European Journal of Medicinal Chemistry 46 (2011) 5598–5608

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

A class of novel N-(1-methyl-β-carboline-3-carbonyl)-N′-(aminoacid-acyl)-hydrazines: Aromatization leaded design, synthesis, *in vitro* anti-platelet aggregation/*in vivo* anti-thrombotic evaluation and 3D QSAR analysis

Chunyu Li, Xiaoyi Zhang, Ming Zhao**, Yuji Wang, Jianhui Wu, Jiawang Liu, Meiqing Zheng, Shiqi Peng*

College of Pharmaceutical Sciences, Capital Medical University, No. 10 You An Men Xitoutiao, Beijing 100069, PR China

ARTICLE INFO

Article history:
Received 30 June 2011
Received in revised form
11 September 2011
Accepted 17 September 2011
Available online 24 September 2011

Keywords:
β-Carboline
Aromatization
Anti-platelet aggregation
Anti-thrombotic activity
3D QSAR

ABSTRACT

High anti-thrombotic activity of aminoacid modified tetrahydro-β-carbolines was generally correlated with a small proximity of the side chain of the aminoacid residue to the carboline-cycle. This paper explored that the aromatization of the tetrahydro-β-carboline-cycle of N-(1-methyl-β-tetrahydrocarboline-3-carbonyl)-N′-(aminoacid-acyl)-hydrazines leaded to N-(1-methyl-β-carboline-3-carbonyl)-N′-(aminoacid-acyl)-hydrazines and decreased the proximity of the side chain of the aminoacid residue to the carboline-cycle. The *in vitro* activities of inhibiting pig platelet aggregation induced by PAF, ADP, and AA, as well as the *in vivo* anti-thrombotic activities of inhibiting rat thrombosis of these aromatized derivatives were generally higher than that of N-(1-methyl-β-tetrahydrocarboline-3-carbonyl)-N′-(aminoacid-acyl)-hydrazines. The understanding was also obtained from the 3D QSAR analysis.

1. Introduction

Deep vein thrombosis, myocardial infarction, pulmonary embolism, and stroke have been the most frequent cardiovascular events, while the intravascular thrombosis has been one of the most prominent causes of morbidity and mortality [1]. The injury of blood vessel closely correlates with the arterial thrombosis [2]. If the vascular endothelium of the injured blood vessel is damaged, the platelets will adhere to the exposed extracellular matrix [3]. Platelet adhesion is not only the primary event that usually associates with the uncontrolled platelet activation and but also culminates in the intravascular thrombosis [4]. The suppression of the platelet adhesion and activation, which is achieved in particularly through targeting such secondary regulatory mechanism, is capable of preventing the thrombosis [5]. Anti-platelet therapy has a definite role for managing the cardiovascular event [6]. Due to the most frequently used anti-platelet drugs lack clinical efficacy, there has been a continuous effort to discover new leads capable of inhibiting platelet activation and aggregation.

Pharmacologically, β-carbolines and tetrahydro-β-carbolines are important indole alkaloids with a wide spectrum of pharmacological actions [7–36]. Harmalol, harmaline, norharmane, harmol, harmine and harmane belong to β-carboline, were only capable of inhibiting the platelet aggregation induced by collagen, and had more than 130 μM of IC_{50} value [7]. In the previous paper N-(1-methyl-β-tetrahydrocarboline-3-carbonyl)-N′-(aminoacid-acyl)-hydrazines extended the inhibition to another three inducers, platelet-activating factor (PAF), adenosine diphosphate (ADP) and arachidonic acid (AA), and had 0.6–722 μM of IC_{50} values [37].

In the conformation analysis we used the QSAR module of Cerius^2, explored the *in vivo* anti-thrombotic activities of aminoacid modified *3S*-tetrahydro-β-carbo- line-3-carboxylic acid, (*3S,12aS*)-hexahydropyrazino[1′,2′:1,6]pyrido [3,4-b]indole- 1,4-dione and N-(1-methyl-β-tetrahydrocarboline-3-carbonyl)-N′-(aminoacid-acyl)-hydrazines depended on the proximity of the side chain of the aminoacid residue to the carboline-cycle, and suggested that reducing the proximity of the side chain of the aminoacid residue to the carboline-cycle was beneficial to increasing the *in vivo* anti-thrombotic activity [37–39]. Following up this clue, this paper compared the proximity of the side chain of the aminoacid residue to the carboline-cycle of N-(1-methyl-β-tetrahydrocarboline-3-carbonyl)-N′-(aminoacid-acyl)-hydrazines with that of their aromatized derivatives, N-(1-methyl-β-carboline-3-carbonyl)-N′-

* Corresponding author. Tel./fax: +86 10 8391 1528.
** Corresponding author. Tel./fax: +86 10 8280 2482.
E-mail addresses: mingzhao@mail.bjmu.edu.cn (M. Zhao), sqpeng@mail.bjmu.edu.cn (S. Peng).

0223-5234/$ – see front matter © 2011 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2011.09.027

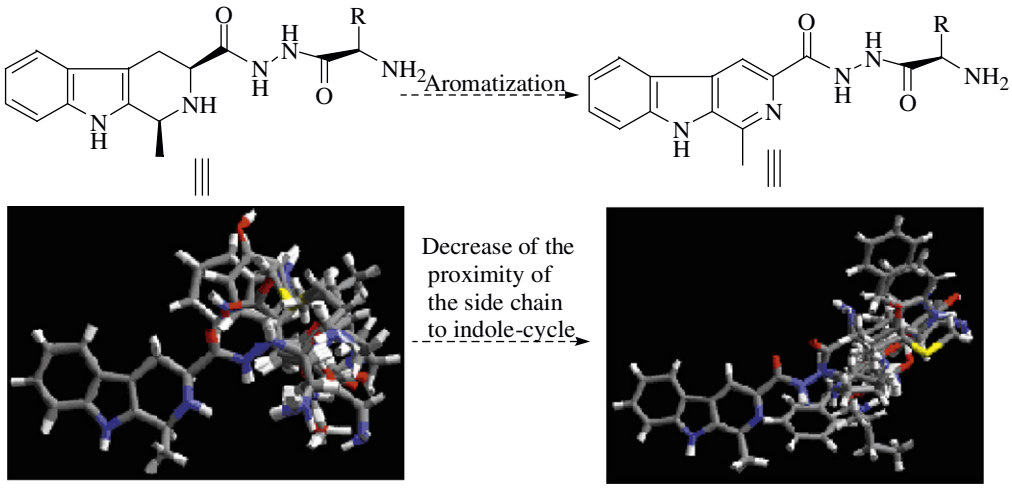


Fig. 1. QSAR module of Cerius[2] derived stereoview of N-(1-methyl-β-tetrahydrocarboline-3-carbonyl)-N′-(aminoacid-acyl)-hydrazines and N-(1-methyl-β-carboline-3 -carbonyl)-N′- (aminoacid-acyl)-hydrazines.

(aminoacid-acyl)-hydrazines. It was found that the aromatization leaded to the decrease of the proximity (Fig. 1).

In this context, this study prepared sixteen novel N-(1-methyl-β-carboline-3-carbonyl)-N′-(aminoacid-acyl)-hydrazines, evaluated their *in vitro* activities of inhibiting pig platelet aggregation induced by PAF, ADP, and AA, tested their *in vivo* anti-thrombotic activities of inhibiting rat thrombosis and analyzed their 3D QSAR.

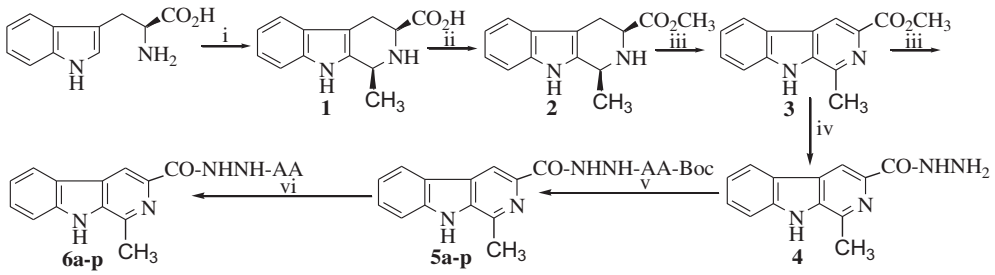
2. Results and discussion

2.1. Synthesis of *6a–p*

The preparation of N-(1-methyl-β-carboline-3-carbonyl)-N′-(aminoacid-acyl)-hydrazines (**6a–p**) was carried out according to the five-step-route depicted in Scheme 1. L-Trp was successively converted into (1S,3S)-1-methyl-1,2,3,4-tetrahydro-β- carboline-3-carboxylic acid (**1**, 79% yield) *via* Pictet-Spengler condensation and (1S,3S)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid methyl ester (**2**, 89% yield) *via* esterification. The oxidation of **2** resulted in 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid methyl ester (**3**) in 65% yield. In the presence of hygrazine hydrate the hydrazinolysis of **3** provided N-(1-methyl-β- carboline-3-carbonyl)-hydrazine (**4**) in 62% yield. In the presence of DCC, HOBt and NMM, **4** coupled with Boc-AA to give N-(1-methyl-β-carboline-3-carbonyl)-N′- (Boc-aminoacid-acyl)-hydrazines (**5a–p**, 13%–52% yield). On removing of the Boc group of **5a–p** gave N-(1-methyl-β-carboline-3-carbonyl)-N′-(aminoacid-acyl)-hydrazines (**6a–p**) in 61–92% yields. To ensure the purity, **5a–p** were purified on a chromatographic column, while **6a–p** were purified repeatedly using ether promoted solidification, and their HPLC purities were more than 97%. These data demonstrate that the used procedures and conditions were suitable for preparing N-(1-methyl-β-caboline-3-carbonyl)-N′-(aminoacid-acyl)-hydrazines with high quality and acceptable yields.

2.2. In vitro anti-platelet aggregation of *6a–p*

The *in vitro* activities of **6a–p** (at a series of concentrations ranging from 1 μM to 1.5 mM) were identified by the anti-platelet aggregation assays. The aggregators were platelet-activating factor (PAF, final concentration 0.1 μM), adenosine diphosphate (ADP, final concentration 10 μM) and arachidonic acid (AA, final concentration 350 μM). The *in vitro* activities of **6a–p** inhibiting the platelet aggregation induced by PAF, ADP, and AA were represented with IC_{50} values and are listed in Table 1. The IC_{50} values of **4** inhibiting the platelet aggregation induced by AA, PAF and ADP are 32.9 μM, 92.8 μM and 110.1 μM, respectively. The IC_{50} values of **6a–p** inhibiting the platelet aggregation induced by AA, PAF and ADP range from 0.2 to 9.0 μM, 1.1 to 52.9 μM, and 20.8 to 74.6 μM, respectively. The *in vitro* anti-platelet aggregation activities of **6a–p** are 1.5–164.5 folds higher than that of **4**. It was reported that the IC_{50} values of N-(1-methyl-β-tetrahydrocarboline-3-carbonyl)-N′-



Scheme 1. Synthetic route of N-(1-methyl-β-caboline-3-carbonyl)-N′-(aminoacid-acyl)-hydrazines. i) CH_3CHO and H_2SO_4; ii) CH_3OH and $SOCl_2$; iii) $KMnO_4$; iv) $NH_2NH_2 \cdot H_2O$; v) DCC, HOBt and NMM; vi) 4N HCl/EtOAc; In **5a** and **6a**, AA = Gly; In **5b** and **6b**, AA = Ala; In **5c** and **6c**, AA = Ile; In **5d** and **6d**, AA = Leu; In **5e** and **6e**, AA = Val; In **5f** and **6f**, AA = Met; In **5g** and **6g**, AA = Pro; In **5h** and **6h**, AA = Tyr; In **5i** and **6i**, AA = Thr; In **5j** and **6j**, AA = Ser; In **5k** and **6k**, AA = Trp; In **5l,** AA = His(Boc); In **6l**, AA = His; In **5m**, AA = Lys(Boc); In **6m**, AA = Lys; In **5n** and **6n**, AA = Asp(OBzl); In **5o** and **6o**, AA = Glu(OBzl); In **5p** and **6p**, AA = Asn.

Table 1
IC_{50} of **6a**–**p** *in vitro* platelet aggregation inhibition in the presence of three aggregators.[a]

Compd.	IC_{50} (μM)		
	ADP	PAF	AA
4	110.1 ± 9.1	92.8 ± 7.9	32.9 ± 2.9
6a	53.3 ± 4.7	52.9 ± 4.5	5.2 ± 0.3
6b	58.9 ± 5.3	47.4 ± 3.6	6.7 ± 0.2
6c	74.0 ± 6.2	5.6 ± 0.5	3.6 ± 0.3
6d	23.0 ± 2.1	1.1 ± 0.2	8.2 ± 0.8
6e	20.8 ± 2.1	2.0 ± 0.2	0.3 ± 0.01
6f	58.9 ± 4.8	13.4 ± 1.2	4.7 ± 0.2
6g	48.7 ± 3.6	44.5 ± 3.5	5.8 ± 0.4
6h	74.6 ± 6.1	37.4 ± 2.7	1.7 ± 0.1
6i	24.8 ± 3.1	20.9 ± 2.1	5.2 ± 0.4
6j	50.9 ± 4.2	52.9 ± 3.9	9.0 ± 1.1
6k	28.9 ± 1.9	19.3 ± 1.5	8.7 ± 0.9
6l	25.7 ± 1.3	42.5 ± 2.9	0.2 ± 0.01
6m	58.9 ± 4.5	52.9 ± 4.3	4.1 ± 0.3
6n	81.7 ± 7.8	52.9 ± 3.5	3.7 ± 0.2
6o	58.9 ± 4.6	64.3 ± 4.2	2.3 ± 0.2
6p	65.8 ± 4.1	39.7 ± 2.8	6.2 ± 0.9

[a] $n = 6$, IC_{50} is represented with mean ± SD μM.

(aminoacid-acyl)-hydrazines inhibiting the platelet aggregation induced by AA, PAF and ADP range from 0.6 to 21.7 μM, 1.2 to 506.8 μM, and 21.7 to 722.1 μM, respectively [37]. This means that the aromatization leads to a general increase of the *in vitro* anti-platelet aggregation activity.

To explain the effect of aromatization on the *in vitro* anti-platelet aggregation the IC_{50} values of aromatized compounds (**6a**–**p**) were compared with those of non-aromatized compounds [37]. The statistical analysis indicates that for ADP induced platelet aggregation the IC_{50} values of 14 aromatized compounds are significantly lower than that of each respective non-aromatized compound, while 2 aromatized compounds are significantly higher than that of each respective non-aromatized compounds. For PAF induced platelet aggregation the IC_{50} values of 11 aromatized compounds are significantly lower than that of each respective non-aromatized compound, 1 of aromatized compound is equal to that of its non-aromatized compound, while 4 of aromatized compounds are significantly higher than that of each respective non-aromatized compound. For AA induced platelet aggregation the IC_{50} values of 11 aromatized compounds are significantly lower than that of each respective non-aromatized compound, while 5 of aromatized compounds are significantly higher than that of each respective non-aromatized compound. These statistical data suggested that a general increase of the *in vitro* anti-platelet aggregation activity was achieved with aromatization.

Table 2
Effect of oral **6a**–**p** on the thrombus weight of the rats after oral administration.[a]

Compd.	Thrombus weight	Compd.	Thrombus weight
NS	31.87 ± 1.71	Aspirin	18.86 ± 1.67[c]
4	26.66 ± 1.19[b]	**6i**	23.65 ± 0.83[c]
6a	23.70 ± 0.85[c]	**6j**	24.78 ± 1.45[c]
6b	24.39 ± 1.65[c]	**6k**	24.56 ± 1.33[c]
6c	23.12 ± 1.23[c]	**6l**	18.10 ± 1.05[c]
6d	24.49 ± 1.29[c]	**6m**	23.30 ± 1.39[c]
6e	18.30 ± 1.28[c]	**6n**	23.23 ± 1.30[c]
6f	23.43 ± 1.32[c]	**6o**	18.80 ± 1.51[c]
6g	24.37 ± 1.25[c]	**6p**	23.97 ± 1.13[c]
6h	18.56 ± 1.46[c]		

[a] Weight of wet thrombus is represented by X ± SD mg, NS = vehicle, $n = 12$; Dose of Aspirin: 160 μmol/kg; Dose of **4**: 10 nmol/kg; Dose of **6a-p**: 1 nmol/kg.
[b] Compared to NS $p < 0.001$.
[c] Compared to NS and **4** $p < 0.001$.

Table 3
Effect of different doses of **6e** on the thrombus weight of the rats after oral administration.[a]

Dose	1 nmol/kg	0.1 nmol/kg	0.01 nmol/kg
6e	18.30 ± 1.28[b]	23.80 ± 1.56[c]	27.12 ± 1.63[d]
NS		31.87 ± 1.71	

[a] Weight of wet thrombus is represented by X ± SD mg, NS = vehicle, $n = 12$.
[b] Compared to NS and 0.1 nmol/kg groups $p < 0.01$.
[c] Compared to NS and 0.01 nmol/kg groups $p < 0.01$.
[d] Compared to NS group $p < 0.01$.

2.3. *In vivo* anti-thrombotic activities of ***6a–p***

On extra-corporeal circulation of arterio-veinos cannula model, the *in vivo* activities of **6a**–**p** were assayed, and the thrombus weights of the treated rats are listed in Table 2. The data indicated that the thrombus weights (18.1 mg–24.8 mg) of 1 nmol/kg of **6a**–**p** treated rats are significantly lower than that (31.9 mg) of normal saline (NS) treated rats. This means that 1 nmol/kg of **6a**–**p** effectively inhibits the rats to form thrombus. Besides, the thrombus weights (18.1 mg–24.8 mg) of 1 nmol/kg of **6a**–**p** treated rats are significantly lower than that (26.7 mg) of 10 nmol/kg of **4** treated rats. This means that the anti-thrombotic activities of **6a-p** are more than 10 folds higher than that of **4**. It was reported that the thrombus weights of 10 nmol/kg of N-(1-methyl-β-tetrahydrocarboline-3-carbonyl)-N′-(aminoacid-acyl)-hydrazines treated rats ranged from 18.6 mg to 24.0 mg (NS, 28.21 mg) [37], which were substantially equal to that of 1 nmol/kg of **6a**–**p** treated rats. This means that the aromatization leads to a 10-fold increase of the *in vivo* anti-thrombotic activity.

2.4. Dose-dependent *in vivo* anti-thrombotic activity of ***6e***

Oral administration of **6e**, the most active compound identified in both *in vitro* and *in vivo* assays, was observed at 1 nmol/kg, 0.1 nmol/kg, and 0.01 nmol/kg of doses produce a possible dose-dependent anti-thrombotic response in rats. The thrombus weights are listed in Table 3, which demonstrated that the thrombus weight was progressively increased with dose decrease. Therefore, **6e** exhibited dose-dependent anti-thrombotic action.

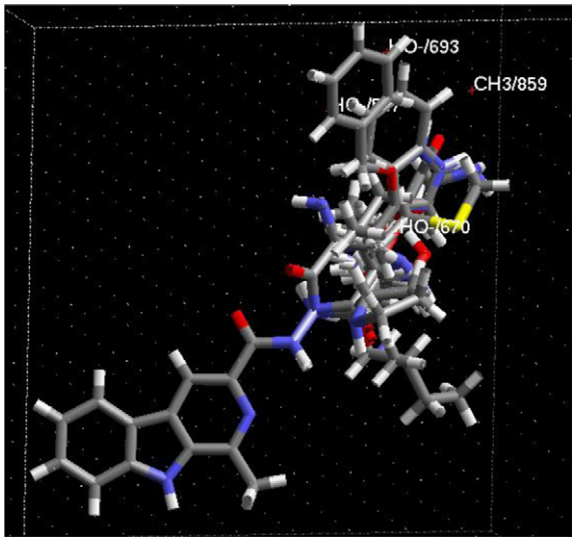


Fig. 2. Alignment stereoview of **6a**–**p** used for molecular field generation.

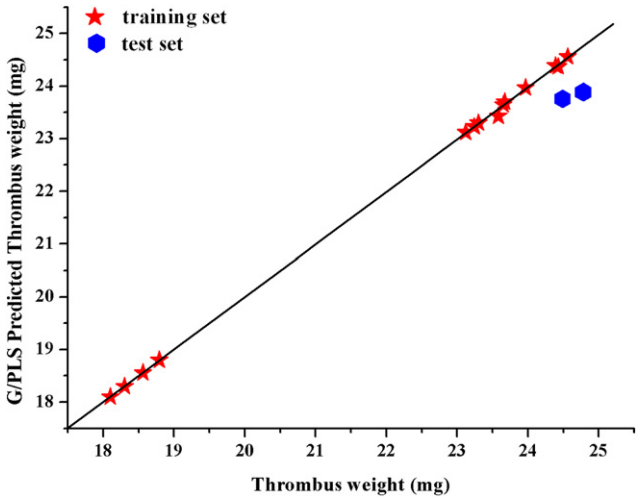


Fig. 3. Graph of tested versus predicted anti-thrombotic activities of **6a**–**p**.

2.5. 3D QSAR analysis of *6a*–*p*

2.5.1. Alignment of *6a*–*p*

Establishing the valid 3D-QSAR models a proper alignment procedure of **6a**–**p** was practiced using the target model align strategy in the align module with Cerius^2. Based on the assumption that each structure of **6a**–**p** exhibits activity at the same binding site of the receptor, they were aligned in a pharmacological active orientation. To obtain a consistent alignment 1-methyl-β-carboline-3-carbonylhydrazine was selected as the template for superposing **6a**–**p**. The method used for performing the alignment was the maximum common subgraph (MCS) [40]. 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 1-methyl-β-carboline-3-carbonylhydrazine that shared by **6a**–**p**. This subset was used for the alignment. A rigid fit of atom pairings was performed to superimpose each structure onto 1-methyl-β-carboline-3-carbonylhydrazine. Stereoview of aligned **6a**–**p** is shown in Fig. 2. The alignment stereoview explores that to superimpose onto 1-methyl-β-carboline-3-carbonylhydrazine the amino acid chain of each structure has to take individual conformation, which will affect on the anti-thrombotic activity.

2.5.2. MFA based Cerius^2 QSAR module of *6a*–*p*

Molecular field analysis (MFA) was performed for **6a**–**c**, **e**–**i**, **k**–**p** using the QSAR module of Cerius^2 [41]. 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 982. Though the spatial and structural descriptors such as dipole moment, polarizability, radius of gyration, number of rotatable bonds, molecular volume, principal moment of intertia, 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 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. The MFA model for the anti-thrombotic activities of **6a**–**c**, **e**–**i**, **k**–**p** in terms of the most relevant descriptors including proton, methyl and hydroxyl anion is expressed by equation (1).

$$\begin{aligned}\text{Thrombus weight} = {} & 23.55 - 0.17(\mathrm{CH_3}/678) - 0.18(\mathrm{CH_3}/859) \\ & + 0.079(\mathrm{HO^-}/597) - 0.063(\mathrm{HO^-}/643) \\ & - 0.017(\mathrm{HO^-}/670) + 0.079(\mathrm{HO^-}/693)\end{aligned} \quad (1)$$

The correlation of the activities tested on the *in vivo* thrombogenesis model and the activities calculated using equation (1) is explained by Fig. 3. In equation (1) the correlation coefficient (r) and square correlation coefficient (r^2) are 0.980 and 0.990, respectively. The tested activities on rat thrombogenesis model and the calculated activities based on equation (1) are shown in Fig. 3. The parameters indicated that equation (1) is able to predict the *in vivo* activity for the analogs of **6a**–**c**, **e**–**i**, **k**–**p**.

Equation (1) contains 2 terms from methyl descriptor and 4 terms from hydroxyl anion descriptor. The term of 0.17 (CH_3/678) and 0.18 (CH_3/859) have negative coefficient, which means that at this position hydrophilic group will decrease the thrombus weight. The term of 0.079 (HO^-/597) and 0.079 (HO^-/693) have positive coefficient, which means that at these position electron donating group will increase the thrombus weight, while term of 0.017 (HO^-/670) has negative coefficient, which means that at this position electron donating group will decrease the thrombus weight.

As examples Fig. 4 gives two representatives **6k**,**l**, of which **6k** has hydrophobic group near CH_3/678 and CH_3/859, electron donating group near HO^-/597 and HO^-/693, thus has lower *in vivo*

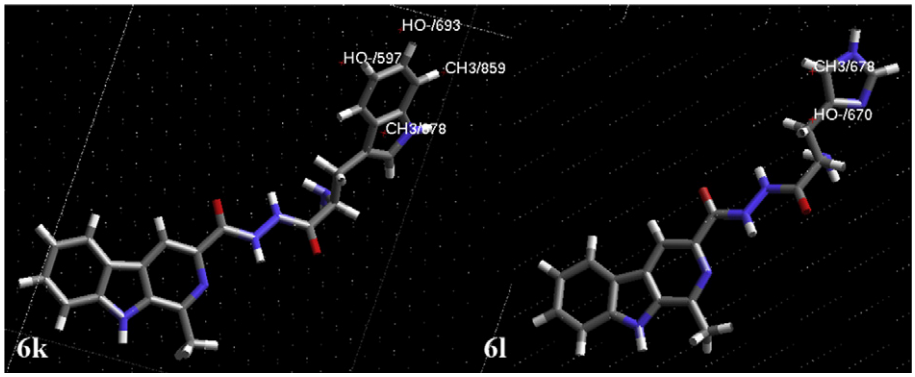


Fig. 4. Electrostatic and environments of **6k** and **6l** within the grid with 3D points of equation (1).

Table 4
Predicted and tested thrombus weight of **6d,j** treated rats.

Compd.	Thrombus weight (mg)			
	Predicted	Tested	Error	Error%
6d	23.76	24.49	−0.73	2.98
6j	23.88	24.78	−0.90	3.63

anti-thrombotic activity. While **6l** has hydrophilic group near CH_3/678 and electron donating group near HO^-/670, thus has higher *in vivo* anti-thrombotic activity.

*2.5.3. Predicting the in vitro anti-thrombotic activity of **6d,j** with equation (1)*

The predict power of equation (1) was demonstrated by comparing the calculated and tested *in vitro* anti-thrombotic activity of **6d,j** (Table 4). The correlations of predict and test values are also shown in Fig. 3. The results indicate that equation (1) rationally gives the thrombus weights for **6d,j** with about 0.7 mg and 0.9 mg of error. The calculated thrombus weights are so approximate to experimental thrombus weights means that equation (1) is practical to accurately predict the thrombus weights of N-(1-methyl-β-carboline-3-carbonyl)-N′-(aminoacid-acyl)hydrazines treated rats.

3. Conclusions

In conclusion, the aromatization of N-(1-methyl-β-tetrahydrocarboline-3-carbonyl)-N′-(aminoacid-acyl)-hydrazines (IC_{50} of *in vitro* anti-platelet aggregation: 0.6–722.1 μM; at 10 nmol/kg dose, *in vivo* thrombus weights based on a same blank control: 20.96–26.83 mg) successively provided sixteen novel anti-thrombotic agents, N-(1-methyl-β-carboline-3-carbonyl)-N′-(aminoacid-acyl)-hydrazines (IC_{50} of *in vitro* anti-platelet aggregation: 0.3–81.7 μM; at 1 nmol/kg dose, *in vivo* thrombus weights based on a same blank control: 18.10–24.78 mg), profoundly increased the *in vitro* anti-platelet aggregation activity and the *in vivo* anti-thrombotic activity, and confirmed that the proximity of the side chain of the amino acid residue to the carboline-cycle of amino acid modified β-carboline-3-carboxylic acids was a key parameter in the molecular design.

4. Experimental section

4.1. 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. In the HPLC analysis, a Waters 2695 HPLC system with a Waters Diode Array Detector was used. The sample was separated on a Symmetry C18 (Waters) reversed-phase column (5 μm, 4.6 × 150 mm). The column thermostat was maintained at 40 °C. Onto the column 10 μl of sample solution was injected for analysis. The mobile phase consisted of solvent A (CH_3OH) and solvent B (H_2O). The gradient elution program consisted of 0–5 min 40% A : 60% B, 5–10 min 45% A : 55% B, 10–15 min 50% A : 50% B and 15–20 min 45% A : 55% B, and the flow rate was 0.6 ml/min. After each run, the column was washed with methol and equilibrated to initial conditions for 15 min. The DAD detector was set to a scanning range of 200–400 nm. ^1H NMR and ^{13}C NMR spectra were recorded on 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.

4.2. Synthesis

*4.2.1. (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-tryptophane, 200 ml of water, 0.2 ml of concentrated H_2SO_4 and 2 ml of aldehyde (40%) was successively added. The reaction mixture was stirred at room temperature for 8 h and TLC (CH_2Cl_2/CH_3OH, 10:1) indicates the complete disappearance of L-tryptophane. 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 powder. 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}; ^1H 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).

*4.2.2. (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 (1*S*,3*S*)- 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) indicates 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 powder. 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}; ^1H 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).

*4.2.3. 1-Methyl-β-carboline-3-carboxylic acid methyl ester (**3**)*

At 0 °C with stirring to the solution of 5.0 g (20.0 mmol) of (1S, 3S)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid methyl ester (**2**) in 50 ml of DMF 4.5 g (28.0 mmol) of potassium permanganate were added, which took 3 h. The reaction mixture was stirred at 0 °C for 1 h and then at room temperature for 14 h, and TLC ($CHCl_3/CH_3OH$, 15:1) indicated the complete disappearance of **2**. On evaporation the residue was dissolved in 10 ml of CH_3OH. After filtration and evaporation under reduced pressure 3.2 g (65%) of the title compound were obtained as yellow powder. Mp 243–244 °C; ESI/MS 241 $[M + H]^+$; IR (KBr): 3310, 2954, 2922, 2901, 2811, 1742, 1600, 1581, 1566, 1450, 1380, 1066, 900 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ/ppm = 9.98 (s, 1 H), 7.43 (d, J = 5.6 Hz, 1 H), 7.33 (d, J = 7.2 Hz, 1 H), 7.16 (d, J = 8.2 Hz, 1 H), 7.14 (t, J = 7.5 Hz, 1 H), 6.95 (t, J = 6.4 Hz, 1 H), 3.76 (s, 3 H), 2.05 (s, 3 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ/ppm = 167.9, 147.8, 141.6, 135.3, 131.3,

122.3, 120.0, 115.3, 111.2, 105.3, 51.6, 19.8. Anal. Calcd for $C_{14}H_{12}N_2O_2$: C, 69.99, H, 5.03, N, 11.66. Found: C, 69.78, H, 4.90, N, 11.43.

4.2.4. 1-Methyl-β-carboline-3-carbonylhydrazine (**4**)

At 60 °C with stirring to the solution of 1.0 g (4.2 mmol) of 1-methyl-β- carboline-3-carboxylic acid methyl ester (**3**) in 20 ml of $CHCl_3$ and 5 ml of CH_3OH 4 ml (80.0 mmol) of hydrazine hydrate was added dropwise, which took 1 h. The reaction mixture was stirred at 60 °C for another 1 h and TLC ($CHCl_3/CH_3OH$,15:1) indicated the complete disappearance of **3**. On evaporation the residue was purified by silica gel chromatography ($CHCl_3/CH_3OH$, 3:1) and 620 mg (62%) of the title compound was obtained as a colorless powder. Mp 294–295 °C; ESI-MS (*m*/*z*) 241 $[M + H]^+$; IR (KBr): 3340, 3335, 2924, 2910, 2900, 1620, 1600, 1585, 1562, 1440, 1380, 1066, 900 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ/ppm = 11.91 (s, 1 H), 8.65 (s, 1 H), 8.33 (d, *J* = 7.8 Hz, 1 H), 7.65 (d, *J* = 8.1 Hz, 1 H), 7.54 (t, *J* = 7.5 Hz, 1 H), 7.28 (t, *J* = 7.5 Hz, 1 H), 4.62 (m, 1 H), 2.80 (s, 3 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ/ppm = 161.6, 150.8, 140.6, 135.2, 131.4, 122.2, 119.6, 113.6, 111.1, 106.1, 51.6, 20.0. Anal. Calcd for $C_{13}H_{12}N_4O$: C, 64.99, H, 5.03, N, 23.32. Found: C, 64.77, H, 4.88, N, 23.54.

4.2.5. N-(1-methyl-β-caboline-3-carbonyl)-N′-(Boc-glycyl)-hydrazine (**5a**)

At 0 °C to the solution of 400 mg (2.29 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 (2.08 mmol) of 1-methyl-β-carboline-3-carbonylhydrazine (**4**) 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 ($CHCl_3/CH_3OH$,15:1) indicated the complete disappearance of **4**. 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 419 mg (50%) of the title compound as yellowing powder. Mp 219–220 °C; ESI-MS (*m*/*z*) 398 $[M + H]^+$; $[\alpha]_D^{20} = -22.72$ (*c* 1.2, CH_3OH); IR (cm^{-1}): 3382, 3354, 3108, 3059, 2978, 2929, 1707, 1686, 1621, 1494, 1466, 1446, 1392, 1368, 1348, 1282, 1249, 1168, 1049, 735; ^1H NMR (300 MHz, DMSO-d_6) δ/ppm = 12.01 (s, 1 H), 10.21 (s, 1 H), 10.17 (s, 1 H), 8.69 (s, 1 H), 8.37 (d, *J* = 6.0 Hz, 1 H), 7.64 (d, *J* = 3.0 Hz, 1 H), 7.60 (t, *J* = 3.0 Hz, 1 H), 7.30 (t, *J* = 6.0 Hz, 1 H), 7.06 (d, *J* = 6.0 Hz, 1 H), 3.73 (d, *J* = 6.0 Hz, 1 H), 2.86 (s, 3 H), 1.41 (s, 9 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 168.7, 163.7, 156.3, 141.7, 141.2, 138.3, 136.6, 128.8, 127.7, 122.7, 121.9, 120.5, 113.1, 112.7, 78.5, 49.1, 28.7, 20.8. Anal. Calcd for $C_{20}H_{23}N_5O_4$: C, 60.44; H, 5.83; N, 17.62. Found: C, 60.21; H, 5.70; N, 17.86.

4.2.6. N-(1-methyl-β-caboline-3-carbonyl)-N′-(Boc-alanyl)-hydrazine (**5b**)

Using a procedure similar to that of preparing N-(1-methyl-β-caboline-3- carbonyl)-N′-(Boc-glycyl)hydrazine (**5a**) from 500 mg (2.08 mmol) of 1-methyl-β- carboline-3-carbonylhydrazine (**4**) and 433 mg (2.29 mmol) of Boc-L-Ala 386 mg (45%) of the title compound were obtained as yellowing powder. Mp 157–158 °C; ESI-MS (*m*/*z*) 412 $[M + H]^+$; $[\alpha]_D^{20} = -74.20$ (*c* 1.1, CH_3OH); IR (cm^{-1}): 3309, 3252, 2978, 2925, 1687, 1621, 1523, 1495, 1450, 1392, 1368, 1348, 1249, 1164, 1049, 861, 726; ^1H NMR (300 MHz, DMSO-d_6) δ/ppm = 12.01 (s, 1 H), 10.22 (s, 2 H), 8.69 (s, 1 H), 8.37 (d, *J* = 6.0 Hz, 1 H), 7.67 (d, *J* = 3.0 Hz, 1 H), 7.60 (t, *J* = 3.0 Hz, 1 H), 7.30 (t, *J* = 6.0 Hz, 1 H), 7.01 (d, *J* = 6.0 Hz, 1 H), 4.19 (m, *J* = 6.0 Hz, 1 H), 2.86 (s, 3 H), 1.41 (s, 9 H), 1.32 (d, *J* = 6.0 Hz, 3 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 172.1, 163.6, 155.4, 141.7, 141.3, 138.3, 136.5, 128.8, 127.8, 122.6, 121.9, 120.5, 113.1, 112.7, 78.5, 55.4, 28.7, 20.8, 19.0. Anal. Calcd for $C_{21}H_{25}N_5O_4$: C, 61.30; H, 6.12; N, 17.02. Found: C, 61.52; H, 6.37; N, 17.27.

4.2.7. N-(1-methyl-β-caboline-3-carbonyl)-N′-(Boc-isoleucyl)-hydrazine (**5c**)

Using a procedure similar to that of preparing N-(1-methyl-β-caboline-3-carbonyl)-N′-(Boc-glycyl)hydrazine (**5a**) from 500 mg (2.08 mmol) of 1-methyl-β-carboline-3-carbonylhydrazine (**4**) and 529 mg (2.29 mmol) of Boc-L-Ile 448 mg (48%) of the title compound were obtained as yellowing powder. Mp 220–221 °C; ESI-MS (*m*/*z*) 454 $[M + H]^+$; $[\alpha]_D^{20} = -51.17$ (*c* 1.2, CH_3OH); IR (cm^{-1}): 3272, 2966, 2929, 2872, 1691, 1666, 1621, 1568, 1495, 1462, 1392, 1368, 1348, 1286, 1249, 1168, 1045, 1012, 874, 735; ^1H NMR (300 MHz, DMSO-d_6) δ/ppm = 12.00 (s, 1 H), 10.23 (s, 2 H), 8.69 (s, 1 H), 8.36 (d, *J* = 7.8 Hz, 1 H), 7.65 (d, *J* = 7.2 Hz, 1 H), 7.59 (t, *J* = 7.5 Hz, 1 H), 7.30 (t, *J* = 7.5 Hz, 1 H), 6.78 (d, *J* = 9.0 Hz, 1 H), 4.00 (m, *J* = 8.7 Hz, 1 H), 2.86 (s, 3 H), 1.75 (m, 1 H), 1.54 (m, 1 H), 1.41 (s, 9 H), 1.15 (s, 1 H), 0.96 (d, *J* = 6.6 Hz, 3 H), 0.86 (t, *J* = 7.2 Hz, 3 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 170.6, 163.7, 155.7, 141.6, 141.2, 138.3, 136.5, 128.8, 127.7, 122.6, 121.9, 120.5, 113.1, 112.7, 78.4, 60.2, 45.9, 28.7, 24.8, 20.8, 15.7, 9.3. Anal. Calcd for $C_{24}H_{31}N_5O_4$: C, 63.56; H, 6.89; N, 15.44. Found: C, 63.35; H, 6.74; N, 15.21.

4.2.8. N-(1-methyl-β-caboline-3-carbonyl)-N′-(Boc-leucyl)-hydrazine (**5d**)

Using a procedure similar to that of preparing N-(1-methyl-β-caboline-3-carbonyl)-N′-(Boc-glycyl)hydrazine (**5a**) from 500 mg (2.08 mmol) of 1-methyl-β-carboline-3-carbonylhydrazine (**4**) and 529 mg (2.29 mmol) of Boc-L-Leu 464 mg (49%) of the title compound were obtained as yellowing powder. Mp 186–188 °C; ESI-MS (*m*/*z*) 454 $[M + H]^+$; $[\alpha]_D^{20} = -15.87$ (*c* 1.2, CH_3OH); IR (cm^{-1}): 3329, 3239, 3043, 2957, 2929, 2871, 1695, 1670, 1625, 1523, 1495, 1446, 1397, 1368, 1348, 1278, 1249, 1168, 1119, 1049, 1025, 878, 730; ^1H NMR (300 MHz, DMSO-d_6) δ/ppm = 12.00 (s, 1 H), 10.23 (s, 1 H), 10.21 (s, 1 H), 8.69 (s, 1 H), 8.35 (d, *J* = 5.1 Hz, 1 H), 7.65 (d, *J* = 5.1 Hz, 1 H), 7.59 (t, *J* = 7.5 Hz, 1 H), 7.30 (t, *J* = 7.2 Hz, 1 H), 6.93 (d, *J* = 8.4 Hz, 1 H), 4.19 (m, *J* = 7.8 Hz, 1 H), 2.86 (s, 3 H), 1.71 (m, 1 H), 1.53 (t, *J* = 6.9 Hz, 2 H), 1.41 (s, 9 H), 0.92 (d, *J* = 5.1 Hz, 6 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 171.9, 163.7, 155.7, 141.6, 141.2, 138.3, 136.5, 128.8, 127.7, 122.6, 121.9, 120.5, 113.1, 112.7, 78.4, 51.7, 41.7, 28.7, 24.6, 23.5, 22.1, 20.8. Anal. Calcd for $C_{24}H_{31}N_5O_4$: C, 63.56; H, 6.89; N, 15.44. Found: C, 63.75; H, 7.04; N, 15.22.

4.2.9. N-(1-methyl-β-caboline-3-carbonyl)-N′-(Boc-valyl)-hydrazine (**5e**)

Using a procedure similar to that of preparing N-(1-methyl-β-caboline-3- carbonyl)-N′-(Boc-glycyl)hydrazine (**5a**) from 500 mg (2.08 mmol) of 1-methyl-β- carboline-3-carbonylhydrazine (**4**) and 497 mg (2.29 mmol) of Boc-L-Val 467 mg (51%) of the title compound were obtained as yellowing powder. Mp 220–221 °C; ESI-MS (*m*/*z*) 440 $[M + H]^+$; $[\alpha]_D^{20} = -59.03$ (*c* 1.1, CH_3OH); IR (cm^{-1}) 3313, 3260, 2969, 2933, 1686, 1662, 1621, 1531, 1495, 1462, 1392, 1368, 1352, 1298, 1249, 1172, 1045, 1025, 870, 735; ^1H NMR (300 MHz, DMSO-d_6) δ/ppm = 12.00 (s, 1 H), 10.23 (s, 1 H), 10.21 (s, 1 H), 8.69 (s, 1 H), 8.35 (d, *J* = 7.8 Hz, 1 H), 7.64 (d, *J* = 8.1 Hz, 1 H), 7.60 (t, *J* = 8.1 Hz, 1 H), 7.30 (t, *J* = 7.8 Hz, 1 H), 6.72 (d, *J* = 9.3 Hz, 1 H), 3.98 (m, *J* = 9.0 Hz, 1 H), 2.86 (s, 3 H), 1.99 (m, 1 H), 1.41 (s, 9 H), 1.00 (d, *J* = 6.9 Hz, 6 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 170.6, 163.8, 155.8, 141.6, 141.2, 138.3, 136.5, 128.8, 127.7, 122.6, 121.9, 120.5, 113.1, 112.7, 78.5, 58.6, 30.9, 28.7, 20.8, 19.7, 18.7. Anal. Calcd for $C_{23}H_{29}N_5O_4$: C, 62.85; H, 6.65; N, 15.93. Found: C, 63.04; H, 6.41; N, 15.70.

4.2.10. N-(1-methyl-β-caboline-3-carbonyl)-N′-(Boc-methionyl)-hydrazine (**5f**)

Using a procedure similar to that of preparing N-(1-methyl-β-caboline-3- carbonyl)-N′-(Boc-glycyl)hydrazine (**5a**) from 500 mg (2.08 mmol) of 1-methyl-β- carboline-3-carbonylhydrazine (**4**) and 570 mg (2.29 mmol) of Boc-L-Met 336 mg (34%) of the title compound were obtained as yellowing powder. Mp 203–205 °C; ESI-MS (*m*/*z*) 472 $[M + H]^+$; $[\alpha]_D^{20} = -36.67$ (*c* 1.2, CH_3OH); IR (cm^{-1}): 3313, 3260, 2978, 2912, 1686, 1653, 1621, 1568, 1523, 1494, 1461, 1446, 1392, 1368, 1348, 1282, 1249, 1168, 1053, 1021, 865, 726; ^1H NMR (300 MHz, DMSO-d_6) δ/ppm = 12.00 (s, 1 H), 10.23 (s, 1 H), 10.19 (s, 1 H), 8.69 (s, 1 H), 8.36 (d, *J* = 8.1 Hz, 1 H), 7.65 (d, *J* = 8.1 Hz, 1 H), 7.59 (t, *J* = 7.5 Hz, 1 H), 7.29 (t, *J* = 7.5 Hz, 1 H), 7.05 (d, *J* = 8.1 Hz, 1 H), 4.19 (m, 1 H), 2.86 (s, 3 H), 2.59 (t, *J* = 7.5 Hz, 1 H), 2.09 (s, 3 H), 1.95 (m, 2 H), 1.41 (s, 9 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 171.1, 163.9, 155.7, 141.6, 141.2, 138.3, 136.5, 128.8, 127.7, 122.6, 121.9, 120.5, 113.1, 112.7, 78.6, 52.7, 32.7, 29.9, 28.7, 20.8, 15.6. Anal. Calcd for $C_{23}H_{29}N_5O_4S$: C, 58.58; H, 6.20; N, 14.85. Found: C, 58.37; H, 6.04; N, 14.62.

4.2.11. N-(1-methyl-β-caboline-3-carbonyl)-N′-(Boc-prolyl)-hydrazine (**5g**)

Using a procedure similar to that of preparing N-(1-methyl-β-caboline-3- carbonyl)-N′-(Boc-glycyl)hydrazine (**5a**) from 500 mg (2.08 mmol) of 1-methyl-β- carboline-3-carbonylhydrazine (**4**) and 492 mg (2.29 mmol) of Boc-L-Pro 476 mg (52%) of the title compound were obtained as yellowing powder. Mp 227–228 °C; ESI-MS (*m*/*z*) 438 $[M + H]^+$; $[\alpha]_D^{20} = -63.25$ (*c* 1.1, CH_3OH); IR (cm^{-1}): 3382, 3301, 3244, 3019, 2974, 2929, 1707, 1683, 1629, 1572, 1523, 1486, 1458, 1442, 1405, 1368, 1352, 1303, 1249, 1168, 1115, 1086, 890, 751; ^1H NMR (300 MHz, DMSO-d_6) δ/ppm = 12.00 (s, 1 H), 10.21 (s, 1 H), 10.14 (s, 1 H), 8.72 (s, 1 H), 8.37 (d, *J* = 7.5 Hz, 1 H), 7.65 (d, *J* = 8.1 Hz, 1 H), 7.59 (t, *J* = 7.2 Hz, 1 H), 7.29 (t, *J* = 7.2 Hz, 1 H), 4.27 (m, 1 H), 3.43 (m, 1 H), 3.30 (m, 1 H), 2.88 (s, 3 H), 2.19 (m, 1 H), 1.80 (m, 3 H), 1.41 (s, 9 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 171.1, 163.6, 157.1, 153.7, 141.6, 141.2, 138.3, 136.5, 128.8, 127.7, 122.6, 121.9, 120.5, 113.1, 112.7, 79.1, 58.7, 47.9, 31.5, 28.6, 24.9, 20.8. Anal. Calcd for $C_{23}H_{27}N_5O_4$: C, 63.14; H, 6.22; N, 14.63. Found: C, 63.36; H, 6.38; N, 14.41.

4.2.12. N-(1-methyl-β-caboline-3-carbonyl)-N′-(Boc-tyrosyl)-hydrazine (**5h**)

Using a procedure similar to that of preparing N-(1-methyl-β-caboline-3- carbonyl)-N′-(Boc-glycyl)hydrazine (**5a**) from 500 mg (2.08 mmol) of 1-methyl-β- carboline-3-carbonylhydrazine (**4**) and 644 mg (2.29 mmol) of Boc-L-Tyr 422 mg (40%) of the title compound were obtained as yellowing powder. Mp 164–166 °C; ESI-MS (*m*/*z*) 504 $[M + H]^+$; $[\alpha]_D^{20} = -9.77$ (*c* 1.1, CH_3OH); IR (cm^{-1}) 3305, 2978, 2925, 1691, 1670, 1621, 1597, 1519, 1495, 1462, 1446, 1388, 1368, 1348, 1249, 1168, 1098, 1049, 1021, 751, 739; ^1H NMR (300 MHz, DMSO-d_6) δ/ppm = 12.05 (s, 1 H), 10.40 (s, 1 H), 10.25 (s, 1 H), 9.18 (s, 1 H), 8.71 (s, 1 H), 8.37 (d, *J* = 7.8 Hz, 1 H), 7.66 (d, *J* = 8.1 Hz, 1 H), 7.59 (t, *J* = 7.2 Hz, 1 H), 7.30 (t, *J* = 7.5 Hz, 1 H), 7.13 (d, *J* = 8.1 Hz, 2 H), 6.90 (d, *J* = 8.7 Hz, 1 H), 6.68 (d, *J* = 8.4 Hz, 2 H), 4.28 (m, 1 H), 4.07 (m, 1 H), 3.02 (m, 1 H), 2.86 (m, 1 H), 1.33 (s, 9 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 171.2, 163.6, 156.2, 155.5, 141.7, 141.2, 138.3, 136.5, 130.7, 128.8, 127.7, 122.6, 121.9, 120.5, 115.3, 113.1, 112.7, 78.4, 56.5, 37.5, 28.6, 20.8. Anal. Calcd for $C_{27}H_{29}N_5O_5$: C, 64.40; H, 5.80; N, 13.91. Found: C, 64.59; H, 5.95; N, 13.69.

4.2.13. N-(1-methyl-β-caboline-3-carbonyl)-N′-(Boc-threonyl)-hydrazine (**5i**)

Using a procedure similar to that of preparing N-(1-methyl-β-caboline-3- carbonyl)-N′-(Boc-glycyl)hydrazine (**5a**) from 500 mg (2.08 mmol) of 1-methyl-β- carboline-3-carbonylhydrazine (**4**) and 501 mg (2.29 mmol) of Boc-L-Thr 348 mg (38%) of the title compound were obtained as yellowing powder. Mp 148–149 °C; ESI-MS (*m*/*z*) 442 $[M + H]^+$; $[\alpha]_D^{20} = -49.83$ (*c* 1.3, CH_3OH); IR (cm^{-1}): 3293, 2978, 2933, 1695, 1625, 1601, 1568, 1495, 1458, 1397, 1368, 1348, 1303, 1282, 1249, 1168, 1086, 1057, 878, 751, 739; ^1H NMR (300 MHz, DMSO-d_6) δ/ppm = 12.00 (s, 1 H), 10.22 (s, 1 H), 10.20 (s, 1 H), 8.70 (s, 1 H), 8.36 (d, *J* = 7.8 Hz, 1 H), 7.66 (d, *J* = 8.1 Hz, 1 H), 7.59 (t, *J* = 7.2 Hz, 1 H), 7.30 (t, *J* = 7.8 Hz, 1 H), 6.39 (d, *J* = 3.9 Hz, 1 H), 4.79 (t, *J* = 3.6 Hz, 1 H), 4.07 (m, 1 H), 4.02 (m, 1 H), 2.86 (s, 3 H), 1.42 (s, 9 H), 1.19 (d, *J* = 3.6 Hz, 3 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 169.7, 163.6, 155.7, 141.7, 141.2, 138.1, 136.5, 128.8, 127.7, 122.6, 121.9, 120.5, 113.1, 112.7, 78.7, 67.6, 65.3, 28.6, 20.8. Anal. Calcd for $C_{22}H_{27}N_5O_5$: C, 59.85; H, 6.16; N, 15.86. Found: C, 59.64; H, 6.01; N, 15.63.

4.2.14. N-(1-methyl-β-caboline-3-carbonyl)-N′-(Boc-seryl)-hydrazine (**5j**)

Using a procedure similar to that of preparing N-(1-methyl-β-caboline-3- carbonyl)-N′-(Boc-glycyl)hydrazine (**5a**) from 500 mg (2.08 mmol) of 1-methyl-β- carboline-3-carbonylhydrazine (**4**) and 469 mg (2.29 mmol) of Boc-L-Ser 324 mg (37%) of the title compound were obtained as yellowing powder. Mp 144–146 °C; ESI-MS (*m*/*z*) 428 $[M + H]^+$; $[\alpha]_D^{20} = -16.10$ (*c* 1.1, CH_3OH); IR (cm^{-1}): 3284, 2982, 2933, 1695, 1625, 1597, 1572, 1495, 1462, 1392, 1368, 1348, 1303, 1282, 1249, 1168, 1061, 898, 755, 735; ^1H NMR (300 MHz, DMSO-d_6) δ/ppm = 12.02 (s, 1 H), 10.30 (s, 1 H), 10.24 (s, 1 H), 8.70 (s, 1 H), 8.36 (d, *J* = 4.8 Hz, 1 H), 7.66 (d, *J* = 4.8 Hz, 1 H), 7.59 (t, *J* = 4.5 Hz, 1 H), 7.30 (t, *J* = 4.5 Hz, 1 H), 6.75 (d, *J* = 5.1 Hz, 1 H), 4.91 (t, *J* = 3.6 Hz, 1 H), 4.21 (dd, *J* = 7.8 Hz, *J* = 3.6 Hz, 1 H), 3.66 (m, 2 H), 2.86 (s, 3 H), 1.41 (s, 9 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 169.7, 163.6, 155.6, 141.7, 141.2, 138.1, 136.5, 128.8, 127.7, 122.6, 121.9, 120.5, 113.1, 112.7, 78.6, 62.6, 56.0, 28.7, 20.8. Anal. Calcd for $C_{21}H_{25}N_5O_5$: C, 59.01; H, 5.90; N, 16.38. Found: C, 58.82; H, 5.75; N, 16.61.

4.2.15. N-(1-methyl-β-caboline-3-carbonyl)-N′-(Boc-trptophanyl)-hydrazine (**5k**)

Using a procedure similar to that of preparing N-(1-methyl-β-caboline-3- carbonyl)-N′-(Boc-glycyl)hydrazine (**5a**) from 500 mg (2.08 mmol) of 1-methyl-β- carboline-3-carbonylhydrazine (**4**) and 696 mg (2.29 mmol) of Boc-L-Trp 445 mg (41%) of the title compound were obtained as yellowing powder. Mp 169–171 °C; ESI-MS (*m*/*z*) 527 $[M + H]^+$; $[\alpha]_D^{20} = -22.43$ (*c* 1.2, CH_3OH); IR (cm^{-1}): 3427, 3407, 3387, 3313, 3051, 2978, 2929, 1691, 1666, 1625, 1601, 1572, 1531, 1495, 1458, 1392, 1368, 1352, 1274, 1249, 1172, 1094, 1049, 1045, 1008, 739; ^1H NMR (300 MHz, DMSO-d_6) δ/ppm = 12.02 (s, 1 H), 10.84 (s, 1 H), 10.47 (s, 1 H), 10.24 (s, 1 H), 8.73 (s, 1 H), 8.38 (d, *J* = 3.0 Hz, 1 H), 7.72 (d, *J* = 6.0 Hz, 1 H), 7.68 (t, *J* = 3.0 Hz, 1 H), 7.60 (t, *J* = 3.0 Hz, 1 H), 7.35 (d, *J* = 3.0 Hz, 1 H), 7.33 (d, *J* = 3.0 Hz, 1 H), 7.29 (s, 1 H), 7.09 (t, *J* = 3.0 Hz, 1 H), 7.03 (t, *J* = 3.0 Hz, 1 H), 6.80 (t, *J* = 3.0 Hz, 1 H), 4.44 (m, 1 H), 3.24 (dd, *J* = 9.0 Hz, *J* = 3.0 Hz, 1 H), 3.03 (t, *J* = 6.0 Hz, 1 H), 2.88 (s, 3 H), 1.34 (s, 9 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 171.2, 163.6, 155.6, 141.7, 141.2, 138.3, 136.5, 128.8, 127.9, 127.8, 124.8, 124.4, 122.6, 121.9, 121.2, 120.9, 120.5, 119.0, 118.6, 113.7, 113.1, 112.7, 111.7, 110.7, 110.4, 78.4, 49.1, 28.6, 20.8. Anal. Calcd for $C_{29}H_{30}N_6O_4$: C, 66.14; H, 5.74; N, 15.96. Found: C, 66.33; H, 5.59; N, 15.74.

4.2.16. N-(1-methyl-β-caboline-3-carbonyl)-N′-(di-Boc-histidyl)-hydrazine (**5l**)

Using a procedure similar to that of preparing N-(1-methyl-β-caboline-3- carbonyl)-N′-(Boc-glycyl)hydrazine (**5a**) from 500 mg (2.08 mmol) of 1-methyl-β- carboline-3-carbonylhydrazine (**4**) and 813 mg (2.29 mmol) of Boc-L-His(Boc) 524 mg (44%) of the title compound were obtained as yellowing powder. Mp 184–185 °C;

ESI-MS (*m*/*z*) 578 $[M + H]^+$; $[\alpha]_D^{20} = -39.90$ (*c* 1.1, CH_3OH); IR (cm^{-1}) 3284, 2978, 2929, 1756, 1690, 1666, 1625, 1596, 1568, 1490, 1457, 1396, 1372, 1348, 1282, 1249, 1164, 1053, 1008, 845, 775, 755, 739; ^1H NMR (300 MHz, DMSO-d_6) δ/ppm = 12.00 (s, 1 H), 10.31 (s, 1 H), 10.21 (s, 1 H), 8.69 (s, 1 H), 8.35 (d, J = 7.8 Hz, 1 H), 8.15 (s, 1 H), 7.66 (d, J = 8.1 Hz, 1 H), 7.59 (t, J = 7.5 Hz, 1 H), 7.30 (t, J = 7.5 Hz, 2 H), 6.96 (d, J = 8.7 Hz, 1 H), 4.42 (m, 1 H), 2.95 (m, 1 H), 2.86 (s, 4 H), 1.57 (s, 9 H), 1.37 (s, 9 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 170.8, 163.7, 155.6, 147.2, 141.7, 141.2, 139.9, 138.3, 137.0, 136.5, 128.8, 127.7, 122.6, 121.9, 120.5, 114.9, 113.1, 112.7, 85.6, 78.6, 54.2, 31.5, 28.6, 20.8. Anal. Calcd for $C_{29}H_{35}N_7O_6$: C, 60.30; H, 6.11; N, 16.97. Found: C, 60.09; H, 6.27; N, 16.74.

4.2.17. *N-(1-methyl-β-caboline-3-carbonyl)-N′-(di-Boc-lysyl)-hydrazine (**5m**)*

Using a procedure similar to that of preparing N-(1-methyl-β-caboline-3- carbonyl)-N′-(Boc-glycyl)hydrazine (**5a**) from 500 mg (2.08 mmol) of 1-methyl-β- carboline-3-carbonylhydrazine (**4**) and 792 mg (2.29 mmol) of Boc-L-Lys(Boc) 392 mg (33%) of the title compound were obtained as yellowing powder. Mp 125–127 °C; ESI-MS (*m*/*z*) 569 $[M + H]^+$; $[\alpha]_D^{20} = -34.07$ (*c* 1.3, CH_3OH); IR (cm^{-1}): 3329, 3268, 2978, 2929, 2864, 1687, 1625, 1601, 1519, 1495, 1458, 1392, 1368, 1348, 1282, 1249, 1168, 1053, 1012, 878, 739; ^1H NMR (300 MHz, DMSO-d_6) δ/ppm = 12.00 (s, 1 H), 10.20 (s, 1 H), 8.35 (d, J = 7.8 Hz, 1 H), 7.65 (d, J = 7.8 Hz, 1 H), 7.65 (t, J = 7.2 Hz, 1 H), 7.29 (d, J = 7.2 Hz, 1 H), 6.89 (d, J = 8.7 Hz, 1 H), 6.79 (m, 1 H), 4.06 (m, 1 H), 2.93 (m, 2 H), 2.86 (s, 3 H), 1.66 (m, 2 H), 1.38 (m, 24 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 171.6, 163.6, 156.1, 155.7, 141.6, 141.2, 138.3, 136.5, 128.8, 127.8, 122.6, 121.9, 120.5, 113.1, 112.7, 78.5, 77.8, 60.2, 53.3, 32.5, 29.7, 28.7, 23.1, 20.8. Anal. Calcd for $C_{29}H_{40}N_6O_6$: C, 61.25; H, 7.09; N, 14.78. Found: C, 61.06; H, 6.93; N, 14.99.

4.2.18. *N-(1-methyl-β-caboline-3-carbonyl)-N′-[Boc-(β-OBzl-aspartyl)]-hydrazine (**5n**)*

Using a procedure similar to that of preparing N-(1-methyl-β-caboline-3- carbonyl)-N′-(Boc-glycyl)hydrazine (**5a**) from 500 mg (2.08 mmol) of 1-methyl-β- carboline-3-carbonylhydrazine (**4**) and 740 mg (2.29 mmol) of Boc-L-Asp(OBzl) 348 mg (31%) of the title compound were obtained as yellowing powder. Mp 130–132 °C; ESI-MS (*m*/*z*) 546 $[M + H]^+$; $[\alpha]_D^{20} = -22.32$ (*c* 1.2, CH_3OH); IR (cm^{-1}): 3309, 3260, 3064, 3031, 2982, 2929, 1719, 1691, 1662, 1625, 1601, 1572, 1499, 1462, 1392, 1372, 1352, 1278, 1249, 1164, 1049, 1025, 865, 751, 739; ^1H NMR (300 MHz, DMSO-d_6) δ/ppm = 11.99 (s, 1 H), 10.17 (s, 1 H), 8.35 (d, J = 7.8 Hz, 1 H), 7.65 (d, J = 7.8 Hz, 1 H), 7.59 (t, J = 7.2 Hz, 1 H), 7.36 (m, 6 H), 7.19 (d, J = 8.1 Hz, 1 H), 5.14 (s, 2 H), 4.56 (m, 1 H), 2.91 (m, 2 H), 2.86 (s, 3 H), 2.71 (m, 2 H), 1.41 (s, 9 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 170.4, 163.7, 155.6, 141.6, 141.2, 138.3, 136.5, 128.8, 127.7, 127.0, 122.6, 121.8, 120.5, 113.1, 112.7, 78.8, 66.1, 50.1, 37.1, 28.7, 20.8. Anal. Calcd for $C_{29}H_{31}N_5O_6$: C, 63.84; H, 5.73; N, 12.84. Found: C, 63.62; H, 5.58; N, 13.06.

4.2.19. *N-(1-methyl-β-caboline-3-carbonyl)-N′-[Boc-(γ-OBzl-glutamoyl)]-hydrazine (**5o**)*

Using a procedure similar to that of preparing N-(1-methyl-β-caboline-3-carbonyl)-N′-(Boc-glycyl)hydrazine (**5a**) from 500 mg (2.08 mmol) of 1-methyl-β-carboline-3-carbonylhydrazine (**4**) and 771 mg (2.29 mmol) of Boc-L-Glu(OBzl) 351 mg (30%) of the title compound were obtained as yellowing powder. Mp 191–193 °C; ESI-MS (*m*/*z*) 560 $[M + H]^+$; $[\alpha]_D^{20} = -21.10$ (*c* 1.3, CH_3OH); IR (cm^{-1}): 3329, 3276, 3064, 3031, 3006, 2982, 2929, 1723, 1687, 1662, 1625, 1593, 1523, 1491, 1462, 1450, 1392, 1368, 1348, 1286, 1249, 1168, 1082, 1066, 1025, 878, 730; ^1H NMR (300 MHz, DMSO-d_6) δ/ppm = 11.99 (s, 1 H), 10.23 (s, 1 H), 10.19 (s, 1 H), 8.69 (s, 1 H), 8.35 (d, J = 7.8 Hz, 1 H), 7.65 (d, J = 7.8 Hz, 1 H), 7.59 (t, J = 7.5 Hz, 1 H), 7.36 (m, 6 H), 7.02 (d, J = 8.1 Hz, 1 H), 5.13 (s, 2 H), 4.16 (m, 1 H), 2.86 (s, 3 H), 2.58 (m, 2 H), 1.97 (m, 2 H), 1.41 (s, 9 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 172.8, 170.9, 163.9, 155.6, 141.6, 141.2, 138.3, 136.7, 128.9, 128.4, 127.7, 122.6, 121.8, 120.5, 113.2, 112.7, 78.6, 65.9, 52.5, 30.4, 28.7, 28.1, 20.8. Anal. Calcd for $C_{30}H_{33}N_5O_6$: C, 64.39; H, 5.94; N, 12.51. Found: C, 64.28; H, 5.80; N, 12.29.

4.2.20. *N-(1-methyl-β-caboline-3-carbonyl)-N′-(Boc-glutaminyl)-hydrazine (**5p**)*

Using a procedure similar to that of preparing N-(1-methyl-β-caboline-3- carbonyl)-N′-(Boc-glycyl)hydrazine (**5a**) from 500 mg (2.08 mmol) of 1-methyl-β- carboline-3-carbonylhydrazine (**4**) and 563 mg (2.29 mmol) of Boc-L-Gln 130 mg (13%) of the title compound were obtained as yellowing powder. Mp 215–216 °C; ESI-MS (*m*/*z*) 469 $[M + H]^+$; $[\alpha]_D^{20} = -21.61$ (*c* 1.1, CH_3OH); IR (cm^{-1}): 3423, 3333, 3268, 2978, 2921, 2851, 1691, 1646, 1519, 1499, 1450, 1392, 1368, 1352, 1319, 1278, 1249, 1172, 1057, 865, 722; ^1H NMR (300 MHz, DMSO-d_6) δ/ppm = 12.01 (s, 1 H), 10.15 (s, 2 H), 8.69 (s, 1 H), 8.36 (d, J = 7.5 Hz, 1 H), 7.65 (d, J = 8.1 Hz, 1 H), 7.59 (t, J = 7.5 Hz, 1 H), 7.29 (m, 2 H), 6.97 (d, J = 8.1 Hz, 1 H), 6.57 (s, 1 H), 4.10 (m, 1 H), 2.86 (s, 3 H), 2.25 (m, 2 H), 1.96 (m, 1 H), 1.73 (m, 1 H), 1.41 (s, 9 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 174.3, 171.1, 163.7, 155.6, 141.7, 141.2, 138.3, 136.5, 128.8, 127.7, 122.6, 121.8, 120.5, 113.1, 112.7, 78.6, 53.0, 31.9, 28.7, 20.8. Anal. Calcd for $C_{23}H_{28}N_6O_5$: C, 58.96; H, 6.02; N, 17.94. Found: C, 58.74; H, 5.86; N, 17.72.

4.2.21. *N-(1-methyl-β-caboline-3-carbonyl)-N′-glycylhydrazine (**6a**)*

At 0 °C to the solution of 100 mg (0.252 mmol) of N-[(1S,3S)-1-methyl-β- caboline-3-carbonyl]-N′-(Boc-glycyl)-hydrazine (**5a**) in 6 ml hydrogen chloride/ethyl acetate (4N) were added dropwise. The reaction solution was stirred at 0 °C for 2 h and ($CHCl_3$/CH_3OH, 10:1) indicated the complete disappearance of **5a**. 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 687 mg (92%) of the title compound as yellowing powder. Mp 231–233 °C; ESI-MS (*m*/*z*) 298 $[M + H]^+$; $[\alpha]_D^{20} = 9.98$ (*c* 1.2, CH_3OH); IR (cm^{-1}): 3423, 2998, 2962, 2925, 2855, 1691, 1625, 1511, 1364, 1245, 755; ^1H NMR (300 MHz, DMSO-d_6) δ/ppm = 12.72 (s, 1 H), 10.85 (s, 1 H), 8.99 (s, 1 H), 8.40 (m, 4 H), 7.72 (d, J = 8.1 Hz, 1 H), 7.67 (t, J = 7.8 Hz, 1 H), 7.37 (t, J = 7.2 Hz, 1 H), 3.74 (m, 2 H), 2.99 (s, 3 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 170.2, 165.7, 162.2, 142.5, 142.1, 136.4, 130.3, 128.9, 123.0, 121.5, 121.3, 114.6, 113.3, 65.4, 19.0. Anal. Calcd for $C_{15}H_{15}N_5O_2$: C, 60.60; H, 5.09; N, 23.56. Found: 60.41; H, 4.93; N, 23.33.

4.2.22. *N-(1-methyl-β-caboline-3-carbonyl)-N′-alanylhydrazine (**6b**)*

Using a procedure similar to that of preparing N-(1-methyl-β-caboline-3-carbonyl)-N′-glycylhydrazine (**6a**) from 100 mg (0.243 mmol) of N-[(1S,3S)-1-methyl-β-caboline-3-carbonyl]-N′-(Boc-alanyl)-hydrazine (**5b**) 71 mg (93%) of the title compound were obtained as yellowing powder. Mp 228–229 °C; ESI-MS (*m*/*z*) 312 $[M + H]^+$; $[\alpha]_D^{20} = -14.57$ (*c* 1.1, CH_3OH); IR (cm^{-1}): 3309, 3252, 2978, 2925, 1687, 1621, 1523, 1495, 1450, 1392, 1368, 1348, 1249, 1164, 1049, 861, 726; ^1H NMR (300 MHz, DMSO-d_6) δ/ppm = 12.53 (s, 1 H), 10.81 (s, 1 H), 10.74 (s, 1 H), 8.40 (m, 4 H), 7.72 (d, J = 8.1 Hz, 1 H), 7.67 (t, J = 7.8 Hz, 1 H), 7.37 (t, J = 7.2 Hz, 1 H), 4.02 (m, J = 7.2 Hz, 1 H), 2.95 (s, 3 H), 1.50 (d, J = 6.9 Hz, 3 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 169.2, 168.9, 162.9, 142.0, 136.3, 129.8, 128.6, 122.9, 121.5, 121.2, 114.1, 113.1, 48.0, 19.7, 17.8. Anal. Calcd for $C_{16}H_{17}N_5O_2$: C, 61.72; H, 5.50; N, 22.49. Found: C, 61.50; H, 5.35; N, 22.71.

*4.2.23. N-(1-methyl-β-caboline-3-carbonyl)-N′-isoleucylhydrazine (**6c**)*

Using a procedure similar to that of preparing N-(1-methyl-β-caboline-3-carbonyl)-N′-glycylhydrazine (**6a**) from 100 mg (0.221 mmol) of N-(1-methyl-β-caboline-3-carbonyl)-N′-(Boc-isoleucyl)-hydrazine (**5c**) 70 mg (90%) of the title compound were obtained as yellowing powder. Mp 211–212 °C; ESI-MS (*m*/*z*) 354 $[M + H]^+$; $[\alpha]_D^{20} = 10.45$ (*c* 1.1, CH_3OH); IR (cm^{-1}): 3440, 3072, 2966, 2921, 2876, 1715, 1674, 1634, 1507, 1364, 1245, 1217, 1057, 755; ^1H NMR (300 MHz, DMSO-d_6) δ/ppm = 12.72 (s, 1 H), 10.89 (s, 1 H), 10.79 (s, 1 H), 8.40 (m, 4 H), 7.75 (d, $J = 8.1$ Hz, 1 H), 7.67 (t, $J = 7.2$ Hz, 1 H), 7.37 (t, $J = 7.5$ Hz, 1 H), 3.82 (m, 1 H), 3.00 (s, 3 H), 1.94 (m, 1 H), 1.68 (m, 1 H), 1.32 (m, 1 H), 1.05 (d, $J = 7.5$ Hz, 3 H), 0.97 (t, $J = 7.5$ Hz, 3 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 167.2, 166.9, 161.7, 142.8, 142.2, 136.1, 130.6, 129.2, 123.0, 121.7, 121.2, 114.9, 113.4, 55.7, 36.9, 24.6, 18.6, 14.6, 11.8. Anal. Calcd for $C_{19}H_{23}N_5O_2$: C, 64.57; H, 6.56; N, 19.82. Found: C, 64.35; H, 6.41; N, 19.61.

*4.2.24. N-(1-methyl-β-caboline-3-carbonyl)-N′-leucylhydrazine (**6d**)*

Using a procedure similar to that of preparing N-(1-methyl-β-caboline-3-carbonyl)-N′-glycylhydrazine (**6a**) from 100 mg (0.221 mmol) of N-(1-methyl-β-caboline-3-carbonyl)-N′-(Boc-leucyl)-hydrazine (**5d**) 70 mg (90%) of the title compound were obtained as yellowing powder. Mp 238–239 °C; ESI-MS (*m*/*z*) 354 $[M + H]^+$; $[\alpha]_D^{20} = 45.97$ (*c* 1.1, CH_3OH); IR (cm^{-1}): 3439, 3411, 3068, 2961, 2929, 2867, 1715, 1679, 1629, 1507, 1364, 1249, 1217, 1029, 755; ^1H NMR (300 MHz, DMSO-d_6) δ/ppm = 12.94 (s, 1 H), 11.08 (s, 1 H), 9.12 (s, 1 H), 8.40 (m, 4 H), 7.76 (d, $J = 8.1$ Hz, 1 H), 7.67 (t, $J = 8.1$ Hz, 1 H), 7.37 (t, $J = 7.2$ Hz, 1 H), 3.92 (m, 1 H), 2.98 (s, 3 H), 1.94 (m, 1 H), 1.68 (m, 2 H), 0.96 (d, 6 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 168.4, 161.7, 142.6, 142.1, 136.0, 130.7, 129.2, 123.0, 121.7, 121.2, 114.8, 113.4, 50.1, 23.9, 22.9, 18.6. Anal. Calcd for $C_{19}H_{23}N_5O_2$: C, 64.57; H, 6.56; N, 19.82. Found: C, 64.76; H, 6.73; N, 20.03.

*4.2.25. N-(1-methyl-β-caboline-3-carbonyl)-N′-valylhydrazine (**6e**)*

Using a procedure similar to that of preparing N-(1-methyl-β-caboline-3-carbonyl)-N′-glycylhydrazine (**6a**) from 100 mg (0.228 mmol) of N-(1-methyl-β-caboline-3-carbonyl)-N′-(Boc-valyl)-hydrazine (**5e**) 71 mg (92%) of the title compound were obtained as yellowing powder. Mp 233–235 °C; ESI-MS (*m*/*z*) 340 $[M + H]^+$; $[\alpha]_D^{20} = 51.73$ (*c* 1.3, CH_3OH); IR (cm^{-1}): 3448, 3068, 2965, 2880, 1715, 1679, 1629, 1507, 1364, 1282, 1249, 1217, 1111, 1035, 869, 755; ^1H NMR (300 MHz, DMSO-d_6) δ/ppm = 12.69 (s, 1 H), 10.86 (s, 1 H), 9.00 (s, 1 H), 8.40 (m, 4 H), 7.73 (d, $J = 7.8$ Hz, 1 H), 7.67 (t, $J = 7.2$ Hz, 1 H), 7.37 (t, $J = 7.2$ Hz, 1 H), 3.78 (m, 1 H), 2.98 (s, 3 H), 2.20 (m, 1 H), 1.02 (d, 6 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 167.2, 162.0, 142.5, 142.1, 136.1, 130.5, 129.1, 123.0, 121.7, 121.2, 114.7, 113.3, 56.5, 30.4, 18.5. Anal. Calcd for $C_{18}H_{21}N_5O_2$: C, 63.70; H, 6.24; N, 22.64. Found: C, 63.91; H, 6.39; N, 22.86.

*4.2.26. N-(1-methyl-β-caboline-3-carbonyl)-N′-methionylhydrazine (**6f**)*

Using a procedure similar to that of preparing N-(1-methyl-β-caboline-3-carbonyl)-N′-glycylhydrazine (**6a**) from 100 mg (0.212 mmol) of N-(1-methyl-β-caboline-3-carbonyl)-N′-(Boc-methionyl)-hydrazine (**5f**) 71 mg (90%) of the title compound were obtained as yellowing powder. Mp 242–243 °C; ESI-MS (*m*/*z*) 372 $[M + H]^+$; $[\alpha]_D^{20} = 25.17$ (*c* 1.1, CH_3OH); IR (cm^{-1}): 3427, 3080, 2978, 2916, 2843, 1711, 1679, 1629, 1511, 1364, 1249, 1221, 869, 755; ^1H NMR (300 MHz, DMSO-d_6) δ/ppm = 12.75 (s, 1 H), 11.06 (s, 1 H), 9.09 (s, 1 H), 8.59 (m, 3 H), 8.37 (d, $J = 8.1$ Hz, 1 H), 7.75 (d, $J = 8.1$ Hz, 1 H), 7.67 (t, $J = 8.1$ Hz, 1 H), 7.39 (t, $J = 7.5$ Hz, 1 H), 4.14 (m, 1 H), 3.01 (s, 3 H), 2.71 (m, 2 H), 2.21 (m, 2 H), 2.12 (s, 3 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 167.8, 162.5, 142.4, 142.1, 136.2, 130.2, 128.8, 122.9, 121.4, 114.5, 113.2, 51.0, 31.6, 28.2, 19.2, 14.9. Anal. Calcd for $C_{18}H_{21}N_5O_2S$: C, 58.20; H, 5.70; N, 18.85. Found: C, 58.01; H, 5.55; N, 18.62.

*4.2.27. N-(1-methyl-β-caboline-3-carbonyl)-N′-prolylhydrazine (**6g**)*

Using a procedure similar to that of preparing N-(1-methyl-β-caboline-3-carbonyl)-N′-glycylhydrazine (**6a**) from 100 mg (0.212 mmol) of N-(1-methyl-β-caboline-3-carbonyl)-N′-(Boc-prolyl)-hydrazine (**5g**) 71 mg (90%) of the title compound were obtained as yellowing powder. Mp 242–243 °C; ESI-MS (*m*/*z*) 372 $[M + H]^+$; $[\alpha]_D^{20} = 25.17$ (*c* 1.1, CH_3OH); IR (cm^{-1}): 3427, 3080, 2978, 2916, 2843, 1711, 1679, 1629, 1511, 1364, 1249, 1221, 869, 755; ^1H NMR (300 MHz, DMSO-d_6) δ/ppm = 12.75 (s, 1 H), 11.06 (s, 1 H), 9.09 (s, 1 H), 8.59 (m, 3 H), 8.37 (d, $J = 8.1$ Hz, 1 H), 7.75 (d, $J = 8.1$ Hz, 1 H), 7.67 (t, $J = 8.1$ Hz, 1 H), 7.39 (t, $J = 7.5$ Hz, 1 H), 4.14 (m, 1 H), 3.01 (s, 3 H), 2.71 (m, 2 H), 2.21 (m, 2 H), 2.12 (s, 3 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 167.8, 162.5, 142.4, 142.1, 136.2, 130.2, 128.8, 122.9, 121.4, 114.5, 113.2, 51.0, 31.6, 28.2, 19.2, 14.9. Anal. Calcd for $C_{18}H_{21}N_5O_2S$: C, 58.20; H, 5.70; N, 18.85. Found: C, 58.41; H, 5.86; N, 19.07.

*4.2.28. N-(1-methyl-β-caboline-3-carbonyl)-N′-tyrosylhydrazine (**6h**)*

Using a procedure similar to that of preparing N-(1-methyl-β-caboline-3-carbonyl)-N′-glycylhydrazine (**6a**) from 100 mg (0.199 mmol) of N-(1-methyl-β-caboline-3-carbonyl)-N′-(Boc-tyrosyl)-hydrazine (**5h**) 72 mg (89%) of the title compound were obtained as yellowing powder. Mp 198–200 °C; ESI-MS (*m*/*z*) 404 $[M + H]^+$; $[\alpha]_D^{20} = 55.20$ (*c* 1.3, CH_3OH); IR (cm^{-1}): 3403, 3158, 3011, 2929, 1687, 1629, 1515, 1364, 1245, 755; ^1H NMR (300 MHz, DMSO-d_6) δ/ppm = 13.10 (s, 1 H), 11.99 (s, 1 H), 8.39 (m, 5 H), 7.78 (d, $J = 8.4$ Hz, 1 H), 7.71 (t, $J = 7.5$ Hz, 1 H), 7.41 (t, $J = 7.5$ Hz, 1 H), 7.25 (d, $J = 8.4$ Hz, 1 H), 6.73 (m, 4 H), 4.17 (m, 1 H), 4.06 (m, 1 H), 3.20 (m, 1 H), 3.05 (m, 3 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 171.5, 168.8, 161.1, 155.2, 142.1, 140.2, 133.8, 131.7, 130.9, 129.0, 128.8, 125.0, 122.4, 121.7, 119.4, 115.8, 61.1, 113.7, 112.3, 53.6, 35.9, 15.9. Anal. Calcd for $C_{22}H_{21}N_5O_3$: C, 65.50; H, 5.25; N, 17.36. Found: C, 65.28; H, 5.10; N, 17.15.

*4.2.29. N-(1-methyl-β-caboline-3-carbonyl)-N′-threonylhydrazine (**6i**)*

Using a procedure similar to that of preparing N-(1-methyl-β-caboline-3-carbonyl)-N′-glycylhydrazine (**6a**) from 100 mg (0.227 mmol) of N-(1-methyl-β-caboline-3-carbonyl)-N′-(Boc-threonyl)-hydrazine (**5i**) 70 mg (90%) of the title compound were obtained as yellowing powder. Mp 214–215 °C; ESI-MS (*m*/*z*) 342 $[M + H]^+$; $[\alpha]_D^{20} = 33.33$ (*c* 1.3, CH_3OH); IR (cm^{-1}): 3391, 3145, 2978, 2924, 1715, 1687, 1629, 1511, 1364, 1278, 1249, 1221, 1111, 1041, 755; ^1H NMR (300 MHz, DMSO-d_6) δ/ppm = 12.81 (s, 1 H), 10.95 (s, 2 H), 9.05 (s, 1 H), 8.39 (m, 4 H), 7.76 (d, $J = 8.4$ Hz, 1 H), 7.69 (t, $J = 7.2$ Hz, 1 H), 7.39 (t, $J = 7.2$ Hz, 1 H), 3.97 (m, 1 H), 3.74 (m, 1 H), 3.00 (m, 3 H), 1.37 (d, $J = 6.3$ Hz, 1 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 166.3, 162.2, 142.6, 142.1, 136.2, 130.3, 128.9, 122.9, 121.5, 114.5, 113.2, 66.5, 57.7, 20.1. Anal. Calcd for $C_{17}H_{19}N_5O_3$: C, 58.81; H, 5.61; N, 20.52. Found: C, 58.60; H, 5.45; N, 20.30.

*4.2.30. N-(1-methyl-β-caboline-3-carbonyl)-N′-serylhydrazine (**6j**)*

Using a procedure similar to that of preparing N-(1-methyl-β-caboline-3-carbonyl)-N′-glycylhydrazine (**6a**) from 100 mg (0.234 mmol) of N-(1-methyl-β-caboline-3-carbonyl)-N′-(Boc-seryl)-hydrazine (**5j**) 73 mg (90%) of the title compound were obtained as yellowing powder. Mp 206–208 °C; ESI-MS (*m*/*z*) 328 $[M + H]^+$; $[\alpha]_D^{20} = 19.23$ (*c* 1.2, CH_3OH); IR (cm^{-1}): 3399, 3325, 3146, 3007, 2929, 2851, 1715, 1679, 1629, 1576, 1507, 1364, 1025, 755; ^1H NMR (300 MHz, DMSO-d_6) δ/ppm = 12.96 (s, 1 H), 10.98 (s, 1 H),

10.93 (s, 1 H), 9.06 (s, 1 H), 8.46 (m, 3 H), 8.37 (d, J = 7.8 Hz, 1 H), 7.76 (d, J = 7.8 Hz, 1 H), 7.69 (t, J = 6.9 Hz, 1 H), 7.39 (t, J = 7.2 Hz, 1 H), 4.04 (m, 1 H), 3.93 (m, 1 H), 3.83 (m, 1 H), 3.00 (s, 3 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 166.5, 162.3, 142.4, 142.1, 136.2, 130.2, 128.8, 122.9, 121.4, 114.5, 113.2, 61.1, 54.0, 19.2. Anal. Calcd for $C_{16}H_{17}N_5O_3$: C, 58.71; H, 5.23; N, 21.39. Found: C, 58.90; H, 5.38; N, 21.60.

4.2.31. N-(1-methyl-β-caboline-3-carbonyl)-N′-trptophanylhydrazine (**6k**)

Using a procedure similar to that of preparing N-(1-methyl-β-caboline-3-carbonyl)-N′-glycylhydrazine (**6a**) from 100 mg (0.190 mmol) of N-(1-methyl-β-caboline-3-carbonyl)-N′-(Boc-trptophanyl)-hydrazine (**5k**) 74 mg (91%) of the title compound were obtained as yellowing powder. Mp 219–220 °C; ESI-MS (m/z) 427 $[M + H]^+$; $[\alpha]_D^{20}$ = 38.47 (c 1.1, CH_3OH); IR (cm^{-1}): 3387, 3145, 2974, 2921, 2847, 1715, 1679, 1625, 1507, 1454, 1364, 1245, 1102, 747; ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm = 12.65 (s, 1 H), 11.12 (s, 1 H), 8.98 (s, 1 H), 8.36 (m, 3 H), 7.84 (d, J = 7.8 Hz, 1 H), 7.74 (d, J = 8.4 Hz, 1 H), 7.67 (t, J = 7.5 Hz, 1 H), 7.40 (m, 3 H), 7.12 (t, J = 7.5 Hz, 1 H), 7.05 (t, J = 7.5 Hz, 1 H), 4.19 (m, 1 H), 3.43 (dd, J = 5.4 Hz, J = 15.0 Hz, 1 H), 3.25 (dd, J = 8.1 Hz, J = 15.0 Hz, 3 H), 2.99 (s, 3 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 167.7, 162.1, 142.4, 142.1, 136.8, 136.2, 130.3, 128.8, 127.7, 125.8, 123.0, 121.5, 1214, 119.1, 118.9, 114.5, 113.2, 111.9, 107.2, 52.1, 28.0, 19.2. Anal. Calcd for $C_{24}H_{22}N_6O_2$: C, 67.59; H, 5.20; N, 19.71. Found: C, 67.37; H, 5.04; N, 19.48.

4.2.32. N-(1-methyl-β-caboline-3-carbonyl)-N′-histidylhydrazine (**6l**)

Using a procedure similar to that of preparing N-(1-methyl-β-caboline-3-carbonyl)-N′-glycylhydrazine (**6a**) from 100 mg (0.173 mmol) of N-(1-methyl-β-caboline-3-carbonyl)-N′-(di-Boc-histidyl)-hydrazine (**5l**) 55 mg (88%) of the title compound were obtained as yellowing powder. Mp 195–197 °C; ESI-MS (m/z) 378 $[M + H]^+$; $[\alpha]_D^{20}$ = 32.77 (c 1.1, CH_3OH); IR (cm^{-1}): 3415, 3121, 2994, 2921, 2851, 1719, 1683, 1629, 1507, 1364, 1249, 1082, 759; ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm = 12.85 (s, 1 H), 11.14 (s, 1 H), 11.06 (s, 1 H), 9.14 (s, 1 H), 9.04 (s, 1 H), 8.77 (s, 3 H), 8.38 (d, J = 8.4 Hz, 1 H), 7.74 (d, J = 7.8 Hz, 1 H), 7.68 (m, 2 H), 7.38 (t, J = 7.5 Hz, 1 H), 4.64 (m, 1 H), 3.48 (m, 2 H), 3.04 (m, 3 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 167.1, 162.6, 142.5, 142.2, 136.2, 134.5, 130.3, 128.8, 126.6, 122.9, 121.5, 121.3, 118.9, 114.6, 113.2, 50.7, 26.8, 21.2. Anal. Calcd for $C_{19}H_{19}N_7O_2$: C, 60.47; H, 5.07; N, 25.98. Found: C, 60.26; H, 4.92; N, 25.77.

4.2.33. N-(1-methyl-β-caboline-3-carbonyl)-N′-lysylhydrazine (**6m**)

Using a procedure similar to that of preparing N-(1-methyl-β-caboline-3-carbonyl)-N′-glycylhydrazine (**6a**) from 100 mg (0.176 mmol) of N-(1-methyl-β-caboline-3-carbonyl)-N′-(di-Boc-lysyl)-hydrazine (**5m**) 59 mg (90%) of the title compound were obtained as yellowing powder. Mp 192–194 °C; ESI-MS (m/z) 369 $[M + H]^+$; $[\alpha]_D^{20}$ = 42.17 (c 1.2, CH_3OH); IR (cm^{-1}): 3472, 2966, 2925, 1715, 1679, 1629, 1503, 1364, 1249, 1041, 759; ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm = 12.71 (s, 1 H), 10.90 (s, 1 H), 8.53 (s, 3 H), 8.37 (d, J = 7.8 Hz, 1 H), 8.15 (s, 3 H), 7.74 (d, J = 8.1 Hz, 1 H), 7.68 (t, J = 7.5 Hz, 1 H), 7.38 (t, J = 7.5 Hz, 1 H), 3.97 (m, 1 H), 2.99 (s, 3 H), 2.78 (m, 2 H), 1.91 (m, 2 H), 1.60 (m, 4 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 169.4, 161.1, 142.0, 140.3, 133.8, 131.7, 129.0, 128.5, 122.3, 121.7, 119.2, 113.7, 112.1, 52.1, 39.1, 30.4, 26.4, 21.4, 15.8. Anal. Calcd for $C_{19}H_{24}N_6O_2$: C, 61.94; H, 6.57; N, 22.81. Found: C, 61.75; H, 6.41; N, 22.68.

4.2.34. N-(1-methyl-β-caboline-3-carbonyl)-N′-(β-OBzl-aspartyl)-hydrazine (**6n**)

Using a procedure similar to that of preparing N-(1-methyl-β-caboline-3-carbonyl)-N′-glycylhydrazine (**6a**) from 100 mg (0.183 mmol) of N-(1-methyl-β-caboline-3-carbonyl)-N′-[Boc-(β-OBzl-aspartyl)]-hydrazine (**5n**) 40 mg (61%) of the title compound were obtained as yellowing powder. Mp 260–261 °C; ESI-MS (m/z) 356 $[M + H]^+$; $[\alpha]_D^{20}$ = 24.97 (c 1.1, CH_3OH); IR (cm^{-1}) 3403, 3084, 2970, 2921, 2855, 1715, 1683, 1625, 1507, 1364, 1245, 1209, 1143, 759; ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm = 13.12 (s, 1 H), 11.14 (s, 1 H), 10.97 (s, 1 H), 9.12 (s, 1 H), 8.55 (s, 3 H), 8.36 (d, J = 8.1 Hz, 1 H), 7.78 (d, J = 8.1 Hz, 1 H), 7.71 (t, J = 7.5 Hz, 1 H), 7.41 (t, J = 7.5 Hz, 1 H), 4.28 (m, 1H), 3.02 (m, 5 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 171.1, 167.5, 161.8, 142.7, 142.2, 136.0, 133.5, 130.5, 129.1, 123.0, 121.6, 121.2, 114.7, 113.3, 65.3, 48.4, 18.7. Anal. Calcd for $C_{17}H_{17}N_5O_4$: C, 57.46; H, 4.82; N, 19.71. Found: C, 57.24; H, 4.66; N, 19.94.

4.2.35. N-(1-methyl-β-caboline-3-carbonyl)-N′-(γ-OBzl-glutamoyl)-hydrazine (**6o**)

Using a procedure similar to that of preparing N-(1-methyl-β-caboline-3-carbonyl)-N′-glycylhydrazine (**6a**) from 100 mg (0.179 mmol) of N-(1-methyl-β-caboline-3-carbonyl)-N′-[Boc-(γ-OBzl-glutamoyl)]-hydrazine (**5o**) 44 mg (65%) of the title compound were obtained as yellowing powder. Mp 202–204 °C; ESI-MS (m/z) 370 $[M + H]^+$; $[\alpha]_D^{20}$ = 19.67 (c 1.1, CH_3OH); IR (cm^{-1}): 3481, 3101, 2966, 2917, 2851, 1707, 1674, 1629, 1507, 1360, 1249, 1209, 1041, 755; ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm = 13.06 (s, 1 H), 11.18 (s, 1 H), 10.97 (s, 1 H), 9.16 (s, 1 H), 8.60 (s, 3 H), 8.36 (d, J = 8.1 Hz, 1 H), 7.78 (d, J = 8.1 Hz, 1 H), 7.71 (t, J = 7.5 Hz, 1 H), 7.41 (t, J = 7.5 Hz, 1 H), 4.04 (m, 1 H), 3.02 (s, 3 H), 2.68 (m, 1 H), 2.11 (m, 3 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 173.9, 167.9, 162.1, 142.7, 142.2, 136.1, 130.5, 129.1, 123.0, 121.6, 121.3, 114.8, 113.3, 50.9, 29.8, 27.0, 18.7. Anal. Calcd for $C_{18}H_{19}N_5O_4$: C, 58.53; H, 5.18; N, 18.96. Found: C, 58.33; H, 5.02; N, 18.74.

4.2.36. N-(1-methyl-β-caboline-3-carbonyl)-N′-(Boc-glutaminyl)-hydrazine (**6p**)

Using a procedure similar to that of preparing N-(1-methyl-β-caboline-3-carbonyl)-N′-glycylhydrazine (**6a**) from 100 mg (0.214 mmol) of N-(1-methyl-β-caboline-3-carbonyl)-N′-(Boc-glutaminyl)-hydrazine (**5p**) 64 mg (81%) of the title compound was obtained as yellowing powder. Mp 212–214 °C; ESI-MS (m/z) 369 $[M + H]^+$; $[\alpha]_D^{20}$ = 46.70 (c 1.1, CH_3OH); IR (cm^{-1}): 3415, 3158, 2974, 2921, 2855, 1666, 1625, 1507, 1364, 1245, 1041, 755; ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm = 12.93 (s, 1 H), 10.94 (s, 1 H), 9.11 (s, 1 H), 8.58 (s, 3 H), 8.38 (d, J = 8.1 Hz, 1 H), 7.76 (d, J = 8.4 Hz, 1 H), 7.70 (t, J = 8.1 Hz, 1 H), 7.53 (s, 1 H), 7.40 (t, J = 7.5 Hz, 1 H), 7.01 (s, 1 H), 4.01 (m, 1 H), 3.02 (s, 3 H), 2.42 (m, 2 H), 2.11 (m, 2 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 173.9, 167.9, 162.3, 142.4, 142.1, 136.2, 134.4, 130.2, 128.8, 123.0, 121.4, 114.5, 113.2, 51.2, 30.7, 27.7, 19.1. Anal. Calcd for $C_{18}H_{20}N_6O_3$: C, 58.69; H, 5.47; N, 22.81. Found: C, 58.90; H, 5.62; N, 22.58.

4.3. 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 1000 rpm for 10 min and the platelet rich plasma (PRP) was collected. The remaining blood was centrifuged for an additional 10 min at 1500 rpm 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 of **6a**–**p** (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 **6a**–**p** 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 10 mM of stock solution of AA in DMSO/NS, 1/10, with NS), or 50 μL of the solution of TH (final concentration 0.1 U/ml, prepared by diluting 100 U/ml of stock solution of TH in DMSO/NS, 1/10, with NS) was added and aggregation was measured at 37 °C for 5 min. The effects of **6a**–**p** (at a series of concentrations ranging from 100 μM to 1 nM) on PAF or ADP or AA or TH-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 (**6a**–**p**) 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 **6a**–**p**)]/(A_m of NS) × 100%. Am% of NS is the value of platelet aggregation induced by PAF, ADP, AA and TH without **6a**–**p** and are 52.30 ± 1.78%, 50.16 ± 3.65%, 49.62 ± 2.90% and 61.20 ± 2.97%, respectively. The concentration vs. inhibition rate curve is plotted to determine the IC_{50} values with GWBASIC.EXE program.

4.4. 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 **6a**–**p** were dissolved in NS before administration and kept in an ice bath. Male Wister rats weighing 250–300 g (purchased from Animal Center of Peking University) were used. The rats were fed with the solution of aspirin in NS (dose, 165 μmol/kg) or each of **6a**–**p** in NS (dose, 1 nmol/kg) or NS alone, then 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 weight of the wet thrombus.

Acknowledgements

This work was completed in the Beijing area major laboratory of peptide and small molecular drugs, supported by PHR (IHLB, KZ 200910025004), the National Natural Scientific Foundation of China (81072522), and Special Project (2011ZX09302-007-01) of China.

Appendix. Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ejmech.2011.09.027.

References

[1] R. Chaireti, C. Jennersjö, T.L. Lindahl, Thromb. Res. 124 (2009) 178–184.
[2] B. Furie, B.C. Furie, N. Engl. J. Med. 359 (2008) 938–949.
[3] T.W. Wakefield, D.D. Myers, P.K. Henke, Arterioscler. Thromb. Vasc. Biol. 28 (2008) 387–391.
[4] W.S. Nesbitt, E. Westein, F.J. Tovar-Lopez, E. Tolouei, A. Mitchell, J. Fu, J. Carberry, A. Fouras, S.P. Jackson, Nat. Med. 15 (2009) 665–673.
[5] N. Mackman, Nature 451 (2008) 914–918.
[6] K.G. Aragam, D.L. Bhatt, J. Cardiovasc. Pharmacol. Ther. 16 (2011) 24–42.
[7] J.-H. Im, Y.-R. Jin, J.-J. Lee, J.-Y. Yu, X.-H. Han, S.-H. Im, J.T. Hong, H.-S. Yoo, M.-Y. Pyo, Y.-P. Yun, Vasc. Pharmacol. 50 (2009) 147–152.
[8] J.I. Trujillo, M.J. Meyers, D.R. Anderson, S. Hegde, M.W. Mahoney, W.F. Vernier, I.P. Buchler, K.K. Wu, S. Yang, S.J. Hartmann, D.B. Reitz, Bioorg. Med. Chem. Lett. 17 (2007) 4657–4663.
[9] P.R. Jenkins, J. Wilson, D. Emmerson, M.D. Garcia, M.R. Smith, S.J. Gray, R.G. Britton, S. Mahale, B. Chaudhuri, Bioorg. Med. Chem. 16 (2008) 7728–7739.
[10] M.D. Garcia, A.J. Wilson, D.P. Emmerson, P.R. Jenkins, Org. Biomol. Chem. 4 (2006) 4478–4484.
[11] T. Herraiz, D. González, C. Ancín-Azpilicueta, V.J. Arán, H. Guillén, Food Chem. Toxicol. 48 (2010) 839–845.
[12] P.A. Barsanti, W. Wang, Z. Ni, D. Duhl, N. Brammeier, E. Martin, Bioorg. Med. Chem. Lett. 20 (2010) 157–160.
[13] M.A. Seefeld, W.H. Miller, K.A. Newlander, W.J. Burgess, D.J. Payne, S.F. Rittenhouse, T.D. Moore, W.E. DeWolf Jr., P.M. Keller, X. Qiu, C.A. Janson, K. Vaidya, A.P. Fosberry, M.G. Smyth, D.D. Jaworski, C. Slater-Radosti, W.F. Huffman, Bioorg. Med. Chem. Lett. 11 (2001) 2241–2244.
[14] Y. Schott, M. Decker, H. Rommelspacherb, J. Lehmanna, Bioorg. Med. Chem. Lett. 16 (2006) 5840–5843.
[15] D.J. Moura, C. Rorig, D.L. Vieira, J.A.P. Henriques, R. Roesler, J. Saffi, J.M. Boeira, Life Sci. 79 (2006) 2099–2104.
[16] R.H. Bahekar, M.R. Jain, P.A. Jadav, A. Goel, D.N. Patel, V.M. Prajapati, A.A. Gupta, H. Modi, P.R. Patel, Bioorg. Med. Chem. 15 (2007) 5950–5964.
[17] S. Nafisi, M. Bonsaii, P. Maali, M.A. Khalilzadeh, F. Manouchehri, J. Photoch. Photobio. B. 100 (2010) 84–91.
[18] Z. Chen, R. Cao, L. Yu, B. Shi, J. Sun, L. Guo, Q. Ma, W. Yi, X. Song, H. Song, Eur. J. Med. Chem. 45 (2010) 4740–4745.
[19] Y.-C. Shen, Y.-T. Chang, C.-L. Lin, C.-C. Liaw, Y.H. Kuo, L.-C. Tu, S.F. Yeh, J.-W. Chern, Mar. Drugs 9 (2011) 256–277.
[20] L.T.D. Tonin, M.R. Panice, C.V. Nakamura, K.J.P. Rocha, A.O. Santos, T. Ueda-Nakamura, W.F. Costa, M. Sarragiotto, Biomed. Pharmacother. 64 (2010) 386–389.
[21] A.E. Wahba, J. Peng, S. Kudrimoti, B.L. Tekwani, M.T. Hamann, Bioorg. Med. Chem. 17 (2009) 7775–7782.
[22] B.K.S. Yeung, B. Zou, M. Rottmann, S.B. Lakshminarayana, S.H. Ang, S.Y. Leong, J. Tan, J. Wong, S. Keller-Maerki, C. Fischli, A. Goh, E.K. Schmitt, P. Krastel, E. Francotte, K. Kuhen, D. Plouffe, K. Henson, T. Wagner, E.A. Winzeler, F. Petersen, R. Brun, V. Dartois, T.T. Diagana, T.H. Keller, J. Med. Chem. 53 (2010) 5155–5164.
[23] C. Ma, R. Cao, B. Shi, S. Li, Z. Chen, W. Yi, W. Peng, Z. Ren, H. Song, Eur. J. Med. Chem. 45 (2010) 1515–1523.
[24] Z. Chen, R. Cao, B. Shi, W. Yi, L. Yu, H. Song, Z. Ren, Chem. Pharm. Bull. 58 (2010) 901–907.
[25] C. Ma, R. Cao, B. Shi, X. Zhou, Q. Ma, J. Sun, L. Guo, W. Yi, Z. Chen, H. Song, Eur. J. Med. Chem. 45 (2010) 5513–5519.
[26] Z. Chen, R. Cao, B. Shi, W. Yi, L. Yu, H. Song, Z. Ren, W. Peng, Bioorg. Med. Chem. Lett. 20 (2010) 3876–3879.
[27] R. Kumar, L. Gupta, P. Pal, S. Khan, N. Singh, S.B. Katiyar, S. Meena, J. Sarkar, S. Sinha, J.K. Kanaujiya, S. Lochab, A.K. Trivedi, P. Chauhan, Eur. J. Med. Chem. 45 (2010) 2265–2276.
[28] R.P. Solyis, V.L. Mcgee, M.R. Smith, Physiol. Behav. 63 (1998) 407–412.
[29] J. Hamann, C. Wernicke, J. Lehmann, H. Reichmann, H. Rommelspacher, G. Gille, Neurochem. Int. 52 (2008) 688–700.
[30] J.F. Miller, E.M. Turner, R.G. Sherrill, K. Gudmundsson, A. Spaltenstein, P. Sethna, K.W. Brown, R. Harvey, K.R. Romines, P. Golden, Bioorg. Med. Chem. Lett. 20 (2010) 256–259.
[31] R. Kumar, S. Khan, A. Verma, S. Srivastava, P. Viswakarma, S. Gupta, S. Meena, N. Singh, J. Sarkar, P.M.S. Chauhan, Eur. J. Med. Chem. 45 (2010) 3274–3280.
[32] S.M. Husbands, R.A. Glennon, S. Gorgerat, R. Gough, R. Tyacke, J. Crosby, D.J. Nutt, J.W. Lewis, A.L. Hudson, Drug Alcohol Depen. 64 (2001) 203–208.
[33] R.A. Glennon, B. Grella, R.J. Tyacke, A. Lau, J. Westaway, A.L. Hudson, Bioorg. Med. Chem. Lett. 14 (2004) 999–1002.
[34] R.D. Fabio, F. Micheli, G. Alvaro, P. Cavanni, D. Donati, T. Gagliardi, G. Fontana, R. Giovannini, M. Maffeis, A. Mingardi, M.E. Tranquillini, G. Vitulli, Bioorg. Med. Chem. Lett. 17 (2007) 2254–2259.
[35] B. Grella, M. Teitler, C. Smith, K. Herrick-Davis, R.A. Glennon, Bioorg. Med. Chem. Lett. 13 (2003) 4421–4425.
[36] G. Dorey, L. Dubois, L.P.d. Carvalho, P. Potier, R.H. Dodd, J. Med. Chem. 38 (1995) 189–198.
[37] K. Yao, M. Zhao, X. Zhang, Y. Wang, L. Li, M. Zheng, S. Peng, Eur. J. Med. Chem. 46 (2011) 3237–3249.
[38] M. Zhao, L. Bi, W. Bi, C. Wang, Z. Yang, J. Jud, S. Penga, Bioorg. Med. Chem. 14 (2006) 4761–4774.
[39] J. Liu, G. Wu, G. Cui, W. Wang, M. Zhao, C. Wang, Z. Zhang, S. Peng, Bioorg. Med. Chem. 15 (2007) 5672–5693.
[40] A.R. Shaikh, M. Ismael, C.A.D. Carpio, H. Tsuboi, M. Koyama, A. Endou, M. Kubo, E.A. Broclawikc, Bioorg. Med. Chem. Lett. 16 (2006) 5917–5925.
[41] M.R. Doddareddy, Y.S. Cho, H.Y. Koh, A.N. Pae, Bioorg. Med. Chem. 12 (2004) 3977–3985.