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A class of oral *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]-*N′*-(amino-acid-acyl)hydrazine: Discovery, synthesis, *in vitro* anti-platelet aggregation/*in vivo* anti-thrombotic evaluation and 3D QSAR analysis

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ABSTRACT

The *in vivo* anti-thrombotic activities of amino acid modified tetrahydro-β-carbolines depended upon the proximity of the side chain of the amino acid residue to the carboline-cycle. Based on this proximity the computerized screening of various tetrahydro-β-carboline derivatives was performed and *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]-*N′*-(amino-acid-acyl)hydrazines were explored having large proximity. The *in vivo* anti-thrombotic assays explored that at a dose of 10 nmol/kg eighteen novel *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]-*N′*-(amino-acid-acyl)hydrazines were orally efficacious.

1. Introduction

Cardiovascular events such as the deep vein thrombosis, myocardial infarction, pulmonary embolism and stroke seriously hazard the health of the human being, while intravascular thrombosis is known to be one of the most prominent causes of morbidity and mortality [1]. The injury of blood vessel is implicated in the arterial thrombosis [2]. Once vascular endothelium of the injured blood vessel is damaged the platelets will adhere to the exposed extracellular matrix [3]. Platelet adhesion primarily associates with uncontrolled platelet activation and culminates in the intravascular thrombosis [4]. The suppression of platelet activation can effectively prevent the thrombosis [5], and the anti-platelet drugs have therapeutic utility [6]. Due to the current anti-platelet therapy usually having limited efficacy, an extra effort has been made to discover new leads, such as β-carbolines and tetrahydro-β-carbolines, capable of more effectively inhibiting the platelet aggregation.

β-Carbolines and tetrahydro-β-carbolines are pharmacologically important indole alkaloids. It has been reported that they have a wide spectrum of pharmacological actions such as a) inhibiting platelet activation [7], mitogen activated protein kinase-activated protein kinase 2 [8], cyclin-dependent kinases 4 [9,10], human monoamine oxidase [11], mitotic kinesin Eg5 [12], bacterial enoyl acyl carrier protein reductase [13], acetyl-cholinesterases and butyrylcholinesterases [14]; b) improving object recognition memory [15]; c) stimulating insulin secretion [16]; d) interacting with DNA [17,18]; e) blocking topoisomerases [19]; f) exhibiting trypanocidal [6,20], antimalarial [21,22], cytotoxic [23–27], cardiovascular [28], neuroprotective and neuron-differentiating [29], antiviral [30], as well as antileishmanial actions [31]; g) binding to imidazoline [32,33], mGluR1 [34], 5-HT_2 serotonin receptors [35], and benzodiazepine receptors [36].

Though a series of β-carbolines such as harmalol, harmaline, norharmane, harmol, harmine and harmane have been known to be able to prevent the platelet activation, and capable of inhibiting collagen-induced platelet aggregation. The IC_{50} values of them to inhibit the platelet aggregation are higher than 130 μM [7]. While (*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acids were reported to be able to effectively inhibit the platelet aggregation induced by four aggregators including platelet-activating factor (PAF), adenosine diphosphate (ADP), arachidonic acid (AA) as well as thrombin (TH). The IC_{50} value of (*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid inhibited the platelet aggregation was lower than 1 μM, and its hydrazine derivative could be a good lead compound [37]. In this context, we modified

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(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carbonylhydrazine with amino acids preparing eighteen novel compounds, evaluated their *in vitro* anti-platelet aggregation and *in vivo* anti-thrombotic activities, and established their 3D QSAR equation.

2. Chemistry

2.1. Discovery of N-[(1S,3S)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carbo nyl]-N′-(amino-acid-acyl)hydrazines

In the analysis of the stereoview derived from the QSAR module of Cerius 2 it was found that the *in vivo* anti-thrombotic activities of amino acid modified *3S*-tetrahydro-β-carboline-3-carboxylic acid and (*3S,12aS*)-hexahydropyrazino-[1′,2′:1,6]pyrido [3,4-b]indole-1,4-dione depended on their stretching conformation [38]. In *3S*-tetrahydro-β-carboline-3-carbonylamino acids (Fig. 1A) the proximity of the side chain of the amino acid residue to the carboline-cycle is larger than that in (*3S,12aS*)-hexahydropyrazino [1′,2′:1,6]pyrido [3,4-b]indole-1,4-diones (Fig. 1B). On the *in vivo* anti-thrombotic model the efficacy of the former is 10-fold lower than that of the latter [37,38]. These observations suggest that for anti-thrombotic *3S*-tetrahydro-β-carboline-3-carboxylic acid derivatives the small proximity of the side chain of the amino acid residue to the carboline-cycle benefits the *in vivo* activity. Following up this clue the proximity of a series of amino acids modified carbolines were estimated, and the side chains of the amino acid residues in *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]-*N*′-(amino-acid-acyl)hydrazines (Fig. 1C) were found been far from the carboline-cycle, and this leaded to the discovery of them as novel anti-thrombotic agents.

2.2. Synthesis of N-[(1S,3S)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]-N′-(amino-acid-acyl)hydrazines

The preparation of *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]-*N*′-(amino-acid-acyl)hydrazines (**5a**–**r**) was carried out according to a five-step-route depicted in Scheme 1. The racemic β-carbolines were usually prepared via Pictet–Spengler condensation. To control the chirality in the Pictet–Spengler condensation for the enantiospecific synthesis of indole alkaloids the effect of substitution on the stereospecificity was investigated [39,40]. When L-tryptophan was used in the Pictet–Spengler condensation *1S,3S*/*1R,3S* diasetereomers were formed in a ratio of 16:1 (79% yield of **1**). After the esterification, *1S,3S* diasetereomer of the carboxylate methyl ester **2** was predominantly generated as precipitates (89% yield). The increase of the stereoselectivity in favor of the *1S,3S* diasetereomer was probably due to the influence of the substituents. In the presence of hydrazine hydrate the hydrazinolysis of **2** provided *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]hydrazine (**3**, 72% yield). In DCC/HOBt/NMM condition the coupling of **3** with Boc-amino acids gave *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]-*N*′-(Boc-aminoacid-acyl)hydrazine (**4a**–**r**, 43–86% yield). On the removal of Boc group of **4a**–**r** *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]-*N*′-(amino-acid-acyl)hydrazine (**5a**–**r**) were obtained in 88–100% yields. To ensure the purity, **4a**–**r** were purified on a chromatographic column, while **5a**–**r** were purified by ether-promoted repeated solidification, and the HPLC purity was more than 97%. These data demonstrated that the used procedures and conditions were suitable for the preparation of **5a**–**r** with high quality and acceptable yield.

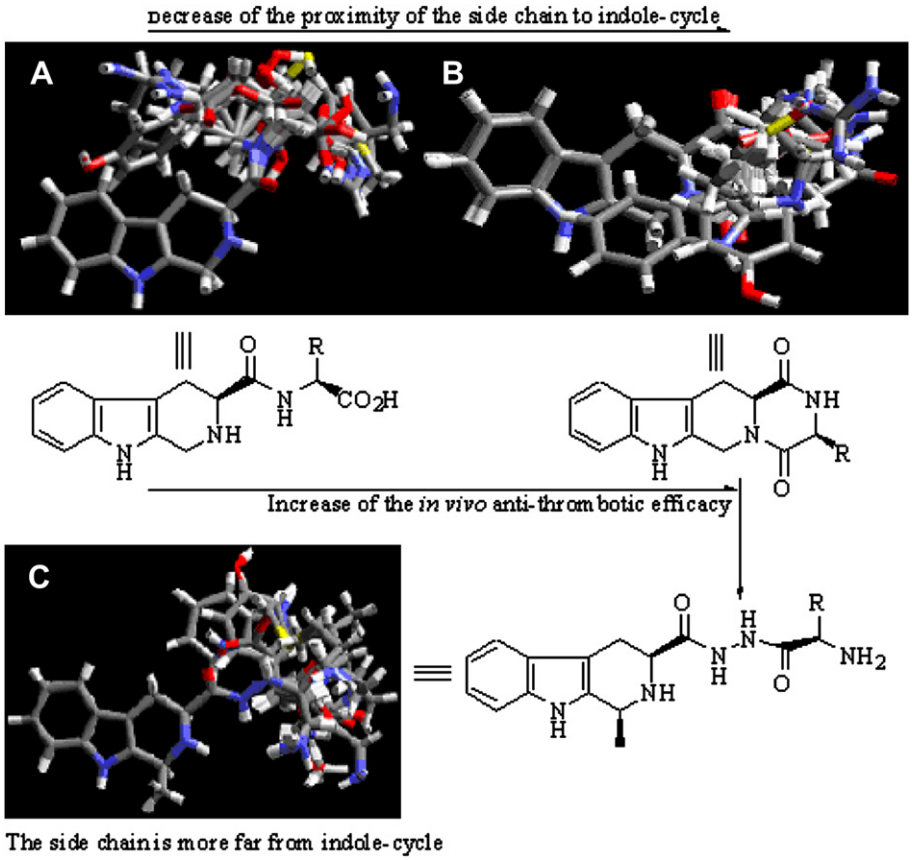
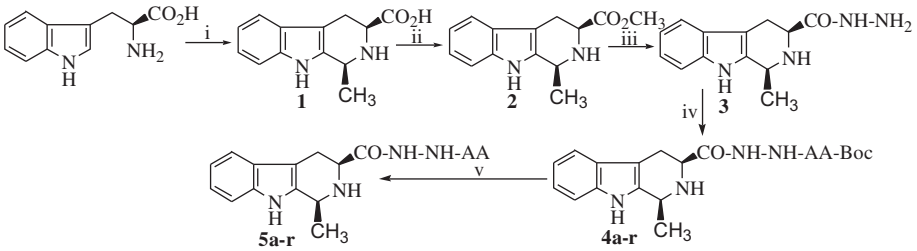


Fig. 1. QSAR module of Cerius 2 derived stereoview of *3S*-tetrahydro-β-carboline-3-carbonyl-amino acids (A), (*3S, 12aS*)-3-(aminoacid-side-chain)-2,3,6,7,12,12a-hexa-hydro-pyrazino-[10,20:1,6]pyrido[3,4-b]indole-1,4-diones (B) and *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]-*N*′-(amino-acid-acyl)hydrazines (C).



Scheme 1. Synthetic route of *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3- carbonyl]-*N′*-(amino-acid-acyl)hydrazine. i) CH_3CHO, H_2SO_4; ii) CH_3OH, $SOCl_2$; iii) $NH_2NH_2 \cdot H_2O$; iv) DCC, HOBt, NMM; v) 4 N HCl/EtOAc. In **4a** and **5a**, AA = Gly; **4b** and **5b**, AA = Ala; **4c** and **5c**, AA = Ile; **4d** and **5d**, AA = Leu; **4e** and **5e**, AA = Val; **4f** and **5f**, AA = Met; **4g** and **5g**, AA = Pro; **4h** and **5h**, AA = Phe; **4i** and **5i**, AA = Tyr; **4j** and **5j**, AA = Thr; **4k** and **5k**, AA = Ser; **4l** and **5l**, AA = Trp; **4m**, AA = His(Boc); **5m**, AA = His; **4n** AA = Lys(Boc); **5n**, AA = Lys; **4o** and **5o**, AA = Asp(OBzl); **4p** and **5p**, AA = Glu(OBzl); **4q** and **5q**, AA = Asn; **4r** and **5r**, AA = Gln.

3. Pharmacology

3.1. In vitro anti-platelet aggregation of 3 and 5a–r

Considering the *in vitro* anti-platelet aggregation assay needs a great quantity of blood, only a great quantity of pig blood is commercially available in Beijing, and the *in vitro* anti-platelet aggregation activity resulted from pig platelets matched the *in vivo* anti-thrombotic activity resulted from rat [37,38], thus pig platelets were used here. The *in vitro* activities of **3** and **5a**–**r** (at a series of concentrations ranging from 1 μM to 1.5 mM) were evaluated by anti-platelet aggregation assay. PAF (final concentration 0.1 μM), ADP (final concentration 10 μM) and AA (final concentration 350 μM) were used as the aggregator. The *in vitro* activities of **3** and **5a**–**r** inhibiting platelet aggregation were represented with IC_{50} values and are listed in Table 1.

The IC_{50} values indicate that **3** selectively inhibits AA-induced *in vitro* platelet aggregation, and the inhibiting sequence is AA > PAF > ADP. The IC_{50} values of **5a**–**r** inhibiting AA-induced platelet aggregation range from 0.6 μM to 21.7 μM. The IC_{50} values of **5a**,**b**,**f**,**g** are equal to that of **3**, while the IC_{50} values of **5c**–**e**,**h**–**r** are significantly lower than that of **3**. The IC_{50} values of **5a**–**r** inhibiting PAF-induced platelet aggregation range from 1.2 μM to 506.8 μM. The IC_{50} value of **5h** is equal to that of **3**, the IC_{50} values of **5c**–**f**,**i**–**n** are significantly lower than that of **3**, while the IC_{50} values of **5a**,**b**,**g**,**o**–**r** are significantly higher than that of **3**. The IC_{50} values of **5a-r** inhibiting ADP-induced platelet aggregation range from 21.7 μM to 722.1 μM. The IC_{50} values of **5q**,**r** are equal to that of **3**, the IC_{50} values of **5b**–**p** are significantly lower than that of **3**, while the IC_{50} value of **5a** is significantly higher than that of **3**. Therefore the IC_{50} values of **5f**,**h** inhibiting the platelet aggregation induced by three aggregators either are equal to or lower than that of **3**, while the IC_{50} values of **5c**–**e**,**i**–**n** inhibiting the platelet aggregation induced by three aggregators are significantly lower than that of **3**.

3.2. In vivo anti-thrombotic activities of 5a–r

To select the dose of **5a**–**r** some preliminary evaluations were performed. The evaluations revealed that the effective dose (0.1 μmol/kg) of *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]hydrazine (**3**) was 50 fold lower than that (5 μmol/kg) of *3S*-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid, while the effective dose of the latter was 10 fold higher than that (0.5 μmol/kg) of some of *3S*-tetrahydro-β-carboline-3-carbonylaminoacids. This means that the introduction of L-amino acid into *3S*-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid can result in 10-fold decrease in the effective dose. Therefore the effective dose of **5a**–**r** could be 500-fold lower than that (5 μmol/kg) of *3S*-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid and should be 10 nmol/kg.

On a model of extra-corporeal circulation of arterio-veinos cannula the thrombus weights of the rats receiving **5a**–**r** were measured, used to represent the *in vivo* anti-thrombotic activities, and are listed in Table 2. As seen, the thrombus weights (18.6–24.0 mg) of the rats receiving 10 nmol/kg of **5a**–**r** are significantly lower than that (28.2 mg) of the rats receiving normal saline (NS). This means that **5a**–**r** effectively inhibit the rats to form thrombus. Besides, the thrombus weights (18.6–19.0 mg) of the rats receiving 10 nmol/kg of **5f**,**j**,**k**,**l**,**n** are significantly lower than that (21.2 mg) of the rats receiving 100 nmol/kg of **3**. This means

Table 1
IC_{50} of **5a**–**r** inhibiting platelet aggregation.[a]

Compd.	IC_{50} (μM)		
	ADP	PAF	AA
3	611.2 ± 59.7	110.9 ± 9.5	21.5 ± 1.0
5a	722.1 ± 34.8	348.1 ± 35.1[n]	20.7 ± 1.5
5b	117.9 ± 5.3[f]	162.4 ± 11.8[k]	20.0 ± 1.3
5c	51.1 ± 4.6[d]	1.4 ± 0.3[b]	2.4 ± 0.2[c]
5d	31.6 ± 1.7[c]	1.2 ± 0.2[b]	3.2 ± 0.3[d]
5e	156.0 ± 10.7	50.0 ± 3.3[g]	3.7 ± 0.3[e]
5f	417.1 ± 18.0[k]	52.9 ± 5.0[g]	21.7 ± 1.1
5g	166.1 ± 5.8[h]	134.2 ± 10.5[j]	20.6 ± 1.7
5h	306.9 ± 26.8[j]	108.9 ± 13.0 [h]	7.1 ± 0.3[i]
5i	152.1 ± 9.3[g]	12.7 ± 1.2[e]	13.0 ± 0.7[j]
5j	531.0 ± 41.2[m]	31.9 ± 1.5[f]	4.7 ± 0.3[f]
5k	276.1 ± 18.0[i]	119.1 ± 9.0[i]	19.9 ± 1.0[l]
5l	21.7 ± 2.2[b]	4.3 ± 0.6[c]	0.6 ± 0.03[b]
5m	94.6 ± 9.4[e]	7.7 ± 0.6[d]	4.8 ± 0.3[f]
5n	321.3 ± 15.3[j]	106.1 ± 5.1[h]	3.4 ± 0.2[d]
5o	117.0 ± 8.6[f]	196.2 ± 7.3[l]	5.2 ± 0.3[g]
5p	524.4 ± 36.2[l]	362.3 ± 22.6[n]	6.3 ± 0.6[h]
5q	609.1 ± 35.5	506.8 ± 21.6	19.7 ± 0.7[l]
5r	598.4 ± 41.1	267.1 ± 8.4[m]	18.5 ± 1.1[k]

For ADP: b) Compare to **3**, **5a**–**k**,**m**–**r** $p < 0.01$; c) Compare to **3**, **5a**–**c**,**e**–**k**,**m**–**r** $p < 0.01$; d) Compare to **3**, **5a**,**b**,**e**–**k**,**m**–**r** $p < 0.01$; e) Compare to **3**, **5a**,**b**,**e**–**k**,**n**–**r** $p < 0.01$; f) Compare to **3**, **5a**,**e**–**k**,**n**,**p**–**r** $p < 0.01$; g) Compare to **3**, **5a**,**e**,**f**,**h**,–**i**,**k**,**n**,**p**–**r** $p < 0.01$ and to **5g** $p < 0.05$; h) Compare to **3**, **5a**,**f**,**h**,**k**,**n**,**p**–**r** $p < 0.01$; i) Compare to **3**, **5a**,**f**,**n**,**p**–**r** $p < 0.01$ and to **5h** $p < 0.05$; j) Compare to **3**, **5a**,**f**,**p**–**r** $p < 0.01$; k) Compare to **3**, **5a**,**p**–**r** $p < 0.01$ and **5o** $p < 0.05$; l) Compare to **3**, **5a**,**q**,**r** $p < 0.01$; m) Compare to **3**, **5a**,**r** $p < 0.01$ and to **5q** $p < 0.05$.

For PAF: b) Compare to **3**, **5a**,**b**,**e**–**r** $p < 0.01$; c) Compare to **3**, **5a**,**b**,**e**–**k**,**m**–**r** $p < 0.01$; d) Compare to **3**, **5a**,**b**,**e**–**k**,**n**–**r** $p < 0.01$; e) Compare to **3**, **5a**,**b**,**e**–**h**,**j**,**k**,**n**–**r** $p < 0.01$; f) Compare to **3**, **5a**,**b**,**e**–**h**,**k**,**n**–**r** $p < 0.01$; g) Compare to **3**, **5a**,**b**,**g**,**h**,**k**,**n**–**r** $p < 0.01$; h) Compare to **3**, **5a**,**b**, **g**,**k**,**o**–**r** $p < 0.01$; i) Compare to **3**, **5a**,**b**,**o**–**r** $p < 0.01$ and to **5g** $p < 0.05$; j) Compare to **3**, **5a**,**b**,**o**–**r** $p < 0.01$; k) Compare to **3**, **5a**,**o**–**r** $p < 0.01$; l) Compare to **3**, **5a**,**p**–**r** $p < 0.01$; m) Compare to **3**, **5a**,**p**,**q** $p < 0.01$; *n*) Compare to **3**, **5a**,**q** $p < 0.01$.

For AA: b) Compare to **3**, **5a**–**k**,**m**–**r** $p < 0.01$; c) Compare to **3**, **5a**,**b**,**d**–**k**,**m**–**r** $p < 0.01$; d) Compare to **3**, **5a**,**b**,**e**–**k**,**m**,**o**–**r** $p < 0.01$; e) Compare to **3**, **5a**,**b**,**d**–**k**,**m**–**r** $p < 0.01$; f) Compare to **3**, **5a**,**b**,**d**–**i**,**k**,**m**,**n**,**q**–**r** $p < 0.01$ and to **5o** $p < 0.05$; g) Compare to **3**, **5a**, **b**,**d**–**i**,**k**,**m**,**n**,**p**–**r** $p < 0.01$; h) Compare to **3**, **5a**,**b**,**d**–**g**,**i**,**k**,**m**,**n**,**q**,**r** $p < 0.01$ and to **5h** $p < 0.05$; i) Compare to **3**, **5a**,**b**,**d**–**g**,**i**,**k**,**m**,**n**,**q**,**r** $p < 0.01$; j) Compare to **3**, **5a**,**b**,**d**–**g**,**k**,**m**,**n**,**q**,**r** $p < 0.01$; k) Compare to **3**, **5a**,**b**,**d**–**g**,**m**,**n** $p < 0.01$ and to **5k**,**q** $p < 0.05$; l) Compare to **3** $p < 0.05$.

[a] $n = 6$, A_m% of platelet aggregation induced by PAF, ADP and AA with of aspirin (1.7 mM) were 33.22 ± 2.02%, 31.14 ± 2.28% and 34.50 ± 2.49%, respectively.

that the anti-thrombotic efficacy of **5f**,**j**,**k**,**l**,**n** is more than 10 fold higher than that of **3**. While the thrombus weights (19.8–21.4 mg) of the rats receiving 10 nmol/kg of **5b**,**c**,**d**,**g**,**h**,**i**,**m**,**o**,**p** are equal to that of the rats receiving 100 nmol/kg of **3**. This means that the anti-thrombotic efficacy of **5b**,**c**,**d**,**g**,**h**,**i**,**m**,**o**,**p** is 10-fold higher than that of **3**. Therefore the introduction of L-amino acid into **3** substantially increases the *in vivo* anti-thrombotic efficacy.

On the other hand, it was also noticed that the *in vivo* activities were not consistent with the *in vitro* activities. *In vivo* each member of **5a**–**r** had to undergo individual absorption, distribution and metabolism, while *in vitro* they did not. This perhaps should be responsible for their inconsistent *in vitro* and *in vivo* activities.

3.3. Dose-dependent in vivo anti-thrombotic activity of *5j*

Oral **5j** was observed at three doses (10, 1 and 0.1 nmol/kg) to produce a possible dose-dependent anti-thrombotic response in rats. The thrombus weights are listed in Table 3. The data demonstrate that the thrombus weight is progressively increased with the decrease of the dose. Therefore, **5j** had a dose-dependent anti-thrombotic action.

4. 3D QSAR analysis of 5a–r

To understand the dependence of the *in vivo* anti-thrombotic activities of **5a**–**r** upon their structures the 3D QSAR analysis was performed. Training set (**5a**–**g**,**i**,**j**,**l**–**r**)/test set (**5h**,**k**) selections were done manually such that they populate the wide range of anti-thrombotic activity in similar proportion. In the analysis, the alignment, MFA based Cerius 2 QSAR module of **5a**–**r** and the electrostatic and environments of them within the grid with 3D points of the equation were involved.

4.1. Alignment of *5a*–*r*

Establishing the valid 3D-QSAR models a proper alignment procedure of **5a**–**r** was practiced using the consensus model align strategy in the align module within Cerius 2. Based on the assumption that each structure of **5a**–**r** exhibits activity at the same binding site of the receptor, they were aligned in a pharmacological active orientation. To obtain a consistent alignment *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]hydrazine was selected as the template for superposing **5a**–**r**. The method used for performing the alignment was the maximum common subgraph (MCS) [41]. MCS looks at molecules as points and lines, and uses the techniques out of graph theory to identify the patterns. Then MCS finds the largest subset of atoms in *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]hydrazine that shared by **5a**–**r**. This subset was used for the alignment. A rigid fit of atom pairings was performed to superimpose each structure onto *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]hydrazine. Stereoview of aligned **5a**–**r** is shown in Fig. 2. The alignment stereoview explores that to superimpose onto *N*-[(*1S,3S*)-1-methyl-1,2,3,4- tetrahydro-β-carboline-3-carbonyl]hydrazine the amino acid chain of each structure has to take individual conformation, which will affect on the anti-thrombotic activity.

Table 2
Effect of oral **5a**–**r** on the thrombus weight of the rats.[a]

Compd.	Thrombus weight	Compd.	Thrombus weight
NS	28.21 ± 1.11	Aspirin	18.24 ± 1.23[b]
3	21.23 ± 2.53[b]	**5j**	18.67 ± 1.51[c]
5a	23.74 ± 2.33[b]	**5k**	19.04 ± 2.51[c]
5b	20.06 ± 2.40[d]	**5l**	18.87 ± 2.71[c]
5c	20.62 ± 2.13[d]	**5m**	19.75 ± 2.85[d]
5d	21.06 ± 2.39[d]	**5n**	18.97 ± 2.61[c]
5e	24.01 ± 1.97[b]	**5o**	19.65 ± 2.40[d]
5f	18.55 ± 2.32[c]	**5p**	21.36 ± 2.01[d]
5g	20.39 ± 2.81[d]	**5q**	20.42 ± 1.81[d]
5h	21.00 ± 2.22[d]	**5r**	21.01 ± 2.85[d]
5i	20.24 ± 2.39[d]		

[a] Weight of wet thrombus is represented by X ± SD mg, NS = vehicle, $n = 12$; Dose of aspirin: 160 μmol/kg; Dose of **3**: 0.1 μmol/kg; Dose of **5a**–**p**: 10 nmol/kg.
[b] Compare to NS $p < 0.001$.
[c] Compare to NS $p < 0.001$ and to **3** $p < 0.05$.
[d] Compare to NS $p < 0.001$ and to **3** $p > 0.05$.

Table 3
Dose-dependent effect of **5j** on the thrombus weight of the rats.[a]

Dose	10 nmol/kg	1 nmol/kg	0.1 nmol/kg
5j	18.67 ± 1.51[b]	22.77 ± 2.47[c]	27.22 ± 3.43[d]
NS	28.21 ± 1.11		

[a] Weight of wet thrombus is represented by X ± SD mg, NS = vehicle, $n = 12$.
[b] Compare to NS and 1 nmol/kg of **5j** $p < 0.01$.
[c] Compare to NS and 0.1 nmol/kg of **5j** $p < 0.01$.
[d] Compared to NS $p > 0.05$.

4.2. MFA based Cerius 2 QSAR module of *5a*–*g*,*i*,*j*,*l*–*r*

Molecular field analysis (MFA) was performed for **5a**–**g**,**i**,**j**,**l**–**r** using the QSAR module of Cerius 2 [42]. 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 grid points generated were 734. Though the spatial and structural descriptors such as dipole moment, polarizability, radius of gyration, number of rotatable bonds, molecular volume, principal moment of inertia, AlogP98, 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 10,000 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. The MFA model for the anti-thrombotic activities of **5a**–**g**,**i**,**j**,**l**–**r** in terms of the most relevant descriptors including proton, methyl and hydroxyl anion is expressed by Equation (1).

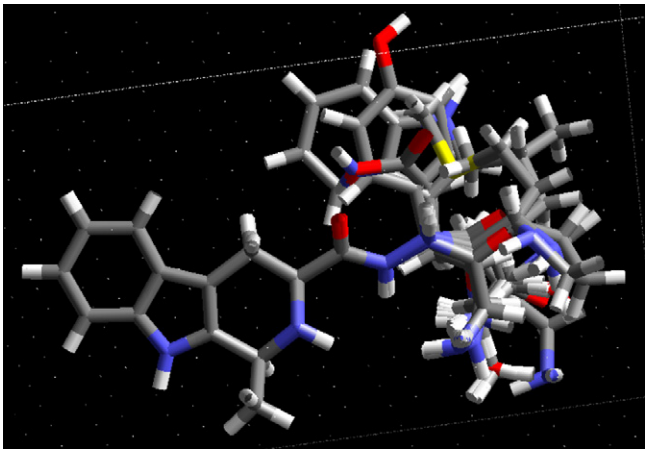


Fig. 2. Alignment stereoview of **5a**–**r** used for molecular field generation.

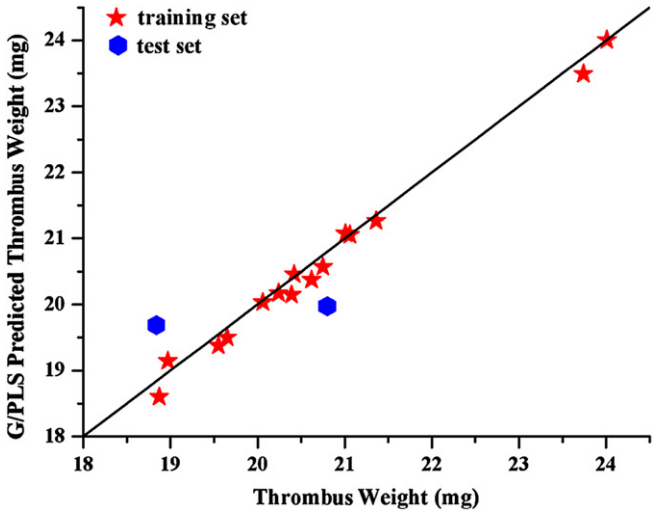


Fig. 3. Graph of tested vs. predicted anti-thrombotic activities of **5a**–**r**.

$$\text{Thrombus weight} = 22.89 - 0.054\left(H^+/394\right) - 0.0054\left(H^+/399\right) + 0.18(CH_3/298) - 0.032(CH_3/362) - 0.030\left(HO^-/320\right) - 0.068\left(HO^-/407\right) \quad (1)$$

In Equation (1) the data points (n), correlation coefficient (r) and square correlation coefficient (r^2) are 16, 0.984 and 0.968, respectively. The parameters indicated that Equation (1) is able to predict the *in vivo* activity for the analogs of **5a**–**g**,**i**,**j**,**l**–**r**. The tested thrombus weights on rat thrombogenesis model and the calculated thrombus weights based on Equation (1) were shown in Fig. 3.

Equation (1) contains 2 terms from proton descriptor, 2 terms from methyl descriptor and 2 terms from hydroxyl anion descriptor. The terms of 0.054 (H^+/394) and 0.0054 (H^+/399) have negative coefficients, which means that at these positions electron-releasing groups will increase the thrombus weight. The term of 0.18 (CH_3/298) has positive coefficient, which means that at this position small group or hydrophilic group will increase the thrombus weight, while term of 0.032 (CH_3/362) has negative coefficient, which means that at this position small groups or hydrophilic group will decrease the thrombus weight. The terms of 0.030 (HO^-/320) and 0.068 (HO^-/407) have negative coefficients, which means that at these positions hydrogen bond forming group will decrease the thrombus weight.

As examples Fig. 4 gives four representatives **5f**,**h**,**k**,**p** of which **5f** has hydrophobic group near CH_3/298 and hydrogen bond forming group near HO^-/320 and decrease the thrombus weight, **5h** has neither hydrophobic group near CH_3/298 nor hydrogen bond forming groups near HO^-/320 and HO^-/407 and increase the thrombus weight, **5k** has hydrophobic groups near CH_3/298 and hydrogen bond forming groups near HO^-/407 and decrease the thrombus weight, while **5p** has electron-releasing group near H^+/399 and hydrophobic group near CH_3/362 thus increase the thrombus weight.

4.3. Predicting the in vivo anti-thrombotic activity of *5h*,*k* with Equation (1)

The predict power of Equation (1) was demonstrated by comparing the calculated and tested *in vivo* anti-thrombotic activity of **5h**,**k** (Table 4). The correlations of the predicting and the testing values are also shown in Fig. 3. The results indicate that Equation (1) rationally gives the thrombus weights of **5h**,**k** treated rats and the errors range from −0.83 to 0.85 mg. The calculated weights are so approximate to the tested weights means that Equation (1) is practical to accurately predict the thrombus weights of *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]-*N′*-(amino-acid-acyl)hydrazine treated rats.

5. Conclusions

The stretching conformation of amino acid modified *3S*-tetrahydro-β-carboline-3-carboxylic acids profoundly benefits the *in vivo* anti-thrombotic activity and is characterized by the smaller proximity of the side chain of the amino acid residue to the carboline-cycle. The insertion of a hydrazine toward (*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carbonylamino acids decrease the proximity of the side chain of the amino acid residue to the carboline-cycle. Comparing to the known *3S*-tetrahydro-β-carboline-3-carbonylamino acids and (*3S,12aS*)-3-(aminoacid-side-chanin)-2,3,6,7,12,12a-hexahydropyrazino[10,20:1,6]pyrido[3,4-b] indole-1,4-diones these novel *N*-[(*1S,3S*)-1-methyl-1,2,3,4-

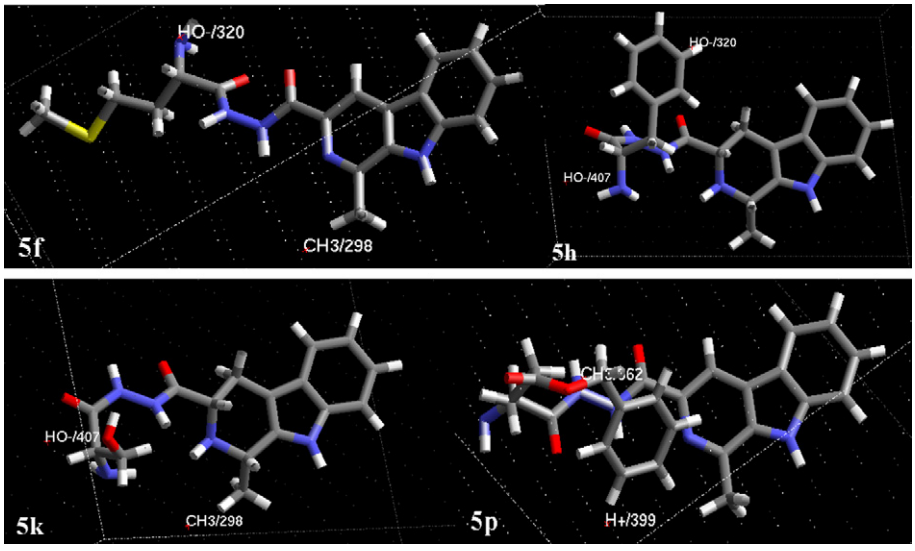


Fig. 4. Electrostatic and environments of **5f**,**h**,**k**,**p** within the grid with 3D points of Equation (1).

Table 4
Predicted and tested thrombus weight of **5h**,**k** treated rats.

Compd.	Thrombus weight (mg)			
	Predicted	Tested	Error	Error%
5h	20.17	21.00	−0.83	−3.95
5k	19.89	19.04	0.85	4.46

tetrahydro-β-carboline-3-carbonyl]-*N*′-(amino-acid-acyl)hydrazine (**5a**–**r**) have smaller proximity and 50-fold–500-fold higher *in vivo* anti-thrombotic activities.

The proximity of the side chain of the amino acid residue to the carboline-cycle is also of critical importance for the *in vivo* anti-thrombotic activity of **5a**–**r** themselves. Fig. 4 clearly shows this importance, in **5f** and **5k** the proximity of the side chains to the carboline-cycle is smaller, while in **5h** and **5p** the proximity of the side chains to the carboline-cycle is larger, and consequently **5f** and **5k** have higher *in vivo* anti-thrombotic activity, **5h** and **5p** have lower *in vivo* anti-thrombotic activity. Therefore the proximity of the side chain of the amino acid residue to the carboline-cycle can be used to predict the *in vivo* anti-thrombotic activity of β-carbolines.

6. Experimental protocols

6.1. General

The protected amino acids with L-configuration were purchased from Sigma Chemical Co. All coupling and deprotective reactions were carried out under anhydrous conditions. Chromatography was performed on Qingdao silica gel H. The purities of the intermediates and the products were confirmed on thin layer chromatography 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). ^{1}H NMR and ^{13}C NMR spectra were recorded by Bruker Advance 300 and 500 spectrometers. FAB-MS was determined by VG-ZAB-MS high resolution GC/MS/DS and HP ES-5989x. Optical rotations were determined with a Schmidt + Haensch Polartromic D instrument. The statistical analysis of all the biological date was carried out by use of ANOVA test with $p < 0.05$ as significant cutoff.

6.2. Synthesis

6.2.1. (1S,3S)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (**1**)

To the mixture of 2.0 g (9.9 mmol) of L-tryptophan, 200 ml of water, 0.2 ml of concentrated H_2SO_4 and 2 ml of ethyl aldehyde (40%) were successively added. The reaction mixture was stirred at room temperature for 8 h and TLC (CH_2Cl_2/CH_3OH, 10:1) indicated the complete disappearance of L-tryptophan. The reaction mixture was adjusted to pH 6–7 with concentrated aqueous solution of ammonia and kept at 4 °C for 2 h. The formed precipitates were collected by filtration and washed with water to give 1.8 g (79%) of the title compound as colorless powders. Mp 287–289 °C; ESI/MS: 231 $[M + H]^+$; IR (KBr): 3101-2405, 2962, 2905, 1703, 1624, 1595, 1506, 1453, 1376, 1072, 904 cm^{-1} ^{1}H NMR (500 MHz, DMSO-d_6) δ/ppm = 11.92 (s, 1 H), 10.97 (s, 1 H), 9.17 (s, 1 H), 7.45 (d, J = 7.5 Hz, 1 H), 7.36 (t, J = 8.0 Hz, 1 H), 7.10 (t, J = 8.0 Hz, 1 H), 7.01 (t, J = 7.5 Hz, 1 H), 4.22 (q, J = 4.8 Hz, 1 H), 3.66 (dd, J = 10.5 Hz, J = 5.0 Hz, 1 H), 3.14 (dd, J = 10.5 Hz, J = 2.4 Hz, 1 H), 2.85 (ddd, J = 10.5 Hz, J = 5.0 Hz, J = 2.4 Hz, 1 H), 1.38 (d, J = 5.0 Hz, 3 H).

6.2.2. (1S,3S)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid methyl ester (**2**)

At 0 °C and with stirring to 30 ml of methanol 1.2 ml of $SOCl_2$ was added. This mixture was stirred for 40 min and then was mixed with 2.0 g (8.7 mmol) of (*1S,3S*)-1-methyl-1,2,3,4-Tetrahydro-β-carboline-3-carboxylic acid (**1**). The reaction mixture was stirred at room temperature for 12 h and TLC (CH_2Cl_2/CH_3OH, 10:1) indicated the complete disappearance of **1**. The reaction mixture was neutralized with aqueous sodium carbonate and then kept at 4 °C for 2 h. The formed precipitates were collected by filtration and washed with water to give 1.9 g (89%) of the title compound as yellowing powders. Mp 75–76 °C; ESI-MS (*m*/*z*) 245 $[M + H]^+$; IR (KBr): 3400, 3204, 2961, 2903, 2814, 1745, 1622, 1594, 1505, 1453, 1384, 1062, 895 cm^{-1} ^{1}H NMR (500 MHz, $CDCl_3$) δ/ppm = 10.06 (s, 1 H), 7.44 (t, J = 6.4 Hz, 1 H), 7.21 (t, J = 8.6 Hz, 1 H), 7.13 (d, J = 7.5 Hz, 1 H), 7.02 (d, J = 6.6 Hz, 1 H), 4.31 (q, J = 6.6 Hz, 1 H), 3.79 (s, 3 H), 3.75 (m, J = 6.9 Hz, 1 H), 3.10 (d, J = 11.2 Hz, 1 H), 2.81 (t, J = 11.2 Hz, 1 H), 2.27 (s, 1 H), 1.44 (d, J = 6.0 Hz, 3 H).

6.2.3. N-[(1S,3S)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]-hydrazine (**3**)

At room temperature with stirring to the solution of 1.0 g (4.1 mmol) of (*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid methyl ester (**2**) in 5 ml of chloroform 4 ml (80.0 mmol, 85%) of hydrazine hydrate was added. The reaction mixture was stirred at 60 °C for 1 h and TLC (CH_2Cl_2/CH_3OH, 10:1) indicated the complete disappearance of **2**. The reaction mixture was evaporated under reduced pressure and the formed residue was dissolved in 50 ml of ethyl acetate. The solution was washed successively with 5% sodium bicarbonate, 5% citric acid, and saturated sodium chloride, and the organic phase was separated and dried over anhydrous sodium sulfate. After filtration and evaporation under reduced pressure, the title compound was provided as colorless powders, 720 mg (72%). Mp 176–177 °C; ESI-MS (*m*/*z*): 245 $[M + H]^+$; $[\alpha]_D^{20} = -103.13$ (*c* 1.01, CH_3OH); IR (cm^{-1}) 3326, 3277, 3019, 2978, 2913, 2843, 1650, 1605, 1687, 1552, 1572, 1454, 1389, 1311, 1204, 1123, 996, 910, 751; ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm = 10.75 (s, 1 H), 9.05 (s, 1 H), 7.37 (d, J = 7.5 Hz, 1 H), 7.30 (m, 2 H), 7.03 (t, J = 7.5 Hz, 1 H), 6.95 (t, J = 7.5 Hz, 1 H), 4.28 (s, 1 H), 4.05 (m, 1 H), 3.45 (dd, J = 10.8 Hz, J = 3.9 Hz, 1 H), 2.86 (dd, J = 15.0 Hz, J = 4.2 Hz, 1 H), 2.60 (m, 1 H), 2.07 (s, 1 H), 1.42 (d, J = 6.6 Hz, 3 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 172.4, 138.3, 136.3, 127.4, 120.9, 118.8, 111.4, 106.7, 65.3, 56.6, 48.7, 26.2, 20.5; Anal. Calcd for $C_{13}H_{16}N_4O$: C, 63.91; H, 6.60; N, 22.93. Found: C, 63.70; H, 6.45; N, 22.71.

6.2.4. N-[(1S,3S)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-N′-(Boc-glycyl)-hydrazine (**4a**)

At 0 °C to the solution of 394 mg (2.25 mmmol) of Boc-Gly, 304 mg (2.25 mmol) of HOBt, 507 mg (2.46 mmol) of DCC and 10 ml of anhydrous THF the solution of 500 mg (2.05 mmol) of *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]hydrazine (**3**) and 10 ml of anhydrous THF was added. The reaction solution was adjusted to pH 9.5 with 0.6 ml of *N*-methylmorpholine, stirred at 0 °C for 1 h and at room temperature for 12 h and TLC (chloroform/methanol, 15:1) indicated the complete disappearance of **3**. The reaction mixture was filtered to remove the resultant precipitates. The filtrate was evaporated under reduced pressure to remove the solvents. The residue was dissolved in 40 mL of ethyl acetate and washed successively with 5% sodium bicarbonate, 5% citric acid, and saturated sodium chloride, and the organic phase was separated and dried over anhydrous sodium sulfate. After filtration and evaporation under reduced pressure the residue was purified on a chromatographic column (CH_2Cl_2/CH_3OH, 20:1) to provide 576 mg (70%) of the title compound as yellowing powders. Mp 146–47 °C; ESI-MS (*m*/*z*): 402 $[M + H]^+$; $[\alpha]_D^{20} = -55.77$ (*c* 1.1, CH_3OH); IR (cm^{-1}) 3350, 3248, 2978, 2929, 1695, 1621, 1491, 1450, 1368, 1290, 1245, 1164, 1049, 861, 743; ^{1}H NMR (300 MHz, DMSO-

d_6) δ/ppm = 10.77 (s, 1 H), 9.94 (s, 2 H), 7.37 (d, J = 7.5 Hz, 1 H), 7.30 (d, J = 7.8 Hz, 1 H), 7.01 (m, 2 H), 6.93 (t, J = 6.9 Hz, 1 H), 4.08 (m, 1 H), 3.64 (d, J = 6.0 Hz, 1 H), 3.57 (dd, J = 10.8 Hz, J = 3.9 Hz, 1 H), 2.88 (dd, J = 15.0 Hz, J = 3.0 Hz, 1 H), 2.63 (t, J = 13.2 Hz, 1 H), 2.55 (m, 2 H), 1.41 (m, 12 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 171.7, 168.6, 156.2, 138.3, 136.3, 127.3, 121.0, 118.8, 117.8, 111.4, 106.5, 78.4, 60.2, 56.3, 48.7, 28.6, 26.3, 20.4. Anal. Calcd for $C_{20}H_{27}N_5O_4$: C, 59.84; H, 6.78; N, 17.44. Found: C, 59.65; H, 6.94; N, 17.66.

6.2.5. *N-[(1S,3S)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-N′-(Boc-alanyl)-hydrazine (**4b**)*

Using a procedure similar to that of preparing *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-*N′*-(Boc-glycyl)-hydrazine (**4a**) from 500 mg (2.05 mmol) of *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]hydrazine (**3**) and 426 mg (2.25 mmol) of Boc-L-Ala 623 mg (73%) of the title compound were obtained as yellowing powders. Mp 128–130 °C; ESI-MS (*m/z*): 416 [M + H]$^+$; $[\alpha]_D^{20} = -97.17$ (*c* 1.3, CH_3OH); IR (cm^{-1}) 3317, 2978, 2929, 1687, 1503, 1454, 1368, 1315, 1249, 1164, 1044, 857, 743; ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm = 10.76 (s, 1 H), 9.95 (s, 1 H), 7.89 (m, 2 H), 7.38 (d, J = 7.5 Hz, 1 H), 7.30 (d, J = 7.5 Hz, 1 H), 7.37 (t, J = 6.9 Hz, 1 H), 6.95 (t, J = 7.5 Hz, 1 H), 4.07 (m, 2 H), 3.57 (dd, J = 10.8 Hz, J = 4.2 Hz, 1 H), 2.88 (dd, J = 15.0 Hz, J = 2.7 Hz, 1 H), 2.65 (m, 1 H), 1.41 (m, 12 H), 1.25 (d, J = 6.9 Hz, 3 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 171.7, 170.7, 155.7, 138.3, 136.3, 130.7, 127.4, 120.9, 118.8, 117.8, 111.4, 106.5, 78.4, 56.3, 48.7, 41.5, 28.6, 26.3, 23.4, 20.4, 14.5. Anal. Calcd for $C_{21}H_{29}N_5O_4$: C, 60.71; H, 7.04; N, 16.86. Found: C, 60.92; H, 7.20; N, 16.63.

6.2.6. *N-[(1S,3S)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-N′-(Boc-isoleu-cyl)hydrazine (**4c**)*

Using a procedure similar to that of preparing *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-*N′*-(Boc-glycyl)-hydrazine (**4a**) from 500 mg (2.05 mmol) of *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]-hydrazine (**3**) and 521 mg (2.25 mmol) of Boc-L-Ile 558 mg (60%) of the title compound were obtained as colorless powders. Mp 137–139 °C; ESI-MS (*m/z*): 458 [M + H]$^+$; $[\alpha]_D^{20} = -87.30$ (*c* 1.2, CH_3OH); IR (cm^{-1}): 3333, 2970, 2933, 2876, 1691, 1625, 1499, 1458, 1368, 1315, 1249, 1164, 1045, 1012, 743; ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm = 10.79 (s, 1 H), 10.02 (s, 2 H), 7.37 (d, J = 7.5 Hz, 1 H), 7.30 (m, 2 H), 7.04 (t, J = 6.9 Hz, 1 H), 6.96 (t, J = 6.9 Hz, 1 H), 6.76 (t, J = 6.0 Hz, 1 H), 4.09 (d, 1 H), 3.94 (t, J = 8.4 Hz, 1 H), 3.56 (dd, J = 10.5 Hz, J = 3.6 Hz, 1 H), 2.88 (dd, J = 15.0 Hz, J = 3.0 Hz, 1 H), 2.63 (t, J = 12.6 Hz, 1 H), 1.69 (m, 2 H), 1.39 (m, 13 H), 0.92 (d, J = 6.9 Hz, 3 H), 0.84 (t, J = 7.5 Hz, 3 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 171.6, 170.6, 155.7, 138.2, 136.2, 127.3, 121.0, 118.8, 117.8, 111.4, 106.5, 78.4, 57.6, 56.5, 48.6, 36.9, 28.6, 26.3, 24.9, 20.4, 15.6, 11.3; Anal. Calcd for $C_{24}H_{35}N_5O_4$: C, 63.00; H, 7.71; N, 15.31. Found: C, 62.81; H, 7.66; N, 15.54.

6.2.7. *N-[(1S,3S)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-N′-(Boc-leucyl)-hydrazine (**4d**)*

Using a procedure similar to that of preparing *N*-[(*1S,3S*)-1-methyl-1,2,3,4- tetrahydro-β-caboline-3-carbonyl]-*N′*-(Boc-glycyl)-hydrazine (**4a**) from 500 mg (2.05 mmol) of *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]-hydrazine (**3**) and 521 mg (2.25 mmol) of Boc-L-Leu 784 mg (84%) of the title compound were obtained as colorless powders. Mp 173–173 °C; ESI-MS (*m/z*) 458 [M + H]$^+$; $[\alpha]_D^{20} = -82.07$ (*c* 1.1, CH_3OH); IR (cm^{-1}): 3329, 2958, 2933, 2872, 1695, 1662, 1499, 1454, 1388, 1367, 1318, 1277, 1253, 1163, 1045, 857, 743. ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm = 10.76 (s, 1 H), 9.98 (s, 2 H), 7.43 (d, J = 7.5 Hz, 1 H), 7.37 (d, J = 7.5 Hz, 1 H), 7.03 (t, J = 6.9 Hz, 1 H), 6.95 (t, J = 7.5 Hz, 1 H), 6.86 (d, J = 8.4 Hz, 1 H), 4.07 (m, 2 H), 3.57 (d, J = 7.2 Hz, 1 H), 2.88 (dd, J = 15.0 Hz, J = 2.7 Hz, 1 H), 2.65 (m, 2 H), 1.71 (m, 1 H), 1.41 (m, 14 H), 0.95 (t, J = 6.6 Hz, 6 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 171.7, 170.7, 155.7, 138.3, 136.3, 130.7, 127.4, 120.9, 118.8, 117.8, 111.4, 106.5, 78.4, 60.2, 56.3, 48.7, 41.5, 28.6, 26.3, 23.4, 20.4, 14.5. Anal. Calcd for $C_{24}H_{35}N_5O_4$: C, 63.00; H, 7.71; N, 15.31. Found: C, 62.81; H, 7.55; N, 15.54.

6.2.8. *N-[(1S,3S)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-N′-(Boc-valyl)-hydrazine (**4e**)*

Using a procedure similar to that of preparing *N*-[(*1S,3S*)-1-methyl-1,2,3,4- tetrahydro-β-caboline-3-carbonyl]-*N′*-(Boc-glycyl)-hydrazine (**4a**) from 500 mg (2.05 mmol) of *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]-hydrazine (**3**) and 489 mg (2.25 mmol) of Boc-L-Val 776 mg (86%) of the title compound were obtained as colorless powders. Mp 183–184 °C; ESI-MS (*m/z*) 444 [M + H]$^+$; $[\alpha]_D^{20} = -83.77$ (*c* 1.0, CH_3OH); IR (cm^{-1}): 3432, 3329, 3211, 2970, 2929, 2847, 1679, 1613, 1527, 1478, 1364, 1315, 1294, 1241, 1160, 1021, 751; ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm = 10.76 (s, 1 H), 9.97 (s, 1 H), 9.86 (s, 1 H), 7.37 (d, J = 7.5 Hz, 1 H), 7.29 (d, J = 7.5 Hz, 1 H), 7.05 (t, J = 6.9 Hz, 1 H), 6.95 (t, J = 6.9 Hz, 1 H), 6.69 (d, J = 9.0 Hz, 1 H), 4.09 (d, J = 5.7 Hz, 1 H), 3.88 (t, J = 8.1 Hz, 1 H), 3.57 (d, J = 10.5 Hz, 1 H), 2.88 (dd, J = 2.7 Hz, J = 15.0 Hz, 1 H), 2.65 (m, 1 H), 1.96 (m, 2 H), 1.41 (m, 12 H), 0.95 (d, J = 6.9 Hz, 3 H), 0.90 (d, J = 6.9 Hz, 3 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 171.7, 170.5, 155.8, 138.3, 136.3, 130.7, 127.4, 120.9, 118.8, 117.8, 111.4, 106.5, 78.4, 58.7, 56.3, 48.7, 31.0, 28.6, 26.3, 20.4, 19.6, 18.8. Anal. Calcd for $C_{23}H_{33}N_5O_4$: C, 62.28; H, 7.50; N, 15.79. Found: C, 62.07; H, 7.34; N, 15.98.

6.2.9. *N-[(1S,3S)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-N′-(Boc-methio-nyl)hydrazine (**4f**)*

Using a procedure similar to that of preparing *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-*N′*-(Boc-glycyl)-hydrazine (**4a**) from 500 mg (2.05 mmol) of *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]-hydrazine (**3**) and 561 mg (2.25 mmol) of Boc-L-Met 591 mg (61%) of the title compound were obtained as colorless powders. Mp 126–127 °C; ESI-MS (*m/z*) 476 [M + H]$^+$; $[\alpha]_D^{20} = -64.03$ (*c* 1.4, CH_3OH); IR (cm^{-1}): 3321, 2978, 2921, 2851, 1687, 1499, 1454, 1368, 1311, 1245, 1164, 1049, 1017, 865, 743; ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm = 10.77 (s, 1 H), 9.99 (s, 2 H), 7.37 (d, J = 7.8 Hz, 1 H), 7.30 (d, J = 7.8 Hz, 1 H), 7.03 (t, 2 H), 6.95 (t, J = 7.5 Hz, 1 H), 4.04 (m, 2 H), 3.57 (d, J = 7.2 Hz, 3 H), 2.90 (d, J = 12.3 Hz, 1 H), 2.62 (d, 1 H), 2.55 (m, 3 H), 2.07 (s, 1 H), 1.86 (m, 2 H), 1.41(m, 12 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 171.8, 171.1, 155.7, 138.3, 136.3, 127.3, 121.0, 118.8, 117.8, 111.4, 106.5, 78.6, 60.2, 56.3, 48.7, 32.4, 30.0, 28.6, 26.3, 20.4, 15.1. Anal. Calcd for $C_{23}H_{33}N_5O_4S$: C, 58.08; H, 6.99; N, 14.73. Found: C, 57.86; H, 6.82; N, 14.96.

6.2.10. *N-[(1S,3S)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-N′-(Boc-prolyl)-hydrazine (**4g**)*

Using a procedure similar to that of preparing ***N-***[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-*N′*-(Boc-glycyl)-hydrazine (**4a**) from 500 mg (2.05 mmol) of *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]-hydrazine (**3**) and 485 mg (2.25 mmol) of Boc-L-Pro 655 mg (72%) of the title compound were obtained as colorless powders. Mp 117–118 °C; ESI-MS (*m/z*) 442 [M + H]$^+$; $[\alpha]_D^{20} = -110.17$ (*c* 1.3, CH_3OH); IR (cm^{-1}): 3284, 2974, 2929, 2876, 1679, 1478, 1454, 1409, 1368, 1315, 1254, 1164, 1045, 739; ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm = 8.92 (s, 1 H), 8.18 (s, 1 H), 7.44 (d, J = 7.5 Hz, 1 H), 7.30 (d, J = 7.5 Hz, 1 H), 7.17 (t, J = 6.9 Hz, 1 H), 7.08 (t, J = 6.9 Hz, 1 H), 6.69 (d, J = 9.0 Hz, 1 H), 4.41 (s, 1 H), 4.18 (m, 1 H), 3.68

(m, 1 H), 3.47 (m, 2 H), 3.20 (dd, J = 15.6 Hz, J = 3.0 Hz, 1 H), 2.75 (m, 2 H), 2.37 (m, 1 H), 1.89 (m, 3 H), 1.49 (m, 12 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 171.1, 169.6, 136.7, 135.9, 127.2, 121.8, 119.6, 118.1, 110.9, 107.9, 80.9, 60.4, 56.9, 49.2, 47.1, 28.4, 25.2, 21.0, 20.1, 14.2; Anal. Calcd for $C_{23}H_{31}N_5O_4$: C, 62.57; H, 7.08; N, 15.86. Found: C, 62.79; H, 7.23; N, 16.07.

6.2.11. *N-[(1S,3S)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-N′-(Boc-phenyla-lanyl)hydrazine (**4h**)*

Using a procedure similar to that of preparing *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-*N′*-(Boc-glycyl)-hydrazine (**4a**) from 500 mg (2.05 mmol) of *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]hydrazine (**3**) and 598 mg (2.25 mmol) of Boc-L-Phe 727 mg (72%) of the title compound were obtained as colorless powder. R_f = 0.41 (CH_2Cl_2: MeOH, 15 : 1), Mp 138–141 °C; ESI-MS (m/z) 492 [M + H]$^+$; $[\alpha]_D^{20}$ = −56.70 (*c* 1.0, MeOH); IR (cm^{-1}) 3333, 3231, 3060, 3027, 2974, 2925, 2859, 1711, 1683, 1625, 1527, 1495, 1454, 1368, 1319, 1278, 1254, 1164, 1025, 1025, 743; ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm = 10.79 (s, 1 H), 10.22 (s, 2 H), 7.35 (m, 6 H), 7.23 (d, J = 6.9 Hz, 1 H), 6.95 (m, 3 H), 4.30 (t, J = 7.5 Hz, 1 H), 4.10 (d, J = 6.6 Hz, 1 H), 3.60 (dd, J = 4.2 Hz, J = 10.5 Hz, 1 H), 3.04 (dd, J = 3.0 Hz, J = 13.5 Hz, 1 H), 2.88 (dd, J = 3.0 Hz, J = 15.0 Hz, 1 H), 2.80 (t, J = 12.3 Hz, 1 H), 2.66 (m, 2 H), 1.48 (d, J = 6.6 Hz, 3 H), 1.30 (s, 9 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 171.6, 171.0, 170.8, 155.7, 138.5, 138.3, 136.3, 129.7, 128.5, 127.3, 126.7, 121.0, 118.8, 117.9, 111.4, 110.5, 106.6, 78.4, 65.4, 56.4, 48.7, 28.6, 26.3, 20.4; Anal. Calcd for $C_{27}H_{33}N_5O_4$: C, 65.97; H, 6.77; N, 14.25.

6.2.12. *N-[(1S,3S)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-N′-(Boc-tyrosyl)-hydrazine (**4i**)*

Using a procedure similar to that of preparing *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-*N′*-(Boc-glycyl)-hydrazine (**4a**) from 500 mg (2.05 mmol) of *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]hydrazine (**3**) and 634 mg (2.25 mmol) of Boc-L-Tyr 523 mg (50%) of the title compound were obtained as colorless powder. Mp 141–142 °C; ESI-MS (m/z) 508 [M + H]$^+$; $[\alpha]_D^{20}$ = −43.43 (*c* 1.1, CH_3OH); IR (cm^{-1}) 3419, 3313, 3248, 2978, 2933, 2847, 1703, 1683, 1617, 1519, 1478, 1454, 1364, 1315, 1249, 1164, 1053, 824, 743. ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm = 10.79 (s, 1 H), 10.17 (s, 2 H), 9.17 (s, 1 H), 7.41 (d, J = 7.5 Hz, 1 H), 7.30 (d, J = 7.8 Hz, 1 H), 7.15 (d, J = 12.3 Hz, 2 H), 7.04 (d, J = 7.5 Hz, 1 H), 6.96 (d, J = 7.5 Hz, 1 H), 6.88 (d, J = 8.7 Hz, 1 H), 6.67 (d, J = 8.4 Hz, 2 H), 4.16 (m, 1 H), 4.10 (d, J = 6.6 Hz, 1 H), 3.60 (dd, J = 9.9 Hz, J = 3.0 Hz, 1 H), 2.88 (m, 3 H), 2.66 (m, 2 H), 1.44 (d, J = 6.6 Hz, 3 H), 1.32 (s, 9 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 171.5, 171.1, 156.2, 155.7, 154.8, 138.2, 136.3, 136.2, 130.6, 128.5, 127.3, 121.0, 118.8, 117.9, 115.3, 111.4, 106.6, 105.9, 78.4, 65.4, 56.4, 48.7, 37.3, 28.6, 26.2, 20.4. Anal. Calcd for $C_{27}H_{33}N_5O_5$: C, 63.89; H, 6.55; N, 13.80. Found: C, 63.68; H, 6.39; N, 13.57.

6.2.13. *N-[(1S,3S)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-N′-(Boc-threo-nyl)hydrazine (**4j**)*

Using a procedure similar to that of preparing *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-*N′*-(Boc-glycyl)-hydrazine (**4a**) from 500 mg (2.05 mmol) of *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]hydrazine (**3**) and 494 mg (2.25 mmol) of Boc-L-Thr 492 mg (54%) of the title compound were obtained as colorless powder. Mp 136–137 °C; ESI-MS (m/z) 446 [M + H]$^+$; $[\alpha]_D^{20}$ = −75.60 (*c* 1.1, CH_3OH); IR (cm^{-1}): 3415, 3338, 2978, 2929, 2847, 1707, 1683, 1572, 1503, 1491, 1450, 1364, 1315, 1254, 1164, 743; ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm = 10.78 (s, 1 H), 10.01 (s, 2 H), 7.37 (d, J = 7.5 Hz, 1 H), 7.30 (d, J = 7.5 Hz, 1 H), 7.03 (t, J = 7.5 Hz, 1 H), 6.95 (t, J = 7.5 Hz, 2 H), 6.39 (d, J = 8.4 Hz, 1 H), 4.79 (s, 1 H), 4.08 (d, J = 5.7 Hz, 1 H), 3.93(m, 2 H), 3.56 (d, J = 7.2 Hz, 1 H), 2.89 (dd, J = 11.7Hz, J = 3.0Hz, 1 H), 2.63 (t, J = 12.0 Hz, 1 H), 1.44 (m, 12 H), 1.14 (d, J = 6.0 Hz, 3 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 171.7, 169.6, 155.7, 138.3, 136.3, 127.3, 121.0, 118.8, 117.8, 111.4, 106.6, 78.7, 67.4, 65.4, 56.3, 48.7, 28.6, 26.3, 20.4. Anal. Calcd for $C_{22}H_{31}N_5O_5$: C, 59.31; H, 7.01; N, 15.72. Found: C, 59.50; H, 7.17; N, 15.92.

6.2.14. *N-[(1S,3S)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-N′-(Boc-seryl)-hydrazine (**4k**)*

Using a procedure similar to that of preparing *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-*N′*-(Boc-glycyl)-hydrazine (**4a**) from 500 mg (2.05 mmol) of *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]hydrazine (**3**) and 462 mg (2.25 mmol) of Boc-L-Ser 500 mg (57%) of the title compound were obtained as colorless powder. Mp 130–132 °C; ESI-MS (m/z) 432 [M + H]$^+$; $[\alpha]_D^{20}$ = −65.00 (*c* 1.2, CH_3OH); IR (cm^{-1}): 3415, 3338, 2978, 2929, 2851, 1703, 1679, 1568, 1503, 1454, 1364, 1315, 1249, 1164, 1057, 747; ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm = 10.77 (s, 1 H), 10.04 (s, 2 H), 7.37 (d, J = 7.5 Hz, 1 H), 7.30 (d, J = 8.1 Hz, 1 H), 7.05 (t, J = 6.9 Hz, 1 H), 6.95 (t, J = 7.5 Hz, 2 H), 6.69 (d, J = 8.1 Hz, 1 H), 4.07 (m, 2 H), 3.58 (m, 3 H), 2.88 (dd, 1 H), 2.62 (m, 2 H), 1.41 (m, 12 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 171.6, 169.5, 155.6, 138.3, 136.3, 127.3, 121.0, 118.8, 117.8, 111.4, 106.5, 78.7, 62.5, 60.2, 56.3, 48.7, 28.6, 26.3, 20.4. Anal. Calcd for $C_{21}H_{29}N_5O_5$: C, 58.45; H, 6.77; N, 16.23. Found: C, 58.64; H, 6.93; N, 16.01.

6.2.15. *N-[(1S,3S)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-N′-(Boc-trpto-phanyl)hydrazine (**4l**)*

Using a procedure similar to that of preparing *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-*N′*-(Boc-glycyl)-hydrazine (**4a**) from 500 mg (2.05 mmol) of *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]hydrazine (**3**) and 686 mg (2.25 mmol) of Boc-L-Trp 795 mg (73%) of the title compound were obtained as colorless powder. Mp 158–160 °C; ESI-MS (m/z) 531 [M + H]$^+$; $[\alpha]_D^{20}$ = −47.80 (*c* 1.2, CH_3OH); IR (cm^{-1}) 3415, 3358, 3329, 3219, 3055, 2974, 2924, 2871, 1702, 1682, 1625, 1576, 1527, 1494, 1457, 1367, 1318, 1273, 1249, 1163, 743; ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm = 10.83 (s, 1 H), 9.97 (s, 1 H), 7.70 (d, J = 7.8 Hz, 1 H), 7.35 (m, 3 H), 7.24 (s, 1 H), 7.05 (m, 4 H), 6.69 (d, J = 7.8 Hz, 1 H), 4.35 (m, 1 H), 4.11 (d, 1 H), 3.60 (d, 1 H), 3.16 (dd, J = 14.4 Hz, J = 3.9 Hz, 1 H), 2.95 (m, 2 H), 2.67 (t, J = 12.6 Hz, 1 H), 1.44 (d, J = 6.6 Hz, 3 H), 1.33 (s, 12 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 171.6, 171.3, 170.8, 155.6, 138.3, 136.5, 136.3, 127.8, 127.3, 124.4, 121.3, 121.0, 119.0, 118.8, 118.6, 117.9, 111.7, 111.4, 110.5, 106.6, 78.4, 60.2, 56.4, 48.7, 28.6, 26.3, 21.2, 20.4. Anal. Calcd for $C_{29}H_{34}N_6O_4$: C, 65.64; H, 6.46; N, 15.84. Found: C, 65.43; H, 6.31; N, 15.62.

6.2.16. *N-[(1S,3S)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-N′-(di-Boc-histi-dyl)-hydrazine (**4m**)*

Using a procedure similar to that of preparing *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-*N′*-(Boc-glycyl)-hydrazine (**4a**) from 500 mg (2.05 mmol) of *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]hydrazine (**3**) and 801 mg (2.25 mmol) of Boc-L-His(Boc) 640 mg (54%) of the title compound were obtained as colorless powder. Mp 154–155 °C; ESI-MS (m/z) 582 [M + H]$^+$; $[\alpha]_D^{20}$ = −36.57 (*c* 1.2, CH_3OH); IR (cm^{-1}) 3346, 3190, 3060, 2978, 2929, 1756, 1695, 1609, 1491, 1458, 1392, 1294, 1254, 1155, 1049, 1012, 841, 743; ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm = 10.79 (s, 1 H), 10.12 (s, 2 H), 8.12 (s, 1 H), 7.37 (d, J = 7.5 Hz, 1 H), 7.30 (d, J = 6.9 Hz, 1 H), 7.03 (t, J = 7.5 Hz, 1 H), 6.96 (t, J = 6.9 Hz, 2 H), 4.35 (m, 1 H), 4.08 (d, J = 6.3 Hz, 1 H), 3.56 (d, J = 6.9 Hz, 1 H), 2.88 (m, 3 H), 2.63 (t, J = 12.3 Hz, 1 H), 1.56 (m, 11 H), 1.44 (d, J = 6.6 Hz, 3 H), 1.35 (s, 9 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 171.7, 170.8, 155.6, 147.2, 139.8, 138.2, 137.0, 136.3,

127.3, 121.0, 118.8, 117.8, 114.9, 111.4, 106.5, 79.6, 78.6, 56.3, 52.8, 48.6, 31.2, 28.6, 27.8, 26.3, 20.4. Anal. Calcd for $C_{29}H_{39}N_7O_6$: C, 59.88; H, 6.76; N, 16.86. Found: C, 59.67; H, 6.61; N, 17.08.

*6.2.17. N-[(1S,3S)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-N′-(di-Boc-lysyl)-hydrazine (**4n**)*

Using a procedure similar to that of preparing *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-*N′*-(Boc-glycyl)-hydrazine (**4a**) from 500 mg (2.05 mmol) of *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]hydrazine (**3**) and 780 mg (2.25 mmol) of Boc-L-Lys(Boc) 541 mg (46%) of the title compound were obtained as colorless powder. Mp 129–130 °C; ESI-MS (*m/z*) 573 $[M + H]^+$; $[\alpha]_D^{20} = -60.37$ (*c* 1.2, CH_3OH); IR (cm^{-1}) 3342, 2974, 2929, 2864, 1691, 1511, 1458, 1392, 1368, 1315, 1274, 1249, 1168, 1045, 1017, 861, 743; ^1H NMR (300 MHz, DMSO-d_6) δ/ppm = 10.78 (s, 1 H), 10.07 (s, 2 H), 7.37 (d, J = 7.5 Hz, 1 H), 7.30 (d, J = 7.8 Hz, 1 H), 7.03 (t, J = 6.9 Hz, 1 H), 6.95 (t, J = 6.9 Hz, 1 H), 6.86 (d, J = 8.1 Hz, 1 H), 6.74 (s, 1 H), 4.09 (d, J = 6.3 Hz, 1 H), 3.99 (m, 1 H), 3.57 (dd, J = 10.5 Hz, J = 3.9 Hz, 1 H), 2.88 (dd, J = 14.4 Hz, J = 3.3 Hz, 3 H), 2.63 (m, 2 H), 1.51 (m, 27 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 171.6, 156.0, 155.7, 138.2, 136.3, 127.3, 121.0, 118.8, 117.8, 111.4, 106.5, 78.6, 77.8, 56.5, 53.3, 49.0, 48.7, 32.2, 29.6, 28.7, 26.2, 23.2, 20.4. Anal. Calcd for $C_{29}H_{44}N_6O_6$: C, 60.82; H, 7.74; N, 14.67. Found: C, 61.03; H, 7.90; N, 14.91.

*6.2.18. N-[(1S,3S)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-N′-[Boc-(β-OBzl-aspartyl)]hydrazine (**4o**)*

Using a procedure similar to that of preparing *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-*N′*-(Boc-glycyl)-hydrazine (**4a**) from 500 mg (2.05 mmol) of *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]hydrazine (**3**) and 728 mg (2.25 mmol) of Boc-L-Asp(OBzl) 487 mg (43%) of the title compound were obtained as colorless powder. Mp 129–130 °C; ESI-MS (*m/z*) 550 $[M + H]^+$; $[\alpha]_D^{20} = -33.13$ (*c* 1.1, CH_3OH); IR (cm^{-1}) 3354, 3182, 3056, 2978, 2925, 2847, 1711, 1683, 1613, 1519, 1495, 1454, 1392, 1368, 1343, 1274, 1254, 1164, 1049, 1025, 857, 739; ^1H NMR (300 MHz, DMSO-d_6) δ/ppm = 10.76 (s, 1 H), 10.01 (s, 2 H), 7.35 (m, 7 H), 7.16 (d, J = 8.1 Hz, 1 H), 7.03 (d, J = 6.9 Hz, 1 H), 6.93 (d, J = 6.9 Hz, 1 H), 5.16 (s, 2 H), 4.51 (m, 1 H), 4.08 (m, 1 H), 3.56 (dd, J = 10.8 Hz, J = 3.9 Hz, 1 H), 2.86 (m, 2 H), 2.67 (m, 3 H), 1.41 (m, 12 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 173.9, 171.8, 170.3, 155.6, 138.3, 136.6, 128.9, 128.5, 128.2, 127.3, 127.0, 121.0, 118.9, 117.8, 111.4, 106.6, 106.3, 78.8, 66.1, 63.4, 56.3, 48.7, 37.0, 28.7, 26.3, 24.9, 20.4; Anal. Calcd for $C_{29}H_{35}N_5O_6$: C, 63.37; H, 6.42; N, 12.74. Found: C, 63.16; H, 6.27; N, 12.96.

*6.2.19. N-[(1S,3S)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-N′-[Boc-(γ-OBzl-glutamoyl)]hydrazine (**4p**)*

Using a procedure similar to that of preparing *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-*N′*-(Boc-glycyl)-hydrazine (**4a**) from 500 mg (2.05 mmol) of *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]hydrazine (**3**) and 760 mg (2.25 mmol) of Boc-L-Glu(OBzl) 495 mg (50%) of the title compound were obtained as colorless powder. Mp 110–111 °C; ESI-MS (*m/z*) 564 $[M + H]^+$; $[\alpha]_D^{20} = -51.87$ (*c* 1.2, CH_3OH); IR (cm^{-1}) 3329, 3031, 2978, 2929, 2851, 1715, 1642, 1499, 1454, 1392, 1368, 1315, 1249, 1164, 1053, 1025, 861, 743; ^1H NMR (300 MHz, DMSO-d_6) δ/ppm = 10.77 (s, 1 H), 10.00 (s, 2 H), 7.37 (m, 7 H), 7.06 (m, 2 H), 6.93 (t, 1 H), 5.16 (s, 2 H), 4.08 (m, 2 H), 3.57 (d, J = 7.2 Hz, 1 H), 2.88 (dd, J = 15.0 Hz, J = 2.7 Hz, 1 H), 2.63 (t, J = 11.4 Hz, 1 H), 2.55 (m, 3 H), 1.90 (m, 2 H), 1.41(m, 12 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 172.7, 171.8, 170.8, 155.6, 138.3, 136.7, 136.3, 128.9, 128.4, 127.3, 121.0, 118.8, 117.8, 111.4, 106.5, 78.6, 65.9, 56.3, 52.6, 48.7, 30.5, 28.6, 27.8, 26.3, 21.2. Anal. Calcd for $C_{30}H_{37}N_5O_6$: C, 63.93; H, 6.62; N, 12.43. Found: C, 63.71; H, 6.47; N, 12.21.

*6.2.20. N-[(1S,3S)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-N′-(Boc-aspara-ginyl)hydrazine (**4q**)*

Using a procedure similar to that of preparing *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-*N′*-(Boc-glycyl)-hydrazine (**4a**) from 500 mg (2.05 mmol) of *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]hydrazine (**3**) and 523 mg (2.25 mmol) of Boc-L-Asn 292 mg (31%) of the title compound were obtained as colorless powder. Mp 172–174 °C; ESI-MS(*m/z*) 459 $[M + H]^+$; $[\alpha]_D^{20} = -60.23$ (*c* 1.3, CH_3OH); IR (cm^{-1}) 3333, 3182, 3056, 2978, 2933, 1695, 1679, 1613, 1499, 1454, 1392, 1368, 1319, 1270, 1254, 1168, 1053, 1025, 857, 743; ^1H NMR (300 MHz, DMSO-d_6) δ/ppm = 10.82 (s, 1 H), 9.50 (s, 2 H), 7.48 (d, J = 6.3 Hz, 1 H), 7.36 (d, J = 7.5 Hz, 2 H), 7.03 (t, J = 6.9 Hz, 1 H), 6.95 (t, J = 6.6 Hz, 2 H), 4.36 (m, J = 8.1 Hz, 1 H), 4.08 (d, J = 6.3 Hz, 1 H), 4.00 (m, 1 H), 3.53 (dd, J = 10.5 Hz, J = 3.9 Hz, 1 H), 3.39 (m, 1 H), 2.85 (dd, J = 14.7 Hz, J = 2.4 Hz, 1 H), 2.62 (t, J = 13.2 Hz, 1 H), 2.47 (m, 2 H), 1.41 (m, 12 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 171.6, 171.1, 155.5, 138.2, 136.2, 127.3, 121.0, 118.8, 117.8, 111.4, 106.5, 78.6, 56.2, 50.4, 48.6, 37.9, 28.6, 28.4, 26.2, 20.4; Anal. Calcd for $C_{22}H_{30}N_6O_5$: C, 57.63; H, 6.59; N, 18.33. Found: C, 57.84; H, 6.75; N, 18.54.

*6.2.21. N-[(1S,3S)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-N′-(Boc-glutami-nyl)hydrazine (**4r**)*

Using a procedure similar to that of preparing *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-*N′*-(Boc-glycyl)-hydrazine (**4a**) from 500 mg (2.05 mmol) of *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]hydrazine (**3**) and 555 mg (2.25 mmol) of Boc-L-Gln 420 mg (43%) of the title compound were obtained as colorless powder. Mp 147–149 °C; ESI-MS (*m/z*) 473 $[M + H]^+$; $[\alpha]_D^{20} = -71.57$ (*c* 1.2, CH_3OH); IR (cm^{-1}) 3329, 2978, 2925, 2851, 1666, 1642, 1507, 1454, 1392, 1368, 1315, 1249, 1160, 1049, 1025, 857, 743; ^1H NMR (300 MHz, DMSO-d_6) δ/ppm = 10.78 (s, 1 H), 10.00 (s, 2 H), 7.06 (d, J = 7.5 Hz, 1 H), 7.28 (m, 2 H), 7.03 (t, J = 6.9 Hz, 1 H), 6.93 (t, J = 6.6 Hz, 2 H), 6.77 (s, 1 H), 4.10 (d, J = 6.6 Hz, 1 H), 4.00 (m, 1 H), 3.57 (dd, J = 10.8 Hz, J = 3.9 Hz, 1 H), 2.88 (dd, J = 15.0 Hz, J = 3.0 Hz, 1 H), 2.79 (t, J = 6.0 Hz, 1 H), 2.19 (m, 3 H), 1.85 (m, 2 H), 1.41 (m, 12 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 174.3, 171.7, 171.1, 155.6, 138.2, 136.3, 127.3, 121.0, 118.8, 117.8, 111.4, 106.5, 78.6, 65.4, 56.3, 48.7, 31.9, 28.6, 28.4, 26.2, 20.4. Anal. Calcd for $C_{23}H_{32}N_6O_5$: C, 58.46; H, 6.83; N, 17.78. Found: C, 58.67; H, 6.99; N, 17.99.

*6.2.22. N-[(1S,3S)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-N′-glycylhydrazine (**5a**)*

At 0 °C to the solution of 100 mg (0.249 mmol) of *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-*N′*-(Boc-glycyl)-hydrazine (**4a**) in 6 ml hydrogen chloride/ethyl acetate (4 N) were added dropwise. The reaction solution was stirred at 0 °C for 2 h and TLC (chloroform/methanol, 10:1) indicated the complete disappearance of **4a**. The reaction mixture was evaporated under reduced pressure. The residue was dissolved in 5 ml of ethyl acetate and then evaporated under reduced pressure. This procedure was repeated for three times to remove the remained hydrogen chloride. The residue was solidified in 20 ml of anhydrous ether to provide 70 mg (93%) of the title compound as yellowing powders. Mp 229–231 °C; ESI-MS (*m/z*) 302 $[M + H]^+$; $[\alpha]_D^{20} = -43.43$ (*c* 1.2, CH_3OH); IR (cm^{-1}): 3187, 2978, 2463, 1723, 1687, 1572, 1478, 1388, 1318, 1274, 1213, 747; ^1H NMR (300 MHz, DMSO-d_6) δ/ppm = 11.50 (s, 1 H), 11.04 (s, 1 H), 9.71 (s, 1 H), 8.45 (m, 3 H), 7.46 (d, J = 7.8 Hz, 1 H), 7.38 (d, J = 7.8 Hz, 1 H), 7.13 (t, J = 7.5 Hz, 1 H), 7.06 (t, J = 7.5 Hz, 1 H), 4.80 (s, 1 H), 4.40 (s, 1 H), 3.71 (s, 2 H), 3.40 (m, 1 H), 3.00 (t, J = 12.9 Hz, 1 H), 1.73 (d, J = 6.6 Hz, 3 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 167.5, 165.4, 136.9, 131.5, 126.1, 122.2, 119.6, 118.3, 120.0, 104.7, 65.3, 55.1, 50.0,

24.0, 16.8; Anal. Calcd for $C_{15}H_{19}N_5O_2$: C, 59.79; H, 6.36; N, 23.24. Found: C, 59.97; H, 6.51; N, 23.01.

6.2.23. *N-[(1S,3S)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-N′-alanylhydrazine (**5b**)*

Using a procedure similar to that of preparing *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-*N′*-glycylhydrazine (**5a**) from 100 mg (0.241 mmol) of *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-*N′*-(Boc-alanyl) hydrazine (**4b**) 70 mg (92%) of the title compound were obtained as yellowing powders. Mp 201–203 °C; ESI-MS (*m/z*) 316 $[M + H]^+$; $[\alpha]_D^{20} = -34.87$ (*c* 1.1, CH_3OH). IR (cm^{-1}) 3175, 2982, 2925, 2472, 1724, 1687, 1569, 1503, 1450, 1389, 1319, 1270, 1209, 751; ^1H NMR (300 MHz, DMSO-d_6) δ/ppm = 11.50 (s, 1 H), 11.02 (s, 1 H), 9.67 (m, 1 H), 8.56 (m, 3 H), 7.44 (d, J = 7.8 Hz, 1 H), 7.38 (d, J = 8.1 Hz, 1 H), 7.15 (t, J = 7.5 Hz, 1 H), 7.03 (t, J = 7.5 Hz, 1 H), 4.80 (s, 1 H), 4.40 (m, 1 H), 4.00 (s, 1 H), 3.40 (m, 1 H), 3.00 (t, J = 13.2 Hz, 1 H), 1.73 (d, J = 6.6 Hz, 3 H), 1.48 (d, J = 6.9 Hz, 3 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 169.0, 167.6, 136.9, 131.5, 126.1, 122.2, 119.6, 118.3, 112.0, 104.8, 65.3, 55.0, 50.0, 24.0, 17.8, 16.8. Anal. Calcd for $C_{16}H_{21}N_5O_2$: C, 60.94; H, 6.71; N, 22.21. Found: C, 61.15; H, 6.88; N, 22.43.

6.2.24. *N-[(1S,3S)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-N′-isoleucylhydrazine (**5c**)*

Using a procedure similar to that of preparing *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-*N′*-glycylhydrazine (**5a**) from 100 mg (0.219 mmol) of *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-*N′*-(Boc-isoleucyl)hydrazine (**4c**) 72 mg (92%) of the title compound were obtained as yellowing powders. Mp 204–206 °C; ESI-MS (*m/z*): 358 $[M + H]^+$; IR (cm^{-1}) 3170, 2970, 2472, 1724, 1683, 1573, 1499, 1454, 1389, 1319, 1279, 1217, 747. $[\alpha]_D^{20} = -22.10$ (*c* 1.0, CH_3OH); ^1H NMR (300 MHz, DMSO-d_6) δ/ppm = 11.48 (s, 1 H), 11.05 (s, 1 H), 10.90 (s, 1 H), 10.41 (d, J = 8.1 Hz, 1 H), 9.65 (m, J = 9.9 Hz, 1 H), 8.53 (s, 3 H), 7.44 (d, J = 7.8 Hz, 1 H), 7.38 (d, J = 8.1 Hz, 1 H), 7.13 (t, J = 7.5 Hz, 1 H), 7.03 (t, J = 7.5 Hz, 1 H), 4.80 (s, 1 H), 4.40 (m, 1 H), 3.78 (s, 1 H), 3.40 (m, 1 H), 3.00 (t, J = 13.2 Hz, 1 H), 1.93 (m, 1 H) , 1.71 (d, J = 6.6 Hz, 3 H), 1.66 (m, 2 H), 1.33 (m, 2 H), 1.01 (d, J = 6.9 Hz, 1 H), 0.89 (t, J = 6.6 Hz, 1 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 167.6, 166.9, 136.9, 131.5, 126.1, 122.2, 119.6, 118.3, 112.0, 104.8, 65.4, 55.0, 49.8, 36.8, 24.6, 24.0, 16.8, 14.6, 11.8; Anal. Calcd for $C_{19}H_{27}N_5O_2$: C, 63.84; H, 7.61; N, 19.59. Found: C, 63.62; H, 7.45; N, 19.35.

6.2.25. *N-[(1S,3S)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-N′-leucylhydra-zine (**5d**)*

Using a procedure similar to that of preparing *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-*N′*-glycylhydrazine (**5a**) from 100 mg (0.219 mmol) of *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-*N′*-(Boc-leucyl)-hydrazine (**4d**) 71 mg (91%) of the title compound were obtained as yellowing powders. Mp 198–200 °C; ESI-MS (*m/z*) 358 $[M + H]^+$; $[\alpha]_D^{20} = -35.47$ (*c* 1.2, CH_3OH); IR (cm^{-1}): 3174, 2962, 2471, 1723, 1683, 1576, 1499, 1450, 1388, 1319, 1274, 1213, 747; ^1H NMR (300 MHz, DMSO-d_6) δ/ppm = 11.48 (s, 1 H), 11.07 (s, 1 H), 11.02 (s, 1 H), 10.44 (d, J = 5.4 Hz, 1 H), 9.65 (m, J = 8.7 Hz, 1 H), 8.60 (s, 3 H), 7.44 (d, J = 7.8 Hz, 1 H), 7.36 (d, J = 6.3 Hz, 1 H), 7.19 (t, J = 7.5 Hz, 1 H), 7.03 (t, J = 7.5 Hz, 1 H), 4.80 (s, 1 H), 4.40 (m, 1 H), 3.88 (s, 1 H), 3.40 (m, 1 H), 3.00 (t, J = 13.2 Hz, 1 H), 2.10 (m, 2 H), 1.68 (m, 6 H), 0.98 (d, J = 6.6 Hz, 1 H), 0.92 (d, J = 6.6 Hz, 1 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 168.1, 167.5, 136.9, 131.5, 126.1, 122.2, 119.6, 118.3, 112.0, 104.8, 65.4, 55.0, 50.0, 23.9, 23.1, 22.8, 16.8; Anal. Calcd for $C_{19}H_{27}N_5O_2$: C, 63.84; H, 7.61; N, 19.59. Found: C, 63.62; H, 7.55; N, 19.83.

6.2.26. *N-[(1S,3S)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-N′-valylhydra-zine (**5e**)*

Using a procedure similar to that of preparing *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-*N′*-glycylhydrazine (**5a**) from 100 mg (0.226 mmol) of *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-*N′*-(Boc-valyl)-hydrazine (**4e**) 72 mg (93%) of the title compound were obtained as yellowing powders. Mp 209–211 °C; ESI-MS (*m/z*) 344 $[M + H]^+$; $[\alpha]_D^{20} = -23.57$ (*c* 1.3, CH_3OH); IR (cm^{-1}): 3170, 2974, 2929, 2472, 1724, 1687, 1573, 1507, 1454, 1385, 1319, 1279, 1217, 747; ^1H NMR (300 MHz, DMSO-d_6) δ/ppm = 11.51 (s, 1 H), 10.42 (d, J = 8.1 Hz, 1 H), 9.67 (m, J = 9.6 Hz, 1 H), 8.54 (s, 3 H), 7.44 (d, J = 7.8 Hz, 1 H), 7.38 (d, J = 8.1 Hz, 1 H), 7.15 (t, J = 7.5 Hz, 1 H), 7.03 (t, J = 7.5 Hz, 1 H), 4.80 (s, 1 H), 4.40 (m, 1 H), 3.74 (s, 1 H), 3.45 (m, 1 H), 3.00 (t, J = 13.2 Hz, 1 H), 2.18 (m, 1 H), 1.71 (d, J = 6.3 Hz, 3 H), 1.08 (d, J = 6.9 Hz, 6 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 167.6, 167.5, 136.9, 131.5, 126.1, 122.2, 119.6, 118.3, 112.0, 104.8, 65.4, 55.0, 50.0, 30.4, 24.0, 18.6, 16.8; Anal. Calcd for $C_{18}H_{25}N_5O_2$: C, 62.95; H, 7.34; N, 20.39. Found: C, 62.74; H, 7.20; N, 20.61.

6.2.27. *N-[(1S,3S)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-N′-methionylhy-drazine (**5f**)*

Using a procedure similar to that of preparing *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-*N′*-glycylhydrazine (**5a**) from 100 mg (0.211 mmol) of *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-*N′*-(Boc-methionyl)hydrazine (**4f**) 72 mg (91%) of the title compound were obtained as yellowing powders. Mp 200–202 °C; ESI-MS (*m/z*) 376 $[M + H]^+$; $[\alpha]_D^{20} = -11.73$ (*c* 1.2, CH_3OH); IR (cm^{-1}): 3175, 2949, 2468, 1724, 1687, 1569, 1495, 1454, 1433, 1385, 1319, 1270, 1213, 747; ^1H NMR (300 MHz, DMSO-d_6) δ/ppm = 11.48 (s, 1 H), 10.42 (d, J = 7.5 Hz, 1 H), 9.65 (m, J = 9.9 Hz, 1 H), 8.68 (s, 3 H), 7.44 (d, J = 7.5 Hz, 1 H), 7.38 (d, J = 8.1 Hz, 1 H), 7.13 (t, J = 7.5 Hz, 1 H), 7.03 (t, J = 7.5 Hz, 1 H), 4.80 (s, 1 H), 4.40 (m, 1 H), 4.03 (m, 1 H), 3.41 (m, 1 H), 3.00 (t, J = 13.2 Hz, 1 H), 2.68 (m, 2 H), 2.18 (m, 2 H), 2.18 (s, 3 H), 1.72 (d, J = 6.9 Hz, 3 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 167.8, 167.6, 136.9, 131.5, 126.1, 122.2, 119.6, 118.3, 112.0, 104.8, 60.2, 55.0, 49.9, 31.6, 28.2, 24.0, 16.8, 14.6. Anal. Calcd for $C_{18}H_{25}N_5O_2S$: C, 57.58; H, 6.71; N, 18.65. Found: C, 57.80; H, 6.88; N, 18.86.

6.2.28. *N-[(1S,3S)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-N′-prolylhy-drazine (**5g**)*

Using a procedure similar to that of preparing *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-*N′*-glycylhydrazine (**5a**) from 100 mg (0.227 mmol) of *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-*N′*-(Boc-prolyl)-hydrazine (**4g**) 72 mg (94%) of the title compound were obtained as yellowing powders. Mp 234–236 °C; ESI-MS (*m/z*) 342 $[M + H]^+$; $[\alpha]_D^{20} = -60.07$ (*c* 1.2, CH_3OH); IR (cm^{-1}): 3170, 2978, 2921, 2733, 2472, 1724, 1687, 1556, 1454, 1389, 1315, 1274, 1221, 747; ^1H NMR (300 MHz, DMSO-d_6) δ/ppm = 11.48 (s, 1 H), 11.02 (s, 1 H), 10.42 (m, 2 H), 9.68 (m, J = 9.6 Hz, 1 H), 8.76 (s, 1 H), 7.44 (d, J = 7.8 Hz, 1 H), 7.38 (d, J = 8.1 Hz, 1 H), 7.15 (t, J = 7.5 Hz, 1 H), 7.03 (t, J = 7.5 Hz, 1 H), 4.80 (s, 1 H), 4.37 (m, 2 H), 3.43 (m, 3 H), 3.00 (t, J = 13.2 Hz, 1 H), 2.38 (m, 1 H), 1.92 (m, 2 H), 1.72 (d, J = 6.9 Hz, 3 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 167.9, 167.6, 136.9, 131.5, 126.0, 122.2, 119.7, 118.3, 112.0, 104.8, 60.2, 55.0, 49.9, 46.0, 30.3, 24.0, 21.6, 16.8; Anal. Calcd for $C_{18}H_{23}N_5O_2$: C, 63.32; H, 6.79; N, 20.51. Found: C, 63.11; H, 6.65; N, 20.74.

6.2.29. *N-[(1S,3S)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-N′-phenylalanylhydrazine (**5h**)*

Using a procedure similar to that of preparing *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-*N′*-glycylhydrazine

(**5a**) from 100 mg (0.204 mmol) of *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-*N′*-(Boc-phenyl-alanyl)hydrazine (**4h**) 73 mg (92%) of the title compound were obtained as yellowing powders. Mp 185–187 °C; ESI-MS (*m/z*): 392 $[M + H]^+$; $[\alpha]_D^{20} = -4.03$ (*c* 1.3, CH_3OH); IR (cm^{-1}) 3178, 2978, 2925, 2472, 1724, 1687, 1577, 1499, 1450, 1389, 1319, 1274, 1213, 747; ^1H NMR (300 MHz, DMSO-d_6) δ/ppm = 11.49 (s, 1 H), 10.45 (s, 1 H), 9.69 (s, 1 H), 8.56 (s, 3 H), 7.38 (m, 7 H), 7.13 (t, *J* = 7.5 Hz, 1 H), 7.03 (t, *J* = 7.5 Hz, 1 H), 4.80 (s, 1 H), 4.39 (m, 1 H), 4.21 (m, 1 H), 3.39 (m, 1 H), 3.25 (dd, *J* = 13.8 Hz, *J* = 6.0 Hz, 1 H), 3.14 (m, *J* = 6.9 Hz, 1 H), 3.00 (t, *J* = 13.2 Hz, 1 H), 1.72 (d, *J* = 6.9 Hz, 3 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 167.4, 167.2, 136.9, 135.1, 131.5, 130.3, 128.9, 127.6, 126.1, 122.2, 119.6, 118.3, 112.0, 104.8, 65.3, 55.0, 49.9, 24.0, 16.8. Anal. Calcd for $C_{22}H_{25}N_5O_2$: C, 67.50; H, 6.44; N, 17.89. Found: C, 67.68; H, 6.59; N, 18.11.

*6.2.30. N-[(1S,3S)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-N′-tyrosylhydra-zine (**5i**)*

Using a procedure similar to that of preparing *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-*N′*-glycylhydrazine (**5a**) from 100 mg (0.197 mmol) of *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-*N′*-(Boc-tyrosyl)-hydrazine (**4i**) 73 mg (90%) of the title compound were obtained as yellowing powders. Mp 216–218 °C; ESI-MS (*m/z*) 408 $[M + H]^+$; $[\alpha]_D^{20} = 3.17$ (*c* 1.2, CH_3OH); IR (cm^{-1}) 3175, 2983, 2929, 2472, 1720, 1695, 1614, 1516, 1450, 1385, 1319, 1270, 1230, 751; ^1H NMR (300 MHz, DMSO-d_6) δ/ppm = 11.48 (s, 1 H), 11.04 (s, 1 H), 10.42 (d, *J* = 7.2 Hz, 1 H), 8.45 (s, 3 H), 7.46 (d, *J* = 7.8 Hz, 1 H), 7.38 (d, *J* = 7.8 Hz, 1 H), 7.20 (d, *J* = 7.8 Hz, 2 H), 7.13 (t, *J* = 7.5 Hz, 1 H), 7.03 (t, *J* = 7.5 Hz, 1 H), 6.75 (d, *J* = 8.4 Hz, 3 H), 4.80 (s, 1 H), 4.39 (m, 1 H), 4.10 (s, 1 H), 3.49 (m, 1 H), 3.15 (dd, *J* = 14.1 Hz, *J* = 5.7 Hz, 1 H), 2.99 (m, 2 H), 1.72 (d, *J* = 6.6 Hz, 3 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 167.4, 167.3, 157.1, 136.9, 131.5, 131.2, 126.1, 124.9, 122.2, 119.6, 118.3, 115.8, 112.0, 103.8, 65.3, 55.0, 49.9, 36.6, 24.0, 16.8. Anal. Calcd for $C_{22}H_{25}N_5O_3$: C, 64.85; H, 6.18; N, 17.19. Found: C, 64.64; H, 6.03; N, 17.42.

*6.2.31. N-[(1S,3S)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-N′-threonylhydrazine (**5j**)*

Using a procedure similar to that of preparing *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-*N′*-glycylhydrazine (**5a**) from 100 mg (0.225 mmol) of *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-*N′*-(Boc-threonyl)-hydrazine (**4j**) 71 mg (92%) of the title compound were obtained as yellowing powders. Mp 221–223 °C; ESI-MS (*m/z*) 346 $[M + H]^+$; $[\alpha]_D^{20} = -40.10$ (*c* 1.1, CH_3OH); IR (cm^{-1}) 3195, 2982, 2933, 2472, 1724, 1683, 1577, 1503, 1458, 1389, 1319, 1279, 1217, 751; ^1H NMR (300 MHz, DMSO-d_6) δ/ppm = 11.50 (s, 1 H), 10.45 (s, 1 H), 9.66 (s, 1 H), 8.45 (s, 3 H), 7.44 (d, *J* = 7.2 Hz, 1 H), 7.38 (d, *J* = 7.8 Hz, 1 H), 7.15 (t, *J* = 7.5 Hz, 1 H), 7.03 (t, *J* = 7.5 Hz, 1 H), 5.70 (s, 1 H), 4.78 (s, 1 H), 4.38 (s, 1 H), 3.99 (m, 1 H), 3.71 (d, *J* = 7.2 Hz, 1 H), 3.41 (m, 1 H), 3.00 (t, *J* = 13.2 Hz, 1 H), 1.72 (d, *J* = 6.6 Hz, 3 H), 1.39 (d, *J* = 6.0 Hz, 3 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 167.6, 166.2, 136.9, 131.5, 126.1, 122.2, 119.6, 118.3, 112.0, 104.8, 66.5, 65.3, 55.0, 49.9, 24.0, 20.1, 16.8; Anal. Calcd for $C_{17}H_{23}N_5O_3$: C, 59.12; H, 6.71; N, 20.28. Found: C, 58.93; H, 6.57; N, 20.52.

*6.2.32. N-[(1S,3S)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-N′-serylhydrazi-ne (**5k**)*

Using a procedure similar to that of preparing *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-*N′*-glycylhydrazine (**5a**) from 100 mg (0.232 mmol) of *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-*N′*-(Boc-seryl)-hydrazine (**4k**) 71 mg (92%) of the title compound were obtained as yellowing powders. Mp 226–228 °C; ESI-MS (*m/z*) 332 $[M + H]^+$; $[\alpha]_D^{20} = -46.33$ (*c* 1.2, CH_3OH); IR (cm^{-1}) 3187, 2982, 2937, 2476, 1724, 1687, 1573, 1503, 1450, 1385, 1319, 1274, 1217, 751; ^1H NMR (300 MHz, DMSO-d_6) δ/ppm = 11.48 (s, 1 H), 10.40 (s, 1 H), 9.68 (s, 1 H), 8.48 (s, 3 H), 7.44 (d, *J* = 7.5 Hz, 1 H), 7.38 (d, *J* = 8.1 Hz, 1 H), 7.15 (t, *J* = 7.5 Hz, 1 H), 7.05 (t, *J* = 7.5 Hz, 1 H), 5.62 (s, 1 H), 4.81 (s, 1 H), 4.40 (s, 1 H), 4.04 (s, 1 H), 3.90 (dd, *J* = 11.4 Hz, *J* = 3.9 Hz, 1 H), 3.78 (m, 1 H), 3.43 (m, 1 H), 3.00 (t, *J* = 13.2 Hz, 1 H), 1.73 (d, *J* = 6.9 Hz, 3 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 167.3, 166.2, 136.9, 131.5, 126.1, 122.2, 119.6, 118.3, 112.0, 104.8, 65.3, 60.9, 55.0, 50.0, 24.0, 16.8. Anal. Calcd for $C_{16}H_{21}N_5O_3$: C, 57.99; H, 6.39; N, 21.13. Found: C, 58.18; H, 6.55; N, 20.90.

*6.2.33. N-[(1S,3S)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-N′-tryptophanyl-hydrazine (**5l**)*

Using a procedure similar to that of preparing *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-*N′*-glycylhydrazine (**5a**) from 100 mg (0.189 mmol) of *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-*N′*-(Boc-trptophanyl)hydrazine (**4l**) 74 mg (91%) of the title compound were obtained as yellowing powders. Mp 213–215 °C; ESI-MS (*m/z*) 431 $[M + H]^+$; $[\alpha]_D^{20} = -9.07$ (*c* 1.1, CH_3OH); IR (cm^{-1}): 3399, 3191, 2982, 2925, 2472, 1724, 1691, 1577, 1491, 1454, 1434, 1385, 1319, 1274, 1234, 747; ^1H NMR (300 MHz, DMSO-d_6) δ/ppm = 11.49 (s, 1 H), 11.12 (s, 1 H), 10.51 (s, 1 H), 9.70 (s, 1 H), 8.45 (s, 3 H), 7.83 (d, *J* = 7.5 Hz, 1 H), 7.46 (d, *J* = 7.5 Hz, 1 H), 7.40 (d, *J* = 7.8 Hz, 3 H), 7.08 (m, 4 H), 4.80 (s, 1 H), 4.39 (m, 1 H), 4.16 (m, 1 H), 3.39 (m, 2 H), 3.23 (dd, *J* = 15.0 Hz, *J* = 7.5 Hz, 1 H), 3.00 (t, *J* = 13.2 Hz, 1 H), 1.72 (d, *J* = 6.6 Hz, 3 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 167.6, 167.2, 136.9, 136.7, 131.5, 127.7, 126.1, 125.6, 122.2, 121.5, 119.6, 119.1, 118.9, 112.0, 111.9, 107.1, 104.8, 60.2, 55.1, 50.0, 27.8, 24.1, 16.8. Anal. Calcd for $C_{24}H_{26}N_6O_2$: C, 66.96; H, 6.09; N, 19.52. Found: C, 66.75; H, 5.93; N, 19.75.

*6.2.34. N-[(1S,3S)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-N′-histidylhydra-zine (**5m**)*

Using a procedure similar to that of preparing *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-*N′*-glycylhydrazine (**5a**) from 100 mg (0.172 mmol) of *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-*N′*-(di-Boc-histidyl)hydrazine (**4m**) 61 mg (94%) of the title compound were obtained as yellowing powders. Mp 213–215 °C; ESI-MS (*m/z*) 382 $[M + H]^+$; $[\alpha]_D^{20} = 25.27$ (*c* 1.3, CH_3OH); IR (cm^{-1}) 3399, 3142, 2987, 2901, 2480, 1720, 1683, 1621, 1556, 1507, 1434, 1385, 1315, 1213, 751; ^1H NMR (300 MHz, DMSO-d_6) δ/ppm = 14.72 (s, 1 H), 11.45 (s, 1 H), 10.34 (s, 1 H), 9.76 (s, 1 H), 9.11 (s, 1 H), 8.30 (s, 3 H), 7.62 (s, 1 H), 7.46 (d, *J* = 5.8 Hz, 1 H), 7.38 (d, *J* = 8.1 Hz, 1 H), 7.13 (t, *J* = 7.5 Hz, 1 H), 7.03 (t, *J* = 7.5 Hz, 1 H), 4.80 (s, 1 H), 4.44 (m, 2 H), 3.39 (m, 3 H), 3.00 (t, *J* = 13.2 Hz, 1 H), 1.72 (d, *J* = 6.6 Hz, 3 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 167.8, 166.8, 136.8, 134.4, 131.4, 126.7, 126.0, 122.3, 119.7, 118.4, 112.0, 104.8, 55.0, 50.5, 49.9, 26.7, 24.0, 16.8. Anal. Calcd for $C_{19}H_{23}N_7O_2$: C, 59.83; H, 6.08; N, 25.70. Found: C, 59.61; H, 5.91; N, 25.93.

*6.2.35. N-[(1S,3S)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-N′-lysylhydra-zine (**5n**)*

Using a procedure similar to that of preparing *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-*N′*-glycylhydrazine (**5a**) from 100 mg (0.175 mmol) of *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-*N′*-(di-Boc-lysyl)-hydrazine (**4n**) 60 mg (93%) of the title compound were obtained as yellowing powders. Mp 216–218 °C; ESI-MS (*m/z*) 373 $[M + H]^+$; $[\alpha]_D^{20} = -20.47$ (*c* 1.1, CH_3OH); IR (cm^{-1}) 3166, 2982, 2938, 2472, 1724, 1695, 1577, 1507, 1454, 1377, 1319, 1270, 751; ^1H NMR (300 MHz, DMSO-d_6) δ/ppm = 11.46 (s, 1 H), 10.39 (d, *J* = 7.8 Hz, 1 H), 9.72 (m, *J* = 9.0 Hz, 1 H), 8.61 (s, 3 H), 8.24 (s, 3 H), 7.44 (d, *J* = 7.8 Hz, 1 H), 7.39 (d, *J* = 8.1 Hz,

1 H), 7.13 (t, J = 7.5 Hz, 1 H), 7.03 (t, J = 7.5 Hz, 1 H), 4.80 (s, 1 H), 4.39 (m, 1 H), 3.94 (m, 3 H), 3.00 (t, J = 13.2 Hz, 1 H), 2.74 (s, 2 H), 1.72 (d, J = 6.6 Hz, 3 H), 1.58 (m, 4 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 170.7, 167.9, 166.7, 136.9, 131.5, 126.0, 119.6, 112.0, 104.8, 60.2, 55.0, 49.9, 38.8, 30.8, 26.7, 24.0, 21.2, 16.8. Anal. Calcd for $C_{19}H_{28}N_6O_2$: C, 61.27; H, 7.58; N, 22.56. Found: C, 61.50; H, 7.74; N, 22.78.

6.2.36. *N-[(1S,3S)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-N′-[(β-OBzl)-as-partyl]hydrazine (**5o**)*

Using a procedure similar to that of preparing *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-*N′*-glycylhydrazine (**5a**) from 100 mg (0.182 mmol) of *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-*N′*-[Boc-(β-OBzl-aspartyl)]hydrazine (**4o**) 59 mg (90%) of the title compound were obtained as yellowing powders. Mp 254–256 °C; ESI-MS (m/z) 360 [M + H]$^+$; $[\alpha]_D^{20}$ = −25.80 (c 1.3, CH_3OH); IR (cm^{-1}) 3182, 2972, 2927, 2475, 1724, 1687, 1507, 1454, 1388, 1319, 1274, 1210, 750; ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm = 11.49 (s, 1 H), 11.08 (s, 1 H), 11.04 (s, 1 H), 10.04 (m, 1 H), 9.73 (m, 1 H), 8.68 (m, 2 H), 7.44 (d, J = 7.5 Hz, 1 H), 7.39 (d, J = 7.8 Hz, 1 H), 7.13 (t, J = 7.5 Hz, 1 H), 7.03 (t, J = 7.5 Hz, 1 H), 4.80 (d, J = 6.3 Hz, 1 H), 4.39 (dd, J = 14.4 Hz, J = 4.2 Hz, 1 H), 4.21 (t, J = 6.0 Hz, 1 H), 3.35 (m, 1 H), 3.00 (t, J = 13.2 Hz, 3 H), 1.71 (d, J = 6.6 Hz, 3 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 170.9, 167.4, 136.9, 131.4, 126.0, 122.2, 119.7, 118.3, 112.0, 104.8, 65.3, 54.8, 49.8, 35.6, 23.9, 16.8. Anal. Calcd for $C_{17}H_{21}N_5O_4$: C, 56.82; H, 5.89; N, 19.49. Found: C, 56.61; H, 5.74; N, 19.71.

6.2.37. *N-[(1S,3S)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-N′-[(γ-OBzl)glutamoyl]hydrazine (**5p**)*

Using a procedure similar to that of preparing *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-*N′*-glycylhydrazine (**5a**) from 100 mg (0.178 mmol) of *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-*N′*-[Boc-(γ-OBzl-glutamoyl)]hydrazine (**4p**) 58 mg (88%) of the title compound were obtained as yellowing powders. Mp 211–213 °C; ESI-MS (m/z) 374 [M + H]$^+$; $[\alpha]_D^{20}$ = −9.30 (c 1.3, CH_3OH); IR (cm^{-1}) 3170, 2978, 2933, 2476, 1720, 1573, 1507, 1450, 1389, 1319, 1274, 1213, 751; ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm = 11.49 (s, 1 H), 11.08 (s, 1 H), 11.04 (s, 1 H), 10.04 (m, 1 H), 9.73 (m, 1 H), 8.68 (m, 2 H), 7.44 (d, J = 7.5 Hz, 1 H), 7.39 (d, J = 8.1 Hz, 1 H), 7.13 (t, J = 7.5 Hz, 1 H), 7.03 (t, J = 7.5 Hz, 1 H), 4.80 (s, 1 H), 4.39 (s, 1 H), 3.98 (s, 1 H), 3.45 (m, 1 H), 3.00 (t, J = 13.2 Hz, 1 H), 2.60 (m, 2 H), 2.11 (m, 2 H), 1.71 (d, J = 6.6 Hz, 3 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 173.8, 167.8, 136.7, 131.4, 126.0, 122.2, 119.7, 118.3, 112.0, 104.8, 65.3, 54.9, 49.8, 29.2, 26.8, 23.9, 16.8. Anal. Calcd for $C_{18}H_{23}N_5O_4$: C, 57.90; H, 6.21; N, 18.76. Found: C, 58.09; H, 6.04; N, 18.52.

6.2.38. *N-[(1S,3S)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-N′-asparaginyl-hydrazine (**5q**)*

Using a procedure similar to that of preparing *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-*N′*-glycylhydrazine (**5a**) from 100 mg (0.218 mmol) of *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-*N′*-(Boc-asparaginyl)]hydrazine (**4q**) 70 mg (90%) of the title compound were obtained as yellowing powders. Mp 253–255 °C; ESI-MS (m/z) 359 [M + H]$^+$; $[\alpha]_D^{20}$ = −31.23 (c 1.2, CH_3OH); IR (cm^{-1}) 3175, 2974, 2806, 2472, 1728, 1675, 1605, 1495, 1450, 1425, 1385, 1315, 1274, 1221, 747; ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm = 11.50 (s, 1 H), 10.44 (s, 1 H), 9.68 (s, 1 H), 8.46 (s, 3 H), 7.87 (s, 1 H), 7.44 (d, J = 7.8 Hz, 1 H), 7.39 (d, J = 8.1 Hz, 1 H), 7.28 (s, 1 H), 7.13 (t, J = 7.5 Hz, 1 H), 7.03 (t, J = 7.5 Hz, 1 H), 4.80 (s, 1 H), 4.37 (s, 1 H), 4.21 (s, 1 H), 3.39 (m, 1 H), 3.00 (t, J = 13.2 Hz, 1 H), 2.78 (m, 2 H), 1.72 (d, J = 6.6 Hz, 3 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 170.8, 167.5, 167.3, 136.9, 131.5, 126.0, 122.2, 119.6, 112.0, 104.8, 65.3, 54.9, 49.9, 35.9, 24.0, 16.8. Anal. Calcd for $C_{17}H_{22}N_6O_3$: C, 56.97; H, 6.19; N, 23.45. Found: C, 56.76; H, 6.04; N, 23.68.

6.2.39. *N-[(1S,3S)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-N′-glutaminyl)-hydrazine (**5r**)*

Using a procedure similar to that of preparing *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-*N′*-glycylhydrazine (**5a**) from 100 mg (0.212 mmol) of *N*-[(*1S,3S*)-1-methyl-1,2,3,4-tetrahydro-β-caboline-3-carbonyl]-*N′*-(Boc-glutaminyl)hydrazine (**4r**) 72 mg (91%) of the title compound were obtained as yellowing powders. Mp 215–217 °C; ESI-MS (m/z) 373 [M + H]$^+$; $[\alpha]_D^{20}$ = −8.57 (c 1.3, CH_3OH); IR (cm^{-1}) 3179, 2983, 2925, 2476, 1724, 1662, 1605, 1507, 1454, 1389, 1319, 1274, 1217, 751; ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm = 11.46 (s, 1 H), 10.44 (d, J = 8.4 Hz, 1 H), 9.68 (m, J = 9.6 Hz, 1 H), 8.63 (s, 3 H), 7.52 (s, 1 H), 7.44 (d, J = 7.8 Hz, 1 H), 7.39 (d, J = 8.1 Hz, 1 H), 7.13 (t, J = 7.5 Hz, 1 H), 7.03 (t, J = 7.5 Hz, 1 H), 6.95 (s, 1 H), 4.80 (s, 1 H), 4.39 (m, 1 H), 3.97 (s, 1 H), 3.39 (m, 1 H), 3.00 (t, J = 13.2 Hz, 1 H), 2.37 (m, 2 H), 2.06 (m, 2 H), 1.72 (d, J = 6.6 Hz, 3 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 173.9, 167.7, 136.9, 131.5, 126.0, 122.2, 119.6, 118.3, 112.0, 104.8, 65.3, 55.0, 49.9, 30.7, 27.5, 24.0, 16.8. Anal. Calcd for $C_{18}H_{24}N_6O_3$: C, 58.05; H, 6.50; N, 22.57. Found: C, 57.84; H, 6.36; N, 22.81.

6.3. *In vitro anti-platelet 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. The pig blood (6 pigs, purchased from Animal Center of Peking University) was centrifuged at 120*g* for 10 min and the platelet rich plasma (PRP) was collected. The remaining blood was centrifuged for an additional 10 min at 850*g* to prepare platelet poor plasma (PPP). The final platelet count of the PRP 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 (1.7 mM) of aspirin in DMSO/NS (1/10) or 5 μL of the solution of **5a**–**r** (in a series of final concentrations of 100, 10, 1, 0.1, 0.01 and 0.001 μM, prepared by diluting 10 mM of stock solutions of **5a**–**r** in DMSO/NS (1/10) with NS) was added. After adjustment of the baseline, 5 μL of the solution of PAF (final concentration 0.1 μM, prepared by diluting 10 mM of stock solution of PAF in DMSO/NS (1/10) with NS or 5 μL of the solution of ADP (final concentration 10 μM, prepared by diluting 10 mM of stock solution of ADP in DMSO/NS (1/10) with NS) or 5 μL of the solution of arachidonic acid in NS (AA, final concentration 350 μM, prepared by diluting 100 mM of stock solution of AA in DMSO/NS (1/10) with NS) was added and aggregation was measured at 37 °C for 5 min. The effects of **5a**–**r** (at a series of concentrations ranging from 100 μM to 1 nM) and aspirin (positive control, 1.7 mM) on PAF or ADP or AA-induced platelet aggregation were observed. All these anti-platelet aggregation tests in sixplicate tubers were carried out. The maximum platelet aggregation (A_m) of control group (NS) or sample group (**5a**–**r**) was represented by the peak height of aggregation curve (equals to the maximum light transmission). The inhibition rate was calculated according to the following formula: Inhibition (%) = [(A_m of NS) − (A_m of **5a**–**r**)]/(A_m of NS) × 100%. A_m% of NS is the value of platelet aggregation induced by PAF, ADP and AA without **5a**–**r** which are 52.30 ± 1.78%, 50.16 ± 3.65% and 49.62 ± 2.90%, respectively. The concentration vs. inhibition rate curve is plotted to determine the IC_{50} values with GWBASIC.EXE program.

6.4. In vivo anti-thrombotic assay

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**–**r** were dissolved in NS before administration and kept in an ice bath. Male Wista rats weighing 250–300 g (purchased from Animal Center of Peking University) were used. Thirty minutes after orally given aspirin (positive control), NS (negative control) or **5a**–**r**, the rats were anesthetized with pentobarbital sodium (80.0 mg/kg, i.p.) 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, 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 wet weight of the thrombus.

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