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Synthesis of new class dipeptide analogues with improved permeability and antithrombotic activity

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Abstract—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 pharmacological property, we designed and synthesized a series of novel dipeptide analogues by incorporating tetrahydro-β-carboline-3-carboxylic acid skeleton as an amino acid surrogate (*Trp). It turned out these dipeptide analogues exhibited good membrane permeability based on in vitro Caco-2 cell monolayers permeability assay. As a result, the overall biological properties of these molecules were significantly improved depending on the nature of the amino acid residues introduced onto the 3-position of the tetrahydro-β-carboline moiety. It was very interesting to notice that these dipeptide analogues (**5b**,**c**,**h**,**i**,**n**,**o**,**p**,**q**) displayed a remarkable dual antiaggregatory activity in both of ADP- and PAF-induced platelet aggregation assay, and their aggregation response was significantly higher than that of aspirin ($p < 0.01$). In addition, these dipeptide analogues were observed for the dose-dependent antithrombotic effect using in vivo rat arterial thrombosis model. The potency of antithrombotic activity of **5h**,**i**,**n**,**p** was significantly higher than that of aspirin ($n = 12$, $p < 0.01$) at equal dose (5 μmol/kg).

1. Introduction

Thromboembolic diseases are the leading cause of mortality and morbidity worldwide.[1] 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.[2–4] Many antithrombotic drug candidates fail to exert their therapeutic potential due to their poor bioavailability. For the oral antithrombotic drug, the major challenge of reaching the action sites is to cross the intestinal epithelial cells to get into the circulation system. Most oral drugs currently on the market rely on passive diffusion to cross cell membranes. Although many factors affect the oral activity of a drug, the limited membrane permeability of antithrombotic drug candidates is thought to be a major barrier leading to the low oral activity of such compounds.[5,6] Various strategies have been attempted to transiently alter the unfavorable physicochemical properties of these compounds to overcome their absorption problem.[7–11] In this regard, a promising strategy for overcoming this problem is to target the intestinal peptide transport system. With this in mind, the drug candidates were designed in the form of di-/tripeptide analogues that can be readily absorbed across the intestinal brush border membrane via the peptide transport system. There are number of reports demonstrating that some orally administered peptide analogues could be effectively absorbed through the intestinal peptide transport system. These studies provided useful information on the structural requirements of substrates for the intestinal peptide transport system. In general, the minimum structural features appearing to be required by the intestinal peptide transport system are: a free C-terminal carboxyl group or a group capable of hydrogen bond formation, either an amino group or a weakly basic

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group at the N-terminus, an overall charge of less than two positive units, and preferably a C-terminal amino acid in its L-configuration.[12–22]

In this study, we have synthesized and evaluated a series of novel dipeptide analogues as a strategy to improve pharmacological and pharmacokinetic properties.[23–32] The main feature of our design is to synthesize small peptide analogues that are metabolically stable and recognized by the intestinal peptide transport system. In this context, we designed and synthesized a series of novel dipeptide analogues by incorporating a tetrahydro-β-carboline-3-carboxylic acid skeleton as an amino acid surrogate (*Trp). 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.[31,33] 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 to a dipeptide analogue for better peptide transport. Herein, we report the design, synthesis, and biological characterization of these unique dipeptide analogues.

2. Results and discussion

2.1. Synthesis of dipeptide analogues based on 3-(*S*)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid scaffold

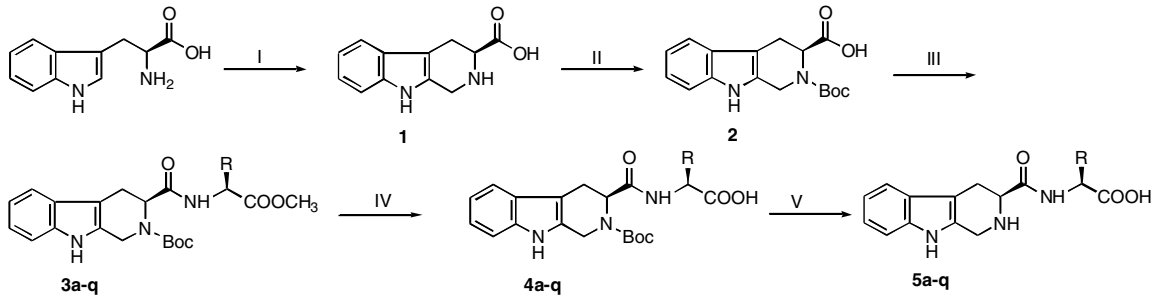
A series of dipeptide analogues were designed and synthesized utilizing 3-(*S*)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid scaffold as L-tryptophane surrogate (*Trp). The synthetic route is outlined in Scheme 1. Starting from the optically active L-tryptophane, compound **1** was synthesized after the Pictect–Spengler reaction, subsequently subject to protection, coupling, and deprotection to generate the desired dipeptide analogues **5a**–**q** with 88–98% yield. In general, these dipeptide analogues fulfilled the minimal structural requirements for the intestinal peptide transport system with a free carboxyl group and one amide group.[22]

2.2. In vitro membrane permeation study

Oral administration is the most commonly used drug-dosing route. Therefore, the ability to predict the extent of absorption of drug candidates after oral administration is crucial during the preclinical evaluation. The oral bioavailability of drug candidates is influenced by many factors, including dissolution, absorption, pre-systemic and systemic metabolism, and elimination. However, the intestinal mucosa is a significant barrier to oral delivery of drugs into the systemic circulation. Caco-2 cells possess many structural and functional similarities to human enterocytes. Recently, Caco-2 cell permeability test has been generally used as a screening tool for assessing drug oral bioavailability during the early stage of drug development.[34–38]

Caco-2 cell monolayers were utilized as an in vitro model of the intestinal mucosa to assess the membrane permeability for these dipeptide analogues. Drug permeability is difficult to measure, thus this assay provides a convenient way to measure permeability based on the apparent permeability coefficient (P_{app}). P_{app} (A → B) is the permeability from the apical to the basolateral side (intestine to blood), and P_{app} (B → A) is the permeability from the basolateral to the apical side (blood to intestine). It was initiated by adding the test solution to the apical or basolateral side of the monolayer. The various dipeptide analogues across Caco-2 cell monolayers were valuated ($n = 3$) in the apical to basolateral (A → B) and basolateral to apical directions (B → A). The influence of efflux carriers on the permeability of the different dipeptide analogues was also examined by comparing the permeability ratio P_{ratio} of absorptive transport P_{app} (A → B) to the secretory one P_{app} (B → A). Permeability coefficients for the synthetic compounds are summarized in Table 1.

According to the previous study, the compound with permeability coefficients $P_{app} < 1 \times 10^{-6}$, $1–10 \times 10^{-6}$, and $>10 \times 10^{-6}$ cm/s was defined as poorly, moderately, and well absorbed, respectively.[36] The parental compound **1**, 3-(*S*)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid, exhibited moderate permeation through the Caco-2 cell monolayer (**1**: 8.95×10^{6} cm/s). In contrast, after converting the parental compound into the dipeptide analogue, the permeability was increased 2- to 3-fold (except for **5f**,**m**). For example, compound Cys-(**5h**), Met-(**5i**), Arg-(**5n**), Lys-(**5p**) *Trp exhibited the considerably increased permeation through Caco-2 cells compared to the parent compound **1** when applied to



Scheme 1. Synthesis of the dipeptide analogues **5a**–**q** based on 3-(*S*)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid scaffold. Reagents and conditions: (I) formaldehyde and sulfuric acid; (II) Boc_2O and triethylamine; (III) L-amino acid methyl ester and DCC; (IV) aqueous NaOH (2 mol/L); (V) hydrogen chloride in ethyl acetate (4 mol/L). In **a**: R = CH_3; **b**: R = $CH_2C_6H_5$; **c**: R = $CH(CH_3)_2$; **d**: R = CH_2OH; **e**: R = $CH(OH)CH_3$; **f**: R = $CH_2C_6H_4$-OH-p; **g**: R = tetrahydropyrrol-2-yl; **h**: R=CH_2SH; **i**: R = $CH_2CH_2SCH_3$; **j**: R = CH_2CH_2COOH; **k**: R = CH_2COOH; **l**: R = 1,3-imidazol-5-methylene; **m**: R = indol-3-methylene; **n**: R = $CH_2(CH_2)_2NHC(NH_2)$=NH; **o**: R = H; **p**: R = $CH_2(CH_2)_3NH_2$; **q**: R = $CH_2CH_2CONH_2$.

Table 1. Apparent permeability coefficients of **1**,**5b–d**,**f**,**h**,**i**,**m–q**

Compound	$P_{app} \times 10^6$ (cm/s)		
	A → B	B → A	A → B/B → A
1	8.95	8.78	1.02
5b	12.52	6.00	2.09
5c	12.35	6.12	2.02
5d	12.27	6.20	2.00
5f	9.33	8.11	1.15
5h	18.20	5.97	3.05
5i	18.54	6.01	3.08
5m	8.96	8.77	1.02
5n	19.54	6.02	3.25
5o	12.55	6.03	2.08
5p	19.82	6.00	3.30
5q	12.64	5.98	2.11

The standard deviations were generally less than 10% ($n = 4$); A → B: From apical side to basolateral side; B → A: From basolateral side to apical side.

the apical side of the monolayer, as shown by the 3-fold increased permeability coefficients P_{app} (A → B) (**5h**: 18.20; **5i**: 18.54; **5n**: 19.54; **5p**: 19.82×10^6 cm/s).

Recent studies indicated that polarized efflux system was a barrier to the absorption of peptides and peptidomimetics.[39] In our study, among the dipeptide analogues tested, the absorptive A → B transport was consistently greater than that observed in the opposite direction (Table 2), and the ratio of the apparent permeability coefficients (P_{app}) of these dipeptide analogues calculated from the A → B and B → A transport of the dipeptides analogues varied between 2.00 and 3.30 (except for **5f**,**m**). Compared to their parental compound **1**, the dipeptide analogues were clearly more permeable to Caco-2 cell monolayers from the A → B direction than their counterpart **1**. The results suggested that the dipeptide analogues imparted greater membrane permeation characteristics than the parental compound **1**. Since P_{app} (A → B) of these compounds was substantially greater than P_{app} (B → A), the asymmetric permeation profile implied that the involvement of apically polarized efflux systems was likely to be excluded.

Moreover, it was noted that the dipeptide analogue, containing polar charged amino acid, that is, Lys-*Trp (**5n**), Arg-*Typ (**5p**), was shown to have increased level of membrane permeability compared to other dipeptide analogues. We speculate that the dipeptide transport system might be preferred to transport polar charged residues better than other types of dipeptide analogues.

Surprisingly, the dipeptide analogues Tyr-*Trp (**5f**) and Trp-*Trp (**5m**) exhibited a slightly improved permeability (**5f**: 9.33×10^6; **5m**: 8.96×10^6 cm/s). This result might imply that factors other than the polarity and charge, for example, spatial arrangement of the molecule, might also influence membrane permeability.

In vitro membrane permeability experiment indicated that this new class of dipeptide analogues was better absorbed in the intestine than its parental compound **1**. In this study, it is suggested that, the membrane permeability of the dipeptide analogue was well correlated to the chemical property of the amino acid residue (i.e., polarity, charge, and spatial arrangement). These compounds will be more likely to target the gastrointestinal transporters involved in the absorption of amino acids and small peptides thereby resulting in improved oral bioavailability.

Although Caco-2 cells have been extensively used to screen the permeation of drug candidates, further validation utilizing in vivo model will be needed due to the differences between in vitro and in vivo models such as the different levels of esterases or the presence of esterases (lipases) in the intestinal lumen.

Table 2. Effect of **5a–q** on ADP-induced platelet aggregation

Compound	Am %($\overline{X} \pm SD$)		
	10^{-7} mol/L	10^{-6} mol/L	10^{-5} mol/L
NS		43.62 ± 2.15	
1	35.43 ± 1.99[a]	30.16 ± 2.76[a]	26.25 ± 2.41[a]
Aspirin	33.46 ± 2.25[a]	29.32 ± 2.38[a]	25.38 ± 2.02[a]
5a	34.37 ± 2.34[a]	29.50 ± 2.80[a]	23.46 ± 2.25[a,b,e]
5b	30.28 ± 2.45[a,b,d]	25.23 ± 2.55[a,b,d]	18.64 ± 2.21[a,b,d]
5c	28.68 ± 2.66[a,b,d]	23.46 ± 2.77[a,b,d]	15.92 ± 2.90[a,b,d]
5d	31.56 ± 2.51[a,b,e]	27.13 ± 3.01[a,c,e]	20.81 ± 2.61[a,b,d]
5e	35.24 ± 2.38[a]	30.78 ± 3.20[a]	25.99 ± 3.02[a]
5f	34.97 ± 3.11[a]	30.11 ± 3.40[a]	25.87 ± 3.15[a]
5g	34.89 ± 3.00[a]	31.01 ± 3.23[a]	25.59 ± 2.85[a]
5h	27.89 ± 2.02[a,b,d]	21.88 ± 2.30[a,b,d]	14.01 ± 2.23[a,b,d]
5i	25.97 ± 2.34[a,b,d]	20.86 ± 2.25[a,b,d]	14.62 ± 1.99[a,b,d]
5j	33.41 ± 2.25[a]	27.82 ± 2.36[a,c]	24.01 ± 2.58[a,c]
5k	32.88 ± 2.57[a,c]	26.69 ± 2.62[a,b,e]	21.74 ± 2.56[a,b,d]
5l	31.40 ± 2.57[a,b,e]	25.45 ± 2.74[a,b,d]	20.77 ± 2.81[a,b,d]
5m	35.01 ± 3.03[a]	30.42 ± 3.29[a]	25.66 ± 3.32[a]
5n	25.22 ± 2.26[a,b,d]	20.04 ± 2.30[a,b,d]	13.84 ± 1.87[a,b,d]
5o	28.50 ± 2.52[a,b,d]	23.12 ± 2.55[a,b,d]	15.55 ± 2.73[a,b,d]
5p	25.01 ± 2.12[a,b,d]	19.94 ± 2.27[a,b,d]	13.62 ± 1.83[a,b,d]
5q	30.05 ± 2.38[a,b,d]	25.10 ± 2.42[a,b,d]	18.26 ± 2.25[a,b,d]

$n = 12$, NS = vehicle.
[a] Compare to NS, $P < 0.01$.
[b] Compare to **1**, $P < 0.01$.
[c] Compare to **1**, $P < 0.05$.
[d] Compare to aspirin, $P < 0.01$.
[e] Compare to aspirin, $P < 0.05$.

2.3. Antiplatelet aggregation in vitro

Antiplatelet aggregation assay was used to evaluate the effects of these analogues utilizing in vitro rabbit platelet aggregation induced by adenosine-5-diphosphate (ADP) or platelet activating factor (PAF). Incubation of dipeptide analogues with ADP or PAF in the presence of platelets for 120 min clearly affected the in vitro induced platelet aggregation at a concentration of 100 nM. The duration of incubation had no significant influence on the antiaggregatory capacity (data not shown).

The initial screening at 100 nM concentration revealed that the most tested compounds were active for anti-aggregation. However, the differences in chemical structure of side chain had influence on ADP- or PAF-induced aggregation. Compound **5h**,**i**,**n**,**p** (Cys-*Trp, Met-*Trp, Arg-*Trp, Lys-*Trp) was found to be the most potent

antiplatelet analogue based on the ADP- or PAF-induced platelet assay (Tables 2 and 3).

At the concentration of 100 nM or above, the dipeptide analogues of Phe-(**5b**), Val-(**5c**), Cys-(**5h**), Met-(**5i**), Arg-(**5n**), Gly-(**5o**), Lys-(**5p**), and Gln-(**5q**) *Trp were observed to have a dual effect against ADP- and PAF-induced platelet aggregation, and the antiaggregatory potency was significantly improved compared to compound **1**, and even considerably higher than that of aspirin (compared to NS, **1**, aspirin, $p < 0.01$, for both). For example, when ADP activated platelets were treated with 10^{-6} mol/L of **5b**,**c**,**h**,**i**,**n**,**o**,**p**,**q**, the platelet aggregation rate (%) was considerably lowered from 43.62 ± 2.15 (for NS, control) to 25.23 ± 2.55 (**5b**), 23.46 ± 2.77 (**5c**), 21.88 ± 2.30 (**5h**), 20.86 ± 2.55 (**5i**), 20.04 ± 2.30 (**5n**), 23.12 ± 2.55 (**5o**), 19.94 ± 2.27 (**5p**), and 25.10 ± 2.42 (**5q**) versus 30.16 ± 2.76 (**1**) and 29.32 ± 2.38 (aspirin), respectively. Interestingly, at the same concentration range, compound **5b**,**c**,**h**,**i**,**n**,**o**,**p**,**q** was detected with comparable antiaggregatory potency in PAF-induced platelet aggregation assay.

To examine the impact of basic amino acid residue (arginine, lysine) within the dipeptide analogues, the neutral residue was introduced by replacing arginine and lysine with neutral amino acid, that is, alanine, valine, and serine. It was noticed that, when the dipeptide analogues containing basic residue, that is, Arg-*Trp (**5n**), Lys-*Trp (**5p**), switched to the neutral dipeptide analogues, that is, Ala-*Trp (**5a**), Val-*Trp (**5c**), Ser-*Trp (**5d**), the antiaggregatory potency was shown to be decreased. For example, when ADP activated platelet was incubated with 10 μM of compound **5a**,**c**,**d**, although antiaggregatory effect was significant (compared to NS, **1**, aspirin, $p < 0.01$ for both), the platelet aggregation was inhibited to a lesser extent compared with that of **5n**,**p** (**5a**: 23.46 ± 2.25; **5c**: 15.92 ± 2.90; **5d**: 20.81 ± 2.61; **5e**: 25.99 ± 3.02 vs **5n**: 13.84 ± 1.87; **5p**: 13.62 ± 1.83).

Since Arg- and Lys-side chain significantly promoted biological activity, we assumed the presence of the positive charged residue in the dipeptide analogue might be enhancing its antiaggregatory property. Compound **5j**,**k** displayed a good antiaggregatory effect but weaker potency than that of Arg-, and Lys-*Trp with the substitution of positive charged amino acid with negative charged residue, that is, Glu-*Trp (**5j**), Asp-*Trp (**5k**) in PAF- or ADP-induced platelet aggregation assay. The results suggest that the basic positive charges in the dipeptide analogues may be important for their biological activity.

More interestingly, the introduction of sulfur-containing side chain (Cys and Met) bestowed the dipeptide analogue (**5h**,**i**) with the considerably enhanced antiaggregatory effect. In ADP-induced platelet assay, at the concentration of 10 μM of **5h**,**i**, the platelet aggregation rate (%) was significantly decreased with 14.01 ± 2.23, 14.62 ± 1.99, respectively, compared to that of **1**: 26.25 ± 2.41 ($p < 0.01$). Similar observation was observed in PAF-induced platelet aggregation assay.

Compared to Lys-, Arg-, Met-, Cys-*Trp, the introduction of amino acid in aromatic rings, that is, Tyr-*Trp (**5f**), Trp-*Trp (**5m**), resulted in a partial loss of antiaggregatory potency in both of PAF- and ADP-induced platelet aggregation assay. In a similar manner, the introduction of a rigid proline ring in the dipeptide analogue generated a moderate anti-aggregation derivative Pro-*Trp (**5g**). We deduced that these conformationally restricted dipeptide analogues (**5f**,**m**,**g**) will be most likely to assume an inappropriate position in the receptor–ligand recognition process thereby leading to a decreased anti-aggregation effect.

It seems that the presence of amino acid residue with aromatic ring or rigid ring might hinder any interaction between molecules and platelets. As a consequence, we attempted to insert a smaller amino acid residue to lower the steric hindrance, that is, Gly-*Trp (**5o**). Interestingly, a positive influence on antiaggregatory activity was observed in these two series studied. For example, in ADP-induced platelet assay, at the concentration of 10 μM, the platelet aggregation rate (%) was significantly decreased with 15.55 ± 2.73, compared to that of **1**: 26.25 ± 2.41 ($p < 0.01$). A similar observation was seen in PAF-induced platelet aggregation assay.

The in vitro assay indicated that the new class of dipeptide analogues was very likely to be a potent platelet aggregation inhibitor based on in vitro induced platelet aggregation. It was interesting to notice that, Val-, Phe-, Cys-, Met-, Arg-, Lys-, Gly-, Gln-*Trp displayed a remarkable dual antiplatelet activity in both of ADP- and PAF-induced platelet aggregation assay. Surprisingly,

Table 3. Effect of **5a**–**q** on PAF-induced platelet aggregation

Compound	Am %($\overline{X} \pm SD$)		
	10^{-7} mol/L	10^{-6} mol/L	10^{-5} mol/L
NS		46.01 ± 2.34	
	36.98 ± 2.43^{a}	32.45 ± 2.28^{a}	29.01 ± 2.50^{a}
Aspirin	36.06 ± 2.38^{a}	31.87 ± 2.44^{a}	27.00 ± 2.52^{a}
5a	36.78 ± 2.44^{a}	32.02 ± 2.67^{a}	27.28 ± 2.33^{a}
5b	$32.67 \pm 2.60^{a,b,d}$	$27.45 \pm 2.62^{a,b,d}$	$21.04 \pm 2.37^{a,b,d}$
5c	$30.48 \pm 3.01^{a,b,d}$	$25.57 \pm 2.69^{a,b,d}$	$18.07 \pm 2.87^{a,b,d}$
5d	$33.27 \pm 2.66^{a,c,e}$	$29.48 \pm 2.95^{a,c,e}$	$23.00 \pm 2.69^{a,b,d}$
5e	35.55 ± 2.78^{a}	31.88 ± 2.95^{a}	29.02 ± 2.97^{a}
5f	36.04 ± 2.89^{a}	32.02 ± 2.89^{a}	28.99 ± 3.21^{a}
5g	36.40 ± 3.11^{a}	33.01 ± 3.37^{a}	29.20 ± 2.77^{a}
5h	$30.30 \pm 2.23^{a,b,d}$	$24.04 \pm 2.72^{a,b,d}$	$16.56 \pm 2.45^{a,b,d}$
5i	$28.04 \pm 2.77^{a,b,d}$	$23.08 \pm 2.90^{a,b,d}$	$17.17 \pm 2.55^{a,b,d}$
5j	36.06 ± 2.64^{a}	$29.38 \pm 2.88^{a,c,e}$	$25.69 \pm 2.73^{a,b}$
5k	$34.22 \pm 2.29^{a,c}$	$29.75 \pm 2.88^{a,c}$	$24.04 \pm 2.66^{a,b,e}$
5l	$33.70 \pm 2.88^{a,c,e}$	$27.80 \pm 2.91^{a,b,d}$	$23.38 \pm 2.60^{a,b,d}$
5m	35.98 ± 2.75^{a}	31.99 ± 2.92^{a}	28.27 ± 3.15^{a}
5n	$26.53 \pm 2.69^{a,b,d}$	$21.88 \pm 2.78^{a,b,d}$	$15.63 \pm 2.48^{a,b,d}$
5o	$30.02 \pm 2.97^{a,b,d}$	$25.22 \pm 2.54^{a,b,d}$	$17.99 \pm 2.65^{a,b,d}$
5p	$25.88 \pm 2.54^{a,b,d}$	$21.38 \pm 2.62^{a,b,d}$	$15.27 \pm 2.44^{a,b,d}$
5q	$32.12 \pm 2.57^{a,b,d}$	$27.07 \pm 2.59^{a,b,d}$	$20.97 \pm 2.41^{a,b,d}$

$n = 12$, NS = vehicle.
[a] Compare to NS, $P < 0.01$.
[b] Compare to **1**, $P < 0.01$.
[c] Compare to **1**, $P < 0.05$.
[d] Compare to aspirin, $P < 0.01$.
[e] Compare to aspirin, $P < 0.05$.

the introduction of Pro-, Trp-, Tyr-side chain was not beneficial to antiplatelet activity in the ADP- and PAF-induced platelet aggregation assay. Thus, the rigid side chain seemed to be detrimental to antiplatelet activity against ADP. In contrast, the presence of the polar side chain rendered the dipeptide analogues, Lys-, Arg-, Cys-, Met-*Trp, to be the most potent compound in this assay. Therefore, it seems reasonable to speculate that, within this series, the nature of the amino acid residues introduced in tetrahydrocarboline skeleton may be crucial to determine the potential biological activity. The aggregation response of these dipeptide analogues was comparable to or better than that of aspirin even when different inducing agents (ADP or PAF) were used ($n = 12$, $p < 0.01$). Longer incubation time has no impact on platelet response, which may be due to a fast metabolization of the test compound (data not shown).

2.4. Evaluation of antithrombotic effect in vivo

The antithrombotic effect of dipeptide analogues was evaluated using in vivo rat model. Initially, the dipeptide analogues were administered at a dose of 1.2, 2.5, and 5 μmol/kg, respectively. From Tables 4 and 5, it was observed that the antithrombotic activity of these dipeptide analogues was following dose-dependent manners. At the dose of 1.2 μmol/kg, only compound **5h**,**i**,**n**,**p** exhibited a weak antithrombotic effect. While at a dose of 2.5 μmol/kg, the antithrombotic activity of all of dipeptide analogues tested was obvious and comparable to that of aspirin (5 μmol/kg). Interestingly, at equivimolar doses, some of the dipeptide analogues (**5h,i,n,p**) presented significant antithrombotic effect. The thrombus weight after the treatment with **5h**,**i**,**n**,**p** was 21.47 ± 2.92, 20.69 ± 3.44, 20.01 ± 3.12, and 19.87 ± 3.01 mg, respectively (NS: 38.67 ± 3.26, $p < 0.01$). The potency of antithrombotic activity of **5h**,**i**,**n**,**p** was significantly higher than that of aspirin (aspirin: 28.95 ± 3.32, $n = 12$, $p < 0.01$) at equal dose (5 μmol/kg).

Table 4. Effect of **5a**–**q** on the thrombus weight

Compound	Wet thrombus ($\overline{X} \pm$ SD mg)	Dry thrombus ($\overline{X} \pm$ SD mg)
NS	38.67 ± 3.26	7.69 ± 1.69
1	29.69 ± 3.22[a]	5.88 ± 1.26[a]
Aspirin	28.95 ± 3.32[a]	5.11 ± 1.99[a]
5a	28.97 ± 3.56[a]	5.13 ± 1.34[a]
5b	24.35 ± 3.28[a,b,d]	4.37 ± 1.88[a,c,e]
5c	23.67 ± 3.18[a,b,d]	4.14 ± 1.77[a,c,e]
5d	24.52 ± 3.25[a,b,d]	4.29 ± 1.54[a,c,e]
5e	29.24 ± 3.18[a]	5.75 ± 1.81[a]
5f	30.22 ± 2.67[a]	5.62 ± 1.53[a]
5g	29.34 ± 3.34[a]	5.56 ± 1.66[a]
5h	21.47 ± 2.92[a,b,d]	3.55 ± 1.07[a,b,d]
5i	20.69 ± 3.44[a,b,d]	3.16 ± 1.55[a,b,d]
5j	29.03 ± 3.31[a]	5.81 ± 1.50[a]
5k	28.78 ± 3.46[a]	5.64 ± 1.26[a]
5l	26.15 ± 3.23[a,c,e]	4.78 ± 1.25[a,c]
5m	31.00 ± 2.59[a]	6.02 ± 1.49[a]
5n	20.01 ± 3.12[a,b,d]	3.00 ± 1.62[a,b,d]
5o	23.22 ± 3.11[a,b,d]	4.00 ± 1.75[a,c,e]
5p	19.87 ± 3.01[a,b,d]	2.89 ± 1.58[a,b,d]
5q	24.11 ± 3.24[a,b,d]	4.12 ± 1.82[a,c,e]

Dosage, 5 μmol/kg; $n = 12$, NS = vehicle.
[a] Compare to NS, $P < 0.01$.
[b] Compare to **1**, $P < 0.01$.
[c] Compare to **1**, $P < 0.05$.
[d] Compare to aspirin, $P < 0.01$.
[e] Compare to aspirin, $P < 0.05$.

Table 5. Effect of **5b**–**d**,**h**,**I**,**n**–**q** at different doses on the thrombus weight

Compound	Dosage/wet thrombus ($\overline{X} \pm$ SD mg)		
	5.0 μmol/kg	2.5 μmol/kg	1.2 μmol/kg
5b	24.35 ± 3.28[a]	29.23 ± 3.11[b]	38.99 ± 3.43
5c	23.67 ± 3.18[a]	28.00 ± 3.04[b]	38.20 ± 3.31
5d	24.52 ± 3.25[a]	29.84 ± 3.26[b]	37.06 ± 3.13
5h	21.47 ± 2.92[a]	27.00 ± 3.02[b]	36.11 ± 3.40
5i	20.69 ± 3.44[a]	26.80 ± 3.30[b]	36.00 ± 3.35
5n	20.01 ± 3.12[a]	26.03 ± 3.22[b]	36.43 ± 3.50
5o	23.22 ± 3.11[a]	27.80 ± 3.00[b]	38.86 ± 3.24
5p	19.87 ± 3.01[a]	26.00 ± 3.06[b]	36.47 ± 3.16
5q	24.11 ± 3.24[a]	29.01 ± 3.14[b]	38.94 ± 3.13

$N = 12$.
[a] Compare to 2.5 μmol/kg group, $P < 0.01$.
[b] Compare to 1.2 μmol/kg group, $P < 0.01$.

3. Conclusion

The in vitro antiplatelet assay and in vitro antithrombotic assessment confirmed that 3-(*S*)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid **1** was responsible for the antithrombotic activity as an important scaffold. After converting into its dipeptide analogue, the parent compound **1** was imparted a significantly enhanced antithrombotic activity, and its anti-aggregation response was also significantly improved.

It was interesting to notice that the nature of the amino acid residues introduced to the 3-position of tetrahydro-β-carboline scaffold apparently had an impact on the antiplatelet and antithrombotic activities of these analogues. The most significant antiplatelet and antithrombotic profile of dipeptide analogues Lys-*Trp, Arg-*Trp could be correlated with the influence of the polar charged side chain. In addition, introduction of sulfur-containing side chain (Cys and Met) bestowed the parent compound **1** with the considerably improved biological activity. Clearly, the polarity, charge, molecular size, and the spatial arrangement of these compounds appeared to be some of the key factors in influencing their biological potencies.

Our results indicated by converting them into dipeptide analogues, it rendered 3-(*S*)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid more easily to penetrate the cell membrane, thereby increasing its penetration ability a cross the cell membrane and leading to the enhanced biological activity. The mechanism of the antithrombotic activity of these compounds has not been elucidated, and their abilities to prevent the aggregation stimulated by different aggregating agents led us to hypothesize that they might intercept a common signaling pathway in the

platelet aggregation cascade. The notable anti-aggregating potency, regardless of the cause of activation, was regarded as an attractive approach to develop novel effective antiplatelet drugs, for instance, for the GPIIb/IIIa antagonists, phosphodiesterase inhibitors, and natural or synthetic platelet activating factor antagonists.

In conclusion, these results suggested that the new class of dipeptide analogues might be a promising antithrombotic agent. The antithrombotic effect of these compounds is most likely based on a direct, inhibitory action on platelets.

4. Experimental

4.1. Chemistry

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 the 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 Hitachi 835-50 instrument. FAB-MS was determined by VG-ZAB-MS high resolution GC/MS/DS and HP ES-5989x. Optical rotations were measured with a Schmidt + Haensch Polartromic D instrument.

4.2. 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), 80 mL of water, and 8 mL of formaldehyde (36–38%) were added. The reaction mixture was stirred at room temperature for 2 h and adjusted to pH 6–7 with a concentrated ammonia solution. The mixture obtained was kept at 0 °C for 12 h and the formed precipitates were collected by filtration. After recrystallization, 3.97 g (75%) of the title compound was obtained as a colorless powder. Mp 280–282 °C; EI/MS: 217 $[M+H]^+$; IR (KBr): 3450, 3200, 3000, 2950, 2850, 1700, 1601, 1452, 1070, 900 cm^{-1}; ^1H NMR (BHSC-500, DMSO-d_6): δ = 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.

4.3. *N*-Boc-3*S*-1,2,3,4-Tetrahydro-β-carboline-3-carboxylic acid (2)

A suspension of 1.1 g (5.0 mmol) of **1** in 15 mL DMF and 1.4 mL of triethylamine was vigorously stirred at room temperature, 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 solution (20%) was added and the solution was extracted with ethyl acetate (3× 30 mL). The separated ethyl acetate layer was dried with anhydrous $MgSO_4$. After removal of $MgSO_4$ by filtration, the filtrate was dried by evaporation. The residue obtained was crystallized in $CHCl_3$ to yield 1.20 g (76%) of the title compound. Mp 165–170 °C; TOF/MS: 317 $[M+H]^+$ 339 $[M+Na]^+$, 355 [M+K]; IR (KBr): 3452, 3205, 3001, 2952, 2848, 1705, 1645, 1600, 1450, 1072, 901 cm^{-1}; ^1H NMR (BHSC-500, DMSO-d_6): δ = 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.

4.4. General procedure for preparation of 3a–q

At 0 °C, to a solution of 2.0 g (6.33 mmol) of **2** in 30 mL of anhydrous THF, 1.2 g (8.9 mmol) of HOBt was added. After stirring for 10 min, 1.75 g (8.5 mmol) of DCC was added. The suspension of 6.96 mmol of HCl·L-AA-OMe in 3 mL of anhydrous THF was adjusted with *N*-methyl morpholine to pH 8–9 and stirred at room temperature for additional 20 min. This suspension was then added to the solution of **2**, and the reaction mixture was continued stirring at 0 °C for 2 h and then at room temperature for 16 h. After evaporation, the residue was dissolved in 30 mL of ethyl acetate. The solution was washed extensively 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, *N*-(*N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxyl)-L-amino acid methylester (**3a**–**q**) was obtained.

4.4.1. *N*-(*N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxyl)-L-alanine methylester (3a). Yield 96%; mp 144–146 °C; ESI/MS 402 $[M+H]^+$; IR (KBr): 3451, 3011, 2949, 2847, 1730, 1604, 1450, 1392, 1370, 1066, 897 cm^{-1}; ^1H NMR (BHSC-500, DMSO-d_6): δ = 9.89 (s, 1H), 7.98 (s, 1H), 7.32 (t, J = 7.5 Hz, 1H), 7.23 (t, J = 7.8 Hz, 1H), 6.97 (d, J = 7.8 Hz, 1H), 6.81 (d, J = 7.5 Hz, 1H), 4.88 (d, J = 5.2 Hz, 1H), 4.59 (m, J = 5.5 Hz, 1H), 4.25 (dd, J = 10.0 Hz, J = 4.7 Hz, 1H), 4.17 (dd, J = 10.1 Hz, J = 3.5 Hz, 1H), 3.64 (s, 3H), 2.94 (d, J = 10.1 Hz, 2H), 1.55 (d, J = 5.2 Hz, 3H), 1.43 (s, 9H). Anal. Calcd for $C_{21}H_{27}N_3O_5$: C, 62.83; H, 6.78; N, 10.47. Found: C, 62.92; H, 6.74; N, 10.30.

4.4.2. *N*-(*N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxyl)-L-phenylalanine methylester (3b). Yield 98%; mp 150–152 °C; ESI/MS 478 $[M+H]^+$; IR (KBr): 3446, 3205, 3006, 2948, 2847, 1731, 1645, 1603, 1451, 1392, 1370, 1069, 904 cm^{-1}; ^1H NMR (BHSC-500, DMSO-d_6): δ = 9.92 (s, 1H), 7.97 (s, 1H), 7.31 (t, J = 7.5 Hz, 1H), 7.28 (t, J = 7.9 Hz, 2H), 7.19 (t, J = 7.6 Hz, 1H), 7.14 (d, J = 7.6 Hz, 2H), 7.02 (t, J = 7.6 Hz, 1H), 6.96 (d, J = 7.8 Hz, 1H), 6.80 (d, J = 7.6 Hz, 1H), 4.93 (d,

J = 5.4 Hz, 1H), 4.82 (t, J = 5.4 Hz, 1H), 4.27 (dd, J = 10.2 Hz, J = 4.5 Hz, 1H), 4.18 (dd, J = 10.2 Hz, J = 3.4 Hz, 1H), 3.62 (s, 3H), 3.17 (d, J = 5.4 Hz, 2H), 2.93 (d, J = 10.2 Hz, 2H), 1.48 (s, 9H). Anal. Calcd for $C_{27}H_{31}N_3O_5$: C, 67.91; H, 6.54; N, 8.80. Found: C, 67.72; H, 6.62; N, 8.67.

4.4.3. *N*-(*N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxyl)-L-valine methylester (3c). Yield 95%; mp 138–140 °C; ESI/MS 430 $[M+H]^+$. IR (KBr): 3443, 3202, 3001, 2951, 2845, 1729, 1648, 1602, 1450, 1392, 1370, 1067, 902 cm^{-1}; ^1H NMR (BHSC-500, DMSO-d_6): δ = 10.04 (s, 1H), 7.96 (s, 1H), 7.29 (t, J = 7.4 Hz, 1H), 7.21 (t, J = 7.7 Hz, 1H), 7.00 (d, J = 7.7Hz, 1H), 6.89 (d, J = 7.4 Hz, 1H), 4.84 (t, J = 5.4 Hz, 1H), 4.42 (d, J = 5.4 Hz, 1H), 4.22 (dd, J = 10.2 Hz, J = 4.5 Hz, 1H), 4.03 (dd, J = 10.2 Hz, J = 3.7 Hz, 1H), 3.62 (s, 3H), 3.10 (m, J = 5.4 Hz, 1H), 2.95 (d, J = 6.7 Hz, 2H), 1.47 (s, 9H), 1.05 (d, J = 5.4 Hz, 6H). Anal. Calcd for $C_{23}H_{31}N_3O_5$: C, 64.32; H, 7.27; N, 9.78. Found: C, 64.43; H, 7.09; N, 9.67.

4.4.4. *N*-(*N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxyl)-L-serine methylester (3d). Yield 92%; mp 139–141 °C; ESI/MS 418 $[M+H]^+$. IR (KBr): 3442, 3200, 3001, 2952, 2845, 1730, 1644, 1606, 1455, 1392, 1370, 1067, 900 cm^{-1}; ^1H NMR (BHSC-500, DMSO-d_6): δ = 9.95 (s, 1H), 7.97 (s, 1H), 7.29 (t, J = 7.6 Hz, 1H), 7.22 (t, J = 7.9 Hz, 1H), 6.99 (d, J = 7.9 Hz, 1H), 6.83 (t, J = 7.6 Hz, 1H), 4.87 (d, J = 5.4 Hz, 1H), 4.52 (t, J = 5.6 Hz, 1H), 4.19 (d, J = 5.2 Hz, 2H), 4.13 (d, J = 5.6 Hz, 2H), 3.63 (s, 3H), 2.95 (d, J = 5.6 Hz, 1H), 2.92 (d, J = 5.6 Hz, 1H), 2.28 (s, 1H), 1.45 (s, 9H). Anal. Calcd for $C_{21}H_{27}N_3O_6$: C, 60.42; H, 6.52; N, 10.07. Found: C, 60.31; H, 6.36; N, 10.24.

4.4.5. *N*-(*N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxyl)-L-threonine methylester (3e). Yield 92%; mp 140–142 °C; ESI/MS 432 $[M+H]^+$; IR (KBr): 3437, 3200, 3002, 2951, 2844, 1735, 1649, 1600, 1450, 1392, 1370, 1065, 901 cm^{-1}; ^1H NMR (BHSC-500, DMSO-d_6): δ = 9.98 (s, 1H), 7.87 (s, 1H), 7.34 (t, J = 7.4 Hz, 1H), 7.25 (t, J = 7.6 Hz, 1H), 6.95 (d, J = 7.6 Hz, 1H), 6.72 (d, J = 7.4 Hz, 1H), 4.87 (t, J = 5.4 Hz, 1H), 4.67 (m, J = 5.6 Hz, 1H), 4.48 (t, J = 5.6 Hz, 1H), 3.99 (m, J = 5.2 Hz, 2H), 3.65 (s, 3H), 2.97 (d, J = 5.7 Hz, 2H), 2.19 (d, J = 3.7 Hz, 1H), 1.47 (s, 9H), 1.19 (d, J = 5.6 Hz, 3H). Anal. Calcd for $C_{22}H_{29}N_3O_6$: C, 61.24; H, 6.77; N, 9.74. Found: C, 61.40; H, 6.91; N, 9.55.

4.4.6. *N*-(*N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxyl)-L-tyrosine methylester (3f). Yield 93%; mp 143–145 °C; ESI/MS 494 $[M+H]^+$; IR (KBr): 3439, 3203, 3001, 2955, 2847, 1732, 1644, 1601, 1453, 1391, 1372, 1062, 903 cm^{-1}; ^1H NMR (BHSC-500, DMSO-d_6): δ = 9.99 (s, 1H), 8.02 (s, 1H), 7.37 (t, J = 7.6 Hz, 1H), 7.22 (t, J = 7.7 Hz, 1H), 7.15 (d, J = 7.5 Hz, 2H), 7.02 (d, J = 7.5 Hz, 1H), 6.96 (d, J = 7.7 Hz, 1H), 6.91 (d, J = 7.5 Hz, 2H), 4.98 (s, 1H), 4.93 (d, J = 5.4 Hz,1H), 4.80 (t, J = 5.6 Hz, 1H), 4.29 (m, J = 5.2 Hz, 2H), 3.64 (s, 3H), 3.15 (d, J = 5.2 Hz, 2H), 2.97 (d, J = 5.0 Hz, 2H), 1.49 (s, 9H). Anal. Calcd for $C_{27}H_{31}N_3O_6$: C, 65.71; H, 6.33; N, 8.51. Found: C, 65.67; H, 6.50; N, 8.67.

4.4.7. *N*-(*N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxyl)-L-proline methylester (3g). Yield 97%; mp 139–141 °C; ESI/MS 428 $[M+H]^+$; IR (KBr): 3435, 3202, 3000, 2950, 2846, 1732, 1645, 1602, 1454, 1390, 1371, 1063, 900 cm^{-1}; ^1H NMR (BHSC-500, DMSO-d_6): δ = 10.01 (s, 1H), 7.35 (t, J = 7.4 Hz, 1H), 7.20 (t, J = 7.7 Hz, 1H), 7.07 (d, J = 7.7 Hz, 1H), 6.91 (d, J = 7.4 Hz, 1H), 4.88 (t, J = 5.4 Hz, 1H), 4.35 (t, J = 5.6 Hz, 1H), 4.22 (d, J = 5.3 Hz, 2H), 3.59 (s, 3H), 3.47 (t, J = 5.6 Hz, 2H), 2.95 (d, J = 5.6 Hz, 2H), 2.29 (d, J = 5.6Hz, 2H), 1.97 (t, J = 4.9 Hz, 2H), 1.45 (s, 9H). Anal. Calcd for $C_{23}H_{29}N_3O_5$: C, 64.62; H, 6.84; N, 9.83. Found: C, 64.57; H, 6.90; N, 9.67.

4.4.8. *N*-(*N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxyl)-L-cysteine methylester (3h). Yield 92%; mp 151–153 °C; ESI/MS 420 $[M+H]^+$; IR (KBr): 3445, 3203, 3000, 2944, 2840, 1731, 1643, 1601, 1453, 1390, 1372, 1061, 898 cm^{-1}; ^1H NMR (BHSC-500, DMSO-d_6): δ = 9.93 (s, 1H), 7.97 (s, 1H), 7.32 (t, J = 7.5 Hz, 1H), 7.22 (t, J = 7.8 Hz, 1H), 7.00 (d, J = 7.8 Hz, 1H), 6.88 (d, J = 7.5 Hz, 1H), 4.93 (t, J = 5.3 Hz, 1H), 4.72 (t, J = 5.5 Hz, 1H), 4.21 (d, J = 5.3 Hz, 2H), 3.68 (s, 3H), 3.16 (d, J = 5.5 Hz, 2H), 2.90 (d, J = 5.6 Hz, 2H), 1.45 (s, 9H), 1.62 (s, 1H). Anal. Calcd for $C_{21}H_{27}N_3O_5S$: C, 58.18; H, 6.28; N, 9.69. Found: C, 58.27; H, 6.33; N, 9.57.

4.4.9. *N*-(*N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxyl)-L-methionine methylester (3i). Yield 97%; mp 159–161 °C; ESI/MS 462 $[M+H]^+$;IR (KBr): 3441, 3203, 3004, 2953, 2847, 1732, 1641, 1603, 1454, 1390, 1372, 1061, 900 cm^{-1}; ^1H NMR (BHSC-500, DMSO-d_6): δ = 10.04 (s, 1H), 7.97 (s, 1H), 7.32 (t, J = 7.5 Hz, 1H), 7.22 (t, J = 7.8 Hz, 1H), 6.99 (d, J = 7.8 Hz, 1H), 6.81 (d, J = 7.5 Hz, 1H), 4.86 (t, J = 5.3 Hz, 1H), 4.45 (t, J = 5.5 Hz, 1H), 4.28 (d, J = 5.1 Hz, 2H), 3.68 (s, 3H), 2.93 (d, J = 5.3 Hz, 2H), 2.42 (t, J = 5.4 Hz, 2H), 2.28 (d, J = 5.6 Hz, 2H), 2.10 (s, 3H), 1.44 (s, 9H). Anal. Calcd for $C_{23}H_{31}N_3O_5S$: C, 59.85; H, 6.77; N, 9.10. Found: C, 59.67; H, 6.59; N, 9.04.

4.4.10. *N*-(*N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxyl)-L-glutamic acid di-methylester (3j). Yield 93%; mp 154–156 °C; ESI/MS 474 $[M+H]^+$; IR (KBr): 3441, 3203, 3000, 2944, 2831, 1731, 1645, 1604, 1455, 1390, 1372, 1067, 903 cm^{-1}; ^1H NMR (BHSC-500, DMSO-d_6): δ = 9.89 (s, 1H), 8.04 (s, 1H), 7.39 (t, J = 7.6 Hz, 1H), 7.28 (t, J = 7.6 Hz, 1H), 7.01 (d, J = 7.7 Hz, 1H), 6.84 (d, J = 7.6 Hz, 1H), 4.90 (d, J = 5.4 Hz, 1H), 4.43 (t, J = 5.6 Hz, 1H), 4.22 (d, J = 5.5 Hz, 2H), 3.66 (s, 3H), 3.64 (s, 3H), 2.96 (d, J = 5.4 Hz, 2H), 2.28 (t, J = 5.6 Hz, 2H), 2.24 (t, J = 5.7 Hz, 2H), 1.43 (s, 9H). Anal. Calcd for $C_{24}H_{31}N_3O_7$: C, 60.88; H, 6.60; N, 8.87. Found: C, 60.73; H, 6.49; N, 8.69.

4.4.11. *N*-(*N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxyl)-L-aspartic acid di-methylester (3k). Yield 90%; mp 158–160 °C; ESI/MS 460 $[M+H]^+$; IR (KBr): 3441, 3210, 3004, 2955, 2841, 1732, 1643, 1604, 1453, 1390, 1371, 1061, 903 cm^{-1}; ^1H NMR (BHSC-500,

DMSO-d_6): δ = 10.05 (s, 1H), 8.05 (s, 1H), 7.37 (t, J = 7.4 Hz, 1H), 7.25 (t, J = 7.4 Hz, 1H), 7.00 (d, J = 7.6 Hz, 1H), 6.95 (d, J = 7.4 Hz, 1H), 4.92 (d, J = 5.5 Hz, 1H), 4.77 (t, J = 5.5 Hz, 1H), 4.24 (d, J = 5.6 Hz, 2H), 3.62 (s, 3H), 3.58 (s, 3H), 2.91 (d, J = 5.2 Hz, 2H), 2.85 (d, J = 5.4 Hz, 2H), 1.49 (s, 9H). Anal. Calcd for $C_{23}H_{29}N_3O_7$: C, 60.12; H, 6.36; N, 9.14. Found: C, 60.03; H, 6.49; N, 8.99.

4.4.12. *N*-(*N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxyl)-L-histidine methylester (3i). Yield 93%; mp 162–164 °C; ESI/MS 454 $[M+H]^+$; IR (KBr): 3442, 3206, 3004, 2949, 2839, 1730, 1643, 1601, 1454, 1391, 1368, 1062, 902 cm^{-1}; ^{1}H NMR (BHSC-500, DMSO-d_6): δ = 12.98 (s, 1H), 9.96 (s, 1H), 8.05 (s, 1H), 7.47 (s, 1H), 6.85 (s, 1H), 7.36 (t, J = 7.4 Hz, 1H), 7.20 (t, J = 7.7 Hz, 1H), 7.16 (d, J = 7.7Hz, 1H), 6.98 (t, J = 7.4 Hz, 1H), 4.93 (t, J = 5.3 Hz, 1H), 4.83 (t, J = 5.4 Hz, 1H), 4.26 (d, J = 5.2 Hz, 2H), 3.64 (s, 3H), 3.19 (d, J = 5.4 Hz, 2H), 2.92 (d, J = 5.2 Hz, 2H), 1.49 (s, 9H). Anal. Calcd for $C_{24}H_{29}N_5O_5$: C, 61.66; H, 6.25; N, 14.98. Found: C, 61.52; H, 6.38; N, 14.77.

4.4.13. *N*-(*N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxyl)-L-tryptophan methylester (3m). Yield 93%; mp 161–163 °C; ESI/MS 517 $[M+H]^+$; IR (KBr): 3442, 3204, 3000, 2948, 2839, 1729, 1642, 1604, 1448, 1391, 1372, 1062, 900 cm^{-1}; ^{1}H NMR (BHSC-500, DMSO-d_6): δ = 9.87 (s, 1H), 9.86 (s, 1H), 8.09 (s, 1H), 7.32 (t, J = 7.5 Hz, 1H), 7.30 (t, J = 7.4 Hz, 1H), 7.12 (d, J = 7.8 Hz, 1H), 7.11 (t, J = 7.8 Hz, 1H), 7.10 (d, J = 7.6 Hz, 1H), 7.09 (t, J = 7.8 Hz, 1H), 7.04 (d, J = 7.6 Hz, 1H), 6.98 (d, J = 7.5 Hz, 1H), 6.83 (s, 1H), 4.94 (d, J = 5.4 Hz, 1H), 4.76 (t, J = 5.3 Hz, 1H), 4.29 (d, J = 5.2 Hz, 2H), 3.64 (s, 3H), 3.19 (d, J = 5.4 Hz, 2H), 2.95 (d, J = 6.4 Hz, 2H), 1.49 (s, 9H). Anal. Calcd for $C_{29}H_{32}N_4O_5$: C, 67.43; H, 6.24; N, 10.85. Found: C, 67.55; H, 6.34; N, 10.72.

4.4.14. *N*-(*N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxyl)-L-arginine methylester (3n). Yield 88%; mp 168–170 °C; ESI/MS 487 $[M+H]^+$; IR (KBr): 3443, 3207, 3001, 2948, 2842, 1731, 1645, 1602, 1453, 1390, 1372, 1061, 904 cm^{-1}; ^{1}H NMR (BHSC-500, DMSO-d_6): δ = 10.22 (s, 1H), 8.45 (s, 2H), 8.27 (s, 1H), 8.22 (s, 1H), 8.01 (s, 1H), 7.29 (t, J = 7.6 Hz, 1H), 7.18 (t, J = 7.7 Hz, 1H), 7.04 (d, J = 7.7 Hz, 1H), 6.96 (d, J = 7.6 Hz, 1H), 4.90 (d, J = 5.3 Hz, 1H), 4.42 (t, J = 4.2 Hz, 1H), 4.25 (d, J = 5.0 Hz, 2H), 3.65 (s, 3H), 2.94 (d, J = 4.1 Hz, 2H), 2.68 (t, J = 5.4 Hz, 2H), 1.92 (m, J = 5.5 Hz, 2H), 1.58 (m, J = 5.5 Hz, 2H), 1.57 (s, 9H). Anal. Calcd for $C_{24}H_{34}N_6O_5$: C, 59.24; H, 7.04; N, 17.27. Found: C, 59.38; H, 7.19; N, 17.31.

4.4.15. *N*-[(3*S*)-*N*-Boc-1,2,3,4-tetrahydro-β-carboline-3-carboxyl]-L-glycine methylester (3o). Yield 97%; mp 133–135 °C; ESI/MS 388 $[M+H]^+$. IR (KBr): 3448, 3010, 2945, 2843, 1732, 1600, 1453, 1390, 1371, 1062, 899 cm^{-1}; ^{1}H NMR (BHSC-500, DMSO-d_6): δ = 9.93 (s, 1H), 8.02 (s, 1H), 7.30 (t, J = 7.5 Hz, 1H), 7.20 (t, J = 7.6 Hz, 1H), 6.95 (d, J = 7.6 Hz, 1H), 6.83 (d, J = 7.6 Hz, 1H), 4.89 (d, J = 5.4 Hz, 1H), 4.22 (dd, J = 10.2 Hz, J = 4.5 Hz, 1H), 4.18 (s, 2H), 4.19 (dd, J = 10.2 Hz, J = 3.7 Hz, 1H), 3.66 (s, 3H), 2.95 (d, J = 10.1 Hz, 2H), 1.45 (s, 9H). $[\alpha]_D^{20}$ −101° (*c* 0.36, $CHCl_3$/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, 62.15; H, 6.68; N, 10.68.

4.4.16. *N*-[(3*S*)-*N*-Boc-1,2,3,4-tetrahydro-β-carboline-3-carboxyl]-L-(Z)lysine methylester (3p). Yield 90%; mp 134–136 °C; ESI/MS: 593 $[M+H]^+$. IR (KBr): 3442, 3007, 2940, 2848, 1730, 1605, 1455, 1391, 1370, 1066, 897 cm^{-1}; ^{1}H NMR (BHSC-500, DMSO-d_6): δ = 9.95 (s, 1H), 8.03 (s, 1H), 7.96 (s, 1H), 7.28 (t, J = 7.6 Hz, 1H), 7.22 (t, J = 7.2 Hz, 1H), 7.19 (t, J = 7.6 Hz, 1H), 7.17 (d, J = 7.2 Hz, 2H), 7.15 (t, J = 7.2 Hz, 2H), 6.96 (d, J = 7.6 Hz, 1H), 6.85 (d, J = 7.6 Hz, 1H), 5.36 (s, 2H), 4.90 (d, J = 5.5 Hz, 1H), 4.41 (t, J = 4.4 Hz, 1H), 4.20 (dd, J = 10.0 Hz, J = 4.5 Hz, 1H), 4.18 (dd, J = 10.0 Hz, J = 3.7 Hz, 1H), 3.64 (s, 3H), 2.98 (t, J = 4.4 Hz, 2H), 2.93 (d, J = 10.0 Hz, 2H), 1.91 (m, J = 4.4 Hz, 2H), 1.55 (m, J = 4.4 Hz, 2H), 1.46 (s, 9H), 1.29 (m, J = 4.4 Hz, 2H). $[\alpha]_D^{20}$ −22° (*c* 0.39, $CHCl_3$/CH_3OH, 1:1, v/v), Anal. Calcd for $C_{32}H_{40}N_4O_7$: C, 64.85; H, 6.80; N, 9.45. Found: C, 64.98; H, 6.69; N, 9.62.

4.4.17. *N*-[(3*S*)-*N*-Boc-1,2,3,4-tetrahydro-β-carboline-3-carboxyl]-L-glutamine methylester (3q). Yield 90%; mp 122–124 °C; ESI/MS: 459 $[M+H]^+$; IR (KBr): 3445, 3200, 3001, 2940, 2835, 1733, 1640, 1602, 1452, 1391, 1370, 1065, 900 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ = 9.91 (s, 1H), 8.00 (s, 1H), 7.29 (t, J = 7.4 Hz, 1H), 7.20 (t, J = 7.4 Hz, 1H), 7.00 (d, J = 7.4 Hz, 1H), 6.80 (d, J = 7.4 Hz, 1H), 6.05 (s, 2H), 4.92 (d, J = 5.5 Hz, 1H), 4.41 (t, J = 5.5 Hz, 1H), 4.24 (d, J = 5.6 Hz, 2H), 3.67 (s, 3H), 2.94 (d, J = 5.4 Hz, 2H), 2.18 (t, J = 5.5 Hz, 2H), 2.14 (t, J = 5.5 Hz, 2H), 1.46 (s, 9H). $[\alpha]_D^{20}$ −56° (*c* 0.38, $CHCl_3$/CH_3OH, 1:1, v/v). Anal. Calcd for $C_{23}H_{30}N_4O_6$: C, 60.25; H, 6.59; N, 12.22. Found: C, 60.73; H, 6.49; N, 8.69.

4.5. General procedure for preparation of 4a–q

At 0 °C to the solution of 1.0 g (2.5 mmol) of **3a–q** in 4 mL of methanol and 2 mL of chloroform, 0.45 g (11.34 mmol) of NaOH was added. The reaction mixture was stirred at 0 °C for 70 min. TLC analysis (chloroform/methanol, 30:1) indicated a complete disappearance of **3a–q**. After evaporation, the residue was dissolved in 30 mL of water and extracted with ethyl acetate (3× 20 mL). The organic phase was washed successively with 5% sodium bicarbonate, 5% citric acid, and saturated sodium chloride. The solution was then dried over anhydrous sodium sulfate. Compounds **4a–q** was obtained after filtration and evaporation under reduced pressure.

4.5.1. *N*-(*N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxyl)-L-alanine (4a). Yield 79%; mp 169–171 °C; ESI/MS 388 $[M+H]^+$. IR (KBr): 3439, 3234, 3215, 3000, 2952, 2847, 1732, 1645, 1602, 1453, 1390, 1373, 1061, 904 cm^{-1}; ^{1}H NMR (BHSC-500, DMSO-d_6): δ = 11.02 (s, 1H), 9.95 (s, 1H), 7.98 (s, 1H), 7.29 (t, J = 7.6 Hz, 1H), 7.17 (t, J = 7.9 Hz, 1H), 7.03 (d, J = 7.9 Hz, 1H),

6.94 (d, J = 7.6 Hz, 1H), 4.93 (d, J = 5.4 Hz, 1H), 4.66 (m, J = 5.4 Hz, 1H), 4.27 (d, J = 6.3 Hz, 2H), 2.97 (d, J = 9.5 Hz, 2H), 1.48 (d, J = 5.4 Hz, 3H), 1.45 (s, 9H). $[\alpha]_D^{20}$ −46° (*c* 0.39, $CHCl_3/CH_3OH$, 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, 62.18; H, 6.39; N, 10.71.

4.5.2. *N*-[(3*S*)-*N*-Boc-1,2,3,4-tetrahydro-β-carboline-3-carboxyl]-L-phenylalanine (4b). Yield 94%; mp 129–131 °C; ESI/MS: 464 $[M+H]^+$. IR (KBr): 3446, 3205, 3006, 2948, 2847, 1731, 1645, 1603, 1451, 1392, 1370, 1069, 904 cm^{-1}; ^1H NMR (DMSO-d_6): δ = 10.94 (s, 1H), 9.93 (s, 1H), 7.97 (s, 1H), 7.30 (t, J = 7.3 Hz, 1H), 7.26 (t, J = 7.4 Hz, 2H), 7.17 (t, J = 7.6 Hz, 1H), 7.15 (d, J = 7.4 Hz, 2H), 7.10 (t, J = 7.4 Hz, 1H), 7.02 (t, J = 7.4 Hz, 1H), 6.97 (d, J = 7.4 Hz, 1H), 4.93 (d, J = 5.2 Hz, 1H), 4.78 (t, J = 5.2 Hz, 1H), 4.27 (d, J = 5.2 Hz, 2H), 3.07 (d, J = 4.5 Hz, 2H), 2.98 (d, J = 5.2 Hz, 2H), 1.49 (s, 9H). $[\alpha]_D^{20}$ −30° (*c* 0.36, $CHCl_3/CH_3OH$, 1:1, v/v), $[\alpha]_D^{20}$ −66° (*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, 67.22; H, 6.39; N, 10.21.

4.5.3. *N*-(*N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxyl)-L-valine (4c). Yield 71%; mp 148–150 °C; ESI/MS: 416 $[M+H]^+$. IR (KBr): 3441, 3236, 3212, 3002, 2951, 2845, 1731, 1643, 1600, 1450, 1392, 1374, 1060, 900 cm^{-1}; ^1H NMR (DMSO-d_6): δ = 10.94 (s, 1H), 9.23 (s, 1H), 7.96 (s, 1H), 7.29 (t, J = 7.5 Hz, 1H), 7.18 (t, J = 7.6 Hz, 1H), 7.06 (d, J = 7.4 Hz, 1H), 6.97 (d, J = 7.5 Hz, 1H), 4.95 (d, J = 5.2 Hz, 1H), 4.48 (d, J = 5.2 Hz, 1H), 4.27 (d, J = 5.1 Hz, 2H), 2.92 (d, J = 5.5 Hz, 2H), 2.78 (m, J = 4.5 Hz, 1H), 1.49 (s, 9H), 1.25 (d, J = 5.6 Hz, 6H). $[\alpha]_D^{20}$ −72° (*c* 0.38, $CHCl_3/CH_3OH$, 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, 63.42; H, 7.19; N, 10.21.

4.5.4. *N*-(*N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxyl)-L-serine (4d). Yield 84%; mp 136–138 °C; ESI/MS: 390 $[M+H]^+$; IR (KBr): 3437, 3236, 3213, 3005, 2950, 2846, 1730, 1641, 1600, 1451, 1392, 1370, 1058, 897 cm^{-1}; ^1H NMR (DMSO-d_6): δ = 10.92 (s, 1H), 9.93 (s, 1H), 7.97 (s, 1H), 7.30 (t, J = 7.3 Hz, 1H), 7.19 (t, J = 7.6 Hz, 1H), 7.06 (d, J = 7.6 Hz, 1H), 6.98 (d, J = 7.3 Hz, 1H), 4.92 (d, J = 5.5 Hz, 1H), 4.46 (t, J = 5.2 Hz, 1H), 4.27 (d, J = 5.0 Hz, 2H), 4.06 (d, J = 5.1 Hz, 2H), 2.93 (d, J = 5.4 Hz, 2H), 2.07 (s, 1H), 1.51 (s, 9H). $[\alpha]_D^{20}$ −70° (*c* 0.35, $CHCl_3/CH_3OH$, 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, 59.42; H, 6.19; N, 10.29.

4.5.5. *N*-(*N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxyl)-L-threonine (4e). Yield 78%; mp 143–145 °C; ESI/MS: 405 $[M+H]^+$; IR (KBr): 3444, 3235, 3217, 3005, 2950, 2843, 1730, 1641, 1600, 1450, 1389, 1370, 1055, 896 cm^{-1}; ^1H NMR (DMSO-d_6): δ = 10.97 (s, 1H), 8.92 (s, 1H), 7.95 (s, 1H), 7.29 (t, J = 7.2 Hz, 1H), 7.15 (t, J = 7.4 Hz, 1H), 7.05 (d, J = 7.4 Hz, 1H), 6.98 (d, J = 7.2 Hz, 1H), 4.89 (d, J = 5.3 Hz, 1H), 4.46 (d, J = 5.4 Hz, 1H), 4.37 (m, J = 5.1 Hz, 1H), 4.27 (d, J = 4.8 Hz, 2H), 2.95 (d, J = 5.5 Hz, 2H), 2.16 (s, 1H), 1.48 (s, 9H), 1.26 (d, J = 5.2 Hz, 3H). $[\alpha]_D^{20}$ −49° (*c* 0.39, $CHCl_3/CH_3OH$, 1:1, v/v). Anal. Calcd for $C_{21}H_{27}N_3O_6$: C, 60.42; H, 6.52; N, 10.07. Found: C, 60.28; H, 6.39; N, 10.16.

4.5.6. *N*-(*N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxyl)-L-tyrosine (4f). Yield 67%; mp 147–149 °C; ESI/MS: 480 $[M+H]^+$; IR (KBr): 3441, 3236, 3217, 3004, 2955, 2846, 1731, 1647, 1600, 1450, 1392, 1371, 1058, 899 cm^{-1}; ^1H NMR (BHSC-500, DMSO-d_6): δ = 10.97 (s, 1H), 9.60 (s, 1H), 8.15 (s, 1H), 7.32 (t, J = 7.2 Hz, 1H),7.14 (t, J = 7.4 Hz, 1H), 7.01 (d, J = 7.2 Hz, 1H), 6.96 (d, J = 7.2 Hz, 1H), 6.94 (d, J = 7.5 Hz, 2H), 6.88 (d, J = 7.2 Hz, 2H), 5.03 (s, 1H), 4.94 (d, J = 5.2 Hz, 1H), 4.84 (d, J = 5.3 Hz, 1H), 4.27 (d, J = 5.2 Hz, 2H), 3.07 (m, J = 3.4 Hz, 2H), 2.92 (d, J = 4.5 Hz, 2H), 1.48 (s, 9H). $[\alpha]_D^{20}$ −61° (*c* 0.36, $CHCl_3/CH_3OH$, 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, 65.28; H, 6.17; N, 8.59.

4.5.7. *N*-(*N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxyl)-L-proline (4g). Yield 83%; mp 136–138 °C; ESI/MS: 414 $[M+H]^+$; IR (KBr): 3440, 3236, 3217, 3004, 2956, 2841, 1735, 1641, 1605, 1450, 1392, 1370, 1044, 897 cm^{-1}; ^1H NMR (DMSO-d_6): δ = 10.95 (s, 1H), 9.70 (s, 1H), 7.32 (t, J = 7.2 Hz, 1H), 7.14 (t, J = 7.5 Hz, 1H), 7.08 (d, J = 7.5 Hz, 1H), 6.96 (d, J = 7.2 Hz, 1H), 4.92 (d, J = 5.1 Hz, 1H), 4.32 (t, J = 5.3 Hz, 1H), 4.22 (m, J = 5.3 Hz, 2H), 3.47 (t, J = 5.2 Hz, 2H), 2.95 (d, J = 5.2 Hz, 2H), 2.17 (dd, J = 5.2 Hz, J = 3.4 Hz, 2H), 1.94 (t, J = 4.5 Hz, 2H), 1.47 (s, 9H). $[\alpha]_D^{20}$ −49° (*c* 0.35, $CHCl_3/CH_3OH$, 1:1, v/v). Anal. Calcd for $C_{22}H_{27}N_3O_5$: C, 63.91; H, 6.58; N, 10.16. Found: C, 64.14; H, 6.47; N, 10.32.

4.5.8. *N*-(*N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxyl)-L-cysteine (4h). Yield 78%; mp 150–152 °C; ESI/MS: 420 $[M+H]^+$; IR (KBr): 3436, 3232, 3217, 3004, 2950, 2844, 1731, 1647, 1600, 1455, 1392, 1375, 1058, 902 cm^{-1}; ^1H NMR (DMSO-d_6): δ = 10.89 (s, 1H), 9.95 (s, 1H), 7.99 (s, 1H), 7.27 (t, J = 7.3 Hz, 1H), 7.15 (t, J = 7.6 Hz, 1H), 7.05 (d, J = 7.6 Hz, 1H), 6.95 (t, J = 7.2 Hz, 1H), 4.91 (t, J = 5.0 Hz, 1H), 4.75 (t, J = 5.2 Hz, 1H), 4.24 (m, J = 5.0 Hz, 2H), 3.06 (d, J = 5.0 Hz, 2H), 2.89 (d, J = 4.5 Hz, 2H), 1.55 (s, 1H), 1.52 (s, 9H). $[\alpha]_D^{20}$ −45° (*c* 0.38, $CHCl_3/CH_3OH$, 1:1, v/v). Anal. Calcd for $C_{20}H_{25}N_3O_5S$: C, 57.26; H, 6.01; N, 10.02. Found: C, 57.19; H, 6.13; N, 10.12.

4.5.9. *N*-(*N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxyl)-L-methionine (4i). Yield 72%; mp 148–150 °C; ESI/MS: 448 $[M+H]^+$; IR (KBr): 3436, 3232, 3213, 3007, 2953, 2849, 1735, 1648, 1600, 1450, 1393, 1375, 1055, 897 cm^{-1}; ^1H NMR (BHSC-500, DMSO-d_6): δ = 11.01 (s, 1H), 9.97 (s, 1H), 8.01 (s, 1H), 7.33 (t, J = 7.0 Hz, 1H), 7.18 (t, J = 7.5 Hz, 1H), 7.05 (d, J = 7.5 Hz, 1H), 6.95 (t, J = 7.0 Hz, 1H), 4.44 (t, J = 5.5 Hz, 1H), 4.25 (m, J = 5.2 Hz, 1H), 4.23 (m, J = 5.0 Hz, 2H), 2.89 (d, J = 4.4 Hz, 2H), 2.46 (t, J = 5.4 Hz, 2H), 2.16 (m, J = 5.6 Hz, 2H), 2.09 (s, 3H), 1.50 (s, 9H). $[\alpha]_D^{20}$ −38° (*c* 0.39, $CHCl_3/CH_3OH$, 1:1, v/v). Anal. Calcd for $C_{22}H_{29}N_3O_5S$: C, 59.04; H, 6.53; N, 9.39. Found: C, 59.20; H, 6.44; N, 9.22.

4.5.10. *N*-(*N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxyl)-L-glutamic acid (4j). Yield 76%; mp 160–162 °C; ESI/MS: 446 $[M+H]^+$; IR (KBr): 3444, 3231, 3212, 3008, 2954, 2843, 1730, 1642, 1600, 1450, 1391, 1371, 1058, 900 cm^{-1}; ^1H NMR (BHSC-500, DMSO-d_6): δ = 11.02 (s, 2H), 9.87 (s, 1H), 8.11 (s, 1H), 7.28 (t, J = 7.2 Hz, 1H), 7.14 (t, J = 7.2 Hz, 1H), 7.06 (d, J = 7.4 Hz, 1H), 6.97 (t, J = 7.2 Hz, 1H), 4.90 (t, J = 5.1 Hz, 1H), 4.42 (t, J = 5.4 Hz, 1H), 4.21 (d, J = 5.1 Hz, 2H), 2.87 (d, J = 5.3 Hz, 2H), 2.22 (t, J = 5.2 Hz, 2H), 2.08 (t, J = 5.2 Hz, 2H), 1.47 (s, 9H). $[\alpha]_D^{20}$ −51° (c 0.33, $CHCl_3$/CH_3OH, 1:1, v/v). Anal. Calcd for $C_{22}H_{27}N_3O_7$: C, 59.32; H, 6.11; N, 9.43. Found: C, 59.24; H, 6.01; N, 9.29.

4.5.11. *N*-(*N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxyl)-L-aspartic acid (4k). Yield 72%; mp 141–143 °C; ESI/MS: 432 $[M+H]^+$; IR (KBr): 3442, 3236, 3217, 3005, 2950, 2843, 1728, 1642, 1600, 1450, 1392, 1370, 1058, 896 cm^{-1}; ^1H NMR (DMSO-d_6): δ = 10.99 (s, 2H), 9.94 (s, 1H), 7.98 (s, 1H), 7.26 (t, J = 7.1 Hz, 1H), 7.11 (t, J = 7.1 Hz, 1H), 7.05 (d, J = 7.2 Hz, 1H), 6.94 (d, J = 7.2 Hz, 1H), 4.87 (t, J = 5.2 Hz, 1H), 4.76 (t, J = 5.3 Hz, 1H), 4.20 (m, J = 4.8 Hz, 2H), 2.89 (d, J = 5.2 Hz, 2H), 2.66 (d, J = 5.2 Hz, 2H), 1.47 (s, 9H). $[\alpha]_D^{20}$ −47° (c 0.35, $CHCl_3$/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, 58.55; H, 6.02; N, 9.91.

4.5.12. *N*-(*N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxyl)-L-histidine (4l). Yield 76%; mp 156–158 °C; ESI/MS: 454 $[M+H]^+$. IR (KBr): 3445, 3231, 3212, 3007, 2947, 2842, 1730, 1642, 1600, 1450, 1388, 1370, 1059, 899 cm^{-1}; ^1H NMR (DMSO-d_6): δ = 11.85 (s, 1H), 11.62 (s, 1H), 9.94 (s, 1H), 7.89 (s, 1H), 7.45 (s, 1H), 7.29 (t, J = 7.2 Hz, 1H), 7.15 (t, J = 7.5 Hz, 1H), 7.06 (d, J = 7.5Hz, 1H), 6.98 (d, J = 7.2 Hz, 1H), 6.82 (s, 1H), 4.89 (t, J = 5.0 Hz,1H), 4.82 (t, J = 5.1 Hz, 1H), 4.20 (m, J = 5.1 Hz, 2H), 3.02 (d, J = 5.0 Hz, 2H), 2.92 (d, J = 5.2 Hz, 2H), 1.50 (s, 9H). $[\alpha]_D^{20}$ −50° (c 0.36, $CHCl_3$/CH_3OH, 1:1, v/v). Anal. Calcd for $C_{23}H_{27}N_5O_5$: C, 60.92; H, 6.00; N, 15.44. Found: C, 61.05; H, 6.09; N, 15.26.

4.5.13. *N*-(*N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxyl)-L-tryptophan (4m). Yield 70%; mp 158–160 °C; ESI/MS: 503 $[M+H]^+$; IR (KBr): 3445, 3238, 3217, 3008, 2951, 2842, 1728, 1641, 1600, 1450, 1393, 1370, 1058, 897 cm^{-1}; ^1H NMR (DMSO-d_6): δ = 10.98 (s, 1H), 9.87 (s, 1H), 9.84 (s, 1H), 8.01 (s, 1H), 7.30 (t, J = 7.1 Hz, 1H), 7.16 (t, J = 7.1 Hz, 1H), 7.14 (t, J = 7.4 Hz, 1H), 7.12 (t, J = 7.2 Hz, 1H), 7.06 (d, J = 7.4 Hz, 1H), 7.05 (d, J = 7.2 Hz, 1H), 6.99 (d, J = 7.1 Hz, 1H), 6.96 (d, J = 7.1 Hz, 1H), 6.82 (s, 1H), 4.89 (t, J = 5.2 Hz, 1H), 4.84 (t, J = 5.1 Hz, 1H), 4.25 (m, J = 5.0 Hz, 2H), 2.91 (d, J = 5.0 Hz, 2H), 2.88 (d, J = 5.1 Hz, 2H), 1.53 (s, 9H). $[\alpha]_D^{20}$ −47° (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, 66.79; H, 5.94; N, 11.27.

4.5.14. *N*-(*N*-Boc-3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxyl)-L-arginine (4n). Yield 81%; mp 144–146 °C; ESI/MS: 473 $[M+H]^+$; IR (KBr): 3441, 3230, 3212, 3007, 2950, 2844, 1730, 1640, 1600, 1450, 1392, 1375, 1057, 896 cm^{-1}; ^1H NMR (BHSC-500, DMSO-d_6): δ = 10.94 (s, 1H), 8.55 (s, 2H), 8.31 (s, 2H), 8.23 (s, 1H), 7.98 (s, 1H), 7.29 (t, J = 7.5 Hz, 1H), 7.11 (t, J = 7.6 Hz, 1H), 7.02 (d, J = 7.6 Hz, 1H), 6.98 (d, J = 7.5 Hz, 1H), 4.87 (t, J = 5.4 Hz, 1H), 4.41 (t, J = 4.3 Hz, 1H), 4.25 (d, J = 4.8 Hz, 2H), 2.89 (d, J = 4.9 Hz, 2H), 2.66 (t, J = 5.2 Hz, 2H), 1.81 (m, J = 5.1 Hz, 2H), 1.59 (m, J = 5.3 Hz, 2H), 1.56 (s, 9H). $[\alpha]_D^{20}$ −66° (c 0.37, $CHCl_3$/CH_3OH, 1:1, v/v). Anal. Calcd for $C_{23}H_{32}N_6O_5$: C, 58.46; H, 6.83; N, 17.78. Found: C, 58.38; H, 6.96; N, 17.57.

4.5.15. *N*-[(3*S*)-*N*-Boc-1,2,3,4-tetrahydro-β-carboline-3-carboxyl]-L-glycine (4o). Yield 95%; mp 148–150 °C; ESI/MS: 374 $[M+H]^+$. IR (KBr): 3444, 3230, 3008, 2942, 2841, 1730, 1602, 1455, 1391, 1370, 1064, 898 cm^{-1}; ^1H NMR (BHSC-500, DMSO-d_6): δ = 11.03 (s, 1H), 9.98 (s, 1H), 8.01 (s, 1H), 7.29 (t, J = 7.5 Hz, 1H), 7.18 (t, J = 7.6 Hz, 1H), 6.97 (d, J = 7.6 Hz, 1H), 6.85 (d, J = 7.6 Hz, 1H), 4.90 (d, J = 5.4 Hz, 1H), 4.24 (dd, J = 10.2 Hz, J = 4.5 Hz, 1H), 4.17 (s, 2H), 4.16 (dd, J = 10.2 Hz, J = 3.7 Hz, 1H), 2.93 (d, J = 10.0 Hz, 2H), 1.46 (s, 9H). $[\alpha]_D^{20}$ (c 0.38, $CHCl_3$/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, 61.30; H, 6.40; N, 11.09.

4.5.16. *N*-[(3*S*)-*N*-Boc-1,2,3,4-tetrahydro-β-carboline-3-carboxyl]-L-(Z)lysine (4p). Yield 92%; mp 155–157 °C; ESI/MS: 579 $[M+H]^+$. IR (KBr): 3438, 3234, 3215, 3009, 2944, 2845, 1733, 1602, 1453, 1390, 1372, 1064, 899 cm^{-1}; ^1H NMR (DMSO-d_6): δ = 10.88 (s, 1H), 9.97 (s, 1H), 8.01 (s, 1H), 7.98 (s, 1H), 7.29 (t, J = 7.5 Hz, 1H), 7.21 (t, J = 7.3 Hz, 1H), 7.18 (t, J = 7.5 Hz, 1H), 7.17 (d, J = 7.3 Hz, 2H), 7.16 (t, J = 7.3 Hz, 2H), 6.98 (d, J = 7.5 Hz, 1H), 6.88 (d, J = 7.5 Hz, 1H), 5.33 (s, 2H), 4.92 (d, J = 5.6 Hz, 1H), 4.45 (t, J = 4.6 Hz, 1H), 4.23 (dd, J = 10.0 Hz, J = 4.5 Hz, 1H), 4.19 (dd, J = 10.0 Hz, J = 3.7 Hz, 1H), 2.94 (t, J = 4.6 Hz, 2H), 2.95 (d, J = 10.0 Hz, 2H), 1.84 (m, J = 4.6 Hz, 2H), 1.54 (m, J = 4.6 Hz, 2H), 1.46 (s, 9H), 1.29 (m, J = 4.6 Hz, 2H). $[\alpha]_D^{20}$ −34° (c 0.36, $CHCl_3$/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, 64.18; H, 6.44; N, 9.83.

4.5.17. *N*-[(3*S*)-*N*-Boc-1,2,3,4-tetrahydro-β-carboline-3-carboxyl]-L-glutamine (4q). Yield 90%; mp 129–131 °C; ESI/MS: 445 $[M+H]^+$; IR (KBr): 3448, 3230, 3215, 3005, 2942, 2833, 1738, 1642, 1604, 1450, 1390, 1372, 1063, 901 cm^{-1}; ^1H NMR (DMSO-d_6): δ = 10.96 (s, 1H), 9.88 (s, 1H), 8.02 (s, 1H), 7.28 (t, J = 7.5 Hz, 1H), 7.21 (t, J = 7.5 Hz, 1H), 7.01 (d, J = 7.5 Hz, 1H), 6.82 (d, J = 7.5 Hz, 1H), 6.02 (s, 2H), 4.90 (d, J = 5.6 Hz, 1H), 4.45 (t, J = 5.4 Hz, 1H), 4.25 (d, J = 5.5 Hz, 2H), 2.92 (d, J = 5.5 Hz, 2H), 2.17 (t, J = 5.5 Hz, 2H), 2.07 (t, J = 5.5Hz, 2H), 1.42 (s, 9H). $[\alpha]_D^{20}$ −54° (c 0.39, $CHCl_3$/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, 59.63; H, 6.20; N, 12.79.

4.6. General procedure for preparation of 5a–o

At 0 °C, solution of 0.7 g (1.85 mmol) of **4a**–**q** in 10 mL of hydrogen chloride/ethyl acetate (4 mol/L) was stirred

for 1 h and TLC analysis (chloroform/methanol, 5:1) indicated complete disappearance of **4a**–**q**. Upon evaporation, the residue was dissolved in ethyl acetate (3× 10 mL) and evaporated under reduced pressure to remove hydrogen chloride. The residue was titrated with 20 mL of ethyl acetate and re-crystallized with butanol/ether to provide **5a**–**q**.

4.6.1. *N*-(3*S*-1,2,3,4-Tetrahydro-β-carboline-3-carboxyl)-L-alanine (5a). Yield 94%; mp 167–169 °C; ESI/MS: 288 $[M+H]^+$; IR (KBr): 3439, 3234, 3215, 3000, 2952, 2847, 1732, 1645, 1602, 1453, 1061, 904 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ = 11.86 (s, 1H), 10.00 (s, 1H), 9.96 (s,1H), 8.02 (s,1H), 7.31 (t, J = 6.6 Hz, 1H), 7.18 (t, J = 7.8 Hz, 1H), 6.87 (d, J = 7.9 Hz, 1H), 6.81 (d, J = 7.6 Hz, 1H), 4.65 (m, J = 5.4 Hz, 1H), 3.95 (m, J = 5.4 Hz, 1H), 3.91 (d, J = 5.3 Hz, 2H), 2.81 (d, J = 5.6 Hz, 2H), 1.48 (d, J = 5.6 Hz, 3H). $[\alpha]_D^{20}$ −80° (*c* 0.39, $CHCl_3$/CH_3OH, 1:1, v/v). Anal. Calcd for $C_{15}H_{17}N_3O_3$: C, 62.71; H, 5.96; N, 14.63. Found: C, 62.55; H, 5.79; N, 14.55.

4.6.2. *N*-(3*S*-1,2,3,4-Tetrahydro-β-carboline-3-carboxyl)-L-phenylalanine (5b). Yield 95%; mp 177–179 °C; ESI/MS: 364 $[M+H]^+$; IR (KBr): 3435, 3231, 3213, 3003, 2950, 2844, 1730, 1642, 1600, 1450, 1060, 901 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ = 11.33 (s, 1H), 10.05 (s, 1H), 9.93 (s, 1H), 8.01 (s, 1H), 7.33 (t, J = 6.8 Hz, 1H), 7.30 (t, J = 6.4 Hz, 2H), 7.20 (t, J = 7.6 Hz, 1H), 7.17 (d, J = 7.8 Hz, 2H), 7.10 (t, J = 6.8 Hz, 1H), 7.02 (d, J = 7.6 Hz, 1H), 6.89 (d, J = 7.4 Hz, 1H), 4.86 (t, J = 5.4 Hz, 1H), 4.00 (m, J = 5.4 Hz, 1H), 3.88 (d, J = 6.2 Hz, 2H), 3.07 (d, J = 6.5 Hz, 2H), 2.81 (d, J = 6.2 Hz, 2H). $[\alpha]_D^{20}$ −30° (*c* 0.41, $CHCl_3$/CH_3OH, 1:1, v/v). Anal. Calcd for $C_{21}H_{21}N_3O_3$: C, 69.41; H, 5.82; N, 11.56. Found: C, 69.56; H, 5.75; N, 11.34.

4.6.3. *N*-(3*S*-1,2,3,4-Tetrahydro-β-carboline-3-carboxyl)-L-valine (5c). Yield 95%; mp 169–171 °C; ESI/MS: 316 $[M+H]^+$; IR (KBr): 3441, 3236, 3212, 3002, 2951, 2845, 1731, 1643, 1600, 1450, 1060, 900 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ = 11.68 (s, 1H), 10.19 (s, 1H), 9.88 (s, 1H), 8.03 (s,1H), 7.32 (t, J = 7.5 Hz, 1H), 7.17 (t, J = 7.8 Hz, 1H), 7.05 (d, J = 7.4Hz, 1H), 6.98 (d, J = 7.4 Hz, 1H), 4.48 (t, J = 5.2 Hz, 1H), 3.94 (t, J = 5.2 Hz, 1H), 3.90 (d, J = 5.2 Hz, 2H), 2.82 (d, J = 5.4 Hz, 2H), 2.80 (m, J = 6.9 Hz, 1H), 1.06 (d, J = 6.9 Hz, 6H). $[\alpha]_D^{20}$ −70° (*c* 0.38, $CHCl_3$/CH_3OH, 1:1, v/v). Anal. Calcd for $C_{17}H_{21}N_3O_3$: C, 64.74; H, 6.71; N, 13.32. Found: C, 64.89; H, 6.85; N, 13.44.

4.6.4. *N*-(3*S*-1,2,3,4-Tetrahydro-β-carboline-3-carboxyl)-L-serine (5d). Yield 89%; mp 162–164 °C; ESI/MS: 304 $[M+H]^+$; IR (KBr): 3437, 3236, 3213, 3005, 2950, 2846, 1730, 1641, 1600, 1451, 1058, 897 cm^{-1}; ^1H NMR(300 MHz, DMSO-d_6): δ = 11.50 (s, 1H), 10.19 (s, 1H), 9.96 (s, 1H), 8.10 (s, 1H), 7.31 (t, J = 7.5 Hz, 1H), 7.18 (t, J = 7.8 Hz, 1H), 7.09 (d, J = 7.8 Hz, 1H), 6.89 (d, J = 7.5 Hz, 1H), 4.59 (t, J = 5.1 Hz, 1H), 4.04 (d, J = 5.1 Hz, 2H), 3.96 (t, J = 5.1 Hz, 1H), 3.81 (d, J = 5.9 Hz, 2H), 2.78 (d, J = 5.5 Hz, 2H), 2.30 (s, 1H). $[\alpha]_D^{20}$ −70° (*c* 0.36, $CHCl_3$/CH_3OH, 1:1, v/v). Anal. Calcd for $C_{15}H_{17}N_3O_4$: C, 59.40; H, 5.65; N, 13.85. Found: C, 59.56; H, 5.81; N, 13.79.

4.6.5. *N*-(3*S*-1,2,3,4-Tetrahydro-β-carboline-3-carboxyl)-L-threonine (5e). Yield 91%; mp 180–182 °C; ESI/MS: 318 $[M+H]^+$; IR (KBr): 3444, 3235, 3217, 3005, 2950, 2843, 1730, 1641, 1600, 1450, 1055, 896 cm^{-1}; ^1H NMR(300 MHz, DMSO-d_6): δ = 11.42 (s, 1H), 10.23 (s, 1H), 9.89 (s, 1H), 8.05 (s, 1H), 7.30 (t, J = 7.5 Hz, 1H), 7.18 (t, J = 8.1 Hz, 1H), 7.10 (d, J = 8.4 Hz, 1H), 7.00 (d, J = 7.8 Hz, 1H), 4.52 (d, J = 5.3 Hz, 1H), 4.37 (m, J = 5.4 Hz, 1H), 3.96 (d, J = 6.4 Hz, 1H), 3.86 (d, J = 5.4 Hz, 2H), 2.80 (d, J = 5.4 Hz, 2H), 2.17 (s, 1H), 1.26 (d, J = 5.2 Hz, 3H). $[\alpha]_D^{20}$ −28° (*c* 0.36, $CHCl_3$/CH_3OH, 1:1, v/v). Anal. Calcd for $C_{16}H_{19}N_3O_4$: C, 60.56; H, 6.03; N, 13.24. Found: C, 60.68; H, 5.88; N, 13.48.

4.6.6. *N*-(3*S*-1,2,3,4-Tetrahydro-β-carboline-3-carboxyl)-L-tyrosine (5f). Yield 84%; mp 171–173 °C; ESI/MS: 380 $[M+H]^+$; IR (KBr): 3441, 3236, 3217, 3004, 2955, 2846, 1731, 1647, 1600, 1450, 1058, 899 cm^{-1}; ^1H NMR(300 MHz, DMSO-d_6): δ = 11.44 (s, 1H), 10.19 (s, 1H), 9.96 (s, 1H), 8.05 (s, 1H), 7.29 (t, J = 7.5 Hz, 1H), 7.18 (t, J = 8.1 Hz, 1H), 7.01 (d, J = 8.1 Hz, 2H), 7.00 (d, J = 7.2 Hz, 2H), 6.97 (d, J = 7.8 Hz, 1H), 6.70 (d, J = 8.4 Hz, 2H), 5.02 (s, 1H), 4.85 (t, J = 5.3 Hz, 1H), 3.97 (t, J = 6.2 Hz, 1H), 3.87 (d, J = 5.6 Hz, 2H), 3.06 (d, J = 5.6 Hz, 2H), 2.78 (d, J = 5.9 Hz, 2H). $[\alpha]_D^{20}$ −88° (*c* 0.36, $CHCl_3$/CH_3OH, 1:1, v/v); Anal. Calcd for $C_{21}H_{21}N_3O_4$: C, 66.48; H, 5.58; N, 11.08. Found: C, 66.48; H, 5.72; N, 11.17.

4.6.7. *N*-(3*S*-1,2,3,4-Tetrahydro-β-carboline-3-carboxyl)-L-proline (5g). Yield 93%; mp 166–168 °C; ESI/MS: 314 $[M+H]^+$; IR (KBr): 3440, 3236, 3217, 3004, 2956, 2841, 1735, 1641, 1605, 1450, 1044, 897 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ = 11.13 (s, 1H), 10.03 (s, 1H), 9.96 (s, 1H), 7.31 (t, J = 8.4 Hz, 1H), 7.18 (t, J = 7.5Hz, 1H), 7.02 (d, J = 7.8 Hz, 1H), 6.95 (d, J = 7.5 Hz, 1H), 4.32 (t, J = 5.1 Hz, 1H), 3.96 (t, J = 6.3 Hz, 1H), 3.86 (d, J = 5.4 Hz, 2H), 3.47 (t, J = 6.3 Hz, 2H), 2.81 (t, J = 6.4 Hz, 2H), 2.26 (t, J = 6.5 Hz, 2H), 1.98 (m, J = 6.6 Hz, 2H). $[\alpha]_D^{20}$ −36° (*c* 0.36, $CHCl_3$/CH_3OH, 1:1, v/v). Anal. Calcd for $C_{17}H_{19}N_3O_3$: C, 65.16; H, 6.11; N, 13.41. Found: C, 65.30; H, 6.20; N, 13.29.

4.6.8. *N*-(3*S*-1,2,3,4-Tetrahydro-β-carboline-3-carboxyl)-L-cysteine (5h). Yield 98%; mp 200–201 °C, ESI/MS: 320 $[M+H]^+$; IR (KBr): 3436, 3232, 3217, 3004, 2950, 2844, 1731, 1647, 1600, 1455, 1058, 902 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ = 11.22 (s, 1H), 10.22 (s, 1H), 9.96 (s, 1H), 8.03 (s, 1H), 7.31 (t, J = 7.6 Hz, 1H), 7.17 (t, J = 7.8 Hz, 1H), 7.02 (d, J = 7.8 Hz, 1H), 6.95 (d, J = 7.2 Hz, 1H), 4.79 (t, J = 5.6 Hz, 1H), 3.96 (t, J = 5.8 Hz, 1H), 3.87 (d, J = 5.7 Hz, 2H), 3.07 (d, J = 6.7 Hz, 2H), 2.84 (d, J = 6.5 Hz, 2H), 1.62 (s, 1H). $[\alpha]_D^{20}$ −86° (*c* 0.36, $CHCl_3$/CH_3OH, 1:1, v/v). Anal. Calcd for $C_{15}H_{17}N_3O_3S$: C, 56.41; H, 5.37; N, 13.16. Found: C, 56.28; H, 5.51; N, 13.03.

4.6.9. *N*-(3*S*-1,2,3,4-Tetrahydro-β-carboline-3-carboxyl)-L-methionine (5i). Yield 83%; mp 186–189 °C; ESI/MS: 348 $[M+H]^+$; IR (KBr): 3436, 3232, 3213, 3007, 2953,

2849, 1735, 1648, 1600, 1450, 1055, 897 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ = 11.21 (s, 1H), 10.11 (s, 1H), 9.96 (s, 1H), 8.14 (s, 1H), 7.29 (t, J = 7.8 Hz, 1H), 7.16 (t, J = 7.8 Hz, 1H), 7.02 (d, J = 7.5 Hz, 1H), 6.93 (d, J = 7.8 Hz, 1H), 4.48 (t, J = 5.5 Hz, 1H), 3.99 (t, J = 5.4 Hz, 1H), 3.89 (d, J = 6.5 Hz, 2H), 2.79 (d, J = 6.4 Hz, 2H), 2.51 (t, J = 5.7 Hz, 2H), 2.24 (m, J = 5.5 Hz, 2H), 2.09 (s, 3H). $[\alpha]_D^{20}$ −86° (c 0.39, $CHCl_3/CH_3OH$, 1:1, v/v). Anal. Calcd for $C_{17}H_{21}N_3O_3S$: C, 58.77; H, 6.09; N, 12.09. Found: C, 58.88; H, 5.89; N, 12.20.

4.6.10. *N*-(3*S*-1,2,3,4-Tetrahydro-β-carboline-3-carboxyl)-L-glutamic acid (5j). Yield 94%; mp 186–189 °C; ESI/MS: 346 $[M+H]^+$; IR (KBr): 3444, 3231, 3212, 3008, 2954, 2843, 1730, 1642, 1600, 1450, 1058, 900cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ = 11.59 (s, 1H), 11.48 (s, 1H), 10.27 (s, 1H), 9.95 (s, 1H), 8.12 (s, 1H), 7.33 (t, J = 7.8 Hz, 1H), 7.18 (t, J = 7.8 Hz, 1H), 7.02 (d, J = 7.5 Hz, 1H), 6.95 (d, J = 7.5 Hz, 1H), 4.52 (t, J = 5.4 Hz, 1H), 3.98 (t, J = 5.5 Hz, 1H), 3.85 (d, J = 6.5 Hz, 2H), 2.81 (d, J = 5.1 Hz, 2H), 2.24 (t, J = 5.1 Hz, 2H), 2.11 (m, J = 5.5 Hz, 2H). $[\alpha]_D^{20}$ −66° (c 0.39, $CHCl_3/CH_3OH$, 1:1, v/v); Anal. Calcd for $C_{17}H_{19}N_3O_5$: C, 59.12; H, 5.55; N, 12.17. Found: C, 59.24; H, 5.46; N, 12.02.

4.6.11. *N*-(3*S*-1,2,3,4-Tetrahydro-β-carboline-3-carboxyl)-L-aspartic acid (5k). Yield 91%; mp 179–181 °C; ESI/MS: 332 $[M+H]^+$; IR (KBr): 3442, 3236, 3217, 3005, 2950, 2843, 1728, 1642, 1600, 1450, 1058, 896 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ = 11.42 (s, 1H), 11.40 (s, 1H), 10.32 (s, 1H), 9.99 (s, 1H), 8.05 (d, J = 5.5 Hz, 1H), 7.31 (d, J = 7.3 Hz, 1H), 7.16 (d, J = 7.4 Hz, 1H), 7.11 (t, J = 7.4 Hz, 1H), 6.95 (t, J = 7.3 Hz, 1H), 4.77 (t, J = 5.5 Hz, 1H), 3.96 (t, J = 5.4 Hz, 1H), 3.87 (d, J = 5.5 Hz, 2H), 2.81 (d, J = 5.2 Hz, 2H), 2.73 (d, J = 5.3 Hz, 2H). $[\alpha]_D^{20}$ −12° (c 0.39, $CHCl_3/CH_3OH$, 1:1, v/v). Anal. Calcd for $C_{16}H_{17}N_3O_5$: C, 58.00; H, 5.17; N, 12.68. Found: C, 57.87; H, 5.09; N, 12.78.

4.6.12. *N*-(3*S*-1,2,3,4-Tetrahydro-β-carboline-3-carboxyl)-L-histidine (5l). Yield 94%; mp 184–186 °C; ESI/MS: 354 $[M+H]^+$; IR (KBr): 3445, 3231, 3212, 3007, 2947, 2842, 1730, 1642, 1600, 1450, 1059, 899 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ = 12.25 (s, 1H), 11.42 (s, 1H), 10.29 (s, 1H), 10.04 (s, 1H), 8.06 (s, 1H), 7.60 (s, 1H), 7.31 (d, J = 7.4 Hz, 1H), 7.17 (d, J = 7.2 Hz, 1H), 7.03 (d, J = 7.2 Hz, 1H), 6.95 (t, J = 7.4 Hz, 1H), 6.94 (s, 1H), 4.97 (t, J = 5.5 Hz, 1H), 3.97 (t, J = 5.5 Hz, 1H), 3.86 (d, J = 5.4 Hz, 2H), 3.05 (d, J = 5.3 Hz, 2H), 2.81 (d, J = 5.0 Hz, 2H). $[\alpha]_D^{20}$ −6° (c 0.37, $CHCl_3/CH_3OH$, 1:1, v/v). Anal. Calcd for $C_{18}H_{19}N_5O_3$: C, 61.18; H, 5.42; N, 19.82. Found: C, 61.23; H, 5.57; N, 19.69.

4.6.13. *N*-(3*S*-1,2,3,4-Tetrahydro-β-carboline-3-carboxyl)-L-tryptophan (5m). Yield 86%; mp 170–172 °C; ESI/MS: 403 $[M+H]^+$; IR (KBr): 3445, 3238, 3217, 3008, 2951, 2842, 1728, 1641, 1600, 1450, 1058, 897 cm^{-1}; ^1H NMR(300 MHz, DMSO-d_6): δ = 11.52 (s, 1H), 11.15 (s, 1H), 10.97 (s, 1H), 10.21 (s, 1H), 8.02 (s, 1H), 7.31 (t, J = 7.2 Hz, 1H), 7.18 (t, J = 7.3 Hz, 1H), 7.14 (t, J = 7.5 Hz, 1H), 7.12 (t, J = 7.5 Hz, 1H), 7.12 (d, J = 7.5 Hz, 1H), 7.10 (d, J = 7.5 Hz, 1H), 7.04 (d, J = 7.5 Hz, 1H), 6.98 (d, J = 7.2 Hz, 1H), 6.85 (s, 1H), 4.87 (t, J = 5.5 Hz, 1H), 3.95 (t, J = 5.5 Hz, 1H), 3.86 (d, J = 5.4 Hz, 2H), 2.94 (d, J = 5.4 Hz, 2H), 2.79 (d, J = 5.5 Hz, 2H). $[\alpha]_D^{20}$ −64° (c 0.39, $CHCl_3/CH_3OH$, 1:1, v/v). Anal. Calcd for $C_{23}H_{22}N_4O_3$: C, 68.64; H, 5.51; N, 13.92. Found: C, 68.55; H, 5.43; N, 13.79.

4.6.14. *N*-(3*S*-1,2,3,4-Tetrahydro-β-carboline-3-carboxyl)-L-arginine (5n). Yield 95%; mp 177–179 °C; ESI/MS: 373 $[M+H]^+$; IR (KBr): 3441, 3230, 3212, 3007, 2950, 2844, 1730, 1640, 1600, 1450, 1057, 896 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ = 11.50 (s, 1H), 10.48 (s, 1H), 10.39 (s, 2H), 10.02 (s, 1H), 9.88 (s, 1H), 8.05 (s, 1H), 7.31 (t, J = 7.5 Hz, 1H), 7.12 (t, J = 7.6 Hz, 1H), 7.06 (d, J = 7.6 Hz, 1H), 6.95 (d, J = 7.5 Hz, 1H), 6.89 (s, 1H), 4.48 (t, J = 5.4 Hz, 1H), 3.96 (t, J = 4.3 Hz, 1H), 3.85 (d, J = 4.8 Hz, 2H), 2.78 (d, J = 4.2 Hz, 2H), 2.66 (t, J = 5.2 Hz, 2H), 1.88 (m, J = 4.1 Hz, 2H), 1.65 (m, J = 4.2 Hz, 2H). $[\alpha]_D^{20}$ −35° (c 0.39, $CHCl_3/CH_3OH$, 1:1, v/v). Anal. Calcd for $C_{18}H_{24}N_6O_3$: C, 58.05; H, 6.50; N, 22.57. Found: C, 58.19; H, 6.34; N, 22.39.

4.7. *N*-[(3*S*)-1,2,3,4-Tetrahydro-β-carboline-3-carboxyl]-L-glycine (5o)

Yield 94%; mp 181–183 °C; ESI/MS: 274 $[M+H]^+$; IR (KBr): 3434, 3230, 3213, 3004, 2950 2844, 1730, 1646, 1601, 1455, 1062, 900 cm^{-1}; ^1H NMR (BHSC-500, DMSO-d_6): δ = 11.05 (s, 1H), 9.94 (s, 1H), 8.00 (s, 1H), 7.27 (t, J = 7.6 Hz, 1H), 7.19 (t, J = 7.6 Hz, 1H), 6.95 (d, J = 7.6 Hz, 1H), 6.87 (d, J = 7.6 Hz, 1H), 4.16 (s, 2H), 4.08 (dd, J = 5.4 Hz, J = 4.5 Hz, 1H), 3.89 (d, J = 5.3 Hz, 2H), 2.83 (d, J = 5.4 Hz, 2H), 2.05 (s, 1H), $[\alpha]_D^{20}$ −104° (c 0.38, $CHCl_3/CH_3OH$, 1:1, v/v). Anal. Calcd for $C_{14}H_{15}N_3O_3$: C, 61.53; H, 5.53; N, 15.38. Found: C, 61.38; H, 5.38; N, 15.51.

4.7.1. *N*-[(3*S*)]-1,2,3,4-Tetrahydro-β-carboline-3-carboxyl]-L-(*Z*)lysine (5p). The solution of 1.5 g (2.50 mmol) of **4p** was mixed with 50 mg of Pd/C (5%) and 15 mL of formic acid in methanol (4.4%), and agitated with hydrogen at room temperature for 24 h. The reaction mixture was filter and evaporated to give a colorless powder. The powder was treated by use of the procedure same as that used for **4a** giving 0.9 g (90%) of the title compound as a pale yellow powder. Mp 166–168 °C; ESI/MS: 479 $[M+H]^+$; IR (KBr): 3440, 3236, 3217, 3004, 2940, 2846, 1736, 1604, 1455, 1062, 898 cm^{-1}; ^1H NMR (BHSC-500, DMSO-d_6): δ = 10.92 (s, 1H), 9.95 (s, 1H), 8.03 (s, 1H), 8.00 (s, 1H), 7.27 (t, J = 7.5 Hz, 1H), 7.20 (t, J = 7.3 Hz, 1H), 7.19 (t, J = 7.5 Hz, 1H), 7.17 (d, J = 7.3 Hz, 2H), 7.14 (t, J = 7.3 Hz, 2H), 6.96 (d, J = 7.5 Hz, 1H), 6.89 (d, J = 7.5 Hz, 1H), 5.35 (s, 2H), 4.46 (t, J = 4.6 Hz, 1H), 3.99 (d, J = 5.6 Hz, 1H), 3.88 (dd, J = 10.0 Hz, J = 4.5 Hz, 1H), 3.86 (dd, J = 10.0 Hz, J = 3.7 Hz, 1H), 2.96 (t, J = 4.6 Hz, 2H), 2.83 (d, J = 10.0 Hz, 2H), 1.80 (m, J = 4.6 Hz, 2H), 2.10 (s, 1H), 1.56 (m, J = 4.6 Hz, 2H), 1.29 (m, J = 4.6 Hz, 2H). $[\alpha]_D^{20}$ −39° (c

0.38, $CHCl_3/CH_3OH$, 1:1, v/v). Anal. Calcd for $C_{26}H_{30}N_4O_5$: C, 65.26; H, 6.32; N, 11.71. Found: C, 65.41; H, 6.49; N, 11.53.

4.7.2. *N*-[(3*S*)-1,2,3,4-Tetrahydro-β-carboline-3-carboxyl]-L-glutamine (5q). Using the procedure same as that used for preparation of **5a**, from 0.9 g (2.0 mmol) of **4q** 0.6 g (91%) of the title compound was obtained as a pale yellow powder. Mp 163–165 °C; ESI/MS: 345 $[M+H]^+$; IR (KBr): 3444, 3233, 3218, 3002, 2945, 2839, 1732, 1645, 1602, 1455, 1060, 900 cm^{-1}; ^1H NMR (BHSC-500, DMSO-d_6): δ = 10.99 (s, 1H), 9.85 (s, 1H), 8.01 (s, 1H), 7.29 (t, J = 7.6 Hz, 1H), 7.20 (t, J = 7.6 Hz, 1H), 7.00 (d, J = 7.6 Hz, 1H), 6.85 (d, J = 7.6 Hz, 1H), 6.00 (s, 2H), 4.46 (t, J = 5.4 Hz, 1H), 3.98 (d, J = 5.8 Hz, 1H), 3.90 (d, J = 5.6 Hz, 1H), 3.82 (d, J = 5.5 Hz, 2H), 2.82 (d, J = 5.8 Hz, 2H), 2.19 (t, J = 5.5 Hz, 2H), 2.07 (t, J = 5.5Hz, 2H), 2.02 (s, 1H). $[\alpha]_D^{20}$ −66° (*c* 0.38, $CHCl_3/CH_3OH$, 1:1, v/v). Anal. Calcd for $C_{17}H_{20}N_4O_4$: C, 59.29; H, 5.85; N, 16.27. Found: C, 59.14; H, 5.68; N, 16.39.

4.8. In vitro anti-platelet aggregation activity assay[27,31]

Platelet-rich plasma was prepared by centrifugation of normal rabbit blood anticoagulation with sodium citrate at a final concentration of 3.8%. The platelet counts were adjusted to 2×10^5/μL by addition of autologous plasma. Platelet aggregation tests were conducted in an aggregometer using the standard turbidimetric technique. The effects of **5a**–**q** on PAF (final concentration 10^{-5}–10^{-7} mol/L) or ADP (final concentration 10^{-5}–10^{-7} mol/L)-induced platelet aggregation were observed. The maximal rate of platelet aggregation (Am%) was represented by the peak height of aggregation curve.

4.9. In vivo antithrombotic activity in rat model[27,31]

The assessments described herein were performed based on a protocol reviewed and approved by the Ethics Committee of Peking University. The committee assures taht the welfare of the animals was maintained in accordance with the requirements of the animal welfare act and according to the guide for care and use of laboratory animals. The tested compound was 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), the right carotid artery and left jugular vein were separated. A 6 cm thread with exact weight was put into the middle of the polyethylene tube. The polyethylene tube was full with heparin sodium (50 IU/mL of NS) and one end was inserted into the left jugular vein. From the other end of the polyethylene tube heparin sodium was injected as anticoagulant, NS or the solution of the tested compound in NS was injected and the polyethylene tube was inserted into the right carotid artery. The blood was flowed from the right carotid artery to the left jugular vein via the polyethylene tube for 15 min. The thread was taken out and weighed 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.

4.10. Apparent permeability coefficient test

Caco-2 cells (from the American Type Culture Collection, Rockville, MD, USA) were cultivated on polycarbonate filters (transwell cell culture inserts, 12 mm in diameter, 3.0 μM in mean pore size) as described elsewhere.[36] Caco-2 cells grown on filter supports for 21 days were used for all transport tests, and the integrity of monolayers was routinely checked by measurements of transepithelial electrical resistance (approximately 700 Ω cm^2). All absorption tests were performed in Hanks' balanced salt solution (HBSS). Compound **1**, **5b**–**d**,**f**,**h**,**i**,**m**–**q** for evaluation was dissolved in HBSS to prepare drug solutions at a final concentration of 4×10^{-3} M. In apical to basolateral direction, transport was initiated by adding drug solutions (total AP volume, 0.5 mL) to the apical compartment of inserts held in transwells containing 1.5 mL of HBSS (basolateral compartment). In basolateral to apical direction, transport was initiated by adding 1.5 mL of the solution of tested compound to basolateral compartment and adding 0.5 mL of HBSS as receiving solution to apical side of the monolayers. The monolayers were incubated in air at 37 °C and 95% relative humidity. At 30, 60, 90, and 120 min, samples were withdrawn from the receiving side, and the concentrations of the samples were determined by HPLC analysis. The resistance of monolayers was checked at the end of each experiment. Apparent permeability coefficients (P_{app}) were calculated according to $P_{app} = dQ/dt \cdot 1/(A \cdot C_0)$, wherein dQ/dt is the permeability rate, C_0 is the initial concentration in the donor chamber, and A is the surface area of monolayer (1 cm^2).

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