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(3*S*)-*N*-(L-Aminoacyl)-1,2,3,4-tetrahydroisoquinolines, a class of novel antithrombotic agents: Synthesis, bioassay, 3D QSAR, and ADME analysis

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ABSTRACT

To increase antithrombotic activity, 3*S*-tetrahydroisoquinoline-3-carboxylic acid (**1**) was modified with natural amino acids to form 19 novel dipeptide analogs, 3*S*-tetrahydroisoquinoline-3-carboxyamino acids (**5a**–**s**), targeting the intestinal peptide transport system. In vitro assay of **5a**–**s** indicated that their potencies for inhibiting adenosine diphosphate (ADP), arachidonic acid (AA), platelet-activating factor (PAF), and thrombin (TH)-induced platelet aggregations were higher than that of **1**. Additionally, in vivo assay of **5a**–**s** indicated that their potencies for inhibiting thrombogenesis in rats were also higher than that of **1**. Among the candidates, **5h** with Ser attachment showed the most impressive features for further development. According to molecular field analysis based Cerius^2 QSAR module, two equations (r, 0.961 and 0.988) correlating the structures with both in vitro and in vivo activities of **5a**–**s** were established. ADMET calculations predict higher intestinal absorption for compounds **5a**–**s**. Further investigation with **5h** as a lead compound is underway.

1. Introduction

Intravascular thrombosis remains one of the most frequent pathological events and a major cause of morbidity and mortality. In the development of acute coronary syndromes, the critical steps are the disruption, rupture, erosion of atherosclerotic plaque with the formation of either partial or complete occlusive thrombus.[1–3] Vascular damage, stimulation of platelets, and activation of the coagulation cascade are the factors contributed to thrombosis. Adherent target of platelets is the 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 antithrombotic therapy of drugs such as anticoagulants, antiplatelet drugs, and thrombolytic drugs, the development of new drug candidates with high potential for improving the prevention and treatment of ischemic symptoms is still urgently needed. Since many antithrombotic drug candidates fail to exert their therapeutic potential mainly due to their poor bioavailability, a great deal of attention has been paid to the small-molecule GPIIb/IIIa antagonists with desirable pharmacodynamic and the pharmacokinetic properties.[8–14] In the development of the small-molecule GPIIb/IIIa antagonists, one of the promising strategies has been to use the intestinal peptide transport system as target to increase bioavailability. The main feature of this strategy is to design drug candidates in the form of dipeptide analogs that can be readily absorbed across the intestinal brush border membrane via the peptide transport system. Accordingly, we report here the design and synthesis of antithrombotic agents with AA modification to improve bioavailability.

To practice this strategy, the naturally occurring small-molecule antagonists such as 3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (THCA) and 3*S*-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (THIQA), have attracted a lot of attentions. THCA, isolated from A. Chinese G. Don, was found possessing moderate antiplatelet aggregation activity and poor bioavailability.[9] While tetrahydroquinolines were reported to exhibit widespread biofunctions, such as cytoxic,[15–18] antimicrobial,[19] antimalarial,[20] hypothermic,[21] phosphatase PTP1B inhibition,[22] increased chloride transport,[23] growth hormone secretagogue,[24] estrogen receptor modulation,[25,26] bradycardic,[27] neuroprotective or neurotoxic,[28] anxiolytic effects,[29] and antiplatelet aggregation.[30–33] As one antiplatelet aggregation derivative of tetrahydroquinolines, THIQA structurally correlates with THCA (Fig. 1), and both possess poor water solubility which limits their intestinal absorption.[9] The modification of THCA with amino acids resulted in the increase of permeability and antithrombotic activity,[9] and the in vitro antiplatelet aggregation assessment indicated the potency of THIQA was significantly higher than that of THCA (Table 1), which inspired us to modify

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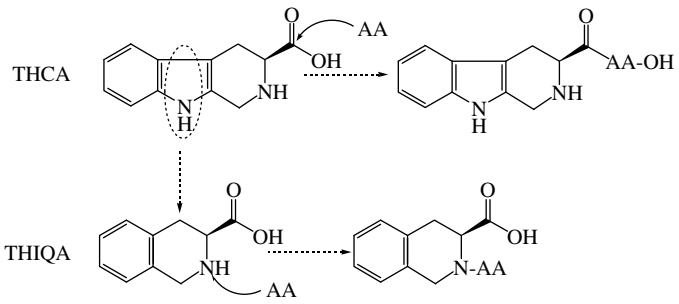


Figure 1. Structural correlation of 3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (THCA) and 3*S*-1,2,3,4-tetrahydroisiqunoline-3-carboxylic acid (THIQA) as well as modification to improve their water solubility and bioactivity.

THIQA much like what was done to THCA to improve its water solubility and bioactivity. In this context, 19 novel 3*S*-*N*-aminoacyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acids with 19 different amino acids (except Cys) were prepared in our laboratory. Their in vitro antiplatelet aggregation activities were evaluated by use of four aggregators, and their in vivo antithrombotic activities were assessed on rat model. The improvement in antithrombotic activities was rationalized by 3D QSAR and ADMET.

2. Results and discussion

2.1. Synthesis of 3*S*-*N*-(aminoacyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acids (5a–s)

The preparation of 3*S*-1,2,3,4-tetrahydroisoquinoline-3-carbonylamino acids (**5a**–**s**) was carried out according to the five-step route depicted in Scheme 1. L-Phe was successively converted into 3*S*-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (**1**, 84% yield) via Pictet–Spengler condensation and 3*S*-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid methylester (**2**, 92% yield) via esterification. Methylester **2** was then conjugated with Boc-AA by the DCC/HOBt/NMM procedure to give 3*S*-*N*-(Boc-aminoacyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid methylester (**3a**–**s**, 45–92% yield). Then **3a**–**s** were saponified to provide 3*S*-*N*-(Boc-aminoacyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (**4a**–**s**, 48–100% yield). Finally the Boc group was removed to give 3*S*–*N*-aminoacyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acids (**5a**–**s**, 48–100% yield). The mild condition and the good yields of the individual reactions suggest that the present synthetic route is suitable for preparing these novel compounds.

2.2. In vitro and in vivo antithrombotic activities of 5a–s

To examine the antithrombotic activities of **5a**–**s**, the in vitro antiplatelet aggregation assays were performed on four aggregators which induced aggregation of pig platelets and the in vivo assays were performed on extra-corporeal circulation of arterio-veinos cannula model of rats. All of the assays consistently indicated that compound **5a**–**s** had in vitro antiplatelet aggregation activities and in vivo antithrombotic activities.

Table 1
IC_{50} of THCA and THIQA against four aggregators induced aggregation of pig platelets[a]

Compound	IC_{50} (nM)			
	ADP	AA	PAF	TH
THCA	3320.42	3429.33	3175.81	3289.10
THIQA	552.15	452.47	960.18	881.64

THCA, 3*S*-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid; THIQA, 3s-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid.

2.2.1. In vitro antiplatelet aggregation of 5a–s

For platelet-activating factor (PAF, final concentration 0.1 μM), adenosine diphosphate (ADP, final concentration 10 μM), arachidonic acid (AA, final concentration 350 μM), and thrombin (TH, final concentration 0.1 U/ml), the in vitro activities of **5a**–**s** (at a series of concentrations ranging from 10 μM to 10 nM) inhibiting PAF, ADP, AA, and TH-induced platelet aggregation were tested, and the IC_{50} values are listed in Table 2.

The IC_{50} values of **1** indicated that it was active against in vitro antiplatelet aggregation with an inhibiting order of AA > ADP > TH > PAF. On the other hand, the data particularly demonstrated that almost all the activities of **5a**–**s** were higher than that of **1**, suggesting that introducing amino acid into the 2-position of **1** generally benefited the in vitro antiplatelet aggregation activity. Except **5a**, **5e**, and **5r**, the IC_{50} values of **5b–d**, **5f–q**, and **5s** inhibiting ADP-induced platelet aggregation were lower than those inhibiting AA, PAF, and TH-induced aggregation, suggesting **5b–d**, **5f–q**, **5s** selectively inhibited ADP-induced platelet aggregation.

2.2.2. In vivo antithrombotic activities of 5a–s

By use of extra-corporeal circulation of arterio-veinos cannula model, the in vivo activities of **5a**–**s** were tested, and the weights of wet thrombi are listed in Table 3. The data indicated that **5a**–**s** (thrombus weights ranging from 18.00 to 22.66 mg, compared to 28.54 mg thrombi of normal saline (NS) receiving rats $p < 0.001$) obviously inhibited the rats to form thrombus. Compared to **1**, the antithrombotic activities of 5 μmol/kg of **5a**, **5e**, **5g**, **5h**, **5l**, **5q**, and **5s** (thrombus weights ranging from 18.00 to 20.30 mg) are significantly higher than that of 15 μmol/kg of **1** (thrombus weight 21.52 mg), while 5 μmol/kg of **5b–d**, **5f**, **5i–k**, **5m–p**, and **5r** possess the same activity (average thrombus weight 21.34 mg) as 15 μmol/kg of **1**. The data demonstrated that introducing amino acid into **1** resulted in at least threefold increases of the antithrombotic activity.

2.2.3. Dose-dependent in vivo antithrombotic activity of 5h

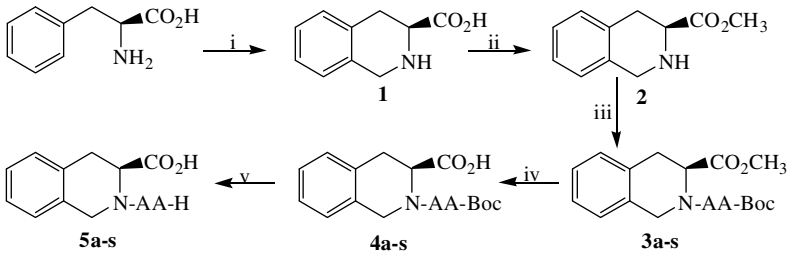
Intravenous injection of **5h** was observed at doses of 5 μmol/kg, 500 nmol/kg, 50 nmol/kg, and 5 nmol/kg to produce a possible dose-dependent antithrombotic response in the rats. The thrombus weights are listed in Table 4, which demonstrated that the thrombus weight was progressively increased with dose decrease. Therefore, **5h** exhibited dose-dependent antithrombotic action.

2.3. 3D QSAR analysis of 5a–s

To understand the dependence of the in vitro antiplatelet aggregation and in vivo antithrombotic activities of **5a**–**s** upon their structures, the corresponding 3D QSAR analysis was performed. In the analysis, the alignment, MFA based Cerius[2] QSAR module of **5a**–**s** and the electrostatic and environments of **5a**–**s** within the grid with 3D points of the equation were involved.

2.3.1. Alignment of 5a–s

For establishing the valid 3D QSAR models, a proper alignment procedure of **5a**–**s** was practiced using the target model align strategy in the align module within Cerius[2]. Based on the assumption that each structure of **5a**–**s** exhibited activity at the same binding site of the receptor, they were aligned in a pharmacological active orientation. To obtain a consistent alignment, the most vasorelaxation potent **5h** was selected as the template for superposing **5a–g** and **5i**–**s**. The method used for performing the alignment was the maximum common subgraph (MCS).[34] MCS looks at molecules as



Scheme 1. Synthetic route of **5a–s**. Reagents and conditions: (i) HCHO and HCl; (ii) $SOCl_2$ and MeOH; (iii) Boc-AA, DCC/HOBt/NMM; (iv) NaOH aqueous (2 N); (v) hydrogen chloride in ethyl acetate (6 N). In **3a–5a** AA = Ala, **3b–5b** AA = Gly, **3c–5c** AA = Val, **3d–5d** AA = Phe, **3e–5e** AA = Leu, **3f–5f** AA = Ile, **3g–5g** AA = Trp, **3h** and **4h** AA = Ser(BZl), **5h** AA = Ser, **3i–5i** AA = Thr, **3j** AA = Tyr(OBzl), **4j** and **5j** AA = Tyr, **3k–5k** AA = Pro, **3l–5l** AA = Met, **3m–5m** AA = Asn, **3n–5n** AA = Gln, **3o–5o** AA = His, **3p** and **4p** AA = Lys(Boc), **5p** AA = Lys, **3q** AA = Asp(OBzl), **4q** and **5q** AA = Asp, **3r** AA = Glu(OBzl), **4r** and **5r** AA = Glu, **3s** and **4s** AA = Arg(NO2), **4s′** and **5s** AA = Arg.

Table 2
IC_{50} of **1** and **5a–s** against four aggregators induced aggregation of pig platelets

Compound	IC_{50} (nM)			
	ADP	AA	PAF	TH
1	552.15	452.47	960.18	881.64
5a	651.34	440.48	945.12	741.17
5b	365.26	422.99	875.34	760.66
5c	388.89	666.24	631.91	653.49
5d	369.72	573.16	526.97	851.46
5e	336.14	238.35	891.64	730.36
5f	314.65	465.10	831.48	674.25
5g	194.01	571.87	422.36	719.92
5h	103.31	214.55	267.43	497.95
5i	240.13	534.69	403.55	879.37
5j	326.96	525.93	668.38	737.59
5k	372.74	382.26	506.97	590.49
5l	265.12	429.75	409.21	606.97
5m	534.45	407.42	360.49	495.34
5n	302.49	350.70	392.43	554.78
5o	323.75	520.87	670.37	684.40
5p	323.08	343.69	472.24	829.37
5q	344.60	368.79	383.37	901.98
5r	494.07	298.02	544.27	589.46
5s	209.81	216.78	516.67	565.26

points and lines and uses the graph theory technique to identify patterns. MCS found the largest subset of atoms in **5h** shared by **5a–g** and **5i–s**. This subset was used for the alignment. A rigid fit of atom pairings was performed to superimpose each structure onto the target model **5h**. A view of aligned **5a–s** is shown in Figure 2. The alignment stereoview explores that to superimpose onto **5h**, the side chains of each structure have to take specific conformations. As seen in Table 2, this individual side chain conformation corresponds to an individual antithrombotic activity.

2.3.2. MFA based Cerius^2 QSAR module of 5a–s

Molecular field analysis (MFA) was performed for **5a–s** using the QSAR module of Cerius^2.[35] A five-step procedure consisted of generating conformers, energy minimization, matching atoms and aligning molecules, setting preferences, and regression analysis was automatically practiced in MFA. Molecular electrostatic and steric fields were created by use of proton, methyl, and hydroxyl anion as probes, respectively. These fields were sampled at each point of a regularly spaced grid of 1 Å. An energy cutoff of ±30.0 kcal/mol was set for both electrostatic and steric fields. The total number of grid points generated was 672. Though the spatial and structural descriptors such as dipole moment, polarizability, radius of gyration, number of rotatable bonds, molecular volume, principal moment of intertia, A log *P*98, number of hydrogen bond donors and acceptors, and molar refractivity were also considered, only the highest variance holder proton and methyl descriptors were used. Regression analysis was carried out using the genetic partial least squares (G/PLS) method consisting of 50,000 generations with a population size of 100. The number of components was set to 5. Cross-validation was performed with the leave-one-out procedure. PLS analysis was scaled, with all variables normalized to a variance of 1.0.

2.3.3. 3D QSAR equation of 5a–s against ADP-induced platelet aggregation

Based on the module, the regions where variations in the steric or electrostatic features of **5a–s** led to the increase or decrease of their in vitro inhibition of ADP-induced platelet aggregation were specified in Figure 3.

In the MFA model, the activities for **5a–s** to inhibit ADP-induced platelet aggregation in terms of the most relevant descriptors including proton, methyl, and hydroxyl anion is expressed by Equation 1

Table 3
Effect of **5a–s** on the thrombus weight of the rats[a]

Compound	Thrombus weight	Compound	Thrombus weight	Compound	Thrombus weight
NS	28.54 ± 2.62	**1**[1]	26.88 ± 1.94	Aspirin[1]	27.60 ± 1.89
Aspirin[2]	13.22 ± 1.67[c]	**1**[2]	21.52 ± 1.49[b]	**5m**	22.66 ± 1.45[b]
5a	20.30 ± 1.33[d]	**5g**	19.47 ± 1.64[d]	**5n**	21.30 ± 1.69[b]
5b	21.17 ± 1.47[b]	**5h**	18.00 ± 1.59[c]	**5o**	21.78 ± 1.96[b]
5c	20.12 ± 1.59[b]	**5i**	20.73 ± 1.53[b]	**5p**	21.31 ± 1.75[b]
5d	22.23 ± 1.72[b]	**5j**	20.92 ± 1.44[b]	**5q**	20.30 ± 1.34[d]
5e	19.12 ± 1.82[d]	**5k**	21.09 ± 1.68[b]	**5r**	20.87 ± 1.45[b]
5f	21.14 ± 1.24[b]	**5l**	19.56 ± 1.72[d]	**5s**	20.06 ± 1.48[d]

[a] Weight of wet thrombus is represented by X ± SD mg, NS = vehicle, $n = 12$; dose of **1**[1], 5 μmol/kg; dose of **1**[2], 15 μmol/kg; dose of aspirin[1], 22 × 5 μmol/kg; dose of aspirin[2], 110 × 5 μmol/kg; dose of **5a–s**, 5 μmol/kg.
[b] Compared to NS and **1**[1] $p < 0.001$.
[c] Compared to NS and **1**[1] $p < 0.001$ and to **1** $p < 0.01$.
[d] Compared to NS and **1**[1] $p < 0.001$ and to **1** $p < 0.05$.

Table 4
Effect of different doses of **5h** on the thrombus weight of the rats[a]

Dose	5 μmol/kg	500 nmol/kg	50 nmol/kg	5 nmol/kg
5h	18.00 ± 1.59[b]	18.31 ± 3.53[b]	22.54 ± 3.40[c]	28.58 ± 1.56[d]
NS	28.54 ± 2.62			

[a] Weight of wet thrombus is represented by X ± SD mg, NS = vehicle, $n = 12$.
[b] Compared to NS and 5 nmol/kg groups $p < 0.01$, to 50 nmol/kg group $p < 0.05$.
[c] Compared to NS and 5 nmol/kg group $p < 0.01$.
[d] Compared to NS group $p > 0.05$.

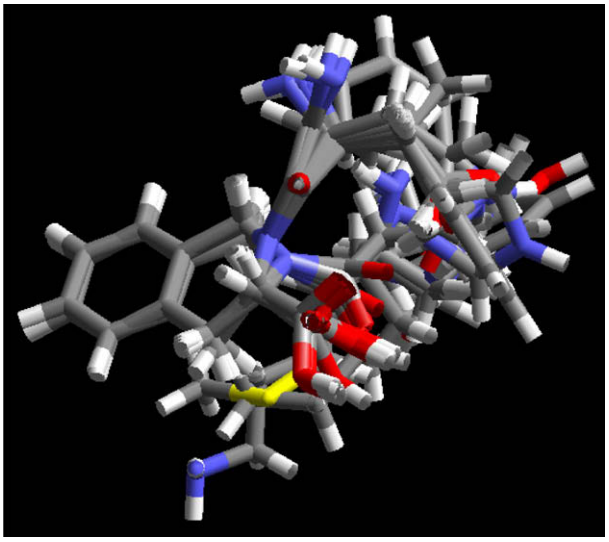


Figure 2. Alignment stereoview of **5a–s** used for molecular field generation.

$$\begin{aligned}\text{Activity} = {} & 535.05 + 2.78808(HO^-/235) \\ & + 1.46836(CH_3/308) - 4.38938(CH_3/316) \\ & + 1.70626(HO^-/227) + 1.00951(CH_3/234) \\ & - 2.1067(H^+/247) + 4.31849(HO^-/310) \\ & - 4.62654(HO^-/292) - 7.84181(HO^-/309) \\ & - 4.30254(HO^-/357) \end{aligned} \quad (1)$$

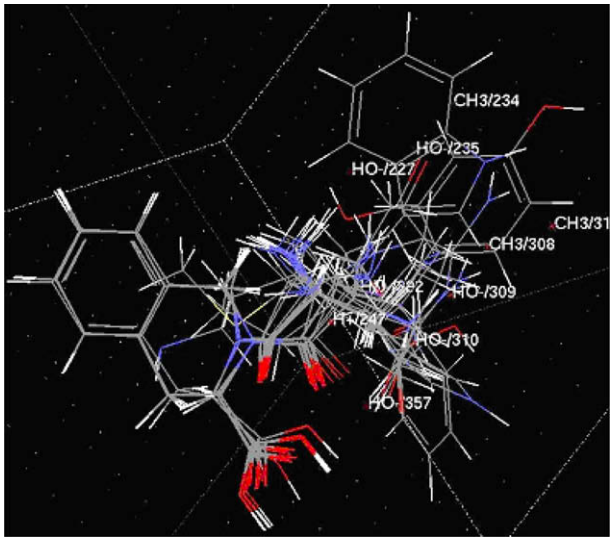


Figure 3. Steric and electrostatic features of **5a–s** leading to different in vitro activities.

The correlation of the activities tested on the in vitro platelet aggregation model and the activities calculated using Equation 1 was explained by Figure 4. In Equation 1, the data points (n), correlation coefficient (r), and square correlation coefficient (r^2) were 17, 0.961, and 0.923, respectively. The tested activities on ADP-induced platelet aggregation model and the calculated activities based on Equation 1 were shown also in Figure 4. The parameters indicated that Equation 1 was able to predict the in vitro activity for the analogs of **5a–s**.

Equation 1 contains one term from proton descriptor, three terms from methyl descriptor, and six terms from hydroxyl anion descriptor. The term of 2.1067(H^+/247) has negative coefficient, which means that at this position an electron-withdrawing group will affect on the activity positively. The terms of 1.46836(CH_3/308) and 1.00951(CH_3/234) have positive coefficients, which means that at these positions large groups will affect on the activity positively, while term of 4.38938(CH_3/316) has negative coefficient, which means that at this position large group will affect on the activity negatively. The terms of 2.78808(HO^-/235), 1.70626(HO^-/227), and 4.31849(HO^-/310) have positive coefficients, which means that at these positions electron-releasing groups will affect on the activity positively, while the terms of 4.62654(HO^-/292), 7.84181(HO^-/309), and 4.30254(HO^-/357) have negative coefficients, which means that at these positions electron-withdrawing groups will affect on the activity positively.

Figure 5 gives four representatives (a) **5a**, **h**, and (b) **5g**, **m**. In Figure 5a, **5a** has no electron-withdrawn group at H^+/247, as a result, it has a low in vitro antiplatelet aggregation activity, while **5h** has an electron-withdrawing hydroxyl at HO^-/292 leading to a higher in vitro antiplatelet aggregation activity. In Figure 5b, **5g** has an electron-releasing indole group at HO^-/235 leading to a higher in vitro antiplatelet aggregation activity, and **5m** has only hydrogen at HO^-/310 with a low in vitro antiplatelet aggregation activity.

2.3.4. 3D QSAR equation of 5a–s inhibiting thrombogenesis

Based on the module, the regions where variations in the steric or electrostatic features of **5a–s** led to the inhibition or enhancement of thrombogenesis on rat model were specified in Figure 6.

In the MFA model, the antithrombotic activities of **5a–s** in terms of the most relevant descriptors including proton, methyl, and hydroxyl anion are expressed by Equation 2.

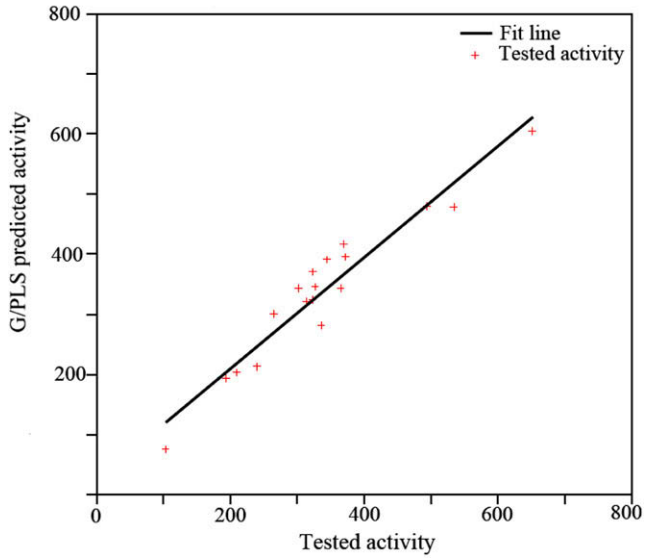


Figure 4. Graph of tested versus predicted antiplatelet aggregation activities of **5a–s**.

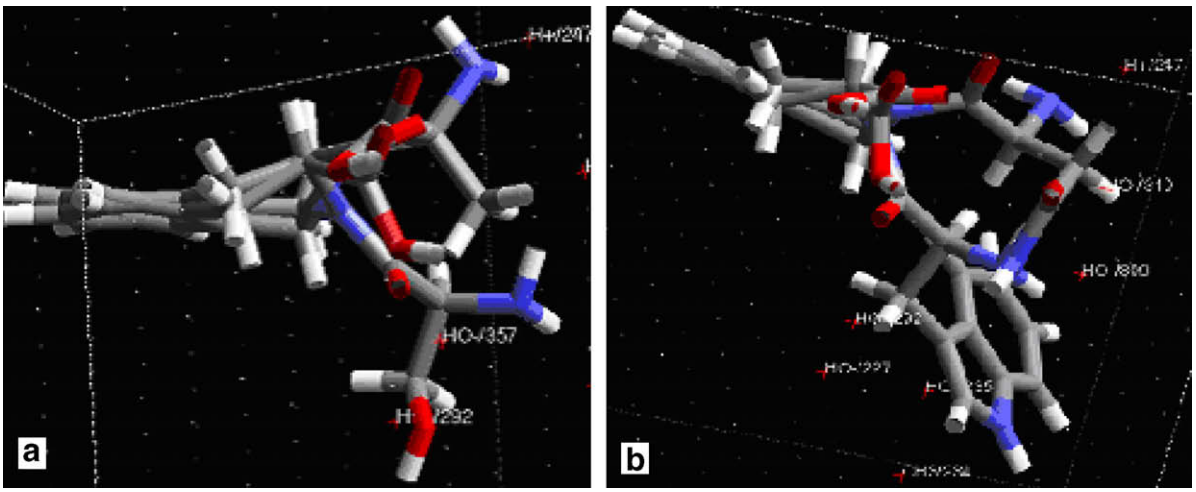


Figure 5. Electrostatic and environments of **5a**, **h** (a) and **5g**, **m** (b) within the grid with 3D points of Equation 1.

$$\begin{aligned}\text{Activity} = {} & 0.302777 + 0.002687(CH_3/236) \\ & - 0.000799(CH_3/300) - 0.000888(HO^-/366) \\ & - 0.000953(CH_3/110) - 0.001743(HO^-/284) \\ & - 0.001437(H^+/227) - 0.000232(HO^-/283) \\ & - 0.001058(CH_3/304) - 0.001545(CH_3/307) \\ & + 0.002148(CH_3/317) \end{aligned} \quad (2)$$

The correlation of the activities tested on the in vivo thrombogenesis model and calculated using Equation 2 is explained by Figure 7. In Equation 2 the data points (n), correlation coefficient (r), and square correlation coefficient (r^2) were 17, 0.988, and 0.976, respectively. The tested activities on rat thrombogenesis model and the calculated activities based on Equation 2 were also shown in Figure 7. The parameters indicated that Equation 2 was able to predict the in vivo activity for the analogs of **5a**–**s**.

Equation 2 contains one term from proton descriptor, six terms from methyl descriptor, and three terms from hydroxyl anion descriptor. The term of 0.001437(H^+/227) has negative coefficient, which means that at this position an electron-withdrawing group will affect the activity positively. The terms of 0.002687(CH_3/236) and 0.002148(CH_3/317) have positive coefficients, which means that at these positions large groups will affect the activity positively, while the terms of 0.000953(CH_3/110), 0.000799(CH_3/300), 0.001058 (CH_3/304), and 0.001545(CH_3/307) have negative coefficients, which means that at these positions small groups will affect the activity positively. The terms of 0.000232(HO^-/283), 0.001743 (HO^-/284), and 0.000888(HO^-/366) have negative coefficients, which means that at these positions electron-withdrawing groups will affect the activity negatively.

Figure 8 gives four representatives **5a**, **5h**, **5g**, and **5m**. In Figure 8a, **5a** has larger L-Ser residue at CH_3/236 leading to a higher in vivo antithrombotic activity, and **5h** has a smaller amido group at CH_3/307, as a result, it has a low in vivo antithrombotic activity. In Figure 8b, **5g** has a larger L-Trp residue at CH_3/236 and an electron-withdrawing amino group at HO^-/366 leading to a higher in vivo antithrombotic activity, while **5m** has an electron-withdrawing amido group at HO^-/366 with a low in vivo antithrombotic activity.

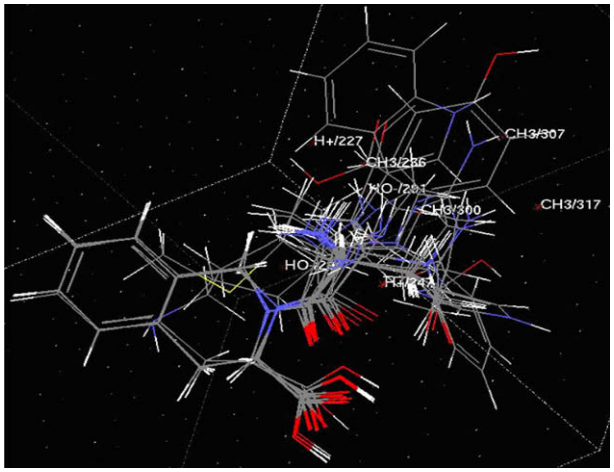


Figure 6. Steric and electrostatic features of **5a**–**s** leading to different in vivo activities.

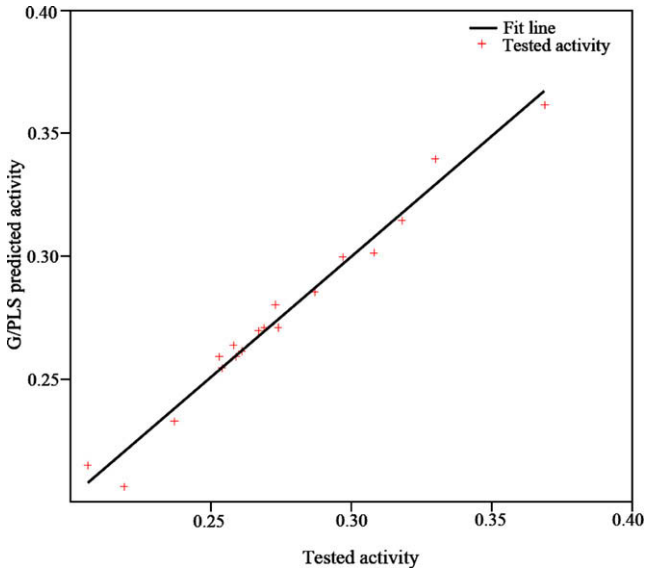


Figure 7. Graph of tested versus predicted antithrombotic activities of **5a**–**s**.

2.4. ADMET defined absorption and aqueous solubility levels of 5a–s

To estimate the prospect of **5a**–**s** as antithrombotic agents, their absorption, aqueous solubility, and drug-likeness were calculated according to absorption, distribution, metabolism, elimination, and toxicity (ADMET) program defined human intestinal absorption (HIA) model, which was developed with 182 compounds as the training set and with Alog*P*98 and 2D polar surface area as the descriptors,[36,37] and aqueous solubility model, which was developed with 775 compounds as the training set.[38] Generally, four levels are defined to predict intestinal absorption of the compounds, namely 0, 1, 2, and 3 as good, moderate, poor, and very poor absorption, respectively, and seven levels are defined to predict aqueous solubility levels of the compounds, namely 0, 1, 2, 3, 4, 5, and 6 as extremely low, very low, low, good, optimal, too soluble, and molecules with one or more unknown Alog*P*98 types, respectively. The prediction levels of **5a**–**s** are listed in Table 5. The data indicate that **5a–l**, **5o**, **5p**, and **5r** are good absorption, **5m**, **5n**, and **5q** are moderate absorption, **5s** is poor absorption, **5c**–**g**, **5j**, and **5k** have good drug-likeness, and **5a**, **5b**, **5h**, **5i**, and **5l**–**s** have optimal drug-likeness. For instance, the neutral, polar, and small hydroxymethyl side chain of Ser residue is preferable. In ADMET related Caco-2 cell monolayer permeability investigation, we explored that the improvement of permeability of amino acid modified THCA varied with the polarity, charge, and spatial arrangement of the side chain of the amino acid.[9] The consistent trend for both theoretical and experimental data suggested that the design of dipeptide analog targeting the intestinal peptide transport system could be a promising way to get the lead compounds and candidates for small antithrombotic molecules.

Table 5
Predicted absorption and drug-likeness of **5a**–**s**

Compound	Absorption level	Solubility	
		Solubility level	Drug-likeness
5a	0 (good)	4	Optimal
5b	0 (good)	4	Optimal
5c	0 (good)	3	Good
5d	0 (good)	3	Good
5e	0 (good)	3	Good
5f	0 (good)	3	Good
5g	0 (good)	3	Good
5h	0 (good)	4	Optimal
5i	0 (good)	4	Optimal
5j	0 (good)	3	Good
5k	0 (good)	3	Good
5l	0 (good)	4	Optimal
5m	1 (moderate)	4	Optimal
5n	1 (moderate)	4	Optimal
5o	0 (good)	4	Optimal
5p	0 (good)	4	Optimal
5q	1 (moderate)	4	Optimal
5r	0 (good)	4	Optimal
5s	2 (poor)	4	Optimal

3. Conclusion

Development of antithrombotic agents with increased bioavailability possesses clinical importance. According to the requirements of the peptide transport system, amino acids were introduced into antiaggregation molecule 3*S*-tetrahydroisoquinoline-3-carboxylic acid to give its dipeptide derivatives based on our new design. Following a five-step route, the 19 novel dipeptide analogs (3*S*-tetrahydroisoquinoline-3-carboxyamino acids, **5a**–**s**) with increased in vitro antiplatelet aggregation activities and in vivo antithrombotic activities compared to compound **1** were synthesized and characterized. The 3D QSAR analysis of **5a**–**s** quantitatively correlates their structures and the in vitro/in vivo activities, which provides valuable data for predicting the in vitro/in vivo activities of new-designed dipeptide analogs. The ADMET calculation of **5a**–**s** quantitatively demonstrated that **5a**–**s** were capable of using the peptide transport system to across the intestinal brush border membrane with improved drug-likeness, which shed the light on the design of antithrombotic agents as well as the prediction of new-designed candidates with ideal bioavailability.

4. Experimental

4.1. General method

All the reactions were carried out under nitrogen (1 bar). ^{1}H (300 and 500 MHz) and ^{13}C (75 and 125 MHz) NMR spectra were recorded on Bruker AMX-300 and AMX-500 spectrometers for solution DMSO-d_6, or $CDCl_3$ with tetramethylsilane as internal standard. IR spectra were recorded with a Perkin-Elmer 983 instrument. FAB/MS was determined on VG-ZAB-MS, and TOF-MS was recorded on MDS SCIEX QSTAR. Melting points were measured on a XT5 hot stage microscope (Beijing key electro-optic factory). All L-amino acids were purchased from China Biochemical Corporation. TLC was made with Qingdao silica gel GF_{254}. Chromatography was performed with Qingdao silica gel H_{60} or Sephadex-LH_{20}. All solvents were distilled and dried before use according to the liter-

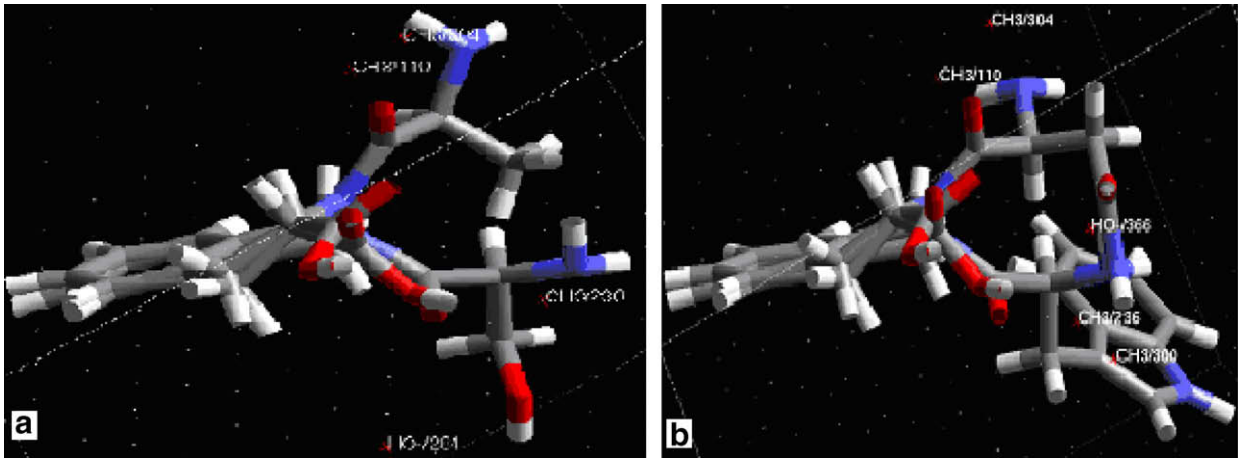


Figure 8. Electrostatic and environments of **5a**, **5h** (a) and **5g**, **5m** (b) within the grid with 3D points of Equation 2.

ature procedures. Optical rotations were determined with a Jasco P-1020 Polarimeter at 20 °C. The statistical analysis of all the biological data was carried out by use of ANOVA test, $p < 0.05$ is considered significant.

4.2. Preparing compounds

4.2.1. (3*S*)-1,2,3,4-Tetrahydroisoquinoline-3-carboxylic acid (1)

To the suspension of 5.0 g (0.03 mmol) of L–Phe–OH in 50 ml of chloroform and 27 ml of formaldehyde 45 ml of concentrated hydrochloric acid was added drop-wise. The reaction mixture was stirred at 80–90 °C for 10 h, and TLC ($CHCl_3/CH_3OH$, 10:1) indicates the complete disappearance of L–Phe–OH. The reaction mixture was cooled to room temperature and the formed precipitates were collected by filtration. The collected solids were successively washed with water (3× 30 ml) and acetone (3× 30 ml) to give 4.5 g (83.9%) of the title compound as a colorless powder. Mp 302–303 °C; $[\alpha]_D^{20}$ −68 (*c* 1.0, H_2O); ESI-MS (*m*/*e*) 178[M+H]$^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ/ppm = 11.0 (s, 1H), 7.25 (m, *J* = 6.4 Hz, 2H), 7.02 (d, *J* = 6.5 Hz, 1H), 6.98 (t, *J* = 6.6 Hz, 1H), 3.80 (m, 3H), 3.03 (d, *J* = 7.5 Hz, 1H), 2.78 (d, *J* = 8.4 Hz, 1H), 2.0 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ/ppm = 174.9, 136.2, 134.2, 127.2, 126.0, 57.6, 47.4, 29.4.

4.2.2. (3*S*)-1,2,3,4-Tetrahydroisoquinoline-3-carboxylic acid methylester (2)

At 0 °C to 20 ml of methanol 5.2 ml of thionyl chloride was added dropwise, which took 10 min. To this mixture 4.27 g (20 mmol) of (3*S*)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid was added. The reaction mixture was stirred at room temperature for 100 h, and TLC (CCl_3/CH_3OH = 10:1, R_f = 0.5) indicated the complete disappearance of (3*S*)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid. The reaction mixture was evaporated under vacuum. The residue was dissolved in 20 ml of methanol and evaporated under vacuum. This procedure was repeated for three times. Then the residue was dissolved in 20 ml of ether and evaporated under vacuum. This procedure was repeated for three times. The residue was crystallized in the mixture of methanol/ether to provide 4.2 g (92%) of the title compound as a colorless powder. ESI-MS (*m*/*e*) 192[M+H]$^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ/ppm = 7.0 (m, 4H), 3.80 (m, 3H), 3.67 (s, 3H), 3.13 (d, *J* = 8.6 Hz, 1H), 2.78 (d, *J* = 7.4 Hz, 1H), 2.0 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ/ppm = 174.9, 136.2, 134.2, 127.2, 126.0, 57.6, 47.4, 29.4.

4.3. General procedure for preparing (3*S*)-*N*-(Boc-aminoacyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid methylesters (3a–s)

At 0 °C and with stirring to the solution of 4.4 mmol of Boc-AA-OH in 10 ml of anhydrous THF 0.594 g (4.4 mmol) of HOBt was added to form reaction mixture A. The solution of 0.91 g (4.0 mmol) of (3*S*)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid methylester in 5 ml of anhydrous THF was adjusted pH 9 with triethylamine and stirred for 30 min to form mixture B. At 0 °C the mixture A and B were mixed and then 1.071 g (5.2 mmol) of DCC was added. The reaction mixture was stirred at 0 °C for 2 h, at room temperature for12 h and TLC (ethyl acetate/petroleum ether, 1:2) indicated the complete disappearance of (3*S*)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid methylester. The formed precipitates of DCU were removed by filtration and the filtrate was evaporated under vacuum. The residue was dissolved in 50 ml of ethyl acetate, the formed solution was washed successively with saturated aqueous solution of $NaHCO_3$ (3× 30 ml), 5% aqueous solution of $KHSO_4$ (3× 30 ml), and saturated aqueous solution of NaCl (3× 30 ml) and dried with anhydrous Na_2SO_4. After filtration the filtrate was evaporated under vacuum to give the title compound.

4.3.1. (3*S*)-*N*-(Boc-L-Alaninyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid methylester (3a)

Yield: 70%. Syrupy. ESI-MS (*m*/*e*) 363 [M+H]$^+$; IR (cm^{-1}) 2968, 1734, 1648, 1497, 1457, 1395, 1380, 1365, 1198, 1111, 750, 600; $[\alpha]_D^{20}$ −28.5 (*c* 1.0, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ/ppm = 8.00 (d, *J* = 6.6 Hz, 1H), 7.25 (m, *J* = 6.4 Hz, 2H), 7.02 (d, *J* = 6.5 Hz, 1H), 6.98 (t, *J* = 6.6 Hz, 1H), 4.83 (d, *J* = 7.5 Hz, 1H), 4.68 (m, 1H), 4.55 (d, *J* = 5.6 Hz, 1H), 4.49 (d, *J* = 8.6 Hz, 1H), 3.63 (s, 3H), 3.30 (d, *J* = 7.5 Hz, 1H), 3.10 (d, *J* = 8.6 Hz, 1H), 1.46 (d, *J* = 5.4 Hz, 3H), 1.41 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ/ppm = 171.6, 172.9, 155.1, 132.1, 131.7, 127.2, 80.8, 55.8, 52.2, 46.9, 44.6, 28.4, 27.0, 19.0. Anal. Calcd for $C_{19}H_{26}N_2O_5$: C, 62.97; H, 7.23; N, 7.73. Found: C, 62.75; H, 7.11; N, 7.50.

4.3.2. (3*S*)-*N*-(Boc-Glycinyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid methylester (3b)

Yield: 72%. Syrupy. ESI-MS (*m*/*e*) 349[M+H]$^+$; IR (cm^{-1}) 2969, 1735, 1648, 1496, 1457, 1395, 1365, 1198, 1111, 750, 599; $[\alpha]_D^{20}$ −11.7 (*c* 1.0, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ/ppm = 8.00 (d, *J* = 5.6 Hz, 1H), 7.22 (m, *J* = 6.4 Hz, 2H), 7.05 (d, *J* = 6.6 Hz, 1H), 6.95 (t, *J* = 6.5 Hz, 1H), 4.80 (d, *J* = 6.4 Hz, 1H), 4.51 (m, 2H), 3.85 (d, *J* = 7.5 Hz, 2H), 3.63 (s, 3H), 3.25 (m, 2H), 1.41 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ/ppm = 171.6, 168.6, 155.8, 131.9, 130.9, 128.4, 127.2, 126.6, 80.8, 55.8, 52.0, 44.6, 43.0, 28.4, 27.0. Anal. Calcd for $C_{18}H_{24}N_2O_5$: C, 62.05; H, 6.94; N, 8.04. Found: C, 61.84; H, 6.82; N, 7.81.

4.3.3. (3*S*)-*N*-(Boc-L-Valinyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid methylester (3c)

Yield: 85%. Syrupy. ESI-MS (*m*/*e*) 391[M+H]$^+$; IR (cm^{-1}) 2970, 1734, 1648, 1498, 1457, 1395, 1385, 1375, 1365, 1198, 1111, 750, 599; $[\alpha]_D^{20}$ −15.8 (*c* 1.0, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ/ppm = 8.00 (d, *J* = 6.6 Hz, 1H), 7.22 (m, *J* = 6.4 Hz, 2H), 7.07 (d, *J* = 6.5 Hz, 1H), 6.96 (t, *J* = 6.6 Hz, 1H), 4.80 (d, *J* = 5.4 Hz, 1H), 4.61 (d, *J* = 6.4 Hz, 1H), 4.51 (m, 2H), 4.24 (m, 1H), 3.63 (s, 3H), 3.25 (m, 2H), 2.0 (s, 1H), 1.41 (s, 9H), 1.21 (d, *J* = 7.8 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ/ppm = 171.6, 168.6, 155.8, 135.9, 133.9, 128.4, 127.2, 126.6, 80.8, 67.8, 58.4, 55.8, 52.0, 44.6, 28.4, 27.0, 18.9. Anal. Calcd for $C_{21}H_{30}N_2O_5$: C, 64.59; H, 7.74; N, 7.17. Found: C, 64.81; H, 7.86; N, 7.41.

4.3.4. (3*S*)-*N*-(Boc-L-Phenylalaninyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid methylester (3d)

Yield: 87%. Syrupy. ESI-MS (*m*/*e*) 439 [M+H]$^+$; IR (cm^{-1}) 2968, 1734, 1648, 1600, 1497, 1457, 1395, 1380, 1365, 1198, 1111, 750, 700, 599; $[\alpha]_D^{20}$ −35.8 (*c* 1.0, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ/ppm = 8.00 (d, *J* = 6.6 Hz, 1H), 7.22 (m, *J* = 7.4 Hz, 2H), 7.15 (d, *J* = 7.2 Hz, 2H), 7.10 (d, *J* = 7.8 Hz, 1H), 7.02 (m, *J* = 8.2 Hz, 2H), 6.95 (d, *J* = 8.8 Hz, 2H), 4.92 (d, *J* = 5.2 Hz, 1H), 4.80 (d, *J* = 8.4 Hz, 1H), 4.51 (d, *J* = 7.1 Hz, 1H), 4.41 (d, *J* = 7.3 Hz, 1H), 3.63 (s, 3H), 3.10 (m, 4H), 1.41 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ/ppm = 171.6, 170.1, 155.8, 139.5, 135.9, 134.2, 128.4, 127.2, 126.6, 79.5, 55.8, 52.0, 44.6, 37.8, 28.4, 27.0. Anal. Calcd for $C_{25}H_{30}N_2O_5$: C, 68.47; H, 6.90; N, 6.39. Found: C, 68.24; H, 6.79; N, 6.61.

4.3.5. (3*S*)-*N*-(Boc-L-Leucinyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid methylester (3e)

Yield: 89%.Syrupy. ESI-MS (*m*/*e*) 405[M+H]$^+$; IR (cm^{-1}) 2970, 1734, 1648, 1497, 1457, 1395, 1385, 1375, 1365, 1198, 1111, 750, 599; $[\alpha]_D^{20}$ −16.3 (*c* 1.0, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ/ppm = 8.00 (d, *J* = 6.6 Hz, 1H), 7.02 (m, *J* = 8.2 Hz, 2H), 6.95 (d,

J = 8.8 Hz, 2H), 4.80 (d, J = 8.4 Hz, 1H), 4.51 (m, 3H), 3.63 (s, 3H), 3.10 (m, 2H), 1.78 (m, 3H), 1.41 (s, 9H), 1.01 (d, J = 7.4 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ/ppm = 171.6, 170.1, 155.8, 135.9, 134.2, 128.4, 127.2, 126.6, 79.5, 55.8, 52.0, 50.7, 44.6, 41.6, 28.4, 27.0, 22.3. Anal. Calcd for $C_{22}H_{32}N_2O_5$: C, 65.32; H, 7.97; N, 6.93. Found: C, 65.53; H, 7.84; N, 6.70.

4.3.6. (3*S*)-*N*-(Boc-L-Isoleucinyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid methylester (3f)

Yield: 89%. Syrupy. ESI-MS (m/e) 405 $[M+H]^+$; IR (cm^{-1}) 2969, 1734, 1648, 1497, 1457, 1395, 1380, 1365, 1198, 1111, 750, 599; $[\alpha]_D^{20}$ −13.4 (c 1.0, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ/ppm = 8.00 (d, J = 7.6 Hz, 1H), 7.02 (m, J = 8.2 Hz, 2H), 6.95 (d, J = 8.8 Hz, 2H), 4.80 (d, J = 5.4 Hz, 1H), 4.51 (m, 3H), 3.63 (s, 3H), 3.10 (m, 2H), 2.5 (m, 1H), 1.41 (s, 9H), 1.29 (m, 2H), 1.06 (d, J = 6.5 Hz, 3H), 0.96 (d, J = 8.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ/ppm = 171.6, 170.1, 155.8, 135.9, 134.2, 128.4, 127.2, 126.6, 79.5, 55.8, 52.0, 44.6, 28.4, 27.0, 24.3, 14.6, 10.9. Anal. Calcd for $C_{22}H_{32}N_2O_5$: C, 65.32; H, 7.97; N, 6.93. Found: C, 65.14; H, 7.85; N, 6.70.

4.3.7. (3*S*)-*N*-(Boc-L-Tryptophanyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid methylester (3g)

Yield: 84%. Syrupy. ESI-MS (m/e) 478 $[M+H]^+$; IR (cm^{-1}) 2968, 1734, 1648, 1497, 1457, 1395, 1380, 1365, 1198, 1111, 750, 599; $[\alpha]_D^{20}$ −6.38 (c 1.0, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ/ppm = 10.10 (s, 1H), 8.00 (d, J = 7.6 Hz, 1H), 7.18 (m, J = 6.4 Hz, 4H), 7.02 (m, J = 8.2 Hz, 2H), 6.95 (d, J = 8.8 Hz, 2H), 6.80 (d, J = 6.4 Hz, 1H), 4.90 (d, J = 8.0 Hz, 1H), 4.81 (d, J = 5.4 Hz, 1H), 4.51 (m, 2H), 3.63 (s, 3H), 3.10 (m, 4H), 1.41 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ/ppm = 171.6, 170.1, 155.8, 135.9, 134.2, 128.4, 127.2, 126.6, 122.9, 119.0, 110.9, 79.5, 55.8, 52.0, 31.5, 44.6, 28.4, 27.0. Anal. Calcd for $C_{27}H_{31}N_3O_5$: C, 67.91; H, 6.54; N, 8.80. Found: C, 67.72; H, 6.43; N, 8.61.

4.3.8. (3*S*)-*N*-(Boc-L-Serinyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid methylester (3h)

Yield: 80%. Syrupy. ESI-MS (m/e) 469 $[M+H]^+$; IR (cm^{-1}) 3411, 2969, 1734, 1648, 1497, 1457, 1395, 1365, 1198, 1111, 750, 599; $[\alpha]_D^{20}$ −7.61 (c 1.0, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ/ppm = 8.00 (d, J = 5.6 Hz, 1H), 7.02 (m, J = 8.2 Hz, 2H), 6.95 (d, J = 8.8 Hz, 2H), 4.83 (m, J = 8.2 Hz, 1H), 4.63 (s, 2H), 4.51 (m, 2H), 3.86 (m, 2H), 3.63 (s, 3H), 3.29 (d, J = 6.5 Hz, 1H), 3.05 (d, J = 8.6 Hz, 1H), 1.41 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ/ppm = 171.6, 170.1, 155.8, 137.2, 135.9, 134.2, 128.4, 127.2, 125.6, 79.5, 74.3, 70.2, 55.8, 52.0, 50.7, 44.6, 28.4, 27.0. Anal. Calcd for $C_{19}H_{26}N_2O_6$: C, 60.30; H, 6.93; N, 7.40. Found: C, 60.11; H, 6.81; N, 7.59.

4.3.9. (3*S*)-*N*-(Boc-L-Threoninyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid methylester (3i)

Yield: 89%. Syrupy, ESI-MS (m/e) 393 $[M+1]^+$; IR (cm^{-1}) 3411, 2969, 1734, 1648, 1497, 1457, 1395, 1380, 1365, 1198, 1111, 750; $[\alpha]_D^{20}$ −20.1 (c 1.0, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ/ppm = 8.00 (d, J = 6.6 Hz, 1H), 7.02 (m, J = 8.2 Hz, 2H), 6.95 (d, J = 8.8 Hz, 2H), 4.80 (t, J = 7.1 Hz, 1H), 4.61 (d, J = 8.2 Hz, 1H), 4.46 (m, 2H), 4.24 (m, 1H), 3.63 (s, 3H), 3.29 (d, J = 5.4 Hz, 1H), 3.05 (d, J = 6.4 Hz, 1H), 2.0 (s, 1H), 1.48 (s, 9H), 1.21 (d, J = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$)/ppm = 171.6, 170.1, 155.8, 135.9, 134.2, 128.4, 127.2, 126.6, 79.5, 67.8, 58.8, 55.0, 51.7, 44.6, 28.4, 27.0, 10.9. Anal. Calcd for $C_{20}H_{28}N_2O_6$: C, 61.21; H, 7.19; N, 7.14. Found: C, 61.40; H, 7.07; N, 7.36.

4.3.10. (3*S*)-*N*-(Boc-L-Tyrocinyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid methylester (3j)

Yield: 82%. Syrupy. ESI-MS (m/e) 545$[M+H]^+$; IR (cm^{-1}) 3411, 2969, 1734, 1648, 1497, 1457, 1395, 1380, 1365, 1198, 1111, 750, 599; $[\alpha]_D^{20}$ −6.86 (c 1.0, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ/ppm = 8.00 (d, J = 7.6 Hz, 1H), 7.02 (m, J = 8.2 Hz, 2H), 6.98 (d, J = 8.8 Hz, 2H), 6.95 (d, J = 8.8 Hz, 2H), 6.72 (d, J = 5.5 Hz, 2H), 5.20 (s, 1H), 4.92 (m, 1H), 4.80 (t, J = 6.4 Hz, 1H), 4.51 (d, J = 7.6 Hz, 1H), 4.41 (d, J = 5.4 Hz, 1H), 3.63 (d, J = 7.5 Hz, 3H), 3.29 (d, J = 6.8 Hz, 1H), 3.05 (d, J = 8.3 Hz, 1H), 2.92 (m, 2H), 1.41 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ/ppm = 171.6, 170.1, 157.9, 155.8, 141.2, 136.5, 134.9, 131.2, 129.0, 128.4, 127.2, 126.6, 114.2, 79.5, 70.9, 55.8, 52.9, 52.0, 44.6, 37.8, 28.4, 27.0. Anal. Calcd for $C_{25}H_{30}N_2O_6$: C, 66.06; H, 6.65; N, 6.16. Found: C, 66.27; H, 6.78; N, 6.38.

4.3.11. (3*S*)-*N*-(Boc-L-Prolinyl)-1,2,3,4-tetrahydroisoquinoline-3- carboxylic acid methylester (3k)

Yield: 92%. Syrupy. ESI-MS (m/e) 389$[M+H]^+$; IR (cm^{-1}) 3410, 2969, 1734, 1648, 1497, 1457, 1395, 1365, 1360, 1198, 1111, 750, 599; $[\alpha]_D^{20}$ −54.6 (c 1.0, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ/ppm = 7.02 (m, J = 8.2 Hz, 2H), 6.95 (d, J = 8.8 Hz, 2H), 4.80 (t, J = 8.4 Hz, 1H), 4.51 (m, 2H), 4.29 (t, J = 7.6 Hz, 1H), 3.63 (s, 3H), 3.20 (m, 4H), 1.71 (m, 2H), 1.60 (m, 2H), 1.41 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ/ppm = 171.6, 170.1, 155.8, 135.9, 134.2, 128.4, 127.2, 126.6, 79.5, 55.8, 52.0, 47.1, 44.6, 29.7, 28.4, 27.0, 22.1. Anal. Calcd for $C_{21}H_{28}N_2O_5$: C, 64.93; H, 7.27; N, 7.21. Found: C, 64.74; H, 7.15; N, 7.00.

4.3.12. (3*S*)-*N*-(Boc-L-Methioninyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid methylester (3l)

Yield: 87%. Syrupy. ESI-MS (m/e) 423$[M+H]^+$; IR (cm^{-1}) 3411, 2969, 1734, 1648, 1497, 1457, 1395, 1380, 1365, 1198, 1111, 750, 599; $[\alpha]_D^{20}$ −17.7 (c 1.0, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ/ppm = 8.00 (d, J = 7.6 Hz, 1H), 7.02 (m, J = 8.2 Hz, 2H), 6.95 (d, J = 8.8 Hz, 2H), 4.80 (t, J = 6.4 Hz, 1H), 4.51 (m, 3H), 3.63 (s, 3H), 3.29 (d, J = 5.3 Hz, 1H), 3.05 (d, J = 7.6 Hz, 1H), 2.44 (m, 2H), 2.16 (t, J = 6.6 Hz, 2H), 2.09 (s, 3H), 1.41 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ/ppm = 171.6, 170.1, 155.8, 135.9, 134.2, 128.4, 127.2, 126.6, 79.5, 55.8, 52.0, 51.6, 44.6, 31.9, 29.3, 28.4, 27.0, 17.4. Anal. Calcd for $C_{21}H_{30}N_2O_5S$: C, 59.69; H, 7.16; N, 6.63. Found: C, 59.49; H, 7.04; N, 6.84.

4.3.13. (3*S*)-*N*-(Boc-L-Asparaginyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid methylester (3m)

Yield: 55%. Syrupy. ESI-MS (m/e) 406$[M+1]^+$; IR (cm^{-1}) 3411, 2969, 1734, 1648, 1497, 1457, 1395, 1380, 1365, 1198, 1111, 750, 599; $[\alpha]_D^{20}$ −10.4 (c 1.0, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ/ppm = 8.00 (d, J = 5.6 Hz, 1H), 7.02 (m, J = 8.2 Hz, 2H), 6.95 (d, J = 8.8 Hz, 2H), 6.0 (s, 2H), 4.78 (m, 2H), 4.51 (d, J = 6.4 Hz, 1H), 4.41 (d, J = 5.4 Hz, 1H), 3.63 (s, 3H), 3.29 (d, J = 7.2 Hz, 1H), 3.05 (d, J = 8.6 Hz, 1H), 2.68 (m, 2H), 1.41 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ/ppm = 174.5, 171.6, 170.1, 155.8, 135.9, 134.2, 128.4, 127.2, 126.6, 79.5, 55.8, 52.0, 51.6, 44.6, 37.9, 28.4, 27.0. Anal. Calcd for $C_{20}H_{26}N_2O_7$: C, 59.10; H, 6.45; N, 6.89. Found: C, 59.31; H, 6.58; N, 6.69.

4.3.14. (3*S*)-*N*-(Boc-L-Glutaminyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid methylesters (3n)

Yield: 45%. Syrupy. ESI-MS (m/e) 420$[M+1]^+$; IR (cm^{-1}) 3411, 2969, 1734, 1648, 1497, 1457, 1395, 1380, 1365, 1198, 1111, 750, 599; $[\alpha]_D^{20}$ −14.7 (c 1.0, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ/ppm = 8.00 (d, J = 5.6 Hz, 1H), 7.02 (m, J = 8.2 Hz, 2H), 6.95 (d, J = 8.8 Hz, 2H), 4.78 (t, J = 6.4 Hz, 1H), 4.48 (m, 3H), 3.63 (s, 3H), 3.29 (d, J = 7.2 Hz, 1H), 3.05 (d, J = 6.6 Hz, 1H), 2.18 (t, J = 8.5 Hz, 2H), 2.07 (m, 2H), 1.41 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ/ppm = 174.5, 171.6, 170.1, 155.8, 135.9, 134.2, 128.4, 127.2, 126.6, 79.5, 55.8, 52.0, 51.6, 44.6, 32.6, 28.4, 27.6, 27.0. Anal. Calcd for $C_{21}H_{28}N_2O_7$: C, 59.99; H, 6.71; N, 6.66. Found: C, 59.78; H, 6.60; N, 6.85.

4.3.15. (3*S*)-*N*-(Boc-L-Histidinyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid methylesters (3o)

Yield: 65%. Syrupy. ESI-MS (*m*/*e*) 429[M+H]$^+$; IR (cm^{-1}) 3411, 2969, 1734, 1648, 1497, 1457, 1395, 1380, 1365, 1198, 1111, 750, 599; $[\alpha]_D^{20}$ −11.2 (*c* 1.0, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ/ppm = 13.40 (d, *J* = 7.4 Hz, 1H), 8.00 (s, 1H), 7.44 (s, 1H), 7.02 (m, *J* = 8.2 Hz, 2H), 6.95 (d, *J* = 8.8 Hz, 2H), 6.80 (d, *J* = 6.3 Hz, 1H), 4.92 (t, *J* = 7.3 Hz, 1H), 4.80 (t, *J* = 5.4 Hz, 1H), 4.51 (d, *J* = 6.2 Hz, 1H), 4.41 (d, *J* = 8.6 Hz, 1H), 3.63 (s, 3H), 3.17 (m, 1H), 2.92 (m, 1H), 1.41 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ/ppm = 171.6, 170.1, 155.8, 135.9, 134.2, 133.5, 128.4, 127.2, 126.6, 119.6, 79.5, 55.8, 52.0, 51.6, 44.6, 30.5, 28.4, 27.0. Anal. Calcd for $C_{22}H_{28}N_4O_5$: C, 61.67; H, 6.59; N, 13.08. Found: C, 61.85; H, 6.71; N, 13.26.

4.3.16. (3*S*)-*N*-(Boc-L-Lysinyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid methylesters (*3p*)

Yield: 67%. Syrupy. ESI-MS (*m*/*e*) 520[M+1]$^+$; IR (cm^{-1}) 3411, 2969, 1734, 1648, 1497, 1457, 1395, 1380, 1365, 1198, 1111, 750, 599; $[\alpha]_D^{20}$ −9.0 (*c* 1.0, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ/ppm = 8.00 (d, *J* = 7.6 Hz, 2H), 7.02 (m, *J* = 8.2 Hz, 2H), 6.95 (d, *J* = 8.8 Hz, 2H), 4.78 (t, *J* = 8.4 Hz, 1H), 4.52 (m, 2H), 4.41 (d, *J* = 6.4 Hz, 1H), 3.63 (s, 3H), 3.29 (d, *J* = 5.6 Hz, 1H), 3.05 (d, *J* = 8.5 Hz, 1H), 2.96 (m, 2H), 1.79 (m, 2H), 1.55 (m, 2H), 1.41 (s, 18 H), 1.29 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ/ppm = 171.6, 170.1, 155.8, 135.9, 134.2, 128.4, 127.2, 126.6, 79.5, 55.8, 52.0, 51.6, 44.6, 41.9, 31.9, 28.4, 27.0, 20.7. Anal. Calcd for $C_{22}H_{33}N_3O_5$: C, 62.99; H, 7.93; N, 10.02. Found: C, 62.78; H, 7.80; N, 10.22.

4.3.17. (3*S*)-*N*-(Boc-L-Aspartyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid methylester (3q)

Yield: 75%. Syrupy. ESI-MS (*m*/*e*)497[M+1]$^+$; IR (cm^{-1}) 3411, 2969, 1734, 1648, 1497, 1457, 1395, 1380, 1365, 1198, 1111, 750, 599; $[\alpha]_D^{20}$ −5.70 (*c* 1.0, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ/ppm = 8.00 (d, *J* = 6.6 Hz, 1H), 7.19 (s, 5H), 7.02 (m, *J* = 8.2 Hz, 2H), 6.95 (d, *J* = 8.8 Hz, 2H), 5.34 (s, 2H), 5.17 (t, *J* = 7.3 Hz, 1H), 4.81 (t, *J* = 8.4 Hz, 1H), 4.51 (d, *J* = 7.3 Hz, 1H), 4.41 (d, *J* = 5.6 Hz, 1H), 3.63 (s, 3H), 3.29 (d, *J* = 6.6 Hz, 1H), 3.05 (d, *J* = 8.6 Hz, 1H), 2.88 (m, 1H), 2.63 (m, 1H), 1.48 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ/ppm = 174.5, 171.6, 170.1, 155.8, 141.2, 135.9, 134.2, 129.4, 127.2, 126.6, 79.5, 68.5, 55.8, 52.0, 51.6, 49.7, 44.6, 37.9, 28.4, 27.0. Anal. Calcd for $C_{20}H_{27}N_3O_6$: C, 59.25; H, 6.71; N, 10.36. Found: C, 59.44; H, 6.83; N, 10.55.

4.3.18. (3*S*)-*N*-(Boc-L-Glutamoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid methylester (3r)

Yield: 78%. Syrupy. ESI-MS (*m*/*e*) 511[M+H]$^+$; IR (cm^{-1}) 3411, 2969, 1734, 1648, 1497, 1457, 1395, 1380, 1365, 1198, 1111, 750, 599; $[\alpha]_D^{20}$ −57.6 (*c* 1.0, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$)/ppm = 8.00 (d, *J* = 6.6 Hz, 1H), 7.19 (s, 5H), 7.02 (m, *J* = 8.2 Hz, 2H), 6.95 (d, *J* = 8.8 Hz, 2H), 5.34 (s, 2H), 4.81 (t, *J* = 8.4 Hz, 1H), 4.53 (m, 1H), 4.51 (d, *J* = 5.4 Hz, 1H), 4.41 (d, *J* = 8.5 Hz, 1H), 3.63 (s, 3H), 3.29 (d, *J* = 7.6 Hz, 1H), 3.05 (d, *J* = 6.5 Hz, 1H), 2.21 (m, 4H), 1.41 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ/ppm = 174.5, 171.6, 170.1, 155.8, 141.2, 135.9, 134.2, 129.4, 127.2, 126.6, 79.5, 68.5, 55.8, 52.0, 51.6, 44.6, 37.9, 28.4, 27.0. Anal. Calcd for $C_{21}H_{29}N_3O_6$: C, 60.13; H, 6.97; N, 10.02. Found: C, 60.34; H, 6.85; N, 10.23.

4.3.19. (3*S*)-*N*-(Boc-L-NO2^G-Argininyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid methylesters (3s)

Yield: 51%. Syrupy. ESI-MS (*m*/*e*) 494[M+H]$^+$; IR (cm^{-1}) 3411, 2969, 1734, 1648, 1497, 1457, 1395, 1380, 1365, 1198, 1111, 750, 599; $[\alpha]_D^{20}$ −2.55 (*c* 1.0, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ/ppm = 8.00 (d, *J* = 7.6 Hz, 2H), 7.02 (m, *J* = 8.2 Hz, 2H), 6.95 (d, *J* = 8.8 Hz, 2H), 4.84 (m, 1H), 4.53 (m, 1H), 4.51 (d, *J* = 7.4 Hz, 1H), 4.41 (d, *J* = 8.6 Hz, 1H), 3.63 (s, 3H), 3.27 (m, 1H), 3.04 (m, 1H), 2.65 (m, 2H), 2.0 (d, *J* = 8.5 Hz, 2H), 1.79 (m, 2H), 1.55 (m, 2H), 1.41 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ/ppm = 171.8, 170.1, 158.2, 155.8, 135.9, 134.2, 129.4, 127.2, 126.6, 79.5, 71.7, 55.8, 53.8, 51.2, 44.6, 37.1, 28.4, 27.0, 24.3. Anal. Calcd for $C_{22}H_{33}N_6O_7$: C, 53.54; H, 6.74; N, 17.03. Found: C, 53.35; H, 6.62; N, 16.85.

4.4. General procedure for preparing (3*S*)-*N*-(Boc-aminoacyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acids (4a–s)

At 0 °C to the solution of 0.362 g (1.0 mmol) of (3*S*)-*N*-(Boc-aminoacyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid methylester in 5 ml of methanol 7 ml of 2 N aqueous of NaOH to adjust pH 11. The reaction mixture was stirred at 0 °C for 3 h and TLC (CCl_3/CH_3OH, 5:1) indicated the complete disappearance of (3*S*)-*N*-(Boc-aminoacyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid methylester. The reaction mixture was adjusted to pH 7 with aqueous solution of $KHSO_4$. The solution was evaporated under vacuum to remove methanol, adjusted pH 2 with aqueous solution of $KHSO_4$ and extracted with ethyl acetate (3× 30 ml). The combined ethyl acetate was successively washed with saturated aqueous solution of NaCl (2× 20 ml) and dried with anhydrous Na_2SO_4. After filtration the filtrate was evaporated to provide the title compound.

4.4.1. (3*S*)-*N*-(Boc-L-Alaninyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (4a)

Yield: 99%. Colorless powder. ESI-MS (*m*/*e*) 347[M−H]$^-$; IR (cm^{-1}) 3411, 2969, 1734, 1648, 1497, 1457, 1395, 1380, 1365, 1198, 1111, 750, 599; $[\alpha]_D^{20}$ −26.7 (*c* 1.0, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ/ppm = 11.00 (s, 1H), 8.00 (d, *J* = 7.6 Hz, 1H), 7.02 (m, *J* = 8.2 Hz, 2H), 6.95 (d, *J* = 8.8 Hz, 2H), 4.83 (d, *J* = 5.4 Hz, 1H), 4.68 (m, 1H), 4.55 (d, *J* = 5.4 Hz, 1H), 4.49 (d, *J* = 7.6 Hz, 1H), 3.20 (d, *J* = 5.5 Hz, 1H), 2.90 (d, *J* = 8.5 Hz, 1H), 1.48 (d, *J* = 6.3 Hz, 3H), 1.41 (s, 9H). Anal. Calcd for $C_{18}H_{24}N_2O_5$: C, 62.05; H, 6.94; N, 8.04. Found: C, 62.24; H, 6.82; N, 8.25.

4.4.2. (3*S*)-*N*-(Boc-Glycinyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (4b)

Yield: 99%. Colorless powder. ESI-MS (*m*/*e*) 333[M−H]$^-$; IR (cm^{-1}) 3411, 2969, 1734, 1648, 1497, 1457, 1395, 1365, 1198, 1111, 750, 599; $[\alpha]_D^{20}$ −16.7 (*c* 1.0, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ/ppm = 11.00 (s, 1H), 8.00 (d, *J* = 7.6 Hz, 1H), 7.02 (m, *J* = 8.2 Hz, 2H), 6.95 (d, *J* = 8.8 Hz, 2H), 4.80 (d, *J* = 5.4 Hz, 1H), 4.51 (m, 2H), 3.85 (d, *J* = 6.2 Hz, 2H), 3.25 (m, 2H), 1.41(s, 9H). Anal. Calcd for $C_{17}H_{22}N_2O_5$: C, 61.07; H, 6.63; N, 8.38. Found: C, 61.28; H, 6.75; N, 8.57.

4.4.3. (3*S*)-*N*-(Boc-L-Valinyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (4c)

Yield: 93%. Colorless powder. ESI-MS (*m*/*e*) 375 [M−H]$^-$; IR (cm^{-1}) 3411, 2969, 1734, 1648, 1497, 1457, 1395, 1384, 1375, 1365, 1198, 1111, 750, 599; $[\alpha]_D^{20}$ −56.4 (*c* 1.0, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ/ppm = 11.00 (s, 1H), 8.00 (d, *J* = 6.6 Hz, 1H), 7.02 (m, *J* = 8.2 Hz, 2H), 6.95 (d, *J* = 8.8 Hz, 2H), 4.80 (d, *J* = 7.4 Hz, 1H), 4.61 (d, *J* = 5.3 Hz, 1H), 4.51 (m, 2H), 4.24 (m, 1H), 3.25 (m, 2H), 2.0 (s, 1H), 1.41 (s, 9H), 1.21 (d, *J* = 8.6 Hz, 3H). Anal. Calcd for $C_{20}H_{28}N_2O_5$: C, 63.81; H, 7.50; N, 7.44. Found: C, 63.62; H, 7.39; N, 7.25.

4.4.4. (3*S*)-*N*-(Boc-L-Phenylalaninyl)-1,2,3,4- tetrahydroisoquinoline-3-carboxylic acid (4d)

Yield: 96%. Colorless powder. ESI-MS (*m*/*e*) 423 [M−H]$^-$; IR(cm^{-1}) 3411, 2969, 1734, 1600, 1648, 1497, 1457, 1395,

1365, 1198, 1111, 750, 599; $[\alpha]_D^{20}$ −53.6 (*c* 1.0, CH_3OH); ^1H NMR (300 MHz, $CDCl_3$) δ/ppm = 11.00 (s, 1H), 8.00 (d, *J* = 7.6 Hz, 1H), 7.12 (m, *J* = 8.2 Hz, 2H), 7.02 (m, *J* = 8.2 Hz, 2H), 6.95 (d, *J* = 8.8 Hz, 2H), 6.80 (d, *J* = 8.8 Hz, 3H), 4.92 (d, *J* = 5.5 Hz, 1H), 4.80 (d, *J* = 6.4 Hz, 1H), 4.51 (d, *J* = 8.6 Hz, 1H), 4.41 (d, *J* = 5.6 Hz, 1H), 3.10 (m, 4H), 1.41 (s, 9H). Anal. Calcd for $C_{24}H_{28}N_2O_5$: C, 67.91; H, 6.65; N, 6.60. Found: C, 67.70; H, 6.52; N, 6.39.

4.4.5. (3*S*)-*N*-(Boc-L-Leucinyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (4e)

Yield: quantitative. Colorless powder. ESI-MS (*m*/*e*) 389[M−H]$^-$; IR (cm^{-1}) 3411, 2969, 1734, 1648, 1497, 1457, 1395, 1375, 1365, 1198, 1111, 750, 599; $[\alpha]_D^{20}$ −24.5 (*c* 1.0, CH_3OH); ^1H NMR (300 MHz, $CDCl_3$) δ/ppm = 11.00 (s, 1H), 8.00 (d, *J* = 8.6 Hz, 1H), 7.02 (m, *J* = 8.2 Hz, 2H), 6.95 (d, *J* = 8.8 Hz, 2H), 4.80 (d, *J* = 7.4 Hz, 1H), 4.51 (m, 3H), 3.10 (m, 2H), 1.78 (m, 3H), 1.41 (s, 9H), 1.01 (d, *J* = 7.6 Hz, 6H). Anal. Calcd for $C_{21}H_{30}N_2O_5$: C, 64.59; H, 7.74; N, 7.17. Found: C, 64.80; H, 7.86; N, 7.40.

4.4.6. (3*S*)-*N*-(Boc-L-Isoleucinyl)-1,2,3,4- tetrahydroisoquinol ine-3-carboxylic acid (4f)

Yield: quantitative. Colorless powder. ESI-MS (*m*/*e*) 389 [M−H]$^-$; IR (cm^{-1}) 3411, 2969, 1734, 1648, 1497, 1457, 1395, 1380, 1365, 1198, 1111, 750, 599; $[\alpha]_D^{20}$ −10.6 (*c* 1.0, CH_3OH); ^1H NMR (300 MHz, $CHCl_3$) δ/ppm = 11.00 (s, 1H), 8.00 (d, *J* = 5.6 Hz, 1H), 7.02 (m, *J* = 8.2 Hz, 2H), 6.95 (d, *J* = 8.8 Hz, 2H), 4.80 (d, *J* = 6.4 Hz, 1H), 4.51 (m, 3H), 3.10 (m, 2H), 2.5 (m, 1H), 1.41 (s, 9H), 1.29 (m, 2H), 1.06 (d, *J* = 7.6 Hz, 3H), 0.96 (d, *J* = 8.5 Hz, 3H). Anal. Calcd for $C_{21}H_{30}N_2O_5$: C, 64.59; H, 7.74; N, 7.17. Found: C, 64.80; H, 7.86; N, 7.40.

4.4.7. (3*S*)-*N*-(Boc-L-Tryptophanyl)-1,2,3,4- tetrahydroisoquinoli ne-3-carboxylic acid (4g)

Yield: 88%. Colorless powder. ESI-MS (*m*/*e*) 462 [M−H]$^-$; IR (cm^{-1}) 3411, 2969, 1734, 1648, 1497, 1457, 1395, 1365, 1198, 1111, 750, 599; $[\alpha]_D^{20}$ −66.5 (*c* 1.0, CH_3OH); ^1H NMR (300 MHz, $CDCl_3$) δ/ppm = 11.00 (s, 1H), 10.10 (s, 1H), 8.00 (d, *J* = 5.6 Hz, 1H), 7.18 (m, *J* = 6.4 Hz, 4H), 7.02 (m, *J* = 8.2 Hz, 2H), 6.95 (d, *J* = 8.8 Hz, 2H), 6.80 (d, *J* = 6.4 Hz, 1H), 4.90 (d, *J* = 7.1 Hz, 1H), 4.81 (d, *J* = 7.4 Hz, 1H), 4.51 (m, 2H), 3.10 (m, 4H), 1.41(s, 9H). Anal. Calcd for $C_{26}H_{29}N_3O_5$: C, 67.37; H, 6.31; N, 9.07. Found: C, 67.55; H, 6.43; N, 9.28.

4.4.8. (3*S*)-*N*-(Boc-L-Serinyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (4h)

Yield: 93%. Colorless powder. ESI-MS (*m*/*e*) 453 [M−H]$^-$; IR (cm^{-1}) 3411, 2969, 1734, 1648, 1497, 1457, 1395, 1365, 1198, 1111, 750, 599; $[\alpha]_D^{20}$ −57.1 (*c* 1.0, CH_3OH); ^1H NMR (300 MHz, $CDCl_3$) δ/ppm = 11.00 (s, 1H), 8.00 (d, *J* = 5.6 Hz, 1H), 7.02 (m, *J* = 8.2 Hz, 2H), 6.95 (d, *J* = 8.8 Hz, 2H), 4.83 (m, 1H), 4.63 (s, 2H), 4.51 (m, 2H), 3.86 (m, 2H), 3.29 (d, *J* = 8.2 Hz, 1H), 3.05 (d, *J* = 6.4 Hz, 1H), 1.41 (s, 9H). Anal. Calcd for $C_{18}H_{24}N_2O_6$: C, 59.33; H, 6.64; N, 7.69. Found: C, 59.14; H, 6.52; N, 7.90.

4.4.9. (3*S*)-*N*-(Boc-L-Threoninyl)-1,2,3,4-tetrahydroisoquin oline-3-carboxylic acid (4i)

Yield: 82%. Colorless powder. ESI-MS (*m*/*e*) 377 [M−H]$^-$; IR (cm^{-1}) 3411, 2969, 1734, 1648, 1497, 1457, 1395, 1365, 1198, 1111, 750, 599; $[\alpha]_D^{20}$ −15.4 (*c* 1.0, CH_3OH); ^1H NMR (300 MHz, $CDCl_3$) δ/ppm = 11.00 (s, 1H), 8.00 (d, *J* = 6.6 Hz, 1H), 7.02 (m, *J* = 8.2 Hz, 2H), 6.95 (d, *J* = 8.8 Hz, 2H), 4.80 (t, *J* = 7.6 Hz, 1H), 4.61 (d, 1H), 4.46 (m, 2H), 4.24 (m, 1H), 3.29 (d, *J* = 8.6 Hz, 1H), 3.05 (d, *J* = 5.5 Hz, 1H), 2.0 (s, 1H), 1.41 (s, 9H), 1.21 (d, *J* = 7.6 Hz, 3H). Anal. Calcd for $C_{19}H_{26}N_2O_6$: C, 60.30; H, 6.93; N, 7.40. Found: C, 60.13; H, 6.81; N, 7.60.

4.4.10. (3*S*)-*N*-(Boc-L-Tyrocinyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (4j)

Yield: 93%. Colorless powder. ESI-MS (*m*/*e*) 439[M−1]$^-$; IR (cm^{-1}) 3411, 2969, 1734, 1648, 1497, 1457, 1395, 1365, 1198, 1111, 750, 599; $[\alpha]_D^{20}$ −35.8 (*c* 1.0, CH_3OH); ^1H NMR (300 MHz, $CDCl_3$) δ/ppm = 11.00 (s, 1H), 8.00 (d, *J* = 8.6 Hz, 1H), 7.02 (m, *J* = 8.2 Hz, 2H), 6.98 (d, *J* = 8.8 Hz, 2H), 6.95 (d, *J* = 8.8 Hz, 2H), 6.72 (d, *J* = 5.5 Hz, 2H), 4.92 (m, 3H), 4.51 (d, *J* = 6.6 Hz, 1H), 4.41 (d, *J* = 8.6 Hz, 1H), 3.29 (d, *J* = 5.3 Hz, 1H), 3.05 (d, *J* = 6.5 Hz, 1H), 2.92 (m, 2H), 1.41(s, 9H). Anal. Calcd for $C_{24}H_{28}N_2O_6$: C, 65.44; H, 6.41; N, 6.36. Found: C, 65.65; H, 6.54; N, 6.59.

4.4.11. (3*S*)-*N*-(Boc-L-Prolinyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (4k)

Yield: 87%. Colorless powder. ESI-MS (*m*/*e*) 373[M−1]$^{-1}$; IR (cm^{-1}) 3411, 2969, 1734, 1648, 1497, 1457, 1395, 1365, 1198, 1111, 750, 599; $[\alpha]_D^{20}$ −87.8 (*c* 1.0, CH_3OH); ^1H NMR (300 MHz, $CDCl_3$) δ/ppm = 11.00 (s, 1H), 7.02 (m, *J* = 8.2 Hz, 2H), 6.95 (d, *J* = 8.8 Hz, 2H), 4.80 (t, *J* = 7.4 Hz, 1H), 4.51 (m, 2H), 4.29 (t, *J* = 8.3 Hz, 1H), 3.20 (m, 4H), 1.71 (m, 2H), 1.60 (m, 2H), 1.41 (s, 9H). Anal. Calcd for $C_{20}H_{26}N_2O_5$: C, 64.15; H, 7.00; N, 7.48. Found: C, 64.38; H, 7.12; N, 7.29.

4.4.12. (3*S*)-*N*-(Boc-L-Methioninyl)-1,2,3,4- tetrahydroisoquino line-3-carboxylic acid (4l)

Yield: 87%. Colorless powder. ESI-MS (*m*/*e*) 407[M−1]$^-$; IR (cm^{-1}) 3411, 2969, 1734, 1648, 1497, 1457, 1395, 1380, 1365, 1198, 1111, 750, 599; $[\alpha]_D^{20}$ −38.6 (*c* 1.0, CH_3OH); ^1H NMR (300 MHz, $CDCl_3$) δ/ppm = 11.00 (s, 1H), 8.00 (d, *J* = 7.6 Hz, 1H), 7.02 (m, *J* = 8.2 Hz, 2H), 6.95 (d, *J* = 8.8 Hz, 2H), 4.80 (t, *J* = 8.4 Hz, 1H), 4.51 (m, 3H), 3.29 (d, *J* = 5.4 Hz, 1H), 3.05 (d, *J* = 7.7 Hz, 1H), 2.44 (m, 2H), 2.16 (t, *J* = 6.4 Hz, 2H), 2.09 (s, 3H), 1.41 (s, 9H). Anal. Calcd for $C_{20}H_{28}N_2O_5S$: C, 58.80; H, 6.91; N, 6.86. Found: C, 58.62; H, 6.80; N, 6.67.

4.4.13. (3*S*)-*N*-(Boc-L-Asparaginyl)-1,2,3,4- tetrahydroisoquino line-3-carboxylic acid (4m)

Yield: 78%. Colorless powder. ESI-MS(*m*/*e*) 390 [M−H]$^-$; IR (cm^{-1}) 3411, 2969, 1734, 1648, 1497, 1457, 1395, 1365, 1198, 1111, 750, 599; $[\alpha]_D^{20}$ −21.6 (*c* 1.0, CH_3OH); ^1H NMR (300 MHz, $CDCl_3$) δ/ppm = 11.00 (s, 1H), 8.00 (d, *J* = 8.6 Hz, 1H), 7.02 (m, *J* = 8.2 Hz, 2H), 6.95 (d, *J* = 8.8 Hz, 2H), 6.0 (s, 2H), 4.78(m, 2H), 4.51 (d, *J* = 7.0 Hz, 1H), 4.41 (d, *J* = 6.5 Hz, 1H), 3.29 (d, *J* = 5.2 Hz, 1H), 3.05 (d, *J* = 7.6 Hz, 1H), 2.68 (m, 2H), 1.41 (s, 9H). Anal. Calcd for $C_{19}H_{24}N_2O_7$: C, 58.16; H, 6.16; N, 7.14. Found: C, 58.00; H, 6.04; N, 7.32.

4.4.14. (3*S*)-*N*-(Boc-L-Glutaminyl)-1,2,3,4-tetrahydroisoquino line-3-carboxylic acid (4n)

Yield: 74%. Colorless powder. ESI-MS (*m*/*e*) 404[M−1]$^-$; IR (cm^{-1}) 3411, 2969, 1734, 1648, 1497, 1457, 1395, 1365, 1198, 1111, 750, 599; $[\alpha]_D^{20}$ −34.1 (*c* 1.0, CH_3OH); ^1H NMR (300 MHz, $CDCl_3$) δ/ppm = 11.00 (s, 1H), 8.00 (d, *J* = 5.6 Hz, 1H), 7.02 (m, *J* = 8.2 Hz, 2H), 6.95 (d, *J* = 8.8 Hz, 2H), 6.0 (s, 2H), 4.78 (t, *J* = 6.4 Hz, 1H), 4.48 (m, 3H), 3.29 (d, *J* = 8.5 Hz, 1H), 3.05 (d, *J* = 7.6 Hz, 1H), 2.18 (t, *J* = 7.1 Hz, 2H), 2.07 (m, 2H), 1.41 (s, 9H). Anal. Calcd for $C_{20}H_{26}N_2O_7$: C, 59.10; H, 6.45; N, 6.89. Found: C, 58.92; H, 6.33; N, 6.67.

4.4.15. (3*S*)-*N*-(Boc-L-Histidinyl)-1,2,3,4-tetrahydroisoquino line-3-carboxylic acid (4o)

Yield: 48%. Colorless powder. ESI-MS (*m*/*e*) 413[M−H]$^-$; IR (cm^{-1}) 3411, 2969, 1734, 1648, 1497, 1457, 1395, 1365, 1198, 1111, 750, 599; $[\alpha]_D^{20}$ −17.6 (*c* 1.0, CH_3OH); ^1H NMR (300 MHz, $CDCl_3$) δ/ppm = 13.40 (d, *J* = 5.6 Hz, 1H), 11.00 (s, 1H), 8.00 (s,

1H), 7.44 (s, 1H), 7.02 (m, J = 8.2 Hz, 2H), 6.95 (d, J = 8.8 Hz, 2H), 6.80 (d, J = 7.6 Hz, 1H), 4.92 (t, J = 8.5 Hz, 1H), 4.80 (t, J = 6.4 Hz, 1H), 4.51 (d, J = 7.1 Hz, 1H), 4.41 (d, J = 5.2 Hz, 1H), 3.17 (m, 1H), 2.92 (m, 1H), 1.41(s, 9H). Anal. Calcd for $C_{21}H_{26}N_4O_5$: C, 60.86; H, 6.32; N, 13.52. Found: C, 61.07; H, 6.44; N, 13.71.

4.4.16. (3*S*)-*N*-(Boc-L-Lysinyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (4p)

Yield: 95%. Colorless powder. ESI-MS (m/e) 504[M−H]$^-$; IR (cm^{-1}) 3411, 2969, 1734, 1648, 1497, 1457, 1395, 1365, 1198, 1111, 750, 599; $[\alpha]_D^{20}$ −16.6 (c 1.0, CH_3OH); ^1H NMR (300 MHz, $CDCl_3$) δ/ppm = 11.00 (s, 1H), 8.00 (d, J = 6.6 Hz, 2H), 7.02 (m, J = 8.2 Hz, 2H), 6.95 (d, J = 8.8 Hz, 2H), 4.78 (t, J = 7.4 Hz, 1H), 4.52 (m, 2H), 4.41 (d, J = 7.1 Hz, 1H), 3.29 (d, J = 6.1 Hz, 1H), 3.05 (d, J = 5.2 Hz, 1H), 2.96 (m, 2H), 1.79 (m, 2H), 1.55 (m, 2H), 1.41 (s, 18 H), 1.29 (m, 2H). Anal. Calcd for $C_{21}H_{31}N_3O_5$: C, 62.20; H, 7.71; N, 10.36. Found: C, 62.01; H, 7.60; N, 10.55.

4.4.17. (3*S*)-*N*-(Boc-L-Aspartyl)-1,2,3,4-tetrahydroisoquinoline-3- carboxylic acid (4q)

Yield: 65%. Colorless powder. ESI-MS (m/e) 391[M−H]$^-$; IR(cm^{-1}) 3411, 2969, 1734, 1648, 1497, 1457, 1395, 1365, 1198, 1111, 750, 599; $[\alpha]_D^{20}$ −25.7 (c 1.0, CH_3OH); ^1H NMR (300 MHz, $CDCl_3$) δ/ppm = 11.00 (s, 2H), 8.00 (d, J = 5.6 Hz, 1H), 7.02 (m, J = 8.2 Hz, 2H), 6.95 (d, J = 8.8 Hz, 2H), 4.85 (m, 2H), 4.51 (d, J = 7.1 Hz, 1H), 4.41 (d, J = 8.0 Hz, 1H), 3.16 (d, J = 8.4 Hz, 1H), 2.88 (m, 2H), 2.63 (d, J = 5.6 Hz, 1H), 1.41 (s, 9H). Anal. Calcd for $C_{19}H_{25}N_3O_6$: C, 58.30; H, 6.44; N, 10.74. Found: C, 58.50; H, 6.55; N, 10.52.

4.4.18. (3*S*)-*N*-(Boc-L-Glutamoyl)-1,2,3,4-tetrahydroisoquino line-3-carboxylic acid (4r)

Yield: 61%. Colorless powder. ESI-MS (m/e) 405[M−H]$^-$; IR (cm^{-1}) 3411, 2969, 1734, 1648, 1497, 1457, 1395, 1365, 1198, 1111, 750, 599; $[\alpha]_D^{20}$ −86.0 (c 1.0, CH_3OH); ^1H NMR (300 MHz, $CDCl_3$) δ/ppm = 11.00 (s, 2H), 8.00 (d, J = 6.6 Hz, 1H), 7.02 (m, J = 8.2 Hz, 2H), 6.95 (d, J = 8.8 Hz, 2H), 4.85 (t, J = 5.4 Hz, 1H), 4.53 (m, 1H), 4.51 (d, J = 7.3 Hz, 1H), 4.41 (d, J = 8.1 Hz, 1H), 3.16 (d, J = 6.4 Hz, 1H), 2.91 (d, J = 5.6 Hz, 1H), 2.11 (m, 4H), 1.41 (s, 9H). Anal. Calcd for $C_{20}H_{27}N_3O_6$: C, 59.25; H, 6.71; N, 10.36. Found: C, 59.06; H, 6.60; N, 10.58.

4.4.19. (3*S*)-*N*-(Boc-L-NO_2^G-Argininyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (4s)

Yield: 86%. Colorless powder. ESI-MS (m/e) 478[M−H]$^-$; IR (cm^{-1}) 3411, 2969, 1734, 1648, 1497, 1457, 1395, 1365, 1198, 1111, 750, 599; $[\alpha]_D^{20}$ −8.8 (c 1.0, CH_3OH); ^1H NMR (300 MHz, $CDCl_3$) δ/ppm = 11.00 (s, 1H), 8.00 (d, J = 6.6 Hz, 2H), 7.02 (m, J = 8.2 Hz, 2H), 6.95 (d, J = 8.8 Hz, 2H), 4.84 (m, 1H), 4.53 (m, 1H), 4.51 (d, J = 8.4 Hz, 1H), 4.41 (d, J = 5.3 Hz, 1H), 3.27 (m, 1H), 3.04 (m, 1H), 2.65 (m, 2H), 2.0 (d, J = 7.6 Hz, 2H), 1.79 (m, 2H), 1.55 (m, 2H), 1.41 (s, 9H). Anal. Calcd for $C_{21}H_{31}N_6O_7$: C, 52.60; H, 6.52; N, 17.53. Found: C, 52.41; H, 6.40; N, 17.75.

4.4.20. (3*S*)-*N*-(Boc-L-Argininyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (4′s)

The solution of 0.400 g (0.84 mmol) of (3*S*)-*N*-(Boc-L-NO_2^G-argininyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (**4s**) in 10 ml of anhydrous ethanol 300 mg of Pt/C (5%) was added. To the suspension hydrogen gas was bubbled for 140 h and TLC(CCl_3/CH_3OH, 5:1) indicated the complete disappearance of **4s**. After filtration the filtrate was evaporated under vacuum to provide 0.256 g (75%) of title compound as a syrupy. ESI-MS (m/e) 432[M−H]$^-$; IR (cm^{-1}) 3411, 2969, 1734, 1648, 1497, 1457, 1395, 1365, 1198, 1111, 750, 599; $[\alpha]_D^{20}$ −9.0 (c 1.0, CH_3OH); ^1H NMR (300 MHz, $CDCl_3$) δ/ppm = 11.00 (s, 1H), 8.00 (d, J = 5.6 Hz, 2H), 7.02 (m, J = 8.2 Hz, 2H), 6.95 (d, J = 8.8 Hz, 2H), 4.84 (m, 1H), 4.53 (m, 1H), 4.51 (d, J = 7.4 Hz, 1H), 4.41 (d, J = 8.2 Hz, 1H), 3.27 (m, 1H), 3.04 (m, 1H), 2.65 (m, 2H), 2.0 (d, J = 6.2 Hz, 2H), 1.79 (m, 2H), 1.55 (m, 2H), 1.41 (s, 9H). Anal. Calcd for $C_{21}H_{31}N_5O_5$: C, 58.18; H, 7.21; N, 16.16. Found: C, 58.00; H, 7.10; N, 16.36.

4.5. IV-2-5 General procedure for preparing (3*S*)-*N*-aminoacyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acids (5a–s)

At 0 °C to the solution of 0.86 mmol of (3*S*)-*N*-(Boc-aminoacyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid in 2 ml of ethyl acetate 6 ml of 4 N solution of hydrogen chloride in ethyl acetate was added. The reaction mixture was stirred at room temperature for 4 h and TLC (CCl_3/CH_3OH, 5:1) indicated the complete disappearance of (3*S*)-*N*-(Boc-aminoacyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid. The reaction mixture was evaporated under vacuum to dry and the residue was dissolved in 5 ml of ethyl acetate. The solution was evaporated under vacuum to dry and the residue was dissolved in 5 ml of ethyl acetate. This procedure was repeated for three times to provide the title compound as colorless powder.

4.5.1. (3*S*)-*N*-(L-Alaninyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (5a)

Yield: 99%. Colorless powder. Mp 125–126 °C. ESI-MS (m/e) 247[M−H]$^-$; IR (cm^{-1}) 3411, 2969, 1734, 1648, 1497, 1457, 1380, 1198, 1111, 750, 599; $[\alpha]_D^{20}$ − 78.5 (c = 1.0, H_2O); ^1H NMR (300 MHz, $CDCl_3$) δ/ppm = 11.00 (s, 1H), 7.02 (m, J = 8.2 Hz, 2H), 6.95 (d, J = 8.8 Hz, 2H), 4.83 (d, J = 7.4 Hz, 1H), 4.51 (d, J = 8.4 Hz, 1H), 4.43 (d, J = 6.3 Hz, 1H), 3.74 (m, 1H), 3.16 (d, J = 5.3 Hz, 1H), 2.91 (d, J = 5.6 Hz, 1H), 2.0 (s, 2H), 1.28 (d, J = 5.6 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ/ppm = 172.9, 171.6, 136.1, 134.7, 127.2, 126.3, 125.8, 58.3, 46.9, 44.6, 26.7, 20.8. Anal. Calcd for $C_{13}H_{16}N_2O_3$: C, 62.89; H, 6.50; N, 11.28. Found: C, 62.70; H, 6.38; N, 11.50.

4.5.2. (3*S*)-*N*-Glycinyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (5b)

Yield: 94%.Colorless powder. Mp 183–184 °C. ESI-MS (m/e) 233 [M−H]$^-$; IR (cm^{-1}) 3411, 2969, 1734, 1648, 1497, 1457, 1375, 1198, 1111, 750, 599; $[\alpha]_D^{20}$ −20.3 (c = 1.0, H_2O); ^1H NMR (300 MHz, $CDCl_3$) δ/ppm = 11.02 (s, 1H), 7.02 (m, J = 8.2 Hz, 2H), 6.95 (d, J = 8.8 Hz, 2H), 4.80 (d, J = 6.4 Hz, 1H), 4.51 (m, 2H), 3.54 (d, J = 8.3 Hz, 2H), 3.16 (d, J = 7.1 Hz, 1H), 2.91 (d, J = 5.6 Hz, 1H), 2.0 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ/ppm = 171.6, 168.6, 135.9, 132.9, 128.4, 127.2, 126.6, 57.8, 52.0, 43.6, 41.5, 27.0. Anal. Calcd for $C_{12}H_{14}N_2O_3$: C, 61.53; H, 6.02; N, 11.96. Found: C, 61.33; H, 5.90; N, 11.77.

4.5.3. (3*S*)-*N*-(L-Valinyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (5c)

Yield: 85%. Colorless powder. Mp 117–121 °C. ESI-MS (m/e) 275 [M−H]$^-$; IR (cm^{-1}) 3411, 2969, 1734, 1648, 1497, 1457, 1385, 1375, 1198, 1111, 750, 599; $[\alpha]_D^{20}$ −26.7 (c = 1.0, H_2O); ^1H NMR (300 MHz, $CDCl_3$) δ/ppm = 11.01 (s, 1H), 7.02 (m, J = 8.2 Hz, 2H), 6.95 (d, J = 8.8 Hz, 2H), 4.80 (d, J = 6.4 Hz, 1H), 4.51 (m, 2H), 3.53 (m, 1H), 3.08 (m, 2H), 2.29 (m, 1H), 2.0 (s, 2H), 1.21 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ/ppm = 171.6, 168.6, 135.9, 133.9, 128.4, 127.2, 126.6, 58.4, 55.8, 44.6, 34.4, 27.0, 17.9. Anal. Calcd for $C_{15}H_{20}N_2O_3$: C, 65.20; H, 7.30; N, 10.14. Found: C, 65.38; H, 7.42; N, 10.36.

4.5.4. (3*S*)-*N*-(L-Phenylalaninyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (5d)

Yield: 92%. Colorless powder. Mp 121–123 °C. ESI-MS (m/e) 323 [M−H]$^-$; IR (cm^{-1}) 3464, 2948, 2769, 2685, 2643, 2498, 1746,

1574; $[\alpha]_D^{20}$ −16.8 (*c* 1.0, H_2O); ^1H NMR (300 MHz, $CDCl_3$) δ/ppm = 11.00 (s, 1H), 7.12 (m, *J* = 8.2 Hz, 2H), 7.02 (m, *J* = 8.2 Hz, 2H), 6.95 (d, *J* = 8.8 Hz, 2H), 6.80 (d, *J* = 8.8 Hz, 3H), 4.85 (d, *J* = 7.4 Hz, 1H), 4.51 (d, *J* = 8.2 Hz, 1H), 4.41 (d, *J* = 7.6 Hz, 1H), 3.95 (m, 1H), 3.16 (d, *J* = 5.6 Hz, 1H), 2.92 (m, 3H), 2.0 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ/ppm = 171.6, 170.1, 139.5, 135.9, 134.2, 128.4, 127.2, 126.6, 57.8, 52.0, 44.6, 40.8, 27.0. Anal. Calcd for $C_{19}H_{20}N_2O_3$: C, 70.35; H, 6.21; N, 8.64. Found: C, 70.26; H, 6.11; N, 8.85.

4.5.5. (3*S*)-*N*-(L-Leucinyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (5e)

Yield: 89%. Colorless powder. Mp 136–137 °C. ESI-MS (*m*/*e*) 289 $[M-H]^-$; IR (cm^{-1}) 3464, 2948, 2769, 2685, 2643, 2498, 1746, 1574, 1395, 1365, 1119, 700, 599; $[\alpha]_D^{20}$ −28.3 (*c* 1.0, H_2O); ^1H NMR (300 MHz, $CDCl_3$) δ/ppm = 11.00 (s, 1H), 7.02 (m, *J* = 8.2 Hz, 2H), 6.95 (d, *J* = 8.8 Hz, 2H), 4.85 (d, *J* = 7.4 Hz, 1H), 4.51 (d, *J* = 8.1 Hz, 1H), 4.41 (d, *J* = 7.0 Hz, 1H), 3.58 (m, 1H), 3.10 (m, 2H), 2.0 (s, 2H), 1.78 (m, 3H), 1.01 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ/ppm = 171.6, 170.1, 135.9, 134.2, 128.4, 127.2, 126.6, 58.3, 50.7, 44.6, 28.4, 27.0, 22.3. Anal. Calcd for $C_{16}H_{22}N_2O_3$: C, 66.18; H, 7.64; N, 9.65. Found: C, 66.00; H, 7.52; N, 9.88.

4.5.6. (3*S*)-*N*-(L-Isoleucinyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (5f)

Yield: 89%. Colorless powder. Mp 126–127 °C. ESI-MS (*m*/*e*) 289 $[M-H]^-$; IR (cm^{-1}) 3414, 2967, 1736, 1648, 1497, 1457, 1378, 1200, 749; $[\alpha]_D^{20}$ −4.6 (*c* 1.0, H_2O); ^1H NMR (300 MHz, $CDCl_3$) δ/ppm = 11.00 (s, 1H), 7.02 (m, *J* = 8.2 Hz, 2H), 6.95 (d, *J* = 8.8 Hz, 2H), 4.80 (m, 1H), 4.51 (m, 2H), 3.53 (d, *J* = 5.2 Hz, 1H), 3.10 (m, 2H), 2.05 (m, 3H), 1.29 (m, 2H), 1.06 (d, *J* = 7.6 Hz, 3H), 0.96 (d, *J* = 8.6 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ/ppm = 171.6, 170.1, 135.9, 134.2, 128.4, 127.2, 126.6, 58.8, 55.0, 44.6, 39.0, 26.4, 24.3, 14.6, 10.9. Anal. Calcd for $C_{16}H_{22}N_2O_3$: C, 66.18; H, 7.64; N, 9.65. Found: C, 66.37; H, 7.77; N, 9.43.

4.5.7. (3*S*)-*N*-(L-Tryptophanyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (5g)

Yield: 91%. Colorless powder. Mp 134–138 °C. ESI-MS (*m*/*e*) 362 $[M-H]^-$; IR (cm^{-1}) 3411, 2969, 1734, 1648, 1497, 1457, 1375, 1198, 1111, 750, 599; $[\alpha]_D^{20}$ −86.2 (*c* 1.0, H_2O); ^1H NMR (300 MHz, $CDCl_3$) δ/ppm = 11.01 (s, 1H), 10.10 (s, 1H), 7.18 (m, *J* = 6.4 Hz, 4H), 7.02 (m, *J* = 8.2 Hz, 2H), 6.95 (d, *J* = 8.8 Hz, 2H), 6.80 (d, *J* = 6.4 Hz, 1H), 4.85 (d, *J* = 7.6 Hz, 1H), 4.51 (m, 2H), 3.95 (m, 1H), 2.9 (m, 4H), 2.0 (d, *J* = 8.6 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ/ppm = 171.6, 170.1, 135.9, 134.2, 128.4, 127.2, 126.6, 122.9, 119.0, 110.9, 57.8, 53.1, 44.6, 34.2, 27.0. Anal. Calcd for $C_{21}H_{23}N_3O_4$: C, 66.13; H, 6.08; N, 11.02. Found: C, 66.34; H, 6.20; N, 11.25.

4.5.8. (3*S*)-*N*-(L-Serinyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (5h)

Yield: 94%. Colorless powder. Mp 111–113 °C. ESI-MS (*m*/*e*) 263 $[M-H]^-$; IR (cm^{-1}) 3411, 2969, 1734, 1648, 1497, 1457, 1375, 1198, 1111, 750, 599; $[\alpha]_D^{20}$ −86.1 (*c* = 1.0, H_2O); ^1H NMR (300 MHz, $CDCl_3$) δ/ppm = 11.00 (s, 1H), 7.02 (m, *J* = 8.2 Hz, 2H), 6.95 (d, *J* = 8.8 Hz, 2H), 4.83 (m, 2H), 4.51 (m, 2H), 3.86 (m, 2H), 3.65 (m, 1H), 3.19 (d, *J* = 6.2 Hz, 1H), 2.92 (d, *J* = 8.5 Hz, 1H), 2.0 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ/ppm = 171.6, 170.1, 137.2, 135.9, 134.2, 128.4, 127.2, 125.6, 65.8, 58.0, 55.7, 44.6, 27.0. Anal. Calcd for $C_{13}H_{16}N_2O_4$: C, 59.08; H, 6.10; N, 10.60. Found: C, 59.26; H, 6.21; N, 10.82.

4.5.9. (3*S*)-*N*-(L-Threoninyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (5i)

Yield: 68%. Colorless powder. Mp 96–99 °C. ESI-MS (*m*/*e*) 277 $[M-H]^-$; IR (cm^{-1}) 3411, 2969, 1734, 1648, 1497, 1457, 1375, 1198, 1111, 750, 599; $[\alpha]_D^{20}$ −10.7 (*c* 1.0, H_2O); ^1H NMR (300 MHz, $CDCl_3$) δ/ppm = 11.00 (s, 1H), 7.02 (m, *J* = 8.2 Hz, 2H), 6.95 (d, *J* = 8.8 Hz, 2H), 4.80 (t, *J* = 7.4 Hz, 1H), 4.46 (m, 2H), 3.85 (m, 1H), 3.64 (t, *J* = 6.5 Hz, 1H), 3.19 (d, *J* = 5.1 Hz, 1H), 3.00 (d, *J* = 8.1 Hz, 1H), 2.0 (s, 3H), 1.21 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ/ppm = 171.6, 170.1, 135.9, 134.2, 128.4, 127.2, 126.6, 70.5, 67.8, 64.2, 58.8, 44.6, 27.0, 18.9. Anal. Calcd for $C_{14}H_{18}N_2O_4$: C, 60.42; H, 6.52; N, 10.07. Found: C, 60.23; H, 6.40; N, 9.84.

4.5.10. (3*S*)-*N*-(L-Tyrocinyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (5j)

Yield: 95%. Colorless powder. Mp 116–118°C. ESI-MS (*m*/*e*) 339 $[M-H]^-$; IR (cm^{-1}) 3411, 2969, 1734, 1648, 1497, 1457, 1375, 1198, 1111, 750, 599; $[\alpha]_D^{20}$ −26.2 (*c* 1.0, H_2O); ^1H NMR (300 MHz, $CDCl_3$) δ/ppm = 11.00 (s, 1H), 7.02 (m, *J* = 8.2 Hz, 2H), 6.98 (d, *J* = 8.8 Hz, 2H), 6.95 (d, *J* = 8.8 Hz, 2H),6.72 (d, *J* = 5.5 Hz, 2H), 5.0 (s, 1H), 4.82 (m, 1H), 4.51 (d, *J* = 7.1 Hz, 1H), 4.41 (d, *J* = 5.1 Hz, 1H), 3.93 (t, *J* = 6.6 Hz, 1H), 3.19 (m, 1H), 2.92 (m, 3H), 2.0 (d, *J* = 8.6 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ/ppm = 171.6, 170.1, 155.9, 136.5, 134.9, 131.2, 129.0, 128.4, 127.2, 126.6, 116.2, 57.8, 52.0, 44.6, 40.8, 27.0. Anal. Calcd for $C_{19}H_{20}N_2O_4$: C, 67.05; H, 5.92; N, 8.23. Found: C, 66.83; H, 5.80; N, 8.00.

4.5.11. (3*S*)-*N*-(L-Prolinyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (5k)

Yield: 87%. Colorless powder. Mp 163–166°C. ESI-MS (*m*/*e*) 273 $[M-H]^-$; IR (cm^{-1}) 3411, 2969, 1734, 1648, 1497, 1457, 1375, 1198, 1111, 750, 599; $[\alpha]_D^{20}$ −114.8 (*c* 1.0, H_2O); ^1H NMR (300 MHz, $CDCl_3$) δ/ppm = 11.01 (s, 1H), 7.02 (m, *J* = 8.2 Hz, 2H), 6.95 (d, *J* = 8.8 Hz, 2H), 4.85 (t, *J* = 6.4 Hz, 1H), 4.51 (m, 2H), 3.69 (m, 1H), 3.00 (m, 2H), 2.75 (m, 2H), 2.0 (m, 1H), 1.71 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ/ppm = 171.6, 170.1, 135.9, 134.2, 128.4, 127.2, 126.6, 59.0, 57.8, 45.1, 44.6, 32.2, 27.0, 24.1. Anal. Calcd for $C_{15}H_{18}N_2O_3$: C, 65.68; H, 6.61; N, 10.21. Found: C, 65.45; H, 6.50; N, 10.46.

4.5.12. (3*S*)-*N*-(L-Methioninyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (5l)

Yield: 88%. Colorless powder. Mp 81–84 °C. ESI-MS (*m*/*e*) 307 $[M-H]^-$; IR (cm^{-1}) 3411, 2969, 1734, 1648, 1497, 1457, 1380, 1198, 1111, 750, 599; $[\alpha]_D^{20}$ −64.2 (*c* 1.0, H_2O); ^1H NMR (300 MHz, $CDCl_3$) δ/ppm = 11.00 (s, 1H), 7.02 (m, *J* = 8.2 Hz, 2H), 6.95 (d, *J* = 8.8 Hz, 2H), 4.85 (t, *J* = 7.4 Hz, 1H), 4.51 (d, *J* = 8.2 Hz, 1H), 4.41 (d, *J* = 6.2 Hz, 1H), 3.56 (m, 2H), 3.15 (m, 1H), 2.91 (m, 1H), 2.44 (t, *J* = 7.6 Hz, 2H), 2.16 (t, *J* = 8.6 Hz, 2H), 2.09 (s, 3H), 2.0 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ/ppm = 171.6, 170.1, 135.9, 134.2, 128.4, 127.2, 126.6, 58.8, 52.0, 44.6, 33.9, 29.3, 27.0, 17.4. Anal. Calcd for $C_{15}H_{20}N_2O_3S$: C, 58.42; H, 6.54; N, 9.08. Found: C, 58.59; H, 6.66; N, 8.87.

4.5.13. (3*S*)-*N*-(L-Asparaginyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (5m)

Yield: 62%. Colorless powder. Mp 107–108 °C. ESI-MS (*m*/*e*) 290 $[M-H]^-$; IR (cm^{-1}) 3411, 2969, 1734, 1648, 1497, 1457, 1375, 1198, 1111, 750, 599; $[\alpha]_D^{20}$ −42.4 (*c* 1.0, H_2O); ^1H NMR (300 MHz, $CDCl_3$) δ/ppm = 11.00 (s, 1H), 7.02 (m, *J* = 8.2 Hz, 2H), 6.95 (d, *J* = 8.8 Hz, 2H), 6.02 (s, 2H), 4.85 (m, 1H), 4.51 (d, *J* = 6.6 Hz, 1H), 4.41 (d, *J* = 8.6 Hz, 1H), 3.79 (d, *J* = 5.6 Hz, 1H), 3.15 (d, *J* = 7.6 Hz, 1H), 2.90 (m, 1H), 2.56 (m, 2H), 2.0 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ/ppm = 174.5, 171.6, 170.1, 135.9, 134.2, 128.4, 127.2, 126.6, 58.2, 48.6, 44.6, 39.9, 27.0. Anal. Calcd for $C_{14}H_{16}N_2O_5$: C, 57.53; H, 5.52; N, 9.58. Found: C, 57.72; H, 5.65; N, 9.79.

4.5.14. (3*S*)-*N*-(L-Glutaminyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (5n)

Yield: 65%. Colorless powder. Mp 90–92 °C. ESI-MS (*m*/*e*) 306 $[M-H]^-$; IR (cm^{-1}) 3411, 2969, 1734, 1648, 1497, 1457, 1375, 1198, 1111, 750, 599; $[\alpha]_D^{20}$ −56.3 (*c* 1.0, H_2O); ^1H NMR (300 MHz, $CDCl_3$) δ/ppm = 11.01 (s, 1H), 7.02 (m, *J* = 8.2 Hz, 2H), 6.95 (d, *J* = 8.8 Hz, 2H), 6.02 (s, 2H), 4.85 (t, *J* = 8.6 Hz, 1H), 4.51 (d, *J* = 5.6 Hz, 1H), 4.41 (d, *J* = 6.4 Hz, 1H), 3.59 (t, *J* = 7.2 Hz, 1H), 3.15 (d, *J* = 6.4 Hz, 1H), 2.88 (d, *J* = 8.6 Hz, 1H), 2.07 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ/ppm = 174.5, 171.6, 170.1, 135.9, 134.2, 128.4, 127.2, 126.6, 57.8, 51.6, 44.6, 32.6, 30.6, 27.0. Anal. Calcd for $C_{15}H_{18}N_2O_5$: C, 58.82; H, 5.92; N, 9.15. Found: C, 58.60; H, 5.80; N, 9.38.

4.5.15. (3*S*)-*N*-(L-Histidinyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (5o)

Yield: 74%. Colorless powder. Mp 127–128 °C. ESI-MS (*m*/*e*) 313 $[M-H]^-$; IR (cm^{-1}) 3411, 2969, 1734, 1648, 1497, 1457, 1375, 1198, 1200, 750; $[\alpha]_D^{20}$ −38.3 (*c* 1.0, H_2O); ^1H NMR (300 MHz, $CDCl_3$) δ/ppm = 13.40 (d, *J* = 5.6 Hz, 1H), 11.00 (s, 1H), 7.44 (s, 1H), 7.02 (m, *J* = 8.2 Hz, 2H), 6.95 (d, *J* = 8.8 Hz, 2H), 6.80 (d, *J* = 5.4 Hz, 1H), 4.85 (t, *J* = 8.2 Hz, 1H), 4.51 (d, *J* = 7.3 Hz, 1H), 4.41 (d, *J* = 6.3 Hz, 1H), 3.95 (t, *J* = 5.1 Hz, 1H), 3.17 (m, 1H), 2.92 (m, 1H), 2.0 (d, *J* = 8.6 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ/ppm = 171.6, 170.1, 135.9, 134.2, 133.5, 128.4, 127.2, 126.6, 119.6, 57.8, 52.0, 44.6, 33.5, 27.0. Anal. Calcd for $C_{16}H_{18}N_4O_3$: C, 61.13; H, 5.77; N, 17.82. Found: C, 61.35; H, 5.89; N, 18.05.

4.5.16. (3*S*)-*N*-(L-Lysinyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (5p)

Yield: 100%. Colorless powder. Mp 121–123 °C. ESI-MS (*m*/*e*) 304 $[M-H]^-$; IR (cm^{-1}) 3411, 2969, 1734, 1648, 1497, 1457, 1375, 1198, 1111, 750, 599; $[\alpha]_D^{20}$ −24.6 (*c* 1.0, H_2O); ^1H NMR (300 MHz, $CDCl_3$) δ/ppm = 11.00 (s, 1H), 7.02 (m, *J* = 8.2 Hz, 2H), 6.95 (d, *J* = 8.8 Hz, 2H), 4.85 (t, *J* = 6.4 Hz, 1H), 4.51 (d, *J* = 7.1 Hz, 1H), 4.41 (d, *J* = 8.1 Hz, 1H), 3.56 (m, 1H), 3.19 (d, *J* = 6.4 Hz, 1H), 2.92 (d, *J* = 5.6 Hz, 1H), 2.65 (m, 2H), 2.0 (m, 4H), 1.79 (m, 2H), 1.55 (m, 2H), 1.29 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ/ppm = 171.6, 170.1, 135.9, 134.2, 128.4, 127.2, 126.6, 57.8, 52.0, 44.6, 41.9, 34.2, 31.9, 27.0, 20.7. Anal. Calcd for $C_{16}H_{23}N_3O_3$: C, 62.93; H, 7.59; N, 13.76. Found: C, 62.70; H, 7.45; N, 13.99.

4.5.17. (3*S*)-*N*-(L-Aspartyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (5q)

Yield: 67%. Colorless powder. Mp 123–126 °C. ESI-MS (*m*/*e*) 291 $[M-H]^-$; IR (cm^{-1}) 3411, 2969, 1734, 1648, 1497, 1457, 1375, 1198, 1111, 750, 599; $[\alpha]_D^{20}$ −36.4 (*c* 1.0, H_2O); ^1H NMR (300 MHz, $CDCl_3$) δ/ppm = 11.01 (s, 2H), 7.02 (m, *J* = 8.2 Hz, 2H), 6.95 (d, *J* = 8.8 Hz, 2H), 4.85 (t, *J* = 6.4 Hz, 1H), 4.51 (d, *J* = 5.6 Hz, 1H), 4.41 (d, *J* = 6.6 Hz, 1H), 3.89 (m, 1H), 3.19 (d, *J* = 7.2 Hz, 1H), 2.92 (d, *J* = 8.3 Hz, 1H), 2.73 (m, 2H), 2.0 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ/ppm = 174.5, 171.6, 170.1, 141.2, 135.9, 134.2, 129.4, 127.2, 126.6, 57.8, 49.7, 44.6, 43.1, 27.0. Anal. Calcd for $C_{14}H_{17}N_3O_4$: C, 57.72; H, 5.88; N, 14.42. Found: C, 57.50; H, 5.76; N, 14.66.

4.5.18. (3*S*)-*N*-(L-Glutamoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (5r)

Yield: 70%. Colorless powder. Mp 108–110 °C. ESI-MS (*m*/*e*) 305 $[M-H]^-$; IR (cm^{-1}) 3411, 2969, 1734, 1648, 1497, 1457, 1375, 1198, 1111, 750, 599; $[\alpha]_D^{20}$ −120.1 (*c* 1.0, H_2O); ^1H NMR (300 MHz, $CDCl_3$) δ/ppm = 11.00 (s, 2H), 7.02 (m, *J* = 8.2 Hz, 2H), 6.95 (d, *J* = 8.8 Hz, 2H), 4.85 (t, *J* = 7.4 Hz, 1H), 4.51 (d, *J* = 5.4 Hz, 1H), 4.41 (d, *J* = 6.5 Hz, 1H), 3.56 (m, 1H), 3.19 (d, *J* = 7.5 Hz, 1H), 2.92 (d, *J* = 8.6 Hz, 1H), 2.21 (m, 2H), 2.03 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ/ppm = 174.5, 171.6, 170.1, 141.2, 135.9, 134.2, 129.4, 127.2, 126.6, 57.8, 52.0, 44.6, 29.4, 27.0. Anal. Calcd for $C_{15}H_{19}N_3O_4$: C, 59.01; H, 6.27; N, 13.76. Found: C, 58.83; H, 6.15; N, 13.99.

4.5.19. (3*S*)-*N*-(L-Argininyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (5s)

Yield: 65%. Colorless powder. Mp 199–202 °C. ESI-MS (*m*/*e*) 334 $[M-H]^-$; IR (cm^{-1}) 3411, 2969, 1734, 1648, 1497, 1457, 1375, 1198, 1111, 750, 599; $[\alpha]_D^{20}$ −10.8 (*c* 1.0, H_2O); ^1H NMR (300 MHz, $CDCl_3$) δ/ppm = 11.00 (s, 1H), 7.02 (m, *J* = 8.2 Hz, 2H), 6.95 (d, *J* = 8.8 Hz, 2H), 4.84 (m, 1H), 4.51 (d, *J* = 7.4 Hz, 1H), 4.41 (d, *J* = 8.5 Hz, 1H), 3.56 (t, *J* = 5.2 Hz, 1H), 3.19 (d, *J* = 6.6 Hz, 1H), 2.92 (d, *J* = 7.2 Hz, 1H), 2.65 (m, 2H), 2.0 (d, *J* = 7.6 Hz, 6H), 1.79 (m, 2H), 1.55 (m, 2H); ^{13}C NMR (75 MHz, $CHCl_3$) δ/ppm = 171.8, 170.1, 158.2, 135.9, 134.2, 129.4, 127.2, 126.6, 58.8, 51.8, 44.6, 37.1, 32.1, 27.0, 24.3. Anal. Calcd for $C_{16}H_{23}N_5O_3$: C, 57.64; H, 6.95; N, 21.01. Found: C, 57.42; H, 6.83; N, 21.25.

4.6. In vitro antiplatelet aggregation activity assay

An H-10 cell counter was used to determine the platelet count, and a two-channel Chronolog aggregometer was used to evaluate platelet aggregation. After collection, the pig blood was centrifuged at 1000*g* for 10 min and the platelet rich plasma (PRP) was removed. The remaining blood was centrifuged for an additional 10 min at 1500*g* to prepare platelet poor plasma (PPP). The final platelet count of the citrated plasma samples was adjusted to 2×10^8 platelets/ml with autologous PPP. To an optical aggregometry testing tuber, 0.5 ml of the adjusted plasma sample and 5 μl of NS or 5 μl of the solution of **5a**–**s** (in a series of final concentrations of a range from 100 μM to 1 nM) was added. After adjustment of the baseline, 5 μl of the solution of platelet-activating factor in NS (PAF, final concentration 0.1 μM) or 5 μl of the solution of adenosine diphosphate in NS (ADP, final concentration 10 μM) or 5 μl of the solution of arachidonic acid in NS (AA, final concentration 350 μM), or 50 μl of the solution of thrombin in NS (TH, final concentration 0.1 U/ml) was added and aggregation was measured at 37 °C for 5 min. The effects of **5a**–**s** (final concentration 10 μM to 1 nM) on PAF or ADP or AA- or TH-induced platelet aggregation were observed. The maximal rate of platelet aggregation (A_m%) was represented by the peak height of aggregation curve. The inhibition rate was calculated by % Inhibition = [(A_m% of NS, 50.16 ± 3.65%) − (A_m% of **5a**–**s**) ÷ (A_m% of NS, 50.16 ± 3.65%). The concentration versus inhibition rate curve was plotted to determine the IC_{50} values via program GWBASIC.EXE.

4.7. In vivo antithrombotic activity in rat model

The assessments described here were performed based on a protocol reviewed and approved by the Ethics Committee of Capital Medical University. The committee assures the welfare of the animals was maintained in accordance to the requirements of the animal welfare act and according to the guide for care and use of laboratory animals. Aspirin and **5a**–**s** were dissolved in NS before administration and kept in an ice bath. Male Wistar rats weighing 250–300 g (purchased from Animal Center of Peking University) were used. After 45 min of the oral administration of proper dose of **5a**–**s** and blank control or positive control the rats 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. From the other end of the polyethylene tube heparin sodium was injected as anticoagulant, then NS or **5a**–**s** were injected, and this end was inserted into the right carotid artery. Blood was allowed

to flow from the right carotid artery to the left jugular vein through the polyethylene tube for 15 min. The thread was removed to obtain the weight of the wet thrombus.

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