95 (s, 1H), 9.98 (s, 1H), 8.01 (s, 1H), 7.45 (s, 1H), 7.35 (t, $J$ = 7.2 Hz, 1H), 7.22 (t, $J$ = 7.3 Hz, 2H), 7.17 (t, $J$ = 7.4 Hz, 1H), 7.15 (d, $J$ = 7.3 Hz, 2H), 7.13 (d, $J$ = 7.4 Hz, 1H), 7.11 (t, $J$ = 7.3 Hz, 1H), 6.95 (t, $J$ = 7.2 Hz, 1H), 6.88 (s, 1H), 5.38 (s, 2H), 4.91 (t, $J$ = 5.2 Hz, 1H), 4.80 (t, $J$ = 5.1 Hz, 1H), 4.22 (d, $J$ = 5.1 Hz, 2H), 3.21 (d, $J$ = 5.2 Hz, 2H), 2.90 (d, $J$ = 5.1 Hz, 2H), 1.47 (s, 9H). Anal. Calcd for $C_{30}H_{33}N_5O_5$ C 66.28, H 6.12, N 12.88. Found C 66.11, H 6.00, N 13.07.

**4.2.13. *N*-[(3*S*)-*N*-Boc-1,2,3,4-tetrahydro-β-carboline-3-carboxyl]-L-tryptophan benzylester (3m).** Yield: 92%. Mp 136–138 °C; ESI/MS (*m*/*z*) 593 $[M+H]^+$. IR (KBr): 3444, 3337, 3208, 3005, 2945, 2835, 1758, 1736, 1645, 1602, 1449, 1390, 1370, 1067, 903 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 9.96 (s, 1H), 9.89 (s, 1H), 8.03 (s, 1H), 7.29 (t, $J$ = 7.3 Hz, 1H), 7.27 (t, $J$ = 7.2 Hz, 1H), 7.22 (t, $J$ = 7.0 Hz, 2H), 7.14 (d, $J$ = 7.0 Hz, 2H), 7.12 (d, $J$ = 7.5 Hz, 1H), 7.11 (t, $J$ = 7.0 Hz, 1H), 7.10 (t, $J$ = 7.5 Hz, 1H), 7.09 (d, $J$ = 7.3 Hz, 1H), 7.07 (t, $J$ = 7.5 Hz, 1H), 7.02 (d, $J$ = 7.3 Hz, 1H), 6.97 (d, $J$ = 7.2 Hz, 1H), 6.85 (s, 1H), 5.36 (s, 2H), 4.96 (d, $J$ = 5.2 Hz, 1H), 4.73 (t, $J$ = 5.1 Hz, 1H), 4.27 (d, $J$ = 5.0 Hz, 2H), 3.17 (d, $J$ = 5.2 Hz, 2H), 2.97 (d, $J$ = 6.2 Hz, 2H), 1.47 (s, 9H). $[\alpha]_D^{20}$ − 77° (*c* 0.36, $CHCl_3/CH_3OH$, 1:1, v/v). Anal. Calcd for $C_{35}H_{36}N_4O_5$ C 70.93, H 6.12, N 9.45. Found C 70.80, H 6.01, N 9.60.

**4.2.14. *N*-(*N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxyl)-L-arginine benzylester (3n).** Yield: 85%. Mp 152–154 °C; ESI/MS (*m*/*z*) 563 $[M+H]^+$; IR (KBr): 3446, 3203, 3005, 2944, 2846, 1725, 1642, 1603, 1450, 1395, 1370, 1064, 900 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 10.20 (s, 1H), 8.42 (s, 2H), 8.25 (s, 1H), 8.20 (s, 1H), 8.00 (s, 1H), 7.27 (t, $J$ = 7.4 Hz, 1H), 7.24 (t, $J$ = 7.2 Hz, 2H), 7.17 (d, $J$ = 7.2 Hz, 2H), 7.15 (t, $J$ = 7.5 Hz, 1H), 7.13 (t, $J$ = 7.2 Hz, 1H), 7.01 (d, $J$ = 7.5 Hz, 1H), 6.93 (d, $J$ = 7.4 Hz, 1H), 5.34 (s, 2H), 4.92 (d, $J$ = 5.2 Hz, 1H), 4.40 (t, $J$ = 4.4 Hz, 1H), 4.27 (d, $J$ = 5.2 Hz, 2H), 2.90 (d, $J$ = 4.4 Hz, 2H), 2.67 (t, $J$ = 5.2 Hz, 2H), 1.95 (m, $J$ = 5.3 Hz, 2H), 1.56 (m, $J$ = 5.3 Hz, 2H), 1.55 (s, 9H). Anal. Calcd for $C_{30}H_{38}N_6O_5$ C 64.04, H 6.81, N 14.94. Found C 64.21, H 6.69, N 14.78.

**4.2.15. *N*-[(3*S*)-*N*-Boc-1,2,3,4-tetrahydro-β-carboline-3-carboxyl]-L-glycine benzylester (3o).** Yield: 95%. Mp 139–141 °C; ESI/MS (*m*/*z*) 464 $[M+H]^+$. IR (KBr): 3444, 3336, 3004, 2940, 2845, 1761, 1730, 1602, 1455, 1392, 1375, 1060, 903 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 9.96 (s, 1H), 8.05 (s, 1H), 7.27 (t, $J$ = 7.4 Hz, 1H), 7.23 (t, $J$ = 7.2 Hz, 2H), 7.18 (t, $J$ = 7.4 Hz, 1H), 7.15 (d, $J$ = 7.2 Hz, 2H), 7.11 (t, $J$ = 7.2 Hz, 1H), 6.93 (d, $J$ = 7.4 Hz, 1H), 6.85 (d, $J$ = 7.4 Hz, 1H), 5.36 (s, 2H), 4.86 (d, $J$ = 5.2 Hz, 1H), 4.25 (dd, $J$ = 10.0 Hz, $J$ = 4.3 Hz, 1H), 4.17 (dd, $J$ = 10.0 Hz, $J$ = 3.4 Hz, 1H), 4.15 (s, 2H), 2.93 (d, $J$ = 10.0 Hz, 2H), 1.48 (s, 9H). $[\alpha]_D^{20}$ − 84° (*c* 0.36, $CHCl_3/CH_3OH$, 1:1, v/v). Anal. Calcd for $C_{26}H_{29}N_3O_5$ C 67.37, H, 6.31, N 9.07. Found C 67.25, H 6.18, N 9.19.

**4.2.16. *N*-[(3*S*)-*N*-Boc-1,2,3,4-tetrahydro-β-carboline-3-carboxyl]-L-lysine(Z) benzylester (3p).** Yield: 92%. Mp 123–125 °C; ESI/MS (*m*/*z*) 669 $[M+H]^+$. IR (KBr): 3445, 3339, 3002, 2942, 2845, 1761, 1735, 1602, 1459, 1392, 1371, 1063, 899 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 9.98 (s, 1H), 8.02 (s, 1H), 7.99 (s, 1H), 7.27 (t, $J$ = 7.3 Hz, 1H), 7.22 (t, $J$ = 7.2Hz, 2H), 7.20 (t, $J$ = 7.0Hz, 1H), 7.17 (t, $J$ = 7.3 Hz, 1H), 7.14 (d, $J$ = 7.0 Hz, 2H), 7.13 (d, $J$ = 7.2Hz, 2H), 7.12 (t, $J$ = 7.0 Hz, 2H), 7.10 (t, $J$ = 7.2Hz, 1H), 6.98 (d, $J$ = 7.3 Hz, 1H), 6.88 (d, $J$ = 7.3 Hz, 1H), 5.35 (s, 2H), 5.33 (s, 2H), 4.93 (d, $J$ = 5.2 Hz, 1H), 4.45 (t, $J$ = 4.2 Hz, 1H), 4.25 (dd, $J$ = 10.2 Hz, $J$ = 4.3 Hz, 1H), 4.15 (dd, $J$ = 10.2 Hz, $J$ = 3.4 Hz, 1H), 2.96 (t, $J$ = 4.2 Hz, 2H), 2.94 (d, $J$ = 10.2 Hz, 2H), 1.93 (m, $J$ = 4.2 Hz, 2H), 1.55 (m, $J$ = 4.4 Hz, 2H), 1.48 (s, 9H), 1.27 (m, $J$ = 4.2 Hz, 2H). $[\alpha]_D^{20}$ − 41° (*c* 0.39, $CHCl_3/CH_3OH$, 1:1, v/v). Anal. Calcd for $C_{38}H_{44}N_4O_7$ C 68.24, H, 6.63, N 8.38. Found C 68.08, H 6.77, N 8.53.

**4.2.17. *N*-[(3*S*)-*N*-Boc-1,2,3,4-tetrahydro-β-carboline-3-carboxyl]-L-glutamine benzylester (3q).** Yield: 87%. Mp 130–132 °C; ESI/MS (*m*/*z*) 535 $[M+H]^+$. IR (KBr): 3441, 3207, 3004, 2942, 2830, 1736, 1642, 1600, 1455, 1390, 1375, 1062, 903 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 9.95 (s, 1H), 8.04 (s, 1H), 7.27 (t, $J$ = 7.3 Hz, 1H), 7.24 (t, $J$ = 7.2 Hz, 2H), 7.18 (t, $J$ = 7.3 Hz, 1H), 7.16 (d, $J$ = 7.2 Hz, 2H), 7.13 (t, $J$ = 7.2 Hz, 1H), 7.04 (d, $J$ = 7.3 Hz, 1H), 6.85 (d, $J$ = 7.3 Hz, 1H), 6.08 (s, 2H), 5.35 (s, 2H), 4.94 (d, $J$ = 5.4 Hz, 1H), 4.43 (t, $J$ = 5.4 Hz, 1H), 4.27 (d, $J$ = 5.5 Hz, 2H), 2.90 (d, $J$ = 5.2 Hz, 2H), 2.15 (t, $J$ = 5.4 Hz, 2H), 2.16 (t, $J$ = 5.4Hz, 2H), 1.49 (s, 9H).

$[\alpha]_D^{20}$ −50° (*c* 0.38, $CHCl_3/CH_3OH$, 1:1, v/v). Anal. Calcd for $C_{29}H_{34}N_4O_6$ C 65.15, H 6.41, N 10.48. Found C 65.32, H 6.52, N 10.31.

**4.2.18. *N*-[(3*S*)-*N*-Boc-1,2,3,4-tetrahydro-β-carboline-3-carboxyl]-L-asparagine benzylester (3r).** Yield: 90%. Mp 142–144 °C; ESI/MS (*m*/*z*) 521 $[M+H]^+$. IR (KBr): 3443, 3207, 3004, 2932, 2833, 1735, 1630, 1604, 1451, 1392, 1375, 1064, 900 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 9.96 (s, 1H), 8.05 (s, 1H), 7.28 (t, *J* = 7.3 Hz, 1H), 7.23 (t, *J* = 7.4 Hz, 2H), 7.19 (t, *J* = 7.3 Hz, 1H), 7.17 (d, *J* = 7.4 Hz, 2H), 7.15 (t, *J* = 7.4 Hz, 1H), 7.06 (d, *J* = 7.3 Hz, 1H), 6.87 (d, *J* = 7.3 Hz, 1H), 6.05 (s, 2H), 5.33 (s, 2H), 4.97 (d, *J* = 5.3 Hz, 1H), 4.45 (t, *J* = 5.3 Hz, 1H), 4.24 (d, *J* = 5.3 Hz, 2H), 2.92 (d, *J* = 5.1 Hz, 2H), 2.17 (t, *J* = 5.1 Hz, 2H), 1.47 (s, 9H). $[\alpha]_D^{20}$ −42° (*c* 0.30, $CHCl_3/CH_3OH$, 1:1, v/v). Anal. Calcd for $C_{28}H_{32}N_4O_6$ C 64.60, H 6.20, N 10.76. Found C 64.78, H 6.29, N 10.57.

**4.2.19. *N*-[(3*S*)-*N*-Boc-1,2,3,4-tetrahydro-β-carboline-3-carboxyl]-L-leucine benzylester (3s).** Yield: 92%. Mp 140–142 °C; ESI/MS (*m*/*z*) 520 $[M+H]^+$. IR (KBr): 3440, 3207, 3005, 2951, 2842, 1735, 1644, 1603, 1452, 1393, 1370, 1060, 904 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 9.95 (s, 1H), 8.06 (s, 1H), 7.27 (t, *J* = 7.3 Hz, 1H), 7.24 (t, *J* = 7.1 Hz, 2H), 7.21 (t, *J* = 7.3 Hz, 1H), 7.15 (d, *J* = 7.1 Hz, 2H), 7.11 (t, *J* = 7.1 Hz, 1H), 7.06 (d, *J* = 7.3 Hz, 1H), 6.82 (d, *J* = 7.3 Hz, 1H), 5.33 (s, 2H), 4.93 (t, *J* = 5.2 Hz, 1H), 4.41 (t, *J* = 5.2 Hz, 1H), 4.26 (dd, *J* = 10.0 Hz, *J* = 4.5 Hz, 1H), 4.09 (dd, *J* = 10.0 Hz, *J* = 3.8 Hz, 1H), 2.91 (d, *J* = 6.2 Hz, 2H), 2.83 (d, *J* = 5.1 Hz, 2H), 1.53 (s, 9H), 1.34 (m, *J* = 5.1 Hz, 1H), 1.07 (d, *J* = 5.3 Hz, 6H). $[\alpha]_D^{20}$ −33° (*c* 0.31, $CHCl_3/CH_3OH$, 1:1, v/v). Anal. Calcd for $C_{30}H_{37}N_3O_5$ C 69.34, H 7.18, N 8.09. Found C 69.25, H 7.08, N 8.26.

**4.2.20. *N*-[(3*S*)-*N*-Boc-1,2,3,4-tetrahydro-β-carboline-3-carboxyl]-L-isoleucine benzylester (3t).** Yield: 90%. Mp 134–136 °C; ESI/MS (*m*/*z*) 520 $[M+H]^+$. IR (KBr): 3445, 3339, 3210, 3010, 2955, 2840, 1758, 1732, 1645, 1600, 1452, 1390, 1371, 1064, 903 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 9.96 (s, 1H), 8.00 (s, 1H), 7.27 (t, *J* = 7.1 Hz, 1H), 7.22 (t, *J* = 7.0 Hz, 2H), 7.20 (t, *J* = 7.1 Hz, 1H), 7.00 (d, *J* = 7.1 Hz, 1H), 7.13 (d, *J* = 7.0 Hz, 2H), 7.10 (t, *J* = 7.0 Hz, 1H), 6.88 (d, *J* = 7.1 Hz, 1H), 5.35 (s, 2H), 4.95 (t, *J* = 5.0 Hz, 1H), 4.42 (t, *J* = 5.0 Hz, 1H), 4.25 (dd, *J* = 10.2 Hz, *J* = 4.0 Hz, 1H), 4.05 (dd, *J* = 10.2 Hz, *J* = 3.5 Hz, 1H), 2.94 (d, *J* = 6.0 Hz, 2H), 2.91 (m, *J* = 5.1 Hz, 1H), 1.47 (s, 9H), 1.30 (m, *J* = 5.2 Hz, 2H), 1.05 (d, *J* = 5.2 Hz, 3H), 0.95 (t, *J* = 5.2 Hz, 3H). $[\alpha]_D^{20}$ −36° (*c* 0.37, $CHCl_3/CH_3OH$, 1:1, v/v). Anal. Calcd for $C_{30}H_{37}N_3O_5$ C 69.34, H 7.18, N 8.09. Found C 69.50, H 7.29, N 8.24.

### 4.3. General procedure for the preparation of hexahydropyrazino[1′,2′:1,6]pyrido-[3,4-*b*]indole-1,4-diones 5a–i,l–o, q–t and 5′j,kp from 3a–t

At 0 °C 2.0 mmol of **3a–t** was dissolved in 10 ml of hydrogen chloride/ethyl acetate (4 mol/L) and stirred for 20 min. Then the reaction mixture was stirred at room temperature for 50 min and TLC(ethyl acetate/petroleum, 5:10) indicated the completion of removing Boc. The reaction mixture was evaporated under vacuum. The residue was diluted in 10 ml of ethyl acetate and then evaporated to dryness, which was repeated for three times to thoroughly remove the free hydrogen chloride. The residue was dissolved in 10 ml of methanol and treated with 1.0 ml of triethylamine. The reaction mixture was stirred at room temperature for 20h and TLC(ethyl acetate/petroleum, 5:10) indicated the completion of reaction. The reaction mixture was evaporated under vacuum and the residue was purified by column of silica gel (ethyl acetate/petroleum, 5:12) to give the title compound as colorless powder.

**4.3.1. (3*S*,12a*S*)-3-Methyl-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido[3,4-*b*]-indole-1,4-dion (5a).** Yield; 91%. Mp 233–235 °C, ESI-MS (*m*/*z*) 270 $[M+H]^+$, IR(KBr): 3337, 2926, 1678, 1455, 1326, 744 $cm^{-1}$; $^1H$ NMR (DMSO-$d^6$, 300 MHz): $\delta$ = 10.97 (s,1H), 8.46 (d, *J* = 2.1Hz, 1H), 7.46 (d, *J* = 7.5 Hz, 1H), 7.35 (t, *J* = 7.5 Hz, 1H), 7.07 (t, *J* = 7.5 Hz, 1H), 6.96 (t, *J* = 7.5 Hz, 1H), 5.33 (d, *J* = 16.8 Hz, lH), 4.73 (m, *J* = 6.9 Hz, 1H), 4.28 (dd, *J* = 4.2 Hz, *J* = 11.7 Hz, 1H), 4.20 (d, *J* = 16.5 Hz, 1H), 3.26 (dd, *J* = 4.2 Hz, *J* = 15.0 Hz, 1H), 2.80 (t, *J* = 4.8 Hz, 1H), 1.35 (d, *J* = 6.9 Hz, 3H). $^{13}C$ NMR (DMSO-$d_6$, 300 MHz) $\delta$ = 167.21, 166.82, 136.02, 134.55, 131.00, 126.26, 121.36, 120.12, 119.15, 111.09, 59.80, 52.98, 42.87, 24.75, 17.31. $[\alpha]_D^{20}$ −150° (*c* 1.0, $CH_3OH$); Anal. Calcd for $C_{15}H_{15}N_3O_2$ C 66.90, H 5.61, N 15.60. Found C 66.70, H 5.41, N 15.81.

**4.3.2. (3*S*,12a*S*)-3-Benzyl-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido[3,4-*b*]-indole-1,4-dione (5b).** Yield: 90%. Mp 242–245 °C; EI-MS (*m*/*z*) 346 $[M+H]^+$; IR(KBr): 3328, 2936, 1669, 1455, 1328, 744 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$, 300MHz) $\delta$ = 10.81 (s,1H), 8.46 (d, *J* = 1.8 Hz, 1H), 7.42 (d, *J* = 7.5 Hz, 1H), 7.31 (t, *J* = 7.5 Hz, 1H), 7.22 (t, *J* = 7.2 Hz, 2H), 7.14 (d, *J* = 7.2 Hz, 2H), 7.06 (t, *J* = 7.2 Hz, 1H), 7.02 (t, *J* = 7.5 Hz, 1H), 6.93 (t, *J* = 7.5 Hz, 1H), 5.32 (d, *J* = 16.5 Hz, 1H), 4.37 (s, 2H), 4.07 (d, *J* = 16.8 Hz, 1H), 3.98 (dd, *J* = 11.7 Hz, *J* = 4.5 Hz, 1H), 3.17 (dd, *J* = 13.2 Hz, *J* = 3.3 Hz, 1H), 2.88 (dd, *J* = 13.5 Hz, *J* = 5.1 Hz, 1H), 2.64 (dd, *J* = 14.7 Hz, *J* = 3.6 Hz, 1H); $[\alpha]_D^{20}$ −53° (*c* 1.0, $CH_3OH$); Anal. Calcd for $C_{21}H_{19}N_3O_2$ C 73.03, H 5.54, N 12.17. Found C 73.30, H 5.76, N 12.01.

**4.3.3. (3*S*,12a*S*)-3-(Isopropyl)-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]-pyrido[3,4-*b*]-indole-1,4-dione (5c).** Yield: 92%. Mp 216–218 °C, ESI-MS (m/z) 298 $[M+H]^+$; IR(KBr): 3331, 2939, 1662, 1457, 1384, 1365, 746 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$, 300 MHz): $\delta$ = 10.82 (s, 1H), 8.60 (d, *J* = 2.7 Hz, 1H), 7.36 (d, *J* = 7.1 Hz, 1H), 7.25 (t, *J* = 7.1 Hz, 1H), 7.02 (t, *J* = 7.1 Hz, 1H), 6.93 (t, *J* = 7.1 Hz, 1H), 5.22 (d, *J* = 15.2 Hz, 1H), 4.21 (dd, *J* = 11.3 Hz, *J* = 4.2 Hz, 1H), 4.19 (d, *J* = 17.0 Hz, 1H), 3.27 (dd, *J* = 14.6 Hz, *J* = 3.7 Hz, 1H), 2.99 (m, *J* = 13.3 Hz, 1H), 2.89 (t, *J* = 13.3 Hz, 1H), 2.74 (m, *J* = 6.2 Hz, 1H), 1.07 (d, *J* = 6.2 Hz, 6H). $^{13}C$ NMR

(DMSO-$d_6$, 300 MHz) $\delta$ = 169.05, 168.48, 135.09, 133.71, 130.93, 122.59, 121.47, 118.99, 113.44, 111.27, 66.99, 63.10, 41.72, 28.75, 24.38, 16.82. $[\alpha]_D^{20}$ − 84° (*c* 1.0, $CH_3OH$). Anal. Calcd for $C_{17}H_{19}N_3O_2$ C 68.67, H 6.44, N 14.13. Found C 68.50, H 6.27, N 14.00.

**4.3.4. (3*S*,12a*S*)-3-Hydroxymethyl-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido-[3,4-*b*]indole-1,4-dione(5d).** Yield: 90%. Mp 264–267 °C, ESI-MS (*m*/*z*) 286 $[M+H]^+$, IR(KBr): 3344, 2926, 1683, 1642, 1463, 1334, 744cm$^{-1}$; $^1$H NMR (DMSO-$d_6$, 300MHz) $\delta$ = 10.92 (s,1H), 8.24 (d, *J* = 2.4 Hz, 1H), 7.43 (d, *J* = 7.5 Hz, 1H), 7.32 (t, *J* = 7.5 Hz, 1H), 7.04 (t, *J* = 7.5 Hz, 1H), 6.93 (t, *J* = 7.5 Hz, 1H), 5.42 (d, *J* = 16.5 Hz, 1H), 5.23 (t, *J* = 4.8 Hz, 1H), 4.25 (dd, *J* = 11.4 Hz, *J* = 4.2 Hz, 1H), 4.15 (d, *J* = 16.5 Hz, 1H), 4.05 (d, *J* = 4.8 Hz, 1H), 3.94 (s, 1H), 3.17 (dd, *J* = 15.0 Hz, *J* = 3.6 Hz, 1H), 2.98 (t, *J* = 13.5 Hz, 2H); $^{13}$C NMR (DMSO-$d_6$, 300 MHz) $\delta$ = 166.88, 163.82, 135.86, 129.77, 126.33, 120.86, 118.56, 117.46, 110.96, 105.82, 62.66, 57.37, 55.82, 27.00. $[\alpha]_D^{20}$ − 141° (*c* 1.0, $CH_3OH$). Anal. Calcd for $C_{15}H_{15}N_3O_3$ C 63.15, H 5.30, N 14.73. Found C 63.48, H 5.52, N 14.54.

**4.3.5. (3*S*,12a*S*)-3-(1′-Hydroxyeth-1′-yl)-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]-pyrido[3,4-*b*]indole-1,4-dione(5e).** Yield: 89%. Mp 242–244 °C; ESI/MS (*m*/*z*) 300 $[M+H]^+$; IR (KBr): 3340, 2929, 1686, 1642, 1465, 1333, 744 cm$^{-1}$; $^1$H NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 10.00 (s, 1H), 7.98 (s, 1H), 7.29 (t, *J* = 7.1 Hz, 1H), 7.25 (t, *J* = 7.1 Hz, 1H), 7.09 (t, *J* = 7.1 Hz, 1H), 6.97 (d, *J* = 7.1 Hz, 1H), 4.81 (t, *J* = 5.3 Hz, 1H), 4.63 (m, *J* = 5.2 Hz, 1H), 4.45 (t, *J* = 5.4 Hz, 2H), 4.22 (m, *J* = 5.6 Hz, 1H), 2.90 (d, *J* = 5.4 Hz, 2H), 2.15 (s, 1H), 1.22 (d, *J* = 5.6 Hz, 3H). $[\alpha]_D^{20}$ − 108.5° (*c* 1.0, $CH_3OH$); Anal. Calcd for $C_{16}H_{17}N_3O_3$ C 64.20, H 5.72, N 14.04. Found C 64.32, H 5.80, N 14.22.

**4.3.6. (3*S*,12a*S*)-3-(*p*-Hydroxyphenylmethyl)-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]-pyrido[3,4-*b*]indole-1,4-dione (5f).** Yield: 88%. Mp 249–251 °C; ESI/MS (*m*/*z*) 362 $[M+H]^+$; IR (KBr): 3342, 2936, 1682, 1643, 1464, 1333, 743 cm$^{-1}$; $^1$H NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 10.02 (s, 1H), 8.01 (s, 1H), 7.31 (t, *J* = 7.0 Hz, 1H), 7.23 (t, *J* = 7.2 Hz, 1H), 7.16 (d, *J* = 7.0 Hz, 2H), 7.13 (d, *J* = 7.0 Hz, 2H), 7.01 (t, *J* = 7.2 Hz, 1H), 6.89 (d, *J* = 7.0 Hz, 1H), 4.97 (s, 1H), 4.85 (d, *J* = 5.2 Hz, 1H), 4.78 (t, *J* = 5.4 Hz, 1H), 4.21 (m, *J* = 5.2 Hz, 2H), 3.13 (d, *J* = 5.2 Hz, 2H), 2.90 (d, *J* = 5.4 Hz, 2H). $[\alpha]_D^{20}$ − 122.3° (*c* 1.0, $CH_3OH$); Anal. Calcd for $C_{21}H_{19}N_3O_3$ C 69.79, H 5.30, N 11.63. Found C 69.61, H 5.41, N 11.47.

**4.3.7. (5a*S*,14a*S*)-1,2,3,5,5a,6,11,12,14,14a-Decahydropyrrolo[1″,2″: 4′5′]pyrazino[1′,2′:1,6]-pyrido[3,4-*b*]indole-1,4-dione(5g).** Yield: 89%. Mp 233–235 °C; ESI/MS (*m*/*z*) 296 $[M+H]^+$; IR (KBr): 3344, 2934, 1684, 1643, 1460, 1332, 743 cm$^{-1}$; $^1$H NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 10.03 (s, 1H), 7.21 (t, *J* = 7.2 Hz, 1H), 7.15 (t, *J* = 7.2 Hz, 1H), 7.05 (t, *J* = 7.2 Hz, 1H), 6.95 (d, *J* = 7.2 Hz, 1H), 4.86 (t, *J* = 5.5 Hz, 1H), 4.33 (t, *J* = 5.4 Hz, 1H), 4.24 (d, *J* = 5.5 Hz, 2H), 3.45 (t, *J* = 5.4 Hz, 2H), 2.93 (d, *J* = 5.3 Hz, 2H), 2.25 (d, *J* = 5.4 Hz, 2H), 1.95 (t, *J* = 5.4 Hz, 2H). $[\alpha]_D^{20}$ − 89.1° (*c* 1.0, $CH_3OH$). Anal. Calcd for $C_{17}H_{17}N_3O_2$ C 69.14, H 5.80, N 14.23. Found C 69.01, H 5.70, N 14.14.

**4.3.8. (3*S*,12a*S*)-3-Mercaptomethyl-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido-[3,4-*b*]indole-1,4-dione (5h).** Yield: 91%. Mp 237–239 °C; ESI/MS (*m*/*z*) 302 $[M+H]^+$; IR (KBr): 3343, 2936, 1647, 1641, 1459, 1333, 741cm$^{-1}$; $^1$H NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 10.03 (s, 1H), 8.00 (s, 1H), 7.28 (t, *J* = 7.0 Hz, 1H), 7.15 (d, *J* = 7.0 Hz, 1H), 7.08 (d, *J* = 7.0 Hz, 1H), 6.89 (d, *J* = 7.0 Hz, 1H), 4.93 (t, *J* = 5.4 Hz, 1H), 4.79 (t, *J* = 5.6 Hz, 1H), 4.29 (d, *J* = 5.2 Hz, 2H), 2.92 (d, *J* = 5.4 Hz, 2H), 3.07 (d, *J* = 5.6 Hz, 2H), 1.65 (s, 1H). $[\alpha]_D^{20}$ − 48.7° (*c* 1.00, $CH_3OH$); Anal. Calcd for $C_{15}H_{15}N_3O_2S$ C 59.78, H 5.02, N 13.94. Found C 59.92, H 5.13, N 13.76.

**4.3.9. (3*S*,12a*S*)-3-Methylmercaptoethyl-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]-pyrido[3,4-*b*]indole-1,4-dione (5i).** Yield: 90%. Mp 216–217 °C; ESI/MS (*m*/*z*) 330 $[M+H]^+$; IR (KBr): 3341, 2934, 1648, 1643, 1458, 1332, 742 cm$^{-1}$; $^1$H NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 10.00 (s, 1H), 7.99 (s, 1H), 7.27 (t, *J* = 7.0 Hz, 1H), 7.19 (t, *J* = 7.0 Hz, 1H), 7.08 (t, *J* = 7.0 Hz, 1H), 6.87 (d, *J* = 7.0 Hz, 1H), 4.87 (t, *J* = 5.4 Hz, 1H), 4.49 (t, *J* = 5.2 Hz, 1H), 4.29 (d, *J* = 5.3 Hz, 2H), 2.94 (d, *J* = 5.4 Hz, 2H), 2.43 (t, *J* = 5.3 Hz, 2H), 2.17 (d, *J* = 5.3 Hz, 2H), 2.10 (s, 3H). $[\alpha]_D^{20}$ − 65.3° (*c* 1.0, $CH_3OH$). Anal. Calcd for $C_{17}H_{19}N_3O_2S$ C 61.98, H 5.81, N 12.76. Found C 62.14, H 5.89, N 12.90.

**4.3.10. (3*S*,12a*S*)-3-Benzyloxycarbonylethyl-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]-pyrido[3,4-*b*]indole-1,4-dione (5′j).** Yield: 92%. Mp 236–238 °C; ESI/MS (*m*/*z*) 417 $[M+H]^+$; IR (KBr): 3342, 3000, 2940, 1675, 1602, 1586, 1457, 1333, 750 cm$^{-1}$; $^1$H NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 9.99 (s, 1H), 8.00 (s, 1H), 7.30 (t, *J* = 7.1 Hz, 1H), 7.22 (t, *J* = 7.1 Hz, 1H), 7.18 (t, *J* = 7.0 Hz, 2H), 7.13 (d, *J* = 7.0 Hz, 2H), 7.08 (t, *J* = 7.0 Hz, 1H), 7.06 (t, *J* = 7.0 Hz, 1H), 7.01 (d, *J* = 7.2 Hz, 1H), 6.85 (d, *J* = 7.2 Hz, 1H), 5.33 (s, 2H), 4.92 (d, *J* = 5.0 Hz, 1H), 4.43 (t, *J* = 5.2 Hz, 1H), 4.21 (d, *J* = 5.1 Hz, 2H), 2.94 (d, *J* = 5.2 Hz, 2H), 2.25 (t, *J* = 5.5 Hz, 2H), 2.22 (t, *J* = 5.5 Hz, 2H). $[\alpha]_D^{20}$ − 109.4° (*c* 1.0, $CH_3OH$); Anal. Calcd for $C_{24}H_{23}N_3O_4$ C 69.05, H 5.55, N 10.07. Found C 68.90, H 5.62, N 10.21.

**4.3.11. (3*S*,12a*S*)-3-Benzyloxycarbonylmethyl-2,3,6,7,12,12a-hexahydro pyrazino[1′,2′:1,6]-pyrido[3,4-*b*]indole-1,4-dione (5′k).** Yield: 90%. Mp 255–257 °C, ESI-MS (*m*/*z*) 404 $[M+H]^+$; (KBr): 3346, 2923, 1678, 1600, 1589, 1507, 1460, 1335, 747 cm$^{-1}$; $^1$H NMR (DMSO-$d_6$, 300 MHz) $\delta$ = 9.98 (s,1H), 8.20 (s,1H), 7.30 (d, *J* = 7.4 Hz, 1H), 7.23 (t, *J* = 7.4 Hz, 1H), 7.20 (t, *J* = 7.2 Hz, 2H), 7.13 (t, *J* = 7.2 Hz, 2H), 7.10 (t, *J* = 7.2 Hz, 1H), 7.03 (t, *J* = 7.4 Hz, 1H), 6.92 (t, *J* = 7.3 Hz, 1H), 5.35 (d, *J* = 16.6 Hz, 1H), 5.33 (s, 2H), 4.22 (dd, *J* = 11.0 Hz, *J* = 3.6 Hz, 1H), 4.17 (d, *J* = 16.5 Hz, 1H), 4.12 (d, *J* = 14.3 Hz, 1H), 3.16 (dd, *J* = 14.0 Hz, *J* = 3.6 Hz, 1H), 2.96 (t, *J* = 13.0 Hz, 1H), 2.81 (d, *J* = 10.0 Hz, 2H). $[\alpha]_D^{20}$ − 70° (*c* 1.0, $CH_3OH$); Anal. Calcd for

$C_{23}H_{21}N_3O_4$ C 68.47, H 5.25, N 10.42. Found C 68.35, H 5.09, N 10.27.

**4.3.12. (3*S*,12a*S*)-3-(1′,3′-Imidazol-5′-methylene)-2,3,6,7,12,12a-hexahydropyrazino-[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4-dione (5l).** Yield: 91%. Mp 207–209 °C; ESI/MS (*m*/*z*) 336 $[M+H]^+$; IR (KBr): 3344, 2925, 1677, 1601, 1587, 1505, 1458, 1333, 751 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 12.99 (s, 1H), 10.03 (s, 1H), 7.98 (s, 1H), 7.45 (s, 1H), 7.33 (t, *J* = 7.0 Hz, 1H), 7.25 (t, *J* = 7.0 Hz, 1H), 7.09 (t, *J* = 7.0 Hz, 1H), 6.99 (t, *J* = 7.0 Hz, 1H), 6.87 (s, 1H), 4.92 (t, *J* = 5.2 Hz, 1H), 4.83 (t, *J* = 5.5 Hz, 1H), 4.27 (d, *J* = 5.5 Hz, 2H), 3.20 (d, *J* = 5.5 Hz, 2H), 2.92 (d, *J* = 5.1 Hz, 2H). $[\alpha]_D^{20}$ − 88.3° (*c* 1.0, $CH_3OH$); Anal. Calcd for $C_{18}H_{17}N_5O_2$ C 64.47, H 5.11, N 20.88. Found C 64.62, H 5.20, N 21.03.

**4.3.13. (3*S*,12a*S*)-3-Indolymethyl-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6] pyrido[3,4-*b*]-indole-1,4-dione (5m).** Yield: 87%. Mp 176–180 °C; ESI-MS (*m*/*z*) 385 $[M+H]^+$; IR(KBr): 3329, 2938, 1683, 1465, 1338, 746 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$, 300 MHz): $\delta$ = 10.76 (s, 1H), 10.74 (s, 1H), 8.43 (d,1H, *J* = 1.8 Hz, 1H), 7.50 (d, *J* = 7.5 Hz, 1H), 7.28 (t, *J* = 7.5 Hz, 1H), 7.26 (t, *J* = 7.2 Hz, 1H), 7.24 (t, *J* = 7.2 Hz, 1H), 7.22 (d, *J* = 7.2 Hz, 1H), 7.20 (t, *J* = 7.2 Hz, 1H), 7.08 (t, *J* = 7.5 Hz, 1H), 6.95 (t,1H, *J* = 7.5 Hz, 1H), 6.82 (s, 1H), 5.27 (d, *J* = 16.2 Hz, 1H), 4.31 (d, *J* = 2.4 Hz, 1H), 4.05 (d, *J* = 16.5 Hz, 1H), 3.95 (dd, *J* = 12.0 Hz, *J* = 4.5 Hz, 1H), 3.28 (dd, *J* = 14.1 Hz, *J* = 3.6, Hz, 1H), 3.07 (dd, *J* = 14.1 Hz, *J* = 4.2 Hz, 1H), 2.93 (d, *J* = 3.5 Hz, 2H); $^{13}C$ NMR (DMSO-$d_6$, 300MHz) $\delta$ = 165.65, 164.64, 135.80, 135.71, 129.11, 127.62, 126.19, 124.00, 120.58, 118.45, 118.36, 118.10, 117.25, 110.81, 108.02, 105.42, 79.06, 55.77, 55.36, 30.17,25.6; $[\alpha]_D^{20}$ − 182° (*c* 0.34, $CHCl_3/CH_3OH$, 1:1, v/v). Anal. Calcd for $C_{23}H_{20}N_4O_2$ C 71.86, H 5.24, N 14.57. Found C 72.04, H 5.56, N 14.33.

**4.3.14. (3*S*,12a*S*)-3-(3′-Guanidinopropyl)-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido-[3,4-*b*]indole-1,4-dione (5n).** Yield: 90%. Mp 240–242 °C; ESI/MS (*m*/*z*) 355 $[M+H]^+$; IR (KBr): 3339, 2941, 1680, 1456, 1342, 743 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 10.03 (s, 1H), 8.44 (s, 2H), 8.20 (s, 1H), 8.16 (s, 1H), 8.01 (s, 1H), 7.23 (t, *J* = 7.0 Hz, 1H), 7.15 (t, *J* = 7.0 Hz, 1H), 7.02 (d, *J* = 7.0 Hz, 1H), 6.95 (d, *J* = 7.0 Hz, 1H), 4.92 (d, *J* = 5.2 Hz, 1H), 4.35 (t, *J* = 4.6 Hz, 1H), 4.23 (d, *J* = 5.2 Hz, 2H), 2.91 (d, *J* = 4.7 Hz, 2H), 2.66 (t, *J* = 5.2 Hz, 2H), 1.95 (m, *J* = 5.2 Hz, 2H), 1.56 (m, *J* = 5.2 Hz, 2H). $[\alpha]_D^{20}$ − 90.6° (*c* 1.0, $CH_3OH$); Anal. Calcd for $C_{18}H_{22}N_6O_2$ C 61.00, H 6.26, N 23.71. Found C 61.18, H 6.33, N 23.53.

**4.3.15. (12a*S*)-2,3,6,7,12,12a-Hexahydropyrazino[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4-dione (5o).** Yield: 92%. Mp 247–249 °C; ESI-MS (*m*/*z*) 256 $[M+H]^+$; IR (KBr) : 3307, 2986, 1646, 1455, 1328, 748 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$, 300 MHz): $\delta$ = 10.94 (s,1 H), 8.26 (s, 1H), 7.43 (d, *J* = 7.5 Hz, 1H), 7.33 (t, *J* = 7.5 Hz, 1H), 7.08 (t, *J* = 7.5 Hz, 1H), 6.96 (t, *J* = 7.5 Hz, 1H), 5.36 (d, *J* = 16.5 Hz, 1H), 4.22 (m, *J* = 13.0 Hz, 2H), 4.05 (d, *J* = 17.7 Hz, 1H), 3.86 (d, *J* = 17.7 Hz, 1H), 3.20 (m, *J* = 13.5 Hz, 1H), 2.88 (t, *J* = 13.5 Hz, 1H). $^{13}C$ NMR (DMSO-$d_6$, 300 MHz) $\delta$ = 168.87, 167.66, 136.11, 134.02, 130.34, 124.63, 121.55, 120.21, 119.44, 111.22, 59.75, 52.76, 42.80, 24.77. $[\alpha]_D^{20}$ − 135 (*c* 1.0, $CH_3OH$). Anal. Calcd for $C_{14}H_{13}N_3O_2$ C 65.87, H 5.13, N 16.46. Found C 65.65, H 5.01, N 16.67.

**4.3.16. (3*S*,12a*S*)-3-(4′-Benzyloxycarbonylaminobutyl)-2,3,6,7,12,12a-hexahydro pyrazino-[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4-dione (5′p).** Yield: 90% Mp 212–214 °C; ESI/MS (*m*/*z*) 461 $[M+H]^+$. IR (KBr): 3445, 3308, 2985, 1601, 1450, 1064, 900 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 10.03 (s, 1H), 8.00 (s, 1H), 7.99 (s, 1H), 7.27 (t, *J* = 7.2 Hz, 1H), 7.21 (t, *J* = 7.1 Hz, 2H), 7.19 (t, *J* = 7.1 Hz, 1H), 7.17 (t, *J* = 7.2 Hz, 1H), 7.16 (d, *J* = 7.1 Hz, 2H), 7.07 (t, *J* = 7.2 Hz, 1H), 6.89 (d, *J* = 7.2 Hz, 1H), 5.32 (s, 2H), 4.93 (d, *J* = 5.2 Hz, 1H), 4.47 (t, *J* = 4.5 Hz, 1H), 4.29 (dd, *J* = 10.0 Hz, *J* = 4.2 Hz, 1H), 4.19 (dd, *J* = 10.0 Hz, *J* = 3.8 Hz, 1H), 2.98 (t, *J* = 4.7 Hz, 2H), 2.95 (d, *J* = 5.2 Hz, 2H), 1.91 (m, *J* = 4.7 Hz, 2H), 1.55 (m, *J* = 4.7 Hz, 2H), 1.29 (m, *J* = 4.7 Hz, 2H). $[\alpha]_D^{20}$ − 44° (*c* 1.0, $CH_3OH$). Anal. Calcd for $C_{26}H_{28}N_4O_4$ C 67.81, H 6.13, N 12.17. Found C 67.96, H 6.21, N 12.33.

**4.3.17. (3*S*,12a*S*)-3-(Propionamide-2′-yl)-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido-[3,4-*b*]indole-1,4-dione (5q).** Yield: 92%. Mp 251–253 °C; ESI/MS (*m*/*z*) 327 $[M+H]^+$. IR (KBr): 3344, 2939, 1684, 1465, 1332, 746 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 10.01 (s, 1H), 7.98 (s, 1H), 7.27 (t, *J* = 7.2 Hz, 1H), 7.19 (t, *J* = 7.2 Hz, 1H), 7.03 (d, *J* = 7.2 Hz, 1H), 6.85 (d, *J* = 7.2 Hz, 1H), 6.11 (s, 2H), 4.93 (d, *J* = 5.5 Hz, 1H), 4.44 (t, *J* = 5.4 Hz, 1H), 4.27 (d, *J* = 5.5 Hz, 2H), 2.92 (d, *J* = 5.2 Hz, 2H), 2.17 (t, *J* = 5.4 Hz, 2H), 2.11 (t, *J* = 5.4 Hz, 2H). $[\alpha]_D^{20}$ − 62° (*c* 1.0, $CH_3OH$); Anal. Calcd for $C_{17}H_{18}N_4O_3$ C 62.57, H 5.56, N 17.17. Found C 62.71, H 5.64, N 17.35.

**4.3.18. (3*S*,12a*S*)-3-(Acetylamine-1′-yl)-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido-[3,4-*b*]indole-1,4-dione (5r).** Yield: 91%. Mp 247–249 °C; ESI/MS (*m*/*z*) 313 $[M+H]^+$. IR (KBr): 3341, 2942, 1681, 1463, 1336, 741 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 10.03 (s, 1H), 8.00 (s, 1H), 7.27 (t, *J* = 7.2 Hz, 1H), 7.19 (t, *J* = 7.2 Hz, 1H), 7.05 (d, *J* = 7.2 Hz, 1H), 6.87 (d, *J* = 7.2 Hz, 1H), 6.06 (s, 2H), 4.93 (d, *J* = 5.3 Hz, 1H), 4.45 (t, *J* = 5.2 Hz, 1H), 4.23 (d, *J* = 5.3 Hz, 2H), 2.92 (d, *J* = 5.4 Hz, 2H), 2.65 (t, *J* = 5.3 Hz, 2H). $[\alpha]_D^{20}$ − 45.2° (*c* 1.0, $CH_3OH$). Anal. Calcd for $C_{16}H_{16}N_4O_3$ C 61.53, H 5.16, N 17.94. Found C 61.38, H 5.08, N 17.76.

**4.3.19. (3*S*,12a*S*)-3-(2′-Methylpropyl)-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido-[3,4-*b*]indole-1,4-dione (5s).** Yield: 89%. Mp 231–233 °C; ESI/MS (*m*/*z*) 312 $[M+H]^+$. IR (KBr): 3343, 2943, 1682, 1465, 1335, 743 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 10.02 (s, 1H), 7.98 (s, 1H), 7.27 (t, *J* = 7.2 Hz, 1H), 7.19 (t, *J* = 7.2 Hz, 1H), 7.03 (d, *J* = 7.2 Hz, 1H), 6.85 (d, *J* = 7.2 Hz, 1H), 4.93 (t, *J* = 5.3 Hz, 1H), 4.45 (t, *J* = 5.2 Hz, 1H), 4.26 (dd, *J* = 10.0 Hz, *J* = 4.4 Hz, 1H), 4.15 (dd, *J* = 10.0 Hz, *J* = 3.9 Hz, 1H), 2.92 (d,

$J$ = 6.2 Hz, 2H), 2.65 (t, $J$ = 5.3 Hz, 2H), 1.83 (m, $J$ = 5.3 Hz, 1H), 1.07 (d, $J$ = 5.3 Hz, 6H). $[\alpha]_D^{20}$ − 52° (*c* 1.0, $CH_3OH$). Anal. Calcd for $C_{18}H_{21}N_3O_2$ C 69.43, H 6.80, N 13.49. Found C 69.60, H 6.71, N 13.66.

**4.3.20. (3*S*,12a*S*)-3-(1′-Methylpropyl)-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido-[3,4-*b*]indole-1,4-dione(5t).** Yield: 87%. Mp 219–220 °C, ESI-MS (*m*/*z*) 312 $[M+H]^+$; IR(KBr): 3328, 2936, 1669, 1455, 1388, 1369, 744 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$, 300 MHz): $\delta$ = 11.12 (s,1H), 8.64 (d, $J$ = 2.1 Hz, 1H), 7.40 (d, $J$ = 7.3 Hz, 1H), 7.30 (t, $J$ = 7.3 Hz, 1H), 7.00 (t, $J$ = 7.3 Hz, 1H), 6.95 (t, $J$ = 7.3 Hz, 1H), 5.30 (d, $J$ = 16.0 Hz, 1H), 4.25 (dd, $J$ = 11.0 Hz, $J$ = 4.0 Hz, 1H), 4.17 (d, $J$ = 17.3 Hz, 1H), 3.22 (dd, $J$ = 15.0 Hz, $J$ = 3.3 Hz, 1H), 2.96 (m, $J$ = 13.0 Hz, 1H), 2.83 (t, $J$ = 13.0 Hz, 1H), 2.61 (m, $J$ = 6.0 Hz, 1H), 1.35 (m, $J$ = 6.0 Hz, 2H), 1.07 (d, $J$ = 6.0 Hz, 3H), 0.97 (t, $J$ = 6.0 Hz, 6H). $^{13}C$ NMR (DMSO-$d_6$, 300 MHz) $\delta$ = 167.00, 166.48, 136.13, 130.37, 126.51, 121.44, 118.96, 117.40, 111.20, 105.81, 56.30, 53.11, 46.12, 26.75, 34.34, 23.30, 14.82, 11.77. $[\alpha]_D^{20}$ − 73.7° (*c* 1.0, $CH_3OH$); Anal. Calcd for $C_{18}H_{21}N_3O_2$ C 9.43, H 6.80, N 13.49. Found C 69.22, H 6.61, N 13.68.

### 4.4. General procedure for the preparation of (3*S*)-*N*-(Boc-aminoacyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid benzylester (8a–t)

At 0 °C to the stirring solution of 2.50 mmol of Boc-AA-OH, 350 mg(2.59 mmol) of HOBt, and 600 mg (2.90 mmol) of DCC in 15 ml of anhydrous dichloromethane, 664 mg (2.17 mmol) of 3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid benzylester (**7**) was added. After stirring at 0 °C for 30 min the reaction mixture was stirred at room temperature for 30 min and TLC (ethyl acetate/petroleum, 5:12) indicated the completion of the reaction. The formed precipitates of DCU were filtered and the filtrate was evaporated. The residue was dissolved in 30 ml of ethyl acetate and the solution was washed successively with saturated aqueous $NaHCO_3$ (30 ml × 3), saturated aqueous NaCl (30 ml × 3), and aqueous $KHSO_4$ (5%, 30 ml × 3). The separated ethyl acetate layer was dried with anhydrous $MgSO_4$, filtered, evaporated, and purified with flash chromatography ($CHCl_3/CH_3OH$, 30:1) to provide the title compound as powder.

**4.4.1. (3*S*)-*N*-(Boc-L-alanyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid benzylester (8a).** Yield: 94%. Mp 130–132 °C; ESI/MS (*m*/*z*) 478 $[M+H]^+$; IR (KBr): 3347, 3005, 2951, 2843, 1745, 1642, 1604, 1457, 1391, 1371, 1071, 902 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 9.97 (s,1H), 8.03 (s,1H), 7.24 (t, $J$ = 7.2 Hz, 1H), 7.20 (t, $J$ = 7.1 Hz, 2H), 7.13 (t, $J$ = 7.2 Hz, 1H), 7.10 (d, $J$ = 7.1 Hz, 2H), 7.07 (t, $J$ = 7.1 Hz, 1H), 6.97 (t, $J$ = 7.2 Hz, 1H), 6.93 (t, $J$ = 7.2 Hz, 1H), 5.33 (s, 2H), 4.75 (m, $J$ = 5.5 Hz, 1H), 4.66 (m, $J$ = 5.4 Hz, 1H), 3.83 (s, 2H), 3.64 (dd, $J$ = 10.1 Hz, $J$ = 5.2 Hz, 1H), 3.25 (dd, $J$ = 10.1 Hz, $J$ = 2.9 Hz, 1H), 1.49 (s, 9H), 1.45 (d, $J$ = 5.1 Hz, 3H). $[\alpha]_D^{20}$ − 1248° (*c* 0.35, $CHCl_3/CH_3OH$, 1:1, v/v). Anal. Calcd for $C_{27}H_{31}N_3O_5$ C 67.91, H 6.54, N 8.80. Found C 67.76, H 6.65, N 8.97.

**4.4.2. (3*S*)-*N*-(Boc-L-phenylalanyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid benzylester (8b).** Yield: 95%. Mp 129–131 °C; ESI/MS (*m*/*z*) 554 $[M+H]^+$; IR (KBr): 3345, 3014, 2942, 2833, 1751, 1647, 1603, 1452, 1389, 1062, 901 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 10.01 (s, 1H), 7.89 (s, 1H), 7.27 (t, $J$ = 7.3 Hz, 1H), 7.20 (t, $J$ = 7.5 Hz, 2H), 7.18 (t, $J$ = 7.1 Hz, 2H), 7.17 (t, $J$ = 7.3 Hz, 1H), 7.13 (d, $J$ = 7.6 Hz, 2H), 7.10 (d, $J$ = 7.1 Hz, 2H), 7.07 (t, $J$ = 7.1 Hz, 1H), 7.05 (t, $J$ = 7.6 Hz, 1H), 6.95 (t, $J$ = 7.2 Hz, 1H), 6.90 (t, $J$ = 7.3 Hz, 1H), 5.33 (s, 2H), 5.01 (t, $J$ = 5.3 Hz, 1H), 4.79 (t, $J$ = 5.4 Hz, 1H), 3.91 (s, 2H), 3.65 (dd, $J$ = 10.1 Hz, $J$ = 5.2 Hz, 1H), 3.23 (dd, $J$ = 10.1 Hz, $J$ = 2.9 Hz, 1H), 3.09 (d, $J$ = 5.3 Hz, 2H), 1.47 (s, 9H). $[\alpha]_D^{20}$ − 80° (*c* 0.35, $CHCl_3/CH_3OH$, 1:1, v/v). Anal. Calcd for $C_{33}H_{35}N_3O_5$ C 71.59, H 6.37, N 7.59. Found C 71.74, H 6.46, N 7.73.

**4.4.3. (3*S*)-*N*-(Boc-L-valinyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid benzylester (8c).** Yield: 89%. Mp 127–129 °C; ESI/MS (*m*/*z*) 506 $[M+H]^+$; IR (KBr): 3343, 3002, 2941, 2844, 1743, 1642, 1600, 1453, 1392, 1375, 1070, 901 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$) $\delta$ = 10.02 (s,1H), 8.03 (s,1H), 7.25 (t, $J$ = 7.2 Hz, 1H), 7.20 (t, $J$ = 7.0 Hz, 2H), 7.13 (t, $J$ = 7.2 Hz, 1H), 7.12 (d, $J$ = 7.0 Hz, 2H), 7.08 (t, $J$ = 7.0 Hz, 1H), 6.96 (t, $J$ = 7.2 Hz, 1H), 6.91 (t, $J$ = 7.2 Hz, 1H), 5.33 (s, 2H), 4.72 (t, $J$ = 5.7 Hz, 1H), 4.53 (d, $J$ = 5.1 Hz, 1H), 3.87 (s, 2H), 3.62 (dd, $J$ = 10.1 Hz, $J$ = 5.3 Hz, 1H), 3.23 (dd, $J$ = 10.1 Hz, $J$ = 3.3 Hz, 1H), 2.65 (m, $J$ = 5.1 Hz, 1H), 1.47 (s, 9H), 1.07 (d, $J$ = 5.3 Hz, 6H). $[\alpha]_D^{20}$ − 58° (*c* 0.39, $CHCl_3/CH_3OH$, 1:1, v/v). Anal. Calcd for $C_{29}H_{35}N_3O_5$ C 68.89, H 6.98, N 8.31. Found C 68.73, H 6.87, N 8.47.

**4.4.4. (3*S*)-*N*-(Boc-L-serinyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid benzylester (8d).** Yield: 90%. Mp 127–129 °C; ESI/MS (*m*/*z*) 494 $[M+H]^+$; IR (KBr): 3345, 3013, 2940, 2844, 1750, 1642, 1600, 1457, 1392, 1073, 902 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 10.01 (s, 1H), 8.03 (s, 1H), 7.25 (t, $J$ = 7.2 Hz, 1H), 7.21 (t, $J$ = 7.0 Hz, 2H), 7.15 (t, $J$ = 7.2 Hz, 1H), 7.11 (d, $J$ = 7.0 Hz, 2H), 7.08 (t, $J$ = 7.0 Hz, 1H), 7.01 (t, $J$ = 7.2 Hz, 1H), 6.90 (t, $J$ = 7.1 Hz, 1H), 5.35 (s, 2H), 4.73 (t, $J$ = 5.6 Hz, 1H), 4.60 (t, $J$ = 5.1 Hz, 1H), 4.05 (d, $J$ = 5.3 Hz, 1H), 3.90 (s, 2H), 3.62 (dd, $J$ = 10.1 Hz, $J$ = 5.3 Hz, 1H), 3.23 (dd, $J$ = 10.1 Hz, $J$ = 2.9 Hz, 1H), 3.10 (d, $J$ = 5.1 Hz, 2H), 1.45 (s, 9H). $[\alpha]_D^{20}$ − 51° (*c* 0.35, $CHCl_3/CH_3OH$, 1:1, v/v). Anal. Calcd for $C_{27}H_{31}N_3O_6$ C 65.71, H 6.33, N 8.51. Found C 65.50, H 6.25, N 8.68.

**4.4.5. (3*S*)-*N*-(Boc-L-threoninyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid benzylester (8e).** Yield: 91%. Mp 131–133 °C; ESI/MS (*m*/*z*) 508 $[M+H]^+$; IR (KBr): 3443, 3336, 3208, 3003, 2950, 2841, 1762, 1735, 1643, 1602, 1455, 1393, 1373, 1062, 904 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 10.02 (s, 1H), 7.87 (s, 1H), 7.27 (t, $J$ = 7.3 Hz, 1H), 7.23 (t, $J$ = 7.3 Hz, 1H), 7.17 (t, $J$ = 7.1 Hz, 2H), 7.13 (d, $J$ = 7.1 Hz, 2H), 7.05 (t, $J$ = 7.1 Hz, 1H), 6.99 (d, $J$ = 7.3 Hz, 1H), 6.87 (t, $J$ = 7.3 Hz, 1H), 5.30 (s, 2H), 4.83 (t, $J$ = 5.4 Hz, 1H), 4.61 (m, $J$ = 5.4 Hz, 1H), 4.43 (t, $J$ = 5.3 Hz, 1H), 3.97

(m, $J$ = 5.5 Hz, 2H), 2.92 (d, $J$ = 5.3 Hz, 2H), 2.17 (s, 1H), 1.46 (s, 9H), 1.20 (d, $J$ = 5.3 Hz, 3H). Anal. Calcd for $C_{28}H_{33}N_3O_6$ C 66.26, H 6.55, N 8.28. Found C 66.12, H 6.43, N 8.42.

**4.4.6. (3*S*)-*N*-(Boc-L-tyrosinyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid benzylester (8f).** Yield: 89%. Mp 133–135 °C; ESI/MS ($m/z$) 570 $[M+H]^+$; IR (KBr): 3442, 3206, 3003, 2954, 2840, 1734, 1642, 1603, 1452, 1391, 1373, 1062, 903 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 10.01 (s, 1H), 8.03 (s, 1H), 7.33 (t, $J$ = 7.2 Hz, 1H), 7.21 (t, $J$ = 7.4 Hz, 1H), 7.14 (d, $J$ = 7.2 Hz, 2H), 7.11 (t, $J$ = 7.2 Hz, 2H), 7.07 (d, $J$ = 7.2 Hz, 2H), 7.03 (t, $J$ = 7.2 Hz, 1H), 7.01 (d, $J$ = 7.2 Hz, 1H), 6.90 (d, $J$ = 7.4 Hz, 1H), 6.88 (d, $J$ = 7.2 Hz, 2H), 5.41 (s, 2H), 4.95 (s, 1H), 4.88 (d, $J$ = 5.3 Hz, 1H), 4.80 (t, $J$ = 5.5 Hz, 1H), 4.23 (m, $J$ = 5.4 Hz, 2H), 3.15 (d, $J$ = 5.4 Hz, 2H), 2.92 (d, $J$ = 5.2 Hz, 2H), 1.48 (s, 9H). Anal. Calcd for $C_{33}H_{35}N_3O_6$ C 69.58, H 6.19, N 7.38. Found C 69.43, H 6.12, N 7.55.

**4.4.7. (3*S*)-*N*-(Boc-L-prolinyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid benzylester (8g).** Yield: 91%. Mp 113–115 °C; ESI/MS ($m/z$) 504 $[M+H]^+$; IR (KBr): 3441, 3209, 3001, 2954, 2840, 1733, 1644, 1603, 1450, 1392, 1370, 1062, 903 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 10.01 (s, 1H), 7.31 (t, $J$ = 7.2 Hz, 1H), 7.19 (t, $J$ = 7.4 Hz, 1H), 7.15 (t, $J$ = 7.0 Hz, 2H), 7.10 (d, $J$ = 7.0 Hz, 2H), 7.07 (t, $J$ = 7.0 Hz, 1H), 7.03 (d, $J$ = 7.4 Hz, 1H), 6.91 (d, $J$ = 7.2 Hz, 1H), 5.33 (s, 2H), 4.84 (t, $J$ = 5.4 Hz, 1H), 4.30 (t, $J$ = 5.6 Hz, 1H), 4.21 (d, $J$ = 5.3 Hz, 2H), 3.46 (t, $J$ = 5.6 Hz, 2H), 2.91 (d, $J$ = 5.4 Hz, 2H), 2.29 (d, $J$ = 5.4 Hz, 2H), 1.93 (t, $J$ = 4.7 Hz, 2H), 1.47 (s, 9H). Anal. Calcd for $C_{29}H_{33}N_3O_5$ C 69.17, H 6.60, N 8.34. Found C 69.32, H 6.68, N 8.49.

**4.4.8. (3*S*)-*N*-(Boc-L-cysteinyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid benzyl-ester (8h).** Yield: 90%. Mp 131–133 °C; ESI/MS ($m/z$) 510 $[M+H]^+$; IR (KBr): 3445, 3201, 3000, 2942, 2845, 1732, 1643, 1601, 1452, 1391, 1373, 1060, 901 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 10.00 (s, 1H), 8.01 (s, 1H), 7.27 (t, $J$ = 7.2 Hz, 1H), 7.21 (t, $J$ = 7.4 Hz, 1H), 7.19 (t, $J$ = 7.2 Hz, 2H), 7.13 (d, $J$ = 7.2 Hz, 2H), 7.08 (t, $J$ = 7.2 Hz, 1H), 7.06 (d, $J$ = 7.4 Hz, 1H), 6.87 (d, $J$ = 7.2 Hz, 1H), 5.33 (s, 2H), 4.96 (t, $J$ = 5.3 Hz, 1H), 4.75 (t, $J$ = 5.5 Hz, 1H), 4.25 (d, $J$ = 5.3 Hz, 2H), 3.12 (d, $J$ = 5.3 Hz, 2H), 3.05 (d, $J$ = 5.4 Hz, 2H), 1.48 (s, 9H), 1.63 (s, 1H). Anal. Calcd for $C_{27}H_{31}N_3O_5S$ C 63.63, H 6.13, N 8.25. Found C 63.79, H 6.20, N 8.11.

**4.4.9. (3*S*)-*N*-(Boc-L-methioninyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid benzylester (8i).** Yield: 90%. Mp 144–146 °C; ESI/MS ($m/z$) 538 $[M+H]^+$; IR (KBr): 3443, 3205, 3002, 2954, 2845, 1733, 1642, 1603, 1455, 1392, 1373, 1061, 902 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 10.02 (s, 1H), 7.97 (s, 1H), 7.30 (t, $J$ = 7.2 Hz, 1H), 7.21 (t, $J$ = 7.4 Hz, 1H), 7.17 (t, $J$ = 7.4 Hz, 2H), 7.14 (d, $J$ = 7.4 Hz, 2H), 7.10 (t, $J$ = 7.4 Hz, 1H), 6.93 (d, $J$ = 7.2 Hz, 1H), 6.85 (d, $J$ = 7.2 Hz, 1H), 5.34 (s, 2H), 4.85 (t, $J$ = 5.2 Hz, 1H), 4.44 (t, $J$ = 5.3 Hz, 1H), 4.23 (d, $J$ = 5.1 Hz, 2H), 2.97 (d, $J$ = 5.2 Hz, 2H), 2.41 (t, $J$ = 5.2 Hz, 2H), 2.21 (d, $J$ = 5.3 Hz, 2H), 2.11 (s, 3H), 1.47 (s, 9H). Anal. Calcd for $C_{29}H_{35}N_3O_5S$ C 64.78, H 6.56, N 7.82. Found C 64.60, H 6.47, N 7.97.

**4.4.10. (3*S*)-*N*-[Boc-L-glutamyl(OBzl)]-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid benzylester (8j).** Yield: 91%. Mp 125–127 °C; ESI/MS ($m/z$) 626 $[M+H]^+$; IR (KBr): 3442, 3206, 3003, 2945, 2823, 1736, 1641, 1604, 1450, 1390, 1373, 1060, 896 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 9.98 (s, 1H), 8.01 (s, 1H), 7.34 (t, $J$ = 7.3 Hz, 1H), 7.24 (t, $J$ = 7.3 Hz, 1H), 7.21 (t, $J$ = 7.1 Hz, 2H), 7.18 (t, $J$ = 7.2 Hz, 2H), 7.17 (d, $J$ = 7.1 Hz, 2H), 7.15 (d, $J$ = 7.2 Hz, 2H), 7.14 (t, $J$ = 7.1 Hz, 1H), 7.12 (t, $J$ = 7.2 Hz, 1H), 7.00 (d, $J$ = 7.3 Hz, 1H), 6.87 (d, $J$ = 7.3 Hz, 1H), 5.36 (s, 2H), 5.34 (s, 2H), 4.91 (d, $J$ = 5.2 Hz, 1H), 4.44 (t, $J$ = 5.4 Hz, 1H), 4.23 (d, $J$ = 5.3 Hz, 2H), 2.97 (d, $J$ = 5.1 Hz, 2H), 2.23 (t, $J$ = 5.4 Hz, 2H), 2.20 (t, $J$ = 5.4 Hz, 2H), 1.47 (s, 9H). Anal. Calcd for $C_{36}H_{39}N_3O_7$ C 69.10, H 6.28, N 6.72. Found C 68.97, H 6.21, N 6.88.

**4.4.11. (3*S*)-*N*-[Boc-L-aspartyl(OBzl)]-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid benzylester (8k).** Yield: 90%. Mp 139–141 °C; ESI/MS ($m/z$) 612 $[M+H]^+$; IR (KBr): 3441, 3342, 3210, 3000, 2954, 2841, 1754, 1732, 1643, 1600, 1452, 1389, 1368, 1061, 900 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 9.97 (s, 1H), 8.00 (s, 1H), 7.28 (t, $J$ = 7.1 Hz, 1H), 7.22 (t, $J$ = 7.1 Hz, 1H), 7.19 (t, $J$ = 6.9 Hz, 2H), 7.17 (t, $J$ = 6.9 Hz, 2H), 7.13 (d, $J$ = 6.9 Hz, 2H), 7.11 (d, $J$ = 6.9 Hz, 2H), 7.09 (t, $J$ = 6.9 Hz, 1H), 7.06 (t, $J$ = 6.9 Hz, 1H), 7.00 (d, $J$ = 7.1 Hz, 1H), 6.96 (d, $J$ = 7.1 Hz, 1H), 5.34 (s, 2H), 5.31 (s, 2H), 4.92 (d, $J$ = 5.1 Hz, 1H), 4.76 (t, $J$ = 5.1 Hz, 1H), 4.25 (d, $J$ = 5.1 Hz, 2H), 2.92 (d, $J$ = 5.1 Hz, 2H), 2.85 (d, $J$ = 5.1 Hz, 2H), 1.45 (s, 9H). Anal. Calcd for $C_{35}H_{37}N_3O_7$ C 68.72, H 6.10, N 6.87. Found C 68.86, H 6.18, N 7.02.

**4.4.12. (3*S*)-*N*-(Boc-L-histidinyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid benzyl-ester (8l).** Yield:91%. Mp 137–139 °C; ESI/MS ($m/z$) 544 $[M+H]^+$; IR (KBr): 3442, 3201, 3004, 2941, 2830, 1732, 1643, 1602, 1455, 1393, 1374, 1062, 901 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 12.97 (s, 1H), 10.01 (s, 1H), 8.03 (s, 1H), 7.47 (s, 1H), 7.33 (t, $J$ = 7.1 Hz, 1H), 7.20 (t, $J$ = 7.1 Hz, 2H), 7.15 (t, $J$ = 7.2 Hz, 1H), 7.13 (d, $J$ = 7.1 Hz, 2H), 7.11 (d, $J$ = 7.2Hz, 1H), 7.05 (t, $J$ = 7.1 Hz, 1H), 6.97 (t, $J$ = 7.1 Hz, 1H), 6.86 (s, 1H), 5.35 (s, 2H), 4.93 (t, $J$ = 5.1 Hz, 1H), 4.81 (t, $J$ = 5.3 Hz, 1H), 4.24 (d, $J$ = 5.2 Hz, 2H), 3.23 (d, $J$ = 5.3 Hz, 2H), 2.93 (d, $J$ = 5.2 Hz, 2H), 1.48 (s, 9H). Anal. Calcd for $C_{30}H_{33}N_5O_5$ C 66.28, H 6.12, N 12.88. Found C 66.13, H 6.04, N 13.04.

**4.4.13. (3*S*)-*N*-(Boc-L-tryptophanyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid benzylester (8m).** Yield: 90%. Mp 144–146 °C; ESI/MS ($m/z$) 593 $[M+H]^+$. IR (KBr): 3441, 3332, 3211, 3002, 2941, 2830, 1755, 1732, 1641, 1600, 1443, 1392, 1373, 1062, 900 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 9.99 (s, 1H), 9.97 (s, 1H), 8.01 (s, 1H), 7.27 (t, $J$ = 7.1 Hz, 1H), 7.25 (t,

$J$ = 7.0 Hz, 1H), 7.21 (t, $J$ = 6.9 Hz, 2H), 7.15 (d, $J$ = 6.9 Hz, 2H), 7.11 (d, $J$ = 7.3 Hz, 1H), 7.07 (t, $J$ = 6.9 Hz, 1H), 7.05 (t, $J$ = 7.3 Hz, 1H), 7.03 (d, $J$ = 7.1 Hz, 1H), 7.00 (t, $J$ = 7.3 Hz, 1H), 6.99 (d, $J$ = 7.1 Hz, 1H), 6.96 (d, $J$ = 7.0 Hz, 1H), 6.83 (s, 1H), 5.32 (s, 2H), 4.93 (d, $J$ = 5.1 Hz, 1H), 4.75 (t, $J$ = 5.2 Hz, 1H), 4.23 (d, $J$ = 5.1 Hz, 2H), 3.15 (d, $J$ = 5.1 Hz, 2H), 2.95 (d, $J$ = 6.0 Hz, 2H), 1.48 (s, 9H). $[\alpha]_D^{20} = -70^\circ$ ($c$ 0.34, $CHCl_3/CH_3OH$, 1:1, v/v). Anal. Calcd for $C_{35}H_{36}N_4O_5$ C 70.93, H 6.12, N 9.45. Found C 71.08, H 6.20, N 9.62.

**4.4.14. (3*S*)-*N*-(Boc-L-argininyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid benzyl-ester (8n).** Yield: 87%. Mp 133–135 °C; ESI/MS ($m/z$) 563 $[M+H]^+$; IR (KBr): 3443, 3205, 3001, 2946, 2842, 1727, 1645, 1600, 1453, 1393, 1372, 1061, 903 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 10.01 (s, 1H), 8.40 (s, 2H), 8.23 (s, 1H), 8.15 (s, 1H), 8.03 (s, 1H), 7.25 (t, $J$ = 7.2 Hz, 1H), 7.22 (t, $J$ = 7.0 Hz, 2H), 7.15 (d, $J$ = 7.0 Hz, 2H), 7.13 (t, $J$ = 7.2 Hz, 1H), 7.11 (t, $J$ = 7.0 Hz, 1H), 7.00 (d, $J$ = 7.2 Hz, 1H), 6.93 (d, $J$ = 7.2 Hz, 1H), 5.36 (s, 2H), 4.90 (d, $J$ = 5.1 Hz, 1H), 4.37 (t, $J$ = 4.5 Hz, 1H), 4.25 (d, $J$ = 5.1 Hz, 2H), 2.93 (d, $J$ = 4.6 Hz, 2H), 2.68 (t, $J$ = 5.1 Hz, 2H), 1.93 (m, $J$ = 5.1 Hz, 2H), 1.54 (m, $J$ = 5.1 Hz, 2H), 1.53 (s, 9H). Anal. Calcd for $C_{30}H_{38}N_6O_5$ C 64.04, H 6.81, N 14.94. Found C 64.19, H 6.90, N 14.79.

**4.4.15. (3*S*)-*N*-(Boc-L-glycinyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid benzyl-ester (8o).** Yield: 93%. Mp 133–135 °C; ESI/MS ($m/z$) 464 $[M+H]^+$. IR (KBr): 3441, 3332, 3001, 2944, 2843, 1760, 1735, 1601, 1459, 1390, 1376, 1062, 900 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 9.99 (s, 1H), 8.02 (s, 1H), 7.25 (t, $J$ = 7.2 Hz, 1H), 7.20 (t, $J$ = 7.0 Hz, 2H), 7.19 (t, $J$ = 7.2 Hz, 1H), 7.13 (d, $J$ = 7.0 Hz, 2H), 7.09 (t, $J$ = 7.0 Hz, 1H), 6.95 (d, $J$ = 7.2 Hz, 1H), 6.87 (d, $J$ = 7.2 Hz, 1H), 5.35 (s, 2H), 4.84 (d, $J$ = 5.1 Hz, 1H), 4.23 (dd, $J$ = 10.1 Hz, $J$ = 4.1 Hz, 1H), 4.15 (dd, $J$ = 10.1 Hz, $J$ = 3.5 Hz, 1H), 4.11 (s, 2H), 2.95 (d, $J$ = 10.1 Hz, 2H), 1.47 (s, 9H). $[\alpha]_D^{20} = -80^\circ$ ($c$ 0.37, $CHCl_3/CH_3OH$, 1:1, v/v). Anal. Calcd for $C_{26}H_{29}N_3O_5$ C 67.37, H, 6.31, N 9.07. Found C 67.49, H 6.39, N 9.23.

**4.4.16. (3*S*)-*N*-[Boc-L-lysinyl(Bzl)]-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid benzyl-ester (8p).** Yield: 90%. Mp 129–131 °C; ESI/MS ($m/z$) 669 $[M+H]^+$. IR (KBr): 3441, 3334, 3005, 2946, 2847, 1763, 1730, 1600, 1454, 1390, 1374, 1062, 902 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 10.01 (s, 1H), 8.01 (s, 1H), 7.97 (s, 1H), 7.25 (t, $J$ = 7.1 Hz, 1H), 7.20 (t, $J$ = 7.0 Hz, 2H), 7.17 (t, $J$ = 6.9 Hz, 1H), 7.15 (t, $J$ = 7.1 Hz, 1H), 7.12 (d, $J$ = 6.9 Hz, 2H), 7.10 (d, $J$ = 7.0 Hz, 2H), 7.08 (t, $J$ = 6.9 Hz, 2H), 7.05 (t, $J$ = 7.0 Hz, 1H), 6.95 (d, $J$ = 7.1 Hz, 1H), 6.87 (d, $J$ = 7.1 Hz, 1H), 5.33 (s, 2H), 5.30 (s, 2H), 4.91 (d, $J$ = 5.1 Hz, 1H), 4.43 (t, $J$ = 4.3 Hz, 1H), 4.27 (dd, $J$ = 10.1 Hz, $J$ = 4.1 Hz, 1H), 4.17 (dd, $J$ = 10.1 Hz, $J$ = 3.6 Hz, 1H), 2.97 (t, $J$ = 4.3 Hz, 2H), 2.93 (d, $J$ = 10.1 Hz, 2H), 1.95 (m, $J$ = 4.1 Hz, 2H), 1.53 (m, $J$ = 4.5 Hz, 2H), 1.47 (s, 9H), 1.25 (m, $J$ = 4.3 Hz, 2H). $[\alpha]_D^{20} = -36^\circ$ ($c$ 0.37, $CHCl_3/CH_3OH$, 1:1, v/v). Anal. Calcd for $C_{38}H_{44}N_4O_7$ C 68.24, H, 6.63, N 8.38. Found C 68.37, H 6.71, N 8.22.

**4.4.17. (3*S*)-*N*-(Boc-L-glutaminyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid benzyl-ester (8q).** Yield: 88%. Mp 137–139 °C; ESI/MS ($m/z$) 535 $[M+H]^+$. IR (KBr): 3443, 3211, 3007, 2940, 2835, 1732, 1644, 1605, 1453, 1393, 1372, 1060, 900 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 9.99 (s, 1H), 8.01 (s, 1H), 7.25 (t, $J$ = 7.1 Hz, 1H), 7.21 (t, $J$ = 7.0 Hz, 2H), 7.16 (t, $J$ = 7.1 Hz, 1H), 7.13 (d, $J$ = 7.0 Hz, 2H), 7.10 (t, $J$ = 7.0 Hz, 1H), 7.01 (d, $J$ = 7.1 Hz, 1H), 6.87 (d, $J$ = 7.1 Hz, 1H), 6.09 (s, 2H), 5.37 (s, 2H), 4.92 (d, $J$ = 5.3 Hz, 1H), 4.41 (t, $J$ = 5.3 Hz, 1H), 4.25 (d, $J$ = 5.4 Hz, 2H), 2.93 (d, $J$ = 5.1 Hz, 2H), 2.17 (t, $J$ = 5.2 Hz, 2H), 2.14 (t, $J$ = 5.2 Hz, 2H), 1.47 (s, 9H). $[\alpha]_D^{20} = -47^\circ$ ($c$ 0.37, $CHCl_3/CH_3OH$, 1:1, v/v). Anal. Calcd for $C_{29}H_{34}N_4O_6$ C 65.15, H 6.41, N 10.48. Found C 65.01, H 6.30, N 10.65.

**4.4.18. (3*S*)-*N*-(Boc-L-asparginyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid benzyl-ester (8r).** Yield: 89%. Mp 136–138 °C; ESI/MS ($m/z$) 521 $[M+H]^+$. IR (KBr): 3441, 3203, 3001, 2935, 2837, 1732, 1634, 1601, 1455, 1393, 1376, 1062, 901 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 9.99 (s, 1H), 8.02 (s, 1H), 7.26 (t, $J$ = 7.1 Hz, 1H), 7.21 (t, $J$ = 7.2 Hz, 2H), 7.17 (t, $J$ = 7.1 Hz, 1H), 7.13 (d, $J$ = 7.2 Hz, 2H), 7.10 (t, $J$ = 7.2 Hz, 1H), 7.02 (d, $J$ = 7.1 Hz, 1H), 6.89 (d, $J$ = 7.1 Hz, 1H), 6.03 (s, 2H), 5.35 (s, 2H), 4.95 (d, $J$ = 5.1 Hz, 1H), 4.43 (t, $J$ = 5.1 Hz, 1H), 4.20 (d, $J$ = 5.1 Hz, 2H), 2.93 (d, $J$ = 5.2 Hz, 2H), 2.15 (t, $J$ = 5.2 Hz, 2H), 1.46 (s, 9H). $[\alpha]_D^{20} = -37^\circ$ ($c$ 0.32, $CHCl_3/CH_3OH$, 1:1, v/v). Anal. Calcd for $C_{28}H_{32}N_4O_6$ C 64.60, H 6.20, N 10.76. Found C 64.75, H 6.27, N 10.89.

**4.4.19. (3*S*)-*N*-(Boc-L-leucinyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid benzyl-ester (8s).** Yield: 91%. Mp 153–155 °C; ESI/MS ($m/z$) 520 $[M+H]^+$. IR (KBr): 3443, 3205, 3002, 2954, 2846, 1733, 1648, 1601, 1455, 1391, 1372, 1063, 900 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 9.99 (s, 1H), 8.02 (s, 1H), 7.25 (t, $J$ = 7.1 Hz, 1H), 7.21 (t, $J$ = 7.0 Hz, 2H), 7.19 (t, $J$ = 7.1 Hz, 1H), 7.13 (d, $J$ = 7.0 Hz, 2H), 7.09 (t, $J$ = 7.0 Hz, 1H), 7.02 (d, $J$ = 7.1 Hz, 1H), 6.80 (d, $J$ = 7.1 Hz, 1H), 5.35 (s, 2H), 4.95 (t, $J$ = 5.1 Hz, 1H), 4.43 (t, $J$ = 5.0 Hz, 1H), 4.24 (dd, $J$ = 10.1 Hz, $J$ = 4.3 Hz, 1H), 4.11 (dd, $J$ = 10.1 Hz, $J$ = 3.7 Hz, 1H), 2.93 (d, $J$ = 6.1 Hz, 2H), 2.80 (d, $J$ = 5.2 Hz, 2H), 1.51 (s, 9H), 1.33 (m, $J$ = 5.2 Hz, 1H), 1.09 (d, $J$ = 5.1 Hz, 6H). $[\alpha]_D^{20} = -39^\circ$ ($c$ 0.33, $CHCl_3/CH_3OH$, 1:1, v/v). Anal. Calcd for $C_{30}H_{37}N_3O_5$ C 69.34, H 7.18, N 8.09. Found C 69.48, H 7.29, N 7.94.

**4.4.20. (3*S*)-*N*-(Boc-L-isoleucinyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid benzyl-ester (8t).** Yield: 89%. Mp 143–145 °C; ESI/MS ($m/z$) 520 $[M+H]^+$. IR (KBr): 3441, 3335, 3216, 3012, 2951, 2844, 1755, 1730, 1641, 1603, 1455, 1392, 1374, 1060, 900 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 10.01 (s, 1H), 8.03 (s, 1H), 7.25 (t, $J$ = 7.0 Hz, 1H), 7.20 (t, $J$ = 6.9 Hz, 2H), 7.17 (t, $J$ = 7.0 Hz, 1H), 7.11 (d, $J$ = 6.9 Hz, 2H),

7.07 (t, $J$ = 6.9 Hz, 1H), 7.00 (d, $J$ = 7.0 Hz, 1H), 6.86 (d, $J$ = 7.0 Hz, 1H), 5.33 (s, 2H), 4.93 (t, $J$ = 5.2 Hz, 1H), 4.40 (t, $J$ = 5.1 Hz, 1H), 4.23 (dd, $J$ = 10.1 Hz, $J$ = 4.2 Hz, 1H), 4.03 (dd, $J$ = 10.1 Hz, $J$ = 3.3 Hz, 1H), 2.95 (d, $J$ = 6.1 Hz, 2H), 2.90 (m, $J$ = 5.2 Hz, 1H), 1.45 (s, 9H), 1.32 (m, $J$ = 5.1 Hz, 2H), 1.03 (d, $J$ = 5.1 Hz, 3H), 0.94 (t, $J$ = 5.3 Hz, 3H). $[\alpha]_D^{20}$ − 37° (*c* 0.35, $CHCl_3/CH_3OH$, 1:1, v/v). Anal. Calcd for $C_{30}H_{37}N_3O_5$ C 69.34, H 7.18, N 8.09. Found C 69.21, H 7.10, N 8.26.

### 4.5. General procedure for the preparation of 9a–t from 8a–t

At 0 °C to the solution of 2.5 mmol of **8a**–**t** in 6 ml of methanol and 3 ml of chloroform, 450 mg (11.34 mmol) of NaOH was added. The reaction mixture was stirred at 0 °C for 50 min and TLC (chloroform/methanol, 30:1) indicated the completion of the reaction. The reaction mixture was adjusted to pH 2 with hydrochloric acid (2 mol/L). On evaporation the residue was dissolved in 30 ml of ethyl acetate. The organic phase was washed with saturated aqueous sodium chloride and then dried over anhydrous sodium sulfate. After filtration, evaporation under reduced pressure, and purification with flash chromatography ($CHCl_3/CH_3OH$, 30:1) the title compounds were obtained as colorless powder.

**4.5.1. (3*S*)-*N*-(Boc-L-alanyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (9a).** Yield: 91%.Mp 160–162 °C; ESI/MS (*m*/*z*) 388 $[M+H]^+$; IR (KBr): 3451, 3343, 3010, 2956, 2847, 1734, 1643, 1600, 1455, 1390, 1070, 906 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 11.30 (s, 1H), 9.96 (s,1H), 8.06 (s,1H), 7.27 (t, $J$ = 7.3Hz, 1H), 7.16 (t, $J$ = 7.3 Hz, 1H), 6.94 (t, $J$ = 7.3 Hz, 1H), 6.90 (t, $J$ = 7.3 Hz, 1H), 4.73 (t, $J$ = 5.6 Hz, 1H), 4.65 (m, $J$ = 5.3 Hz, 1H), 3.86 (s, 2H), 3.60 (dd, $J$ = 10.0 Hz, $J$ = 5.0 Hz, 1H), 3.22 (dd, $J$ = 10.0 Hz, $J$ = 2.9 Hz,1H), 1.46 (d, $J$ = 5.6 Hz, 3H), 1.48 (s, 9H). $[\alpha]_D^{20}$ − 128° (*c* 0.38, $CHCl_3/CH_3OH$, 1:1, v/v). Anal. Calcd for $C_{20}H_{25}N_3O_5$ C 62.00, H 6.50, N 10.85. Found C 62.19, H 6.68, N 10.67.

**4.5.2. (3*S*)-*N*-(Boc-L-phenylalanyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (9b).** Yield: 90%. Mp 167–169 °C; ESI/MS (*m*/*z*) 464 $[M+H]^+$; IR (KBr): 3448, 3339, 3004, 2947, 2845, 1732, 1645, 1605, 1453, 1392, 1067, 905 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$= 11.29 (s, 1H), 9.98 (s, 1H), 7.99 (s, 1H), 7.28 (t, $J$ = 7.3Hz, 1H), 7.22 (t, $J$ = 7.5Hz, 2H), 7.14 (t, $J$ = 7.2 Hz, 1H), 7.13 (d, $J$ = 7.5Hz, 2H), 7.09 (t, $J$ = 7.5Hz, 1H), 6.97 (t, $J$ = 7.2 Hz, 1H), 6.93 (t, $J$ = 7.2 Hz, 1H), 4.98 (t, $J$ = 5.2 Hz, 1H), 4.77 (t, $J$ = 5.3 Hz, 1H), 3.96 (s, 2H), 3.62 (dd, $J$ = 10.3 Hz, $J$ = 5.0 Hz, 1H), 3.23 (dd, $J$ = 10.3 Hz, $J$ = 2.6 Hz, 1H), 3.04 (d, $J$ = 5.3 Hz, 2H), 1.46 (s, 9H). $[\alpha]_D^{20}$ − 75° (*c* 0.37, $CHCl_3/CH_3OH$, 1:1, v/v). Anal. Calcd for $C_{26}H_{29}N_3O_5$ C 67.37, H 6.31, N 9.07. Found C 67.23, H 6.20, N 9.25.

**4.5.3. (3*S*)-*N*-(Boc-L-valinyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (9c).** Yield: 92%.Mp 157–159 °C; ESI/MS (*m*/*z*) 416 $[M+H]^+$; IR (KBr): 3446, 3345, 3007, 2950, 2843, 1729, 1645, 1600, 1452, 1393, 1370, 1072, 904 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 11.37 (s, 1H), 9.94 (s,1H), 8.03 (s,1H), 7.27 (t, $J$ = 7.4 Hz, 1H), 7.15 (t, $J$ = 7.4 Hz, 1H), 6.97 (t, $J$ = 7.4 Hz, 1H), 6.94 (t, $J$ = 7.4 Hz, 1H), 4.75 (t, $J$ = 5.2 Hz, 1H), 4.55 (d, $J$ = 5.4 Hz, 1H), 3.89 (s, 2H), 3.65 (dd, $J$ = 10.1 Hz, $J$ = 5.3 Hz, 1H), 3.25 (dd, $J$ = 10.1 Hz, $J$ = 3.0 Hz, 1H), 2.68 (m, $J$ = 5.4 Hz, 1H), 1.49 (s, 9H), 1.05 (d, $J$ = 5.2 Hz, 6H). $[\alpha]_D^{20}$ − 58° (*c* 0.35, $CHCl_3/CH_3OH$, 1:1, v/v). Anal. Calcd for $C_{22}H_{29}N_3O_5$ C 63.60, H 7.04, N 10.11. Found C 63.76, H 7.16, N 9.99.

**4.5.4. (3*S*)-*N*-(Boc-L-serinyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (9d).** 90%. Mp 170–172 °C; ESI/MS (*m*/*z*) 404 $[M+H]^+$; IR (KBr): 3441, 3342, 3010, 2942, 2847, 1732, 1647, 1600, 1454, 1393, 1070, 905 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 11.21 (s, 1H), 10.00 (s, 1H), 8.00 (s, 1H), 7.27 (t, $J$ = 7.3 Hz, 1H), 7.16 (t, $J$ = 7.2 Hz, 1H), 7.00 (t, $J$ = 7.2 Hz, 1H), 6.93 (t, $J$ = 7.2 Hz, 1H), 4.75 (t, $J$ = 5.3 Hz, 1H), 4.66 (t, $J$ = 5.3 Hz, 1H), 4.07 (d, $J$ = 5.3 Hz, 2H), 3.64 (dd, $J$ = 10.3 Hz, $J$ = 5.2 Hz, 1H), 3.26 (dd, $J$ = 10.3 Hz, $J$ = 2.9 Hz, 1H), 3.11 (d, $J$ = 5.3 Hz, 2H), 2.20 (s, 1H), 1.49 (s, 9H). $[\alpha]_D^{20}$ − 54° (*c* 0.39, $CHCl_3/CH_3OH$, 1:1, v/v). Anal. Calcd for $C_{20}H_{25}N_3O_6$ C 59.54, H 6.25, N 10.42. Found C 59.37, H 6.12, N 10.61.

**4.5.5. (3*S*)-*N*-(Boc-L-threoninyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (9e).** Yield: 89%. Mp 155–157 °C; ESI/MS (*m*/*z*) 418 $[M+H]^+$; IR (KBr): 3440, 3345, 3008, 2952, 2845, 1733, 1646, 1602, 1456, 1393, 1370, 1063, 901 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$= 11.12 (s, 1H), 9.99 (s, 1H), 8.00 (s, 1H), 7.27 (t, $J$ = 7.1 Hz, 1H), 7.20 (t, $J$ = 7.1 Hz, 1H), 6.92 (d, $J$ = 7.2 Hz, 1H), 6.73 (d, $J$ = 7.1 Hz, 1H), 4.77 (t, $J$ = 5.1 Hz, 1H), 4.60 (m, $J$ = 5.1 Hz, 1H), 4.42 (t, $J$ = 5.3 Hz, 1H), 4.00 (m, $J$ = 5.2 Hz, 2H), 2.93 (d, $J$ = 5.2 Hz, 2H), 2.23 (d, $J$ = 3.7 Hz, 1H), 1.47 (s, 9H), 1.24 (d, $J$ = 5.2 Hz, 3H). Anal. Calcd for $C_{21}H_{27}N_3O_6$ C 60.42, H 6.52, N 10.07. Found C 60.59, H 6.62, N 9.92.

**4.5.6. (3*S*)-*N*-(Boc-L-tyrosinyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (9f).** Yield: 88%. Mp 161–163 °C; ESI/MS (*m*/*z*) 480 $[M+H]^+$; IR (KBr): 3442, 3335, 3001, 2943, 2846, 1750, 1643, 1602, 1450, 1393, 1371, 1072, 900 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 11.24 (s, 1H), 10.00 (s, 1H), 8.02 (s, 1H), 7.23 (t, $J$ = 7.2 Hz, 1H), 7.15 (t, $J$ = 7.2 Hz, 1H), 6.93 (d, $J$ = 7.3 Hz, 2H), 6.85 (d, $J$ = 7.2 Hz, 1H), 6.82 (d, $J$ = 7.2 Hz, 1H), 6.66 (d, $J$ = 7.3 Hz, 2H), 5.00 (s, 1H), 4.90 (t, $J$ = 5.3 Hz, 1H), 4.81 (t, $J$ = 5.3 Hz, 1H), 3.90 (s, 2H), 3.61 (dd, $J$ = 10.1 Hz, $J$ = 5.3 Hz, 1H), 3.34 (dd, $J$ = 10.1 Hz, $J$ = 2.9 Hz, 1H), 3.05 (d, $J$ = 5.2 Hz, 2H), 1.49 (s, 9H). $[\alpha]_D^{20}$ − 37° (*c* 0.34, $CHCl_3/CH_3OH$, 1:1, v/v). Anal. Calcd for $C_{26}H_{29}N_3O_6$ C 65.12, H 6.10, N 8.76. Found C 65.00, H 6.01, N 8.93.

**4.5.7. (3*S*)-*N*-(Boc-prolinyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (9g).** Yield: 89%. Mp 140–142 °C; ESI/MS (*m*/*z*) 414 $[M+H]^+$; IR (KBr): 3445, 3430, 3203,

3007, 2952, 2840, 1733, 1641, 1602, 1453, 1391, 1372, 1062, 901 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$= 11.04 (s, 1H), 9.99 (s, 1H), 7.30 (t, $J$ = 7.0 Hz, 1H), 7.20 (t, $J$ = 7.2 Hz, 1H), 7.01 (d, $J$ = 7.2 Hz, 1H), 6.90 (d, $J$ = 7.0 Hz, 1H), 4.83 (t, $J$ = 5.1 Hz, 1H), 4.33 (t, $J$ = 5.4 Hz, 1H), 4.22 (d, $J$ = 5.2 Hz, 2H), 3.41 (t, $J$ = 5.4 Hz, 2H), 2.95 (d, $J$ = 5.4 Hz, 2H), 2.27 (d, $J$ = 5.3 Hz, 2H), 1.93 (t, $J$ = 5.2 Hz, 2H), 1.49 (s, 9H). Anal. Calcd for $C_{22}H_{27}N_3O_5$ C 63.91, H 6.58, N 10.16. Found C 63.98, H, 6.61, N 10.20.

**4.5.8. (3*S*)-*N*-(Boc-cysteinyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (9h).** Yield: 91%. Mp 151–153 °C; ESI/MS (*m*/*z*) 420 $[M+H]^+$; IR (KBr): 3442, 3433, 3201, 3002, 2943, 2845, 1730, 1643, 1602, 1453, 1391, 1372, 1061, 903 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 10.96 (s, 1H), 10.00 (s, 1H), 8.01 (s, 1H), 7.27 (t, $J$ = 7.1 Hz, 1H), 7.18 (t, $J$ = 7.3 Hz, 1H), 7.00 (d, $J$ = 7.3 Hz, 1H), 6.87 (d, $J$ = 7.1 Hz, 1H), 4.87 (t, $J$ = 5.3 Hz, 1H), 4.71 (t, $J$ = 5.1 Hz, 1H), 4.20 (d, $J$ = 5.3 Hz, 2H), 3.15 (d, $J$ = 5.1 Hz, 2H), 2.97 (d, $J$ = 5.4 Hz, 2H), 1.66 (s, 1H), 1.49 (s, 9H). Anal. Calcd for $C_{20}H_{25}N_3O_5S$ C 57.26, H 6.01, N 10.02. Found C 57.12, H 6.10, N 10.19.

**4.5.9. (3*S*)-*N*-(Boc-methioninyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (9i).** Yield: 90%. Mp 154–156 °C; ESI/MS (*m*/*z*) 448 $[M+H]^+$; IR (KBr): 3445, 3440, 3205, 3002, 2953, 2841, 1732, 1646, 1600, 1450, 1393, 1372, 1062, 901 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 11.01 (s, 1H), 9.98 (s, 1H), 7.96 (s, 1H), 7.28 (t, $J$ = 7.1 Hz, 1H), 7.17 (t, $J$ = 7.3 Hz, 1H), 6.99 (d, $J$ = 7.3 Hz, 1H), 6.85 (d, $J$ = 7.1 Hz, 1H), 4.81 (t, $J$ = 5.0 Hz, 1H), 4.45 (t, $J$ = 5.1 Hz, 1H), 4.25 (d, $J$ = 5.1 Hz, 2H), 2.93 (d, $J$ = 5.2 Hz, 2H), 2.43 (t, $J$ = 5.1 Hz, 2H), 2.22 (d, $J$ = 5.1 Hz, 2H), 2.13 (s, 3H), 1.49 (s, 9H). Anal. Calcd for $C_{22}H_{29}N_3O_5S$ C 59.04, H 6.53, N 9.39. Found C 59.21, H 6.62, N 9.56.

**4.5.10. (3*S*)-*N*-(Boc-glutamyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (9j).** Yield: 92%. Mp 149–151 °C; ESI/MS (*m*/*z*) 446 $[M+H]^+$; IR (KBr): 3445, 3440, 3211, 3001, 2943, 2830, 1732, 1640, 1603, 1450, 1392, 1371, 1060, 899 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 11.03 (s, 1H), 11.00 (s, 1H), 9.96 (s, 1H), 8.00 (s, 1H), 7.32 (t, $J$ = 7.0 Hz, 1H), 7.23 (t, $J$ = 7.0 Hz, 1H), 7.02 (d, $J$ = 7.2 Hz, 1H), 6.89 (d, $J$ = 7.0 Hz, 1H), 4.85 (d, $J$ = 5.2 Hz, 1H), 4.47 (t, $J$ = 5.3 Hz, 1H), 4.25 (d, $J$ = 5.2 Hz, 2H), 2.95 (d, $J$ = 5.2 Hz, 2H), 2.25 (t, $J$ = 5.3 Hz, 2H), 2.21 (t, $J$ = 5.5 Hz, 2H), 1.48 (s, 9H). Anal. Calcd for $C_{22}H_{27}N_3O_7$ C 59.32, H 6.11, N 9.43. Found C 59.49, H 6.19, N 9.26.

**4.5.11. (3*S*)-*N*-(Boc-L-aspartyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (9k).** Yield: 90%. Mp 159–161 °C; ESI/MS (*m*/*z*) 432 $[M+H]^+$; IR (KBr): 3446, 3441, 3343, 3002, 2943, 2842, 1730, 1648, 1602, 1452, 1390, 1075, 901 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 11.39 (s, 1H), 11.06 (s, 1H), 9.97 (s, 1H), 8.05 (s, 1H), 7.27 (t, $J$ = 7.4 Hz, 1H), 7.19 (t, $J$ = 7.3 Hz, 1H), 7.17 (t, $J$ = 7.4 Hz, 1H), 6.99 (t, $J$ = 7.3 Hz, 1H), 5.16 (t, $J$ = 5.3 Hz, 1H), 4.75 (t, $J$ = 5.4 Hz, 1H), 3.60 (dd, $J$ = 10.0 Hz, $J$ = 5.3 Hz, 1H), 3.27 (dd, $J$ = 10.0 Hz, $J$ = 2.9 Hz, 1H), 3.02 (d, $J$ = 5.3 Hz, 2H), 2.73 (d, $J$ = 5.2 Hz, 2H), 1.49 (s, 9H). $[\alpha]_D^{20}$ − 69° (*c* 0.39, $CHCl_3/CH_3OH$, 1:1, v/v). Anal. Calcd for $C_{21}H_{25}N_3O_7$ C 58.46, H 5.84, N 9.74. Found C 58.31, H 5.77, N 9.92.

**4.5.12. (3*S*)-*N*-(Boc-L-histidinyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (9l).** Yield: 91%. Mp 162–164 °C; ESI/MS (*m*/*z*) 454 $[M+H]^+$; IR (KBr): 3447, 3441, 3211, 3002, 2942, 2834, 1733, 1640, 1600, 1455, 1390, 1372, 1063, 904 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 12.97 (s, 1H), 11.02 (s, 1H), 10.01 (s, 1H), 8.00 (s, 1H), 7.43 (s, 1H), 7.28 (t, $J$ = 7.0 Hz, 1H), 7.15 (t, $J$ = 7.2 Hz, 1H), 7.12 (d, $J$ = 7.2 Hz, 1H), 6.97 (t, $J$ = 7.0 Hz, 1H), 6.85 (s, 1H), 4.92 (t, $J$ = 5.0 Hz, 1H), 4.83 (t, $J$ = 5.1 Hz, 1H), 4.21 (d, $J$ = 5.0 Hz, 2H), 3.13 (d, $J$ = 5.0 Hz, 2H), 2.94 (d, $J$ = 5.0 Hz, 2H), 1.49 (s, 9H). Anal. Calcd for $C_{24}H_{29}N_5O_5$ C 60.92, H 6.00, N 15.44. Found C 61.10, H 6.08, N 15.59.

**4.5.13. (3*S*)-*N*-(Boc-L-tryptophanyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (9m).** Yield: 87%. Mp 141–143 °C; ESI/MS (*m*/*z*) 503 $[M+H]^+$; IR (KBr): 3443, 3339, 3010, 2945, 2846, 1730, 1642, 1600, 1455, 1393, 1070, 906 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 11.32 (s, 1H), 9.95 (s, 1H), 9.93 (s, 1H), 8.05 (s, 1H), 7.27 (t, $J$ = 7.3 Hz, 1H), 7.22 (t, $J$ = 7.2 Hz, 1H), 7.20 (t, $J$ = 7.2 Hz, 1H), 7.19 (t, $J$ = 7.2 Hz, 1H), 7.18 (t, $J$ = 7.2 Hz, 1H), 7.13 (t, $J$ = 7.3 Hz, 1H), 7.03 (t, $J$ = 7.3 Hz, 1H), 6.92 (t, $J$ = 7.3 Hz, 1H), 6.84 (s, 1H), 4.93 (t, $J$ = 5.2 Hz, 1H), 4.88 (t, $J$ = 5.1 Hz, 1H), 3.96 (s, 2H), 3.60 (dd, $J$ = 10.3 Hz, $J$ = 5.2 Hz, 1H), 3.35 (dd, $J$ = 10.3 Hz, $J$ = 2.6 Hz, 1H), 2.95 (t, $J$ = 5.3 Hz, 2H), 1.46 (s, 9H). $[\alpha]_D^{20}$ − 55° (*c* 0.38, $CHCl_3/CH_3OH$, 1:1, v/v). Anal. Calcd for $C_{28}H_{30}N_4O_5$ C 66.92, H 6.02, N 11.15. Found C 67.09, H 6.17, N 11.01.

**4.5.14. (3*S*)-*N*-(Boc-L-argininyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (9n).** Yield: 90%.Mp 148–150 °C; ESI/MS (*m*/*z*) 473 $[M+H]^+$; IR (KBr): 3443, 3441, 3211, 3006, 2942, 2841, 1735, 1644, 1603, 1450, 1391, 1374, 1061, 903 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 11.03 (s, 1H),10.04 (s, 1H), 8.41 (s, 2H), 8.22 (s, 1H), 8.20 (s, 1H), 8.01 (s, 1H), 7.25 (t, $J$ = 7.2 Hz, 1H), 7.16 (t, $J$ = 7.3 Hz, 1H), 7.00 (d, $J$ = 7.3 Hz, 1H), 6.95 (d, $J$ = 7.2 Hz, 1H), 4.93 (d, $J$ = 5.2 Hz, 1H), 4.42 (t, $J$ = 4.5 Hz, 1H), 4.29 (d, $J$ = 5.2 Hz, 2H), 2.93 (d, $J$ = 4.5 Hz, 2H), 2.64 (t, $J$ = 5.4 Hz, 2H), 1.91 (m, $J$ = 5.4 Hz, 2H), 1.55 (m, $J$ = 5.4 Hz, 2H), 1.50 (s, 9H). Anal. Calcd for $C_{23}H_{32}N_6O_5$ C 58.46, H 6.83, N 17.78. Found C 58.61, H 6.92, N 17.61.

**4.5.15. (3*S*)-*N*-(Boc-glycyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (9o).** Yield: 92%. Mp 171–173 °C; ESI/MS (*m*/*z*) 374 $[M+H]^+$; IR (KBr): 3446, 3340, 3233, 3008, 2942, 2841, 1730, 1644, 1605, 1456, 1392, 1066, 990 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$= 11.22 (s, 1H), 9.95 (s, 1H), 8.07 (s,1H), 7.27 (t, $J$ = 7.3 Hz, 1H), 7.17 (t, $J$ = 7.3 Hz, 1H), 6.97 (t, $J$ = 7.3 Hz, 1H), 6.93 (t, $J$ = 7.3 Hz, 1H), 4.79 (t, $J$ = 5.3 Hz, 1H), 4.53 (d, $J$ = 4.7 Hz, 2H), 3.88 (s, 2H), 3.67 (dd, $J$ = 10.1 Hz,

$J$ = 5.2 Hz, 1H), 3.13 (dd, $J$ = 10.1 Hz, $J$ = 2.6 Hz,1H),1.47 (s, 9H). $[\alpha]_D^{20}$ −123° (*c* 0.34, $CHCl_3$/$CH_3OH$, 1:1, v/v). Anal. Calcd for $C_{19}H_{23}N_3O_5$ C 61.11, H 6.21, N 11.25. Found C 61.28, H 6.39, N 11.07.

**4.5.16. (3*S*)-*N*-[Boc-L-lysinyl(Bzl)]-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (9p).** YiEld: 90%. ESI/MS (*m*/*z*) 579 $[M+H]^+$; IR (KBr): 3448, 3340, 3001, 2943, 2841, 1750, 1643, 1600, 1452, 1391, 1372, 1071, 900 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 11.18 (s, 1H), 10.02 (s, 1H), 8.03 (s, 1H), 8.01 (s, 1H), 7.25 (t, $J$ = 7.2 Hz, 1H), 7.19 (t, $J$ = 7.0 Hz, 1H), 7.13 (t, $J$ = 7.2 Hz, 1H), 7.11 (d, $J$ = 7.0 Hz, 2H), 7.07 (t, $J$ = 7.0 Hz, 2H), 7.01 (t, $J$ = 7.2 Hz, 1H), 6.84 (t, $J$ = 7.2 Hz, 1H), 5.33 (s, 2H), 4.72 (t, $J$ = 5.5 Hz, 1H), 4.51 (t, $J$ = 5.5 Hz, 1H), 3.65 (dd, $J$ = 10.1 Hz, $J$ = 5.0 Hz, 1H), 3.32 (dd, $J$ = 10.0 Hz, $J$ = 2.9 Hz, 1H), 3.09 (d, $J$ = 5.4 Hz, 2H), 2.93 (t, $J$ = 5.2 Hz, 2H), 1.77 (t, $J$ = 5.4 Hz, 2H), 1.56 (t, $J$ = 5.2 Hz, 2H), 1.47 (s, 9H), 1.29 (m, $J$ = 5.5 Hz, 2H). $[\alpha]_D^{20}$ −24° (*c* 0.35, $CHCl_3$/$CH_3OH$, 1:1, v/v). Anal. Calcd for $C_{31}H_{38}N_4O_7$ C 64.34, H 6.62, N 9.68. Found C 64.21, H 6.51, N 9.83.

**4.5.17. (3*S*)-*N*-(Boc-L-glutaminyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (9q).** 92%. Mp 161–163 °C; ESI/MS (*m*/*z*) 445 $[M+H]^+$; IR (KBr): 3344, 3438, 3010, 2944, 2841, 1752, 1643, 1601, 1452, 1393, 1369, 1070, 901 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 11.03 (s, 1H), 9.98 (s, 1H), 8.00 (s, 1H), 7.27 (t, $J$ = 7.3 Hz, 1H), 7.15 (t, $J$ = 7.3 Hz, 1H), 6.82 (d, $J$ = 7.3 Hz, 1H), 6.80 (d, $J$ = 7.3 Hz, 1H), 6.10 (s, 2H), 4.93 (t, $J$ = 5.2 Hz, 1H), 4.57 (t, $J$ = 5.2 Hz, 1H), 3.91 (s, 2H), 3.60 (dd, $J$ = 10.2 Hz, $J$ = 5.0 Hz, 1H), 3.30 (dd, $J$ = 10.0 Hz, $J$ = 2.7 Hz, 1H), 2.17 (t, $J$ = 4.8 Hz, 2H), 2.00 (m, $J$ = 4.9 Hz, 2H), 1.45 (s, 9H). $[\alpha]_D^{20}$ = −32° (*c* 0.36, $CHCl_3$/$CH_3OH$, 1:1, v/v). Anal. Calcd for $C_{22}H_{28}N_4O_6$ C 59.45, H 6.35, N 12.60. Found C 59.61, H 6.26, N 12.77.

**4.5.18. (3*S*)-*N*-(Boc-L-asparaginyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (9r).** Yield: 91%. Mp 166–168 °C; ESI/MS (*m*/*z*) 431 $[M+H]^+$. IR (KBr): 3446, 3339, 3207, 3003, 2930, 2838, 1730, 1633, 1600, 1452, 1390, 1373, 1064, 903 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 11.03 (s, 1H), 9.99 (s, 1H), 8.03 (s, 1H), 7.20 (t, $J$ = 7.1 Hz, 1H), 7.11 (t, $J$ = 7.0 Hz, 1H), 7.00 (d, $J$ = 7.1 Hz, 1H), 6.87 (d, $J$ = 7.1 Hz, 1H), 6.00 (s, 2H), 4.93 (d, $J$ = 5.2 Hz, 1H), 4.43 (t, $J$ = 5.2 Hz, 1H), 4.26 (d, $J$ = 5.1 Hz, 2H), 2.90 (d, $J$ = 5.1 Hz, 2H), 2.57 (t, $J$ = 5.1 Hz, 2H), 1.45 (s, 9H). $[\alpha]_D^{20}$ −31° (*c* 0.32, $CHCl_3$/$CH_3OH$, 1:1, v/v). Anal. Calcd for $C_{21}H_{26}N_4O_6$ C 58.59, H 6.09, N 13.02. Found C 59.77, H 6.18, N 13.20.

**4.5.19. (3*S*)-*N*-(Boc-L-leucyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (9s).** Yield: 89%. Mp 147–149 °C; ESI/MS (*m*/*z*) 430 $[M+H]^+$; IR (KBr): 3445, 3347, 3001, 2953, 2845, 1741, 1642, 1601, 1455, 1393, 1371, 1072, 903 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 10.97 (s, 1H), 9.87 (s, 1H), 8.03 (s, 1H), 7.25 (t, $J$ = 7.2 Hz, 1H), 7.13 (t, $J$ = 7.2 Hz, 1H), 6.97 (t, $J$ = 7.4 Hz, 1H), 6.93 (t, $J$ = 7.2 Hz, 1H), 4.73 (t, $J$ = 5.7 Hz, 1H), 4.57 (d, $J$ = 5.4 Hz, 1H), 3.86 (s, 2H), 3.62 (dd, $J$ = 10.0 Hz, $J$ = 5.0 Hz, 1H), 3.24 (dd, $J$ = 10.1 Hz, $J$ = 2.7 Hz, 1H), 1.89 (m, $J$ = 5.3 Hz, 1H), 1.76 (t, $J$ = 5.2 Hz, 2H), 1.49 (s, 9H), 1.05 (d, $J$ = 5.4 Hz, 6H). $[\alpha]_D^{20}$ −46° (*c* 0.35, $CHCl_3$/$CH_3OH$, 1:1, v/v). Anal. Calcd for $C_{23}H_{31}N_3O_5$ C 64.32, H 7.27, N 9.78. Found C 64.50, H 7.37, N 9.61.

**4.5.20. (3*S*)-*N*-(Boc-L-isoleucyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (9t).** Yield: 87%. Mp 144–146 °C; ESI/MS (*m*/*z*) 430 $[M+H]^+$; IR (KBr): 3447, 3346, 3004, 2952, 2846, 1742, 1643, 1600, 1450, 1393, 1372, 1071, 903 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 10.97 (s,1H), 9.96 (s,1H), 8.01 (s,1H), 7.25 (t, $J$ = 7.2 Hz, 1H), 7.13 (t, $J$ = 7.2 Hz, 1H), 6.97 (t, $J$ = 7.2 Hz, 1H), 6.93 (t, $J$ = 7.2 Hz, 1H), 4.73 (t, $J$ = 5.7 Hz, 1H), 4.52 (d, $J$ = 5.3 Hz, 1H), 3.85 (s, 2H), 3.63 (dd, $J$ = 10.1 Hz, $J$ = 5.2 Hz, 1H), 3.25 (dd, $J$ = 10.1 Hz, $J$ = 2.7 Hz, 1H), 2.44 (m, $J$ = 5.2 Hz, 1H), 1.35 (m, $J$ = 5.1 Hz, 2H), 1.47 (s, 9H), 1.08 (d, $J$ = 5.2 Hz, 3H), 1.04 (t, $J$ = 5.2 Hz, 3H). $[\alpha]_D^{20}$ −47° (*c* 0.36, $CHCl_3$/$CH_3OH$, 1:1, v/v). Anal. Calcd for $C_{23}H_{31}N_3O_5$ C 64.32, H 7.27, N 9.78. Found C 64.48, H 7.36, N 9.63.

### 4.6. General procedure for the preparation of hexahydropyrazino[1′,2′:1,6]pyrido[3,4-*b*]-indole-1,4-diones 5a–t from 8a–t

At 0 °C 2.0 mmol of **8a**–**t** was dissolved in 10 ml of hydrogen chloride/ethyl acetate (4 mol/L) and stirred for 10 min. Then the reaction mixture was stirred at room temperature for 20–25 min and TLC(ethyl acetate/petroleum, 5:12) indicated the completion of the reaction. The reaction mixture was evaporated under vacuum. The residue was diluted in 10 ml of ethyl acetate and then evaporated to dryness, which was repeated for three times to give the title compound as colorless powder in 84–90% yields.

### 4.7. General procedure for the preparation of hexahydropyrazino[1′,2′:1,6]pyrido[3,4-*b*]-indole-1,4-diones 5a–t from 9a–t

At 0 °C 2.0 mmol of **9a**–**t** was dissolved in 10 ml of hydrogen chloride/ethyl acetate (4 mol/L) and stirred for 10 min. Then the reaction mixture was stirred at room temperature for 100–140 min and TLC(ethyl acetate/petroleum, 5:12) indicated the completion of the reaction. The reaction mixture was evaporated under vacuum. The residue was diluted in 10 ml of ethyl acetate and then evaporated to dryness, which was repeated for three times to give the title compound as colorless powder in 86–91% yields.

**4.7.1. (3*S*,12a*S*)-3-Carboxyethyl-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]-pyrido[3,4-*b*]-indole-1,4-dione (5j).** A colorless powder of 834 mg (2.0 mmol) of **5j′** was mixed with 50 mg of Pd/C (5%) and 25 ml of formic acid in methanol (4.4%), and agitated with hydrogen at room temperature for 24h. The reaction mixture was filtered and evaporated to give 634 mg (97%) of the title

compound as colorless powder. Mp 255–257 °C; ESI/MS (*m*/*z*) 328 $[M+H]^+$; IR (KBr): 3445, 3332, 2940, 1684, 1338, 746 $cm^{-1}$; $^1H$ NMR (BHSC-500, DMSO-$d_6$): $\delta$ = 11.02 (s, 1H), 10.01 (s, 1H), 8.02 (s, 1H), 7.29 (t, *J* = 7.0 Hz, 1H), 7.21 (t, *J* = 7.0 Hz, 1H), 7.03 (d, *J* = 7.0 Hz, 1H), 6.83 (d, *J* = 7.0 Hz, 1H), 4.90 (d, *J* = 5.2 Hz, 1H), 4.41 (t, *J* = 5.3 Hz, 1H), 4.25 (d, *J* = 5.2 Hz, 2H), 2.93 (d, *J* = 5.3 Hz, 2H), 2.27 (t, *J* = 5.3 Hz, 2H), 2.02 (q, *J* = 5.3 Hz, 2H). $[\alpha]_D^{20}$ − 100.1° (*c* 1.0, $CH_3OH$); Anal. Calcd for $C_{17}H_{17}N_3O_4$ C 62.38, H 5.23, N 12.84. Found C 62.55, H 5.32, N 12.71.

**4.7.2. (3*S*,12a*S*)-3-Carboxymethyl-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido-[3,4-*b*]indole-1,4-dione (5k).** Using the same procedure for **5j** from 806 mg (2.0 mmol) of **5′k** 601 mg (96%) of the title compound was obtained as colorless powder. Mp 271–273 °C, ESI-MS (*m*/*z*) 314 $[M+H]^+$; (KBr): 3442, 3336, 2943, 1688, 1337, 743 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$, 300 MHz) $\delta$ = 11.00 (s, 1H), 9.99 (s,1H), 8.01 (s,1H), 7.28 (d, *J* = 7.2 Hz, 1H), 7.20 (t, *J* = 7.2 Hz, 1H), 7.05 (t, *J* = 7.2 Hz, 1H), 6.90 (t, *J* = 7.2 Hz, 1H), 5.05 (t, *J* = 6.6 Hz, 1H), 4.85 (t, *J* = 5.3 Hz, 1H), 4.24 (d, *J* = 5.6 Hz, 2H), 2.96 (t, *J* = 5.6 Hz, 2H), 2.85 (d, *J* = 5.3 Hz, 2H). $[\alpha]_D^{20}$ − 59° (*c* 1.0, $CH_3OH$); Anal. Calcd for $C_{16}H_{15}N_3O_4$ C 61.34, H 4.83, N 13.41. Found C 61.27, H 4.77, N 13.59.

**4.7.3. (3*S*,12a*S*)-3-Aminobutyl-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido[3,4-*b*] indole-1,4-dione (5p).** Using the same procedure for **5j** from 922 mg (2.0 mmol) of **5′p** 619 mg (95%) of the title compound was obtained as colorless powder. Mp 216–218 °C, EI-MS (*m*/*z*) 327 $[M+H]^+$; IR(KBr): 3324, 2936, 1683, 1463, 1336, 745 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$, 300 MHz) $\delta$ = 9.92 (s,1H), 8.21 (d, *J* = 1.8 Hz, 1H), 7.41 (d, *J* = 7.5 Hz, 1H), 7.28 (t, *J* = 7.5 Hz, 1H), 7.08 (t, *J* = 7.5 Hz, 1H), 6.95 (t,1H, *J* = 7.5 Hz, 1H), 5.36 (d. *J* = 16.5 Hz, 1H), 4.60 (t, *J* = 6.8 Hz, 1H), 4.30 (dd, *J* = 11.7 Hz, *J* = 4.5 Hz, 1H), 4.13 (dd, *J* = 11.7 Hz, *J* = 4.5 Hz, 1H), 3.26 (dd, *J* = 13.2 Hz, *J* = 3.3 Hz, 1H), 2.79 (dd, *J* = 13.5 Hz, *J* = 5.1 Hz, 1H), 2.70 (t, *J* = 4.8 Hz, 2H), 2.01 (s, 2H), 1.80 (m, *J* = 4.8 Hz, 2H), 1.60 (m, *J* = 4.8 Hz, 2H), 1.32 (m, *J* = 4.8 Hz, 2H). $[\alpha]_D^{20}$ − 48° (*c* 1.0, $CH_3OH$); Anal. Calcd for $C_{18}H_{22}N_4O_2$ C 66.24, H 6.79, N 17.17. Found C 66.01, H 6.58, N 17.41.

### 4.8. Determination of the retention time of 5a–t

Hexahydropyrazino[1′,2′:1,6]pyrido-[3,4-*b*]indole-1,4-dions **5a**–**t** were dissolved in aqueous methanol (50%) to prepare sample solution of 10 μM. The HPLC analysis was carried out on Agilent 1100 series, the column was a reversed-phase C18 column (Agilent Zorbax Extend-C18, 4.6 × 15 mm, 5 μm). After 20 μl of the sample (10 μM) was loaded, the column was eluted with 50% solution of MeOH as the mobile phase for 40 min. The flow rate was 1 ml/min. The peak of **5a**–**t** in the sample was monitored with UV detector at 254.8 nm and the retention time ($t_R$) corresponding to its peak was recorded. With the same HPLC conditions the retention time of acetone peak was recorded as $t_0$. In order to offset the influence of the solvent on the appearance time of the peak of **5a**–**t**, the appearance time of acetone peak ($t_0$, 1.591 min) was used as a control. As an alternative representation of $t_R$, the parameter log *K* was defined based on the equation $\log K = \log[(t_R - t_0)/t_0]$.

### 4.9. In vitro anti-platelet aggregation activity assay

Platelet-rich plasma was prepared by centrifugation of pig blood anticoagulation with sodium citrate at a final concentration of 0.38%. The platelet counts were adjusted to $2 \times 10^8$/ml by addition of autologous plasma. Platelet aggregation tests were conducted in an aggregometer using the standard turbidimetric technique. The effects of **5a**–**t** (in a series of concentrations of a range from 1 μmol/L to 0.01 μmol/L) on PAF (final concentration $10^{-7}$ mol/L) or ADP (final concentration $10^{-5}$ mol/L) induced platelet aggregation were observed. The maximal rate of platelet aggregation (Am %) was represented by the peak height of aggregation curve.

### 4.10. In vivo anti-thrombotic activity in rat model

The assessments of in vivo anti-thrombotic activity were performed based on a protocol reviewed and approved by the Ethics Committee of Peking University. The committee assures the welfare of the animals which were maintained in accordance to the requirements of the animal welfare act and according to the guide for care and use of laboratory animals.

### 4.11. Intravenous administration

Aspirin, **5a**–**t** were dissolved in NS just before use and kept in an ice bath. Male Wistar rats weighing 250–300 g (purchased from Animal Center of Peking University) were anesthetized with pentobarbital sodium (80.0 mg/kg, ip), and the right carotid artery and left jugular vein were separated. A weighed 6 cm thread was inserted into the middle of a polyethylene tube. The polyethylene tube was filled with heparin sodium (50 IU/ml in NS) and one end was inserted into the left Jugular vein. When the rats were intravenously administered from the other end of the polyethylene tube heparin sodium was injected as anticoagulant, then NS, Aspirin, **5a**–**t** were injected, and this end was inserted into the right carotid artery. Blood flowed from the right carotid artery to the left jugular vein through the polyethylene tube for 15 min. The thread was removed. The weight of the wet thrombus thread was recorded. The thread was kept in a desiccator for 2 weeks and the weight of the dry thrombus was recorded.

### 4.12. Oral administration

The solution of Aspirin, **5a**–**t** in NS was fed to Male Wistar rats (weighing 250–300 g, purchased from Animal Center of Peking University) orally. Then the rats were anesthetized with pentobarbital sodium (80.0 mg/kg, ip). Thirty min later the right carotid artery and left jugular vein of the rat were separated. A weighed 6-cm thread was inserted into the middle of a polyethylene tube. The polyethylene tube was filled with heparin sodium (50 IU/ml in NS) and one end was inserted into

the left jugular vein while another end was inserted into the right carotid artery. Blood flowed from the right carotid artery to the left jugular vein through the polyethylene tube for 15 min. The thread was taken out and the weight of the wet thrombus was recorded. The thread was kept in a desiccator for 2 weeks and the weight of the dry thrombus was recorded.

### 4.13. Dosage

For intravenous anti-thrombotic assay, **5a**–**t** were given to the rats at a dose of 0.5 μmol/kg. For dose-dependent experiments, **5b**,**c**,**n** were given to the rats intravenously at three doses 0.5 μmol/kg, 5 nmol/kg, and 0.5 nmol/kg, respectively. For oral anti-thrombotic assay, **5k**,**o**,**p** were given to the rats at a dose of 0.5 μmol/kg.

### 4.14. Deducing the cyclization product of *N*-[(3*S*)]-1,2,3,4-tetrahydro-β-carboline-3-carboxyl]-L-lysine in rat plasma

Male Wistar rats weighing 250 ± 10 g (purchased from Animal Center of Peking University) were anesthetized with pentobarbital sodium (80.0 mg/kg, ip), and the left jugular vein was separated. A 6-cm polyethylene tube was inserted into the left jugular vein and the whole blood was drawn into a heparin sodium containing centrifugal tube. The whole blood was centrifuged at 0 °C and 3500 g for 5 min to isolate the plasma. To the plasma (15 ml) a solution of 3.5 mg of *N*-[(3*S*)]-1,2,3,4-tetrahydro-β-carboline-3-carboxyl]-L-lysine in 100 μl of normal saline was added. At 37 °C the mixture was incubated and shaken in a shaker. Thirty minutes later the incubated mixture was centrifuged at 0 °C and 3500 g for 5 min, and the supernatant was exposed to a gentle nitrogen-flow to evaporate. The obtained residue was dissolved in 100 μl of aqueous methanol (50%, v/v) to prepare sample solution of HPLC-MS analysis on HP ES-5989x instrument.

In the HPLC-MS analysis the column was a reversed-phase C18 column (Agilent Zorbax Extend-C18, 4.6 × 15 mm, 5 μm). After 20 μl of the sample solution was loaded, the column was eluted with 50% solution of MeOH as the mobile phase for 40 min. The flow rate was 1 ml/min. The formed metabolite in the sample was monitored with UV detector at 254.8 nm. The MS analysis was carried out using ESI-model (cone, 30 V; source temperature, 120 °C; desolvation HPLC-MS analysis desolvation gas, 350 L/h; cone gas, 50 L/h).

### 4.15. Deducing the cyclization product of *N*-[(3*S*)]-1,2,3,4-tetrahydro-β-carboline-3-carboxyl]-L-lysine in 5% acetic acid aqueous

To water (15 ml) a solution of 3.5 mg of *N*-[(3*S*)]-1,2,3,4-tetrahydro-β-carboline-3-carboxyl]-L-lysine in 100 μl of normal saline was added. At 37 °C the mixture was incubated and shaken in a shaker. Thirty minutes latter the incubated mixture was centrifuged at 0 °C and 3500 g for 5 min, and the supernatant was exposed to a gentle nitrogen-flow to evaporate. The obtained residue was dissolved in 100 μl of aqueous methanol (50%, v/v) to prepare sample solution of HPLC-MS analysis on HP ES-5989x instrument.

In the HPLC-MS analysis the column was a reversed-phase C18 column (Agilent Zorbax Extend-C18, 4.6 × 15 mm, 5 μm). After 20 μl of the sample solution was loaded, the column was eluted with 50% solution of MeOH as the mobile phase for 40 min. The flow rate was 1 ml/min. The formed metabolite in the sample was monitored with UV detector at 254.8 nm. The MS analysis was carried out using ESI-model (cone, 30 V; source temperature, 120 °C; desolvation HPLC-MS analysis desolvation gas, 350 L/h; cone gas, 50 L/h).

### 4.16. QSAR analysis

To perform a QSAR analysis, first the SMILES formats of compounds **5a**–**t** were generated using JME server (http://www.molinspiration.com/cgi-bin/properties). Moreover, the obtained SMILES files were submitted to e-dragon server (http://www.vcclab.org/lab/edragon/) to calculate molecular descriptors. Finally, the 1664 different molecular parameters for each compound were obtained.

The MLR method was employed to derive the QSAR equation of **5a**–**t**. Concerning the anti-thrombotic effect of a molecule, the weight of wet thrombus (i.e., WWT) was considered in the current QSAR analysis. As the number of compounds is only 20, the optimum number of molecular descriptors used in the QSAR analysis is around 4. To obtain the best regression equation, a two-step descriptor selection procedure was employed. The first step consisted in the elimination of the descriptors that are not able to provide useful statistical information. For instance, the absolute value of the correlation coefficient between the selected descriptor ($X_i$) and the activity ($WWT$) under investigation should be larger than 0.2. At the second step, the descriptor subset was optimized via a genetic algorithm. Finally, the optimal QSAR equation in describing the anti-thrombotic effects of **5a**–**t** was determined.

To systematically assess a QSAR model, a reliable validation is required. Usually, a QSAR model is evaluated by the predictive results for a training dataset (resubstitution test) and a testing dataset (cross-validation), respectively. Of the various cross-validation tests, the LOO test is thought to be a reliable one. By the LOO test, the WWT value of each compound in the dataset is predicted by the rules derived using all other compounds except the one that is being predicted. Both tests of resubstitution and LOO were used to evaluate the QSAR model in the current work. In the resubstitution test, three statistical parameters ($R^2$, $S$, and $\bar{e}$) were used to evaluate the performance. The squared correlation coefficient $R^2$ is defined as follows:

$$R^2 = 1 - \frac{\sum_{i=1}^{N}(y_i - \hat{y}_i)^2}{\sum_{i=1}^{N}(y_i - \bar{y})^2} \qquad (2)$$

where $y_i$, $\hat{y}_i$, and $\bar{y}$ are predicted, actual, and mean values of the activity, respectively. The $N$is the number of

compounds in the resubstitution test. The root mean square of errors $S$ was calculated with the following equation:

$$S = \sqrt{\frac{\sum_{i=1}^{N}(y_i - \hat{y}_i)^2}{N}} \quad (3)$$

To illustrate the predictive accuracy more explicitly, the $\bar{e}$ (absolute average error) is defined as follows:

$$\bar{e} = \frac{\sum_{i=1}^{N}|y_i - \hat{y}_i|}{N} \quad (4)$$

In the LOO test, the above three statistical parameters, that is $R^2_{\mathrm{LOO}}, S_{\mathrm{LOO}}$, and $\bar{e}_{\mathrm{LOO}}$, were also calculated.

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