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2-Substituted (*S*)-2-(3,3-dimethyl-1-oxo-10,10a-dihydroimidazo[1,5-*b*]isoquinolin-2(1*H*,3*H*,5*H*)-yl)acetic acids: Conformational prediction, synthesis, anti-thrombotic and vasodilative evaluation

Hong Wang, Le Peng, Ming Zhao *, Jiawang Liu, Xiaoyi Zhang, Yuji Wang, Jianhui Wu, Li Li, Shiqi Peng *

College of Pharmaceutical Sciences, Capital Medical University, Beijing 100069, PR China

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ABSTRACT

(*S*)-1,2,3,4-Tetrahydroisoquinoline-3-carboxylic acid (TIC) can inhibit thrombosis by inhibiting platelet aggregation. The investigation of amino acids modified TIC reveals that a stretching conformation is critical for high anti-thrombotic activity. The conformational modeling shows that introducing a ring into amino acid modified TIC results in a desirable stretching conformation. According to this hypothesis, we synthesized seventeen novel 2-substituted (*S*)-2-(3,3-dimethyl-1-oxo-10,10a-dihydroimidazo[1,5-*b*]isoquinolin-2(1*H*,3*H*,5*H*)-yl)acetic acids (**5a**–**q**). In the in vitro anti-platelet aggregation assay, for ADP-induced platelet aggregation the IC_{50} values of **5a**–**q** are 1.8–3.4-folds lower than that of TIC. In the in vivo anti-thrombotic assay, the effective dose of **5a**–**q** was 167-folds lower than that of TIC. The vessel strip assay showed that **5a**–**q** had mild vasorelaxation activity.

1. Introduction

In the most frequent cardiovascular events such as deep vein thrombosis, myocardial infarction, pulmonary embolism, and stroke, the intravascular thrombosis is one of the most prominent causes of morbidity and mortality.[1] The injury of blood vessel has been correlated with the arterial thrombosis.[2] Upon the damage of the vascular endothelium of the injured blood vessel, the platelets adhere to the exposed extracellular matrix.[3] Platelet adhesion is the primary event that usually associates with the uncontrolled platelet activation and culminates in the intravascular thrombosis.[4] The suppression of the platelet adhesion and activation, particularly through targeting such secondary regulatory mechanism is effective in the prevention of the thrombosis.[5] In the management of the cardiovascular event, anti-platelet therapy has an established role.[6] Therefore, a continuous effort has been made to discover new leads capable of inhibiting platelet activation and aggregation. Tetrahydroisoquinoline is one of the reported platelet-aggregation inhibitors. Besides anti-platelet aggregation action, tetrahydroisoquinolines have a number of additional bioactivities.[7–12] The anti-platelet aggregation mechanism of tetrahydroisoquinolines includes the β-adrenergic/α_2-adrenergic receptor system,[13] and the thromboxane A_2/prostaglandin H_2 receptor system.[14–16] Based on these receptor systems the design of tetrahydroisoquinolines attracts many interests.[17–20]

In our previous work, (*S*)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (TIC) was used as a lead and C- and/or N-terminal amino acid modifications were performed.[21–23] In this continuing work on the modification of TIC with a substituted-dihydroimidazole functional group, seventeen novel 2-substituted (*S*)-2-(3,3-dimethyl-1-oxo-10,10a-dihydroimidazo[1,5-*b*]isoquinolin-2(1*H*,3*H*,5*H*)-yl)acetic acids (**5a–q**) were synthesized, and evaluated in an in vitro anti-platelet aggregation assay, and an in vivo anti-thrombotic model.

2. Results and discussion

2.1. Stretching conformation of 5a–q

The SAR analysis revealed that the in vivo anti-thrombotic activity of amino acids modified TIC derivatives depended on the stretching level of their conformation.[21] As seen in the stereoview derived from the QSAR module of Cerius,[2] the in vivo anti-thrombotic activities of *N*-(L-aminoacyl)-1,2,3,4-tetrahydroisoquinolines (A), 1,2,3,4-tetrahydroisoquinoline-3-carboxylamino acids (B) and *N*-(L-amino-acyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxyl amino acids (C) are enhanced with the increase of the stretching level of the conformation (Fig. 1). This means that to get derivatives with stretching conformation is a key step to TIC modification. In the stretching conformation screening, it was found that (*S*)-2-(3,3-dimethyl-1-oxo-10,10a-dihydroimidazo[1,5-*b*]isoquinolin-2(1*H*,3*H*,5*H*)-yl)acetic acids (**5a–q**) derived from the cyclization of 3S-*N*-(L-aminoacyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylamino acids (B) had desirable stretching conformation (Fig. 1, D). Consequently

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

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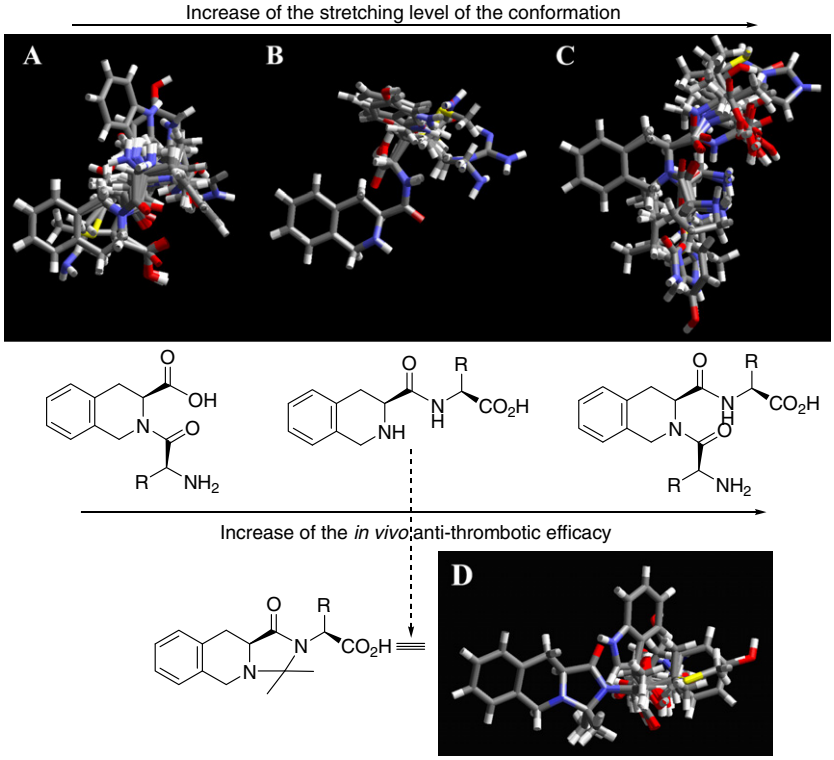


Figure 1. The correlation of in vivo anti-thrombotic activity with the stretching level of the conformation and the desirable stretching conformation of 2-substituted (*S*)-2-(3,3-dimethyl-1-oxo-10,10a-dihydroimidazo[1,5-*b*]isoquinolin-2(1*H*,3*H*,5*H*)-yl)-acetic acids (**5a–q**).

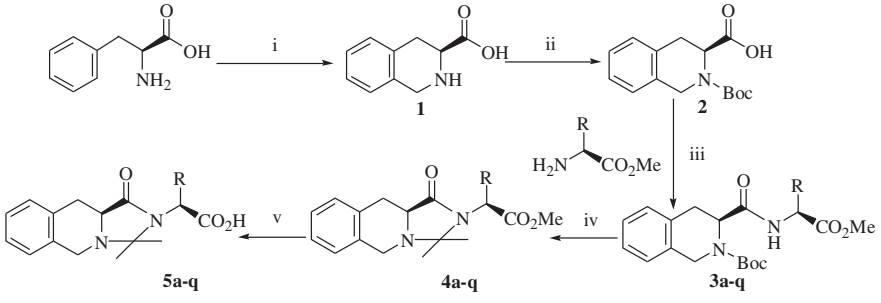
the stretching conformation of **5a–q** was correlated with the desirable anti-thrombotic activity.

2.2. Synthesis of 5a–q

The preparation of **5a–q** was achieved by following the route of Scheme 1. Via Pictet–Spengler condensation L-Phe-OH was converted into (*S*)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (TIC, **1**, 84% yield). After Boc-protection the Boc-TIC (**2**) was coupled with L-amino acid methylester to form seventeen Boc-TIC-L-aminoacyl-OCH_3s (**3a–q**) in 45–95% yield. The reaction of **3a–q** with acetone provided dihydroimidazo[1,5-*b*]isoquinolinyl carboxylic acid methylesters (**4a–q**) in 37–66% yield. After removing methylester group, **4a–q** were converted into dihydroimidazo [1,5-*b*]isoquinolinyl carboxylic acids (**5a–q**) in 85%–94% yield. Therefore, seventeen novel compounds **5a–q** were smoothly prepared via this route.

2.3. In vitro anti-platelet aggregation of 5a–q

In the in vitro assays, the inhibition of **5a–q** (final concentrations ranging from 10 μM to 10 nM) to platelet-activating factor (PAF, final concentration 0.1 μM), adenosine diphosphate (ADP, final concentration 10 μM), arachidonic acid (AA, final concentration 350 μM), and thrombin (TH, final concentration 0.1 U/mL) induced platelet aggregation was tested. The anti-aggregation activity was represented by IC_{50} values listed in Table 1. The IC_{50} values demonstrate that the activities of **5a–q** are significantly higher than that of TIC (**1**). This means that introducing tricyclic unit into TIC could generally increase the in vitro anti-platelet aggregation activity. Besides, the IC_{50} values reflect an inhibition order of ADP > AA > PAF > TH. The differences of the IC_{50} values suggest that introducing tricyclic unit into TIC benefits the inhibition of ADP induced platelet aggregation in particular. Therefore, ADP induced platelet



Scheme 1. Synthetic route of 2-substituted (*S*)-2-(3,3-dimethyl-1-oxo-10,10a-dihydroimidazo-[1,5-*b*] isoquinolin-2(1*H*,3*H*,5*H*)-yl)-acetic acids. Reagents: (i) HCHO and HCl; (ii) $(Boc)_2O$ and $NaHCO_3$; (iii) L-AA-OCH_3, DCC and NMM; (iv) 4 N HCl/EtOAc, methanol, acetone and triethylamine; (v) NaOH in methanol (2 N). In **3a–5a**, R = $CH_2CH(CH_3)_2$; **3b–5b**, R = $CH(CH_3)_2$; **3c–5c**, R = $CH(CH_3)CH_2CH_3$; **3d–5d**, R = CH_3; **3e–5e**, R = H; **3f–5f**, R = $CH_2C_6H_5$; **3g–5g**, R = $CH_2C_6H_4$–OH-*p*; **3h–5h**, R = indole-5-yl–CH_2; **3i** & **4i**, R = $CH_2CO_2CH_3$; **5i**, R = CH_2CO_2H; **3j** & **4j**, R = $CH_2CH_2CO_2CH_3$; **5j**, R = $CH_2CH_2CO_2H$; **3k** & **4k**, R = $CH_2CH_2CH_2CH_2NHCOOCH_2C_6H_5$; **5k**, R = $CH_2CH_2CH_2CH_2NH_2$; **3l–5l**, R = $CH(OH)CH_3$; **3m–5m**, R = CH_2CONH_2; **3n–5n**, R = $CH_2CH_2CONH_2$; **3o** & **4o**, R = $CH_2CH_2CH_2NHC(NH)NHNO_2$; **5o**, R = $CH_2CH_2CH_2NHC(NH)NH_2$; **3p–5p**, R = $CH_2CH_2SCH_3$; **3q–5q**, R = CH_2SH.

Table 1
IC_{50} of **5a–q** against four aggregators induced aggregation of pig platelets[a]

Compd	IC_{50} (nM)			
	ADP	AA	PAF	TH
1	541.7 ± 36.6	481.4 ± 34.0	943.8 ± 63.4	980.3 ± 65.3
5a	171.0 ± 25.4	287.3 ± 28.2	514.9 ± 36.0	598.9 ± 41.9
5b	175.2 ± 30.3	292.4 ± 32.3	524.7 ± 36.8	610.3 ± 40.1
5c	158.5 ± 27.5	271.1 ± 31.0	485.8 ± 34.0	565.0 ± 39.6
5d	199.5 ± 32.6	324.3 ± 32.8	576.0 ± 40.0	677.3 ± 47.7
5e	172.9 ± 29.0	289.8 ± 29.7	519.4 ± 36.4	604.1 ± 42.3
5f	163.1 ± 28.1	277.0 ± 30.5	496.5 ± 34.8	577.5 ± 40.4
5g	272.9 ± 31.7	419.7 ± 33.4	752.4 ± 52.7	875.1 ± 61.3
5h	198.0 ± 32.2	322.4 ± 32.4	577.8 ± 40.1	672.1 ± 47.1
5i	293.9 ± 33.1	447.1 ± 34.5	801.3 ± 56.1	932.0 ± 65.2
5j	248.3 ± 30.5	387.8 ± 36.0	695.0 ± 48.7	808.4 ± 56.6
5k	190.7 ± 32.1	312.9 ± 32.7	560.8 ± 39.3	652.3 ± 45.7
5l	207.4 ± 31.6	334.6 ± 32.9	599.7 ± 42.0	697.6 ± 48.8
5m	283.3 ± 32.8	433.3 ± 33.7	776.6 ± 54.4	903.2 ± 63.2
5n	182.0 ± 31.2	301.6 ± 30.9	540.6 ± 37.8	628.7 ± 44.0
5o	158.8 ± 27.1	271.1 ± 29.6	485.8 ± 34.0	566.7 ± 40.1
5p	264.2 ± 31.0	408.5 ± 32.7	499.1 ± 35.0	851.5 ± 59.6
5q	179.7 ± 30.8	298.6 ± 29.1	535.2 ± 37.5	622.5 ± 43.6

[a] IC_{50} was represented by X ± SD nM, $n = 6$.

aggregation is more sensitive to **5a–q**, and **5c**,**o** are the most potential compounds.

2.4. In vivo anti-thrombotic activity of 5a–q

On an extra-corporeal circulation of arterio-veinos cannula model the in vivo assays were performed. In these assays 10 nmol/kg of **5a–q** were intravenously administered and the thrombus weights of the treated rats are listed in Table 2. The thrombus weights of **5a–q** treated rats (ranged from 12.99 to 21.41 mg) are significantly lower than that of normal saline (NS) treated rats (24.09 mg, p <0.01), which indicates that **5a–q** effectively inhibit the rats to form thrombus. On the other hand, except the thrombus weights (20.10–21.41 mg) of 10 nmol/kg of **5g**,**i**,**m** treated rats, which equal that of 15 μmol/kg of **1** treated rats (22.01 mg), the thrombus weights of 10 nmol/kg of **5a–f**, **h**,**j–l**,**n–q** treated rats are significantly lower than that of 15 μmol/kg of **1** treated rats. These comparisons mean that the present modification benefits the in vivo anti-thrombotic efficacy. Table 2 further indicates that most compounds with lower in vivo anti-thrombotic activity have polar side chain, while most compounds with higher in vivo anti-thrombotic activity have apolar side chain.

2.5. Dose-dependent in vivo anti-thrombotic activity of 5a

Intravenously injection of **5a** was observed at the doses of 100, 10 and 1 nmol/kg to produce a possible dose-dependent anti-thrombotic response in the rats. The thrombus weights are listed in Table 3, which demonstrate that the thrombus weight is progressively increased with dose decrease. Therefore, **5a** exhibited dose-dependent anti-thrombotic action.

2.6. Vasorelaxation activities of 4a–q and 5a–q

Vasoconstriction may disturb the vascular tone and blood flow, and subsequently influence the supply of oxygen and nutrients for the organs. In the discovery of the novel vasodilators, (11a*S*)-1,2,3,5,11,11a-hexahydro-3,3-dimethyl-1-oxo-6*H*-imidazo-[3′,4′:1,2]pyridin[3,4-*b*]indol-2-substituted acetates (Fig. 2, E) were reported.[24] As is shown in Figure 2, the removing of a pyrrole ring from E derives **4a–q**. Based on this structural correlation, the vasorelaxation assays of **4a–q** were performed and the data are listed in Table 4. The data indicate that 3×10^{-4} M of **4a–q** exhibit moderate vasorelaxation, and the effective concentration is 6-folds higher than that of E (5×10^{-5} M). This great increase of the effective concentration emphasizes that the pyrrole ring has essential contribution to the vasorelaxation of E.

To clarify the contribution of the methylester group to the vasorelaxation of **4a–q** the vasorelaxation assays of **5a–q** were

Table 3
Effect of different doses of **5a** on the thrombus weight of the rats[a]

Dose	100 nmol/kg	10 nmol/kg	1 nmol/kg
Thrombus weight	10.02 ± 2.07[b]	12.99 ± 2.05[c]	20.67 ± 2.16[d]
NS		22.09 ± 3.33	

[a] Weight of wet thrombus is represented by X ± SD mg, NS = vehicle, $n = 12$.
[b] Compared to 1 nmol/kg of **5a** p <0.01, to 10 nmol/kg group p <0.05.
[c] Compared to NS and 1 nmol/kg groups p <0.01.
[d] Compared to NS group p >0.05.

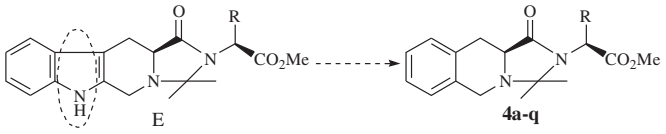


Figure 2. Structural correlation of (11a*S*)-1,2,3,5,11,11a-hexahydro-3,3-dimethyl-1-oxo-6*H*-imidazo[3′,4′:1,2]pyridin[3,4-*b*]indol-2-substituted acetates (*E*) with **4a–q**.

Table 2
Effect of **5a–q** on the thrombus weight of the rats[a]

Compd	Dose	Thrombus weight	Compd	Dose	Thrombus weight
NS		24.09 ± 2.33	Aspirin	167 μmol/kg	13.22 ± 1.67[c]
1	15 μmol/kg	22.01 ± 2.14[e]	**5i**	10 nmol/kg	21.41 ± 2.12[d]
5a	10 nmol/kg	13.79 ± 2.02 [b]	**5j**	10 nmol/kg	18.57 ± 2.21[b]
5b	10 nmol/kg	14.01 ± 2.10[b]	**5k**	10 nmol/kg	14.98 ± 1.94[b]
5c	10 nmol/kg	12.99 ± 2.05[b]	**5l**	10 nmol/kg	16.04 ± 2.01[b]
5d	10 nmol/kg	15.45 ± 1.89[b]	**5m**	10 nmol/kg	20.75 ± 2.49[d]
5e	10 nmol/kg	13.89 ± 2.00[b]	**5n**	10 nmol/kg	14.39 ± 2.29[b]
5f	10 nmol/kg	13.27 ± 2.27[b]	**5o**	10 nmol/kg	13.01 ± 2.44[b]
5g	10 nmol/kg	20.10 ± 2.36[d]	**5p**	10 nmol/kg	19.56 ± 2.10[c]
5h	10 nmol/kg	15.43 ± 1.89[b]	**5q**	10 nmol/kg	14.36 ± 2.14[b]

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

Table 4
Effect of 3×10^{-4} M of **4a–q** and **5a–q** on NE induced vasoconstriction[a]

Compd	Vasorelaxation	Compd	Vasorelaxation
4a	74.68 ± 6.72	**5a**	30.14 ± 2.75
4b	54.99 ± 4.70	**5b**	16.00 ± 1.91
4c	99.00 ± 7.00	**5c**	34.29 ± 2.23
4d	34.10 ± 2.04	**5d**	8.41 ± 1.25
4e	75.35 ± 3.52	**5e**	21.11 ± 2.20
4f	32.18 ± 3.34	**5f**	26.61 ± 2.71
4g	15.56 ± 2.78	**5g**	1.59 ± 0.40
4h	28.08 ± 2.44	**5h**	11.86 ± 1.38
4i	64.09 ± 5.12	**5i**	2.89 ± 0.62
4j	42.48 ± 4.72	**5j**	4.33 ± 0.65
4k	68.21 ± 4.62	**5k**	12.01 ± 1.41
4l	35.21 ± 2.22	**5l**	9.98 ± 1.07
4m	47.89 ± 4.61	**5m**	3.89 ± 0.39
4n	38.61 ± 3.73	**5n**	12.24 ± 1.72
4o	6.78 ± 0.40	**5o**	32.58 ± 1.92
4p	43.06 ± 9.36	**5p**	5.58 ± 0.49
4q	96.30 ± 3.84	**5q**	16.57 ± 1.81
NS	0		

[a] Vasorelaxation is represented by X ± SD%, $n = 6$.

performed and the data are listed in Table 4. The data indicate that at the dose of 3×10^{-4} M **5a–q** exhibit a weak vasorelaxation. Except **5f**, the vasorelaxation of **5a–q** is significantly lower than that of **4a–q**. This great decrease of the vasorelaxation implies that the methylester group has important contribution to the vasorelaxation of **4a–q**. However, the data further indicate that the vasorelaxation order of **5a–q** is substantially similar to that of their in vivo anti-thrombotic activities. This similarity suggests that the vasorelaxation may enhance the anti-thrombotic activity.

3. Conclusion

The introduction of an imidazole ring into the amino acid modified TIC is optionally correlated with conformation rigidity design. Based on conformational rigidity, seventeen novel dihydroimidazo[1,5-*b*]isoquinolinyl carboxylic acids (**5a–q**) do have desirable stretching conformation and consequently do have high in vitro anti-platelet aggregation and in vivo anti-thrombotic activities. The pharmacological benefit of the stretching conformation is not only reflected by the in vivo anti-thrombotic activity of the cyclic derivatives (**5a–q**) over that of their parents (C-terminal modified TICs, B), but also reflected by the in vivo anti-thrombotic activities of **5c**,**a**,**o**,**f**,**e**,**q** over that of **5l**,**j**,**p**,**g**,**m**,**i**. The stretching level of the conformation of **5c**,**a**,**o**,**f**,**e**,**q** is higher than that of **5l**,**j**,**p**,**g**,**m**,**i**, which was visualized in Figure 3. Besides, a week vasorelaxation of **5a–q** contributes to their in vivo anti-thrombotic activity. This provides an additional approach for the design of the derivatives of **5a–q**.

4. Experimental section

4.1. Synthesis

4.1.1. 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 cut-off.

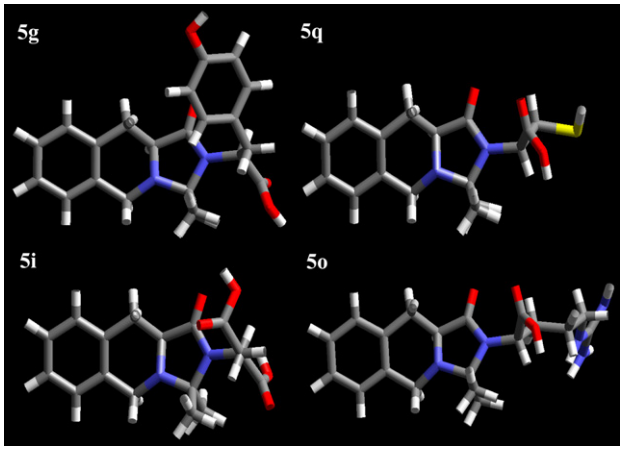


Figure 3. The comparison of the stretching level of **5o**,**q** with high activity to that of **5g**,**i** with low activity.

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

To the suspension of 5.0 g (0.03 mmol) of L-Phe in 50 mL of chloroform and 27 ml of formaldehyde, 45 mL of concentrated hydrochloric acid was added drop-wise. The reaction mixture was stirred at 80–90 °C for 10 h, and TLC ($CHCl_3$/CH_3OH, 10:1) indicates the complete disappearance of L-Phe. The reaction mixture was cooled to room temperature and the formed precipitates were collected by filtration. The collected solids were successively washed with water (30 mL × 3) and acetone (30 mL × 3) to give 4.5 g (84%) of the title compound as a colorless powder. Mp 302–303 °C; $[\alpha]_D^{20} = -68$ (*c* 1.0, H_2O); ESI-MS (*m*/*e*) 178 $[M+H]^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ/ppm = 11.0 (s, 1H), 7.25 (m, J = 6.4 Hz, 2H), 7.02 (d, J = 6.5 Hz, 1H), 6.98 (t, J = 6.6 Hz, 1H), 3.80 (m, 3H), 3.03 (d, J = 7.5 Hz, 1H), 2.78 (d, J = 8.4 Hz, 1H), 2.0 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ/ppm = 174.9, 136.2, 134.2, 127.2, 126.0, 57.6, 47.4, 29.4.

4.1.3. (*S*)-2-*tert*-Butoxycarbonyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (2)

To the suspension of 2.49 g (62.2 mmol) of NaOH, 62.2 mL of water, and 10.0 g (56.5 mmol) of 3S-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, the solution of 14.8 g (67.8 mmol) of Boc_2O in 40 mL of THF was added at 0 °C. The suspension was stirred at room temperature for 48 h to form a clean solution, and TLC (ethyl acetate/petroleum ether, 1:3) indicated complete disappearance of 3S-1,2,3,4-tetra-hydroisoquinoline-3-carboxylic acid. The reaction mixture was evaporated under vacuum, and the residue was dissolved in 100 ml of ethyl acetate. The solution was washed successively with 5% aqueous solution of $KHSO_4$ (30 mL × 3) and saturated aqueous solution of NaCl (30 mL × 3), and dried with anhydrous Na_2SO_4. After filtration, the filtrate was evaporated under vacuum and the residue was triturated with petroleum ether to give 12.5 g (80%) of the title compound as a colorless powder. ESI-MS (*m*/*e*) 278 $[M+H]^+$; $[\alpha]_D^{20} = -6.78$ (*c* 1.0, methanol); ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm = 11.2 (s, 1H), 7.56 (d, J = 7.5 Hz, 1H), 7.44 (d, J = 8.0 Hz, 1H), 7.23 (t, J = 8.2 Hz, 1H), 7.22 (t, J = 7.3 Hz, 1H), 4.14 (m, J = 5.2 Hz, 3H), 3.13 (m, J = 4.1 Hz, 2H), 2.72 (m, J = 4.5 Hz, 2H), 1.42 (s, 9H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 176.8, 169.8, 137.5, 132.8, 129.0, 127.8, 126.9, 125.2, 82.9, 60.7, 56.9, 51.3, 28.2, 25.5.

4.1.4. General procedure for preparing (*S*)-2-Boc-1,2,3,4-tetrahydroisoquinoline-3-carbonylamino acid methyl esters (3a–q)

At 0 °C and with stirring, to the solution of 0.256 g (0.924 mmol) of (*S*)-2-Boc-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, 2 mL of anhydrous THF 0.151 g (0.109 mmol) of HOBt was added, and stirred for 10 min. Then, 0.228 g (0.108 mmol) of DCC was added to form reaction mixture A. To the suspension of 0.102 mmol of HCl·AA-OMe in 4 ml of anhydrous THF, 1 mL of *N*-methylmorpholine was added and stirred at room temperature for 35 min to form reaction mixture B (pH 9). The reaction mixture A and B were combined, stirred at room temperature for 12 h, and TLC (ethyl acetate/petroleum ether, 1:3) indicated the complete disappearance of 3S-2-Boc-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid. The formed precipitate of DCU was removed by filtration, and the filtrate was evaporated under vacuum. The residue was dissolved in 50 ml of ethyl acetate, the formed solution was washed successively with saturated aqueous solution of $NaHCO_3$ (30 mL × 3), 5% aqueous solution of $KHSO_4$ (30 mL × 3) and saturated aqueous solution of NaCl (30 mL × 3), and dried with anhydrous Na_2SO_4. After filtration, the filtrate was evaporated under vacuum to give **3a–q**.

4.1.4.1. *N*-((*S*)-2-*tert*-Butoxycarbonyl-1,2,3,4-tetrahydroisoquinoline-3-carbonyl)-L-leucine methylester (3a). Yield: 91%, colorless powder, mp 81–83 °C; ESI-MS (*m/e*) 405 $[M+H]^+$; $[\alpha]_D^{20} = -14.1$ (*c* 1.0, methanol); ^1H NMR (300 MHz, DMSO-d_6) δ/ppm = 8.72 (d, *J* = 8.4 Hz, 1H), 7.55 (d, *J* = 7.5 Hz, 1H), 7.34 (d, *J* = 8.1 Hz, 1H), 7.24 (t, *J* = 7.3 Hz, 1H), 7.14 (t, *J* = 8.2 Hz, 1H), 4.25 (t, *J* = 5.4 Hz, 1H), 4.14 (m, *J* = 5.5 Hz, 6H), 3.14 (m, *J* = 4.3 Hz, 2H), 2.72 (m, *J* = 5.3 Hz, 2H), 1.47 (s, 9H), 1.44 (s, 9H), 0.93 (m, *J* = 4.1 Hz, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 171.6, 170.2, 168.2, 137.2, 133.9, 128.6, 127.8, 127.3, 125.9, 80.2, 68.9, 55.9, 53.4, 51.8, 49.5, 41.0, 29.5, 27.9, 22.9, 22.2.

4.1.4.2. *N*-((*S*)-2-*tert*-Butoxycarbonyl-1,2,3,4-tetrahydroisoquinoline-3-carbonyl)-L-valine methylester (3b). Yield: 86%, colorless powder, mp 87–88 °C; ESI-MS (*m/e*) 391 $[M+H]^+$; $[\alpha]_D^{20} = -37.57$ (*c* 1.0, methanol); ^1H NMR (300 MHz, DMSO-d_6) δ/ppm = 8.78 (d, *J* = 8.4 Hz, 1H), 7.66 (d, *J* = 7.5 Hz, 1H), 7.56 (d, *J* = 8.0 Hz, 1H), 7.31 (t, *J* = 7.7 Hz, 1H), 7.22 (t, *J* = 8.2 Hz, 1H), 4.45 (m, *J* = 5.4 Hz, 6H), 4.19 (t, *J* = 5.6 Hz, 1H), 3.42 (m, *J* = 5.3 Hz, 2H), 2.91 (m, *J* = 4.4 Hz, 2H), 2.17 (dd, *J* = 5.7 Hz, 2H), 1.44 (s, 9H), 0.97 (m, *J* = 3.2 Hz, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 171.8, 171.2, 169.6, 137.2, 133.6, 128.9, 127.8, 127.5, 125.6, 82.0, 68.9, 56.8, 56.0, 53.4, 52.0, 30.1, 29.3, 27.6, 17.6, 17.3.

4.1.4.3. *N*-((*S*)-2-*tert*-Butoxycarbonyl-1,2,3,4-tetrahydroisoquinoline-3-carbonyl)-L-isoleucine methylester (3c). Yield: 89%, colorless powder, mp 73–74 °C; ESI-MS (*m/e*) 405 $[M+H]^+$; $[\alpha]_D^{20} = -12.39$ (*c* 1.0, methanol); ^1H NMR (300 MHz, DMSO-d_6) δ/ppm = 8.73 (d, *J* = 8.4 Hz, 1H), 7.55 (d, *J* = 7.3 Hz, 1H), 7.34 (d, *J* = 8.5 Hz, 1H), 7.27 (t, *J* = 7.3 Hz, 1H), 7.13 (t, *J* = 8.2 Hz, 1H), 4.33 (m, *J* = 4.7 Hz, 6H), 4.29 (t, *J* = 5.6 Hz, 1H), 3.13 (m, *J* = 4.1 Hz, 2H), 2.74 (m, *J* = 4.8 Hz, 3H), 1.29 (dd, *J* = 5.7 Hz, 2H), 1.56 (s, 9H), 0.94 (m, *J* = 3.9 Hz, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 172.8, 171.8, 169.9, 137.2, 133.4, 128.9, 127.9, 127.3, 125.6, 81.2, 68.9, 57.0, 53.4, 53.2, 51.8, 36.4, 30.0, 27.2, 26.2, 15.8, 11.5.

4.1.4.4. *N*-((*S*)-2-*tert*-Butoxycarbonyl-1,2,3,4-tetrahydroisoquinoline-3-carbonyl)-L-alanine methylester (3d). Yield: 89%, colorless powder, mp 84–85 °C; ESI-MS (*m/e*) 363 $[M+H]^+$; $[\alpha]_D^{20} = -47.46$ (*c* 1.0, methanol); ^1H NMR (300 MHz, DMSO-d_6) δ/ppm = 8.72 (d, *J* = 8.4 Hz, 1H), 7.55 (d, *J* = 7.4 Hz, 1H), 7.34 (d, *J* = 8.1 Hz, 1H), 7.22 (t, *J* = 7.1 Hz, 1H), 7.12 (t, *J* = 7.3 Hz, 1H), 4.44 (t, *J* = 5.4 Hz, 1H), 4.42 (m, *J* = 5.1 Hz, 6H), 3.13 (m, *J* = 4.4 Hz, 2H), 2.75 (m, *J* = 5.5 Hz, 2H), 1.41 (s, 9H), 1.38 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 172.8, 168.9, 137.2, 132.4, 129.0, 127.5, 127.1, 124.9, 83.2, 69.5, 57.2, 52.0, 53.1, 49.8, 29.2, 25.6, 16.6.

4.1.4.5. *N*-((*S*)-2-*tert*-Butoxycarbonyl-1,2,3,4-tetrahydroisoquinoline-3-carbonyl)-glycine methylester (3e). Yield: 85%, colorless powder, mp 125–126 °C; ESI-MS (*m/e*) 349 $[M+H]^+$; $[\alpha]_D^{20} = -15.83$ (*c* 1.0, methanol); ^1H NMR (300 MHz, DMSO-d_6) δ/ppm = 8.82 (d, *J* = 8.6 Hz, 1H), 7.58 (d, *J* = 7.9 Hz, 1H), 7.54 (d, *J* = 8.6 Hz, 1H), 7.43 (t, *J* = 7.4 Hz, 1H), 7.15 (t, *J* = 8.5 Hz, 1H), 4.29 (t, *J* = 6.3 Hz, 1H), 4.15 (m, *J* = 4.3 Hz, 6H), 3.25 (m, *J* = 5.5 Hz, 2H), 2.95 (m, *J* = 4.6 Hz, 2H), 1.52 (s, 9H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 171.1, 169.1, 168.9, 138.2, 132.3, 128.6, 128.1, 127.6, 124.6, 82.0, 68.1,57.5, 54.8, 51.9, 41.2, 31.0, 26.2.

4.1.4.6. *N*-((*S*)-2-*tert*-Butoxycarbonyl-1,2,3,4-tetrahydroisoquinoline-3-carbonyl)-L-phenylalanine methylester (3f). Yield: 87%, colorless powder, mp 91–93 °C; ESI-MS (*m/e*) 439 $[M+H]^+$; $[\alpha]_D^{20} = -7.61$ (*c* 1.0, methanol); ^1H NMR (300 MHz, DMSO-d_6) δ/ppm = 9.06 (d, *J* = 8.4 Hz, 1H), 7.25 (m, *J* = 7.5 Hz, 9H), 4.25 (t, *J* = 5.6 Hz, 1H), 4.15 (m, *J* = 5.2 Hz, 6H), 3.02 (m, *J* = 4.2 Hz, 4H), 2.93 (m, *J* = 5.6 Hz, 2H), 1.45 (s, 9H), 1.41 (m, *J* = 4.1 Hz, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 169.6, 133.3, 128.2, 128.1, 127.9, 127.5, 127.3, 126.2, 125.7, 80.2, 69.5, 53.9, 53.5, 52.0, 37.5, 29.3, 28.5, 27.5.

4.1.4.7. *N*-((*S*)-2-*tert*-Butoxycarbonyl-1,2,3,4-tetrahydroisoquinoline-3-carbonyl)-L-tyrosine methylester (3g). Yield: 82%, colorless powder, mp 62–65 °C; ESI-MS (*m/e*) 455 $[M+H]^+$; $[\alpha]_D^{20} = -23.26$ (*c* 1.0, methanol); ^1H NMR (300 MHz, DMSO-d_6) δ/ppm = 7.73 (d, *J* = 8.6 Hz, 1H), 7.56 (d, *J* = 7.4 Hz, 1H), 7.39 (d, *J* = 8.3 Hz, 1H), 7.23 (t, *J* = 7.7 Hz, 1H), 7.13 (t, *J* = 8.2 Hz, 5H), 4.30 (t, *J* = 4.4 Hz, 1H), 6.70 (d, *J* = 8.4 Hz, 2H), 4.28 (m, *J* = 4.2 Hz, 6H), 3.13 (m, *J* = 4.3 Hz, 2H), 2.94 (m, *J* = 4.8 Hz, 3H), 1.43 (s, 9H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 171.9, 171.6, 167.1, 156.8, 133.1, 132.4, 129.9, 129.6, 128.1, 127.8, 125.1, 127.3, 116.1, 115.7, 83.2, 69.1, 57.2, 53.8, 53.1, 52.0, 37.8, 37.2, 29.7, 27.2.

4.1.4.8. *N*-((*S*)-2-*tert*-Butoxycarbonyl-1,2,3,4-tetrahydroisoquinoline-3-carbonyl)-L-tryptophan methylester (3h). Yield: 87%, colorless powder, mp 71–72 °C; ESI-MS(*m/e*) 478 $[M+H]^+$; $[\alpha]_D^{20} = -7.99$ (*c* 1.0, methanol); ^1H NMR (300 MHz, DMSO-d_6) δ/ppm = 10.97 (s, 1H), 9.06 (d, *J* = 8.4 Hz, 1H), 7.72 (m, *J* = 7.5 Hz, 9H), 4.38 (m, *J* = 5.3 Hz, 6H), 4.30 (t, *J* = 5.1 Hz, 1H), 3.13 (m, *J* = 4.5 Hz, 2H), 2.69 (m, *J* = 4.0 Hz, 2H), 1.65 (s, 9H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 172.9, 171.9, 168.9, 137.6, 136.8, 128.4, 127.9, 127.3, 125.8, 122.9, 122.6, 122.1, 119.8, 112.0, 110.9, 81.2, 68.9, 57.2, 54.3, 51.8, 30.9, 29.4, 27.6.

4.1.4.9. *N*-((*S*)-2-*tert*-Butoxycarbonyl-1,2,3,4-tetrahydroisoquinoline-3-carbonyl)-aspartic acid dimethylester (3i). Yield: 75%, colorless powder, mp 106–107 °C; ESI-MS (*m/e*) 407 $[M+H]^+$; $[\alpha]_D^{20} = -3.23$ (*c* 1.0, methanol); ^1H NMR (300 MHz, DMSO-d_6) δ/ppm = 8.72 (d, *J* = 8.4 Hz, 1H), 7.55 (d, *J* = 7.6 Hz, 1H), 7.35 (d, *J* = 8.3 Hz, 1H), 7.28 (t, *J* = 7.7 Hz, 1H), 7.13 (t, *J* = 8.2 Hz, 1H), 4.31 (m, *J* = 4.3 Hz, 3H), 4.24 (t, *J* = 4.4 Hz, 1H), 3.73 (m, *J* = 4.6 Hz, 6H), 3.12 (m, *J* = 4.6 Hz, 2H), 2.89 (m, *J* = 4.3 Hz, 3H), 2.76 (m, *J* = 4.5 Hz, 2H), 1.42 (s, 9H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 173.1, 172.8, 171.6, 169.6, 137.9, 133.6, 127.6, 127.0, 126.9, 124.1, 81.8, 69.5, 56.7, 53.8, 51.7, 48.2, 37.9, 29.1, 27.6.

4.1.4.10. *N*-((*S*)-2-*tert*-Butoxycarbonyl-1,2,3,4-tetrahydroisoquinoline-3-carbonyl)-L-glutamic acid dimethylester (3j). Yield: 78%, colorless powder, mp 107–109 °C; ESI-MS (*m/e*) 420 $[M+H]^+$; $[\alpha]_D^{20} = -4.05$ (*c* 1.0, methanol); ^1H NMR (300 MHz, DMSO-d_6) δ/ppm = 8.72 (d, *J* = 8.5 Hz, 1H), 7.57 (d, *J* = 7.6 Hz, 1H), 7.33 (d, *J* = 8.7 Hz, 1H), 7.28 (t, *J* = 7.7 Hz, 1H), 7.12 (t, *J* = 8.2 Hz,

1H), 4.45 (m, J = 5.6 Hz, 3H), 4.29 (t, J = 5.5 Hz, 1H), 3.53 (m, J = 4.6 Hz, 6H), 3.12 (m, J = 4.5 Hz, 2H), 2.72 (m, J = 4.8 Hz, 2H), 1.96 (m, J = 5.2 Hz, 4H), 1.42 (s, 9H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 173.2, 172.8, 171.6, 169.6, 137.9, 133.6, 127.6, 127.0, 126.9, 124.1, 81.8, 69.5, 56.7, 53.8, 51.6, 50.7, 29.1, 27.8, 27.6, 26.6.

4.1.4.11. *N*-((*S*)-2-*tert*-Butoxycarbonyl-1,2,3,4-tetrahydroisoquinoline-3-carbonyl)-L-lysine(Z) benzylester (3k). Yield: 95%. Syrupy. ESI-MS (m/e) 630 $[M+H]^+$; $[\alpha]_D^{20}$ = −19.9 (c 1.0, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ/ppm = 8.72 (d, J = 8.4 Hz, 1H), 6.99–7.60 (m, 14H), 5.34 (d, J = 6.3 Hz, 2H), 4.28 (t, 1H), 4.18 (m, 9H), 3.12 (m, 2H), 2.82 (m, 2H), 2.21 (m, 5H), 1.36 (m, 13H); ^{13}C NMR (75 MHz, $CDCl_3$) δ/ppm = 171.9, 171.6, 169.6, 142.8, 137.6, 136.5, 133.7, 125.2–128.9, 82.7, 68.8, 67.8, 56.8, 54.8, 53.7, 52.8, 49.5, 32.0, 30.2, 28.7, 27.9, 21.7.

4.1.4.12. *N*-((*S*)-2-*tert*-Butoxycarbonyl-1,2,3,4-tetrahydroisoquinoline-3-carbonyl)-L-threonine methylester (3l). Yield: 89%, colorless powder, mp 80–81 °C; ESI-MS (m/e) 393 $[M+H]^+$; $[\alpha]_D^{20}$ = −42.43 (c 1.0, methanol); ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm = 8.73 (d, J = 8.4 Hz, 1H), 7.56 (d, J = 7.5 Hz, 1H), 7.33 (d, J = 8.1 Hz, 1H), 7.22 (t, J = 7.7 Hz, 1H), 7.14 (t, J = 8.6 Hz, 1H), 4.28 (t, J = 4.5 Hz, 1H), 4.29 (m, J = 4.3 Hz, 6H), 3.16 (m, J = 4.2 Hz, 2H), 2.74 (m, J = 4.1 Hz, 3H), 2.15 (s, 1H), 1.52 (s, 9H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 172.8, 171.6, 167.9, 137.1, 129.1, 127.9, 127.2, 125.6, 83.1, 68.2, 67.9, 58.2, 53.7, 51.2, 28.2, 27.8, 18.6.

4.1.4.13. *N*-((*S*)-2-*tert*-Butoxycarbonyl-1,2,3,4-tetrahydroisoquinoline-3-carbonyl)-L-asparagine methylester (3m). Yield: 55%, colorless powder, mp 101–102 °C; ESI-MS (m/e) 407 $[M+H]^+$; $[\alpha]_D^{20}$ = −6.50 (c 1.0, methanol); ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm = 8.72 (d, J = 8.4 Hz, 1H), 7.55 (d, J = 7.6 Hz, 1H), 7.34 (d, J = 8.2 Hz, 1H), 7.23 (t, J = 7.7 Hz, 1H), 7.12 (t, J = 8.2 Hz, 1H), 6.20 (s, 1H), 4.41 (m, J = 5.4 Hz, 6H), 4.29 (t, J = 4.3 Hz, 1H), 3.12 (m, J = 4.5 Hz, 2H), 2.74 (m, J = 4.5 Hz, 4H), 1.42 (s, 9H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 174.5, 171.8, 168.9, 137.2, 133.7, 128.6, 127.5, 127.3, 125.9, 82.9, 69.0, 53.3, 52.0, 37.2, 17.8.

4.1.4.14. *N*-((*S*)-2-*tert*-Butoxycarbonyl-1,2,3,4-tetrahydroisoquinoline-3-carbonyl)-L-glutamine methylester (3n). Yield: 45%, colorless powder, mp 100–102 °C; ESI-MS (m/e) 420 $[M+H]^+$; $[\alpha]_D^{20}$ = −28.78 (c 1.0, methanol); ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm = 8.72 (d, J = 8.5 Hz, 1H), 8.12 (s, 1H), 7.55 (d, J = 7.7 Hz, 1H), 7.34 (d, J = 8.2 Hz, 1H), 7.24 (t, J = 7.7 Hz, 1H), 7.13 (t, J = 8.2 Hz, 1H), 4.33 (m, J = 5.6 Hz, 6H), 4.24 (t, J = 4.3 Hz, 1H), 3.13 (m, J = 4.8 Hz, 2H), 2.73 (m, J = 4.3 Hz, 6H), 1.46 (s, 9H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 173.9, 171.9, 171.6, 168.9, 137.1, 133.5, 127.5, 127.2, 125.8, 82.3, 68.2, 56.1, 53.4, 51.3, 51.2, 34.2, 28.9, 27.2, 26.8.

4.1.4.15. *N*-((*S*)-2-*tert*-Butoxycarbonyl-1,2,3,4-tetrahydroisoquinoline-3-carbonyl)-L-arginine methylester (3o). Yield: 51%, colorless powder, mp 151–152 °C; ESI-MS (m/e) 448 $[M+H]^+$; $[\alpha]_D^{20}$ = −11.56 (c 1.0, methanol); ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm = 8.99 (s, 3H), 8.72 (d, J = 8.5 Hz, 1H), 7.54 (d, J = 7.3 Hz, 1H), 7.35 (d, J = 8.4 Hz, 1H), 7.24 (t, J = 7.7 Hz, 1H), 7.13 (t, J = 8.3 Hz, 1H), 4.24 (t, J = 5.3 Hz, 1H), 4.12 (m, J = 4.3 Hz, 6H), 3.53 (m, J = 4.6 Hz, 6H), 3.15 (m, J = 4.6 Hz, 2H), 2.73 (m, J = 4.3 Hz, 4H), 2.11 (m, J = 4.3 Hz, 1H), 1.96 (m, J = 3.9 Hz, 1H), 1.45 (s, 9H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 171.9, 171.6, 169.6, 149.7, 137.2, 133.6, 128.9, 127.9, 127.2, 125.6, 83.2, 68.5, 57.2, 53.7, 52.7, 51.0, 37.2, 29.1, 28.7, 26.7, 24.5.

4.1.4.16. *N*-((*S*)-2-*tert*-Butoxycarbonyl-1,2,3,4-tetrahydroisoquinoline-3-carbonyl)-L-methionine methylester (3p). Yield: 49%, colorless powder, mp 143–144 °C; ESI-MS (m/e) 409 $[M+H]^+$; $[\alpha]_D^{20}$ = −12.90 (c 1.0, methanol); ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm = 8.72 (d, J = 8.7 Hz, 1H), 7.55 (d, J = 7.6 Hz, 1H), 7.34 (d, J = 8.3 Hz, 1H), 7.23 (t, J = 7.7 Hz, 1H), 7.12 (t, J = 8.2 Hz, 1H), 4.32 (t, J = 4.5 Hz, 1H), 4.25 (m, J = 4.9 Hz, 6H), 3.12 (m, J = 4.3 Hz, 2H), 2.81 (m, J = 4.9 Hz, 2H), 2.35 (m, J = 4.6 Hz, 2H), 1.42 (s, 9H), 1.27 (m, J = 4.2 Hz, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 171.8, 171.6, 169.2, 137.6, 128.9, 127.6, 127.2, 125.8, 81.3, 69.1, 56.7, 53.2, 52.9, 52.3, 31.2, 29.1, 27.9, 17.8.

4.1.4.17. *N*-((*S*)-2-*tert*-Butoxycarbonyl-1,2,3,4-tetrahydroisoquinoline-3-carbonyl)-L-cysteine methylester (3q). Yield: 50%, colorless powder, mp 107–110 °C; ESI-MS (m/e) 395 $[M+H]^+$; $[\alpha]_D^{20}$ = −25.12 (c 1.0, methanol); ^{1}H NMR (300 MHz, $CDCl_3$) δ/ppm = 8.00 (d, J = 7.6 Hz, 1H), 7.02 (m, J = 8.2 Hz, 2H), 6.95 (d, J = 8.8 Hz, 2H), 4.90 (t, J = 8.4 Hz, 1H), 4.71 (m, 1H), 4.20 (t, J = 8.3 Hz, 2H), 3.65 (s, 3H), 3.07 (m, J = 5.4 Hz, 4H), 1.5 (t, J = 6.4 Hz, 1H), 1.41 (s, 9H); ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm = 174.8, 172.6, 159.2, 139.6, 137.1, 128.1, 127.4, 126.2, 125.6, 70.1, 66.1, 63.2, 52.1, 46.3, 31.8, 29.0, 26.8.

4.1.5. General procedure for preparing 2-substituted (*S*)-2-(3,3-dimethyl-1-oxo-10,10a-dihydroimidazo[1,5-*b*]isoquinolin-2(1*H*,3*H*,5*H*)-yl)-acetic acid methylesters (4a–q)

At 0 °C to the solution of 4.51 mmol of substituted (*S*)-2-Boc-1,2,3,4-tetrahydro-isoquinoline-3-carbonylamino acid methylesters (**3**) in 8 mL of ethyl acetate 16 mL of hydrogen chloride/ethyl acetate (4 N) were added drop-wise. The reaction solution was stirred at 0 °C for 90 min and evaporated under reduced pressure. The residue was dissolved in 60 mL of methanol and 20 mL of acetone. With triethylamine, the reaction solution was adjusted to pH 9, at room temperature and in dark stirred for 240 h, and thin layer chromatography (TLC) ($CHCl_3$/MeOH, 10:1) indicated the complete disappearance of **3**. On evaporation, the residue was dissolved in 200 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 residue was dissolved in 5 mL of methanol to crystallize **4a–q**.

4.1.5.1. (*S*)-2-((*S*)-3,3-Dimethyl-1-oxo-10,10a-dihydroimidazo-[1,5-*b*]isoquinolin-2(1*H*,3*H*,5*H*)-yl)-4-methylpentanoic acid methylester (4a). Yield: 46%, colorless powder, mp 186–188 °C. $[\alpha]_D^{20}$ = −58.73 (c 0.25, $CHCl_3$); ESI/MS: 345 $[M+H]^+$; IR (KBr disk): 3456, 2957, 1744, 1697, 1457, 1425, 1367, 1262, 1245, 1191, 750 cm^{-1}. ^{1}H NMR (500 MHz, DMSO) δ_H = 7.16 (t, J = 7.6 Hz, 1H), 7.12 (d, J = 7.6 Hz, 1H), 7.10 (d, J = 7.6 Hz, 1H), 7.09 (t, J = 7.6 Hz, 1H), 4.05 (t, J = 10.0 Hz, 1H), 3.95 (t, J = 15.0 Hz, 1H), 3.74 (s, 2H), 3.63 (s, 3H), 2.90 (dd, J = 5.0 Hz, J = 15.0 Hz, 1H), 2.65 (dd, J = 10.0 Hz, J = 15.0 Hz, 1H), 1.90 (m, 2H), 1.65 (m, 1H), 1.41 (s, 3H), 1.26 (s, 3H), 0.94 (d, J = 5.0 Hz, 3H), 0.92 (d, J = 5.0 Hz, 3H); ^{13}C NMR (125 MHz, DMSO) δ_C = 19.33, 22.62, 22.82, 25.09, 31.00, 46.69, 52.48, 52.57, 52.90, 56.38, 78.79, 126.26, 126.60, 127.38, 129.91, 133.78, 135.16, 171.19, 171.66. Elemental Anal. Calcd for $C_{20}H_{28}N_2O_3$: C, 69.74; H, 8.19; N, 8.13. Found: C, 69.96; H, 8.01; N, 8.36.

4.1.5.2. (*S*)-2-((*S*)-3,3-Dimethyl-1-oxo-10,10a-dihydroimidazo-[1,5-*b*]isoquinolin-2(1*H*,3*H*,5*H*)-yl)-3-methylbutanoic acid methylester (4b). Yield: 66%, colorless powder, mp 181–183 °C. $[\alpha]_D^{20}$ = −23.68 (c 0.25, $CHCl_3$); ESI/MS: 331 $[M+H]^+$; IR (KBr disk): 3468, 2965, 1722, 1668, 1445, 1415, 1358, 1268, 1191, 749 cm^{-1}. ^{1}H NMR (500 MHz, DMSO) δ_H = 7.15 (t, J = 7.5 Hz, 1H), 7.11 (d, J = 7.5 Hz, 1H), 7.08 (d, J = 7.5 Hz, 1H), 7.06 (t, J = 7.5 Hz, 1H), 3.95 (d, J = 15.0 Hz, 1H), 3.92 (d, J = 5.0 Hz, 1H), 3.71 (d, J = 15.0 Hz, 1H), 3.64 (s, 3H), 3.30 (dd, J = 4.0 Hz, J = 10.0 Hz, 1H), 2.85 (dd,

J = 4.0 Hz, J = 15.0 Hz, 1H), 2.61 (dd, J = 10.0 Hz, J = 15.0 Hz, 1H), 1.85 (m, 1H), 1.39 (s, 3H), 1.24 (s, 3H), 0.98 (t, J = 6.0 Hz, 6H); ^{13}C NMR (125 MHz, DMSO) δ_C = 21.12, 21.22, 22.49, 23.40, 28.07, 52.64, 56.45, 56.76, 65.38, 81.74, 127.37, 127.50, 128.57, 128.69, 132.94, 133.34, 171.18, 171.77. Elemental Anal. Calcd for $C_{19}H_{26}N_2O_3$: C, 69.06; H, 7.93; N, 8.48. Found: C, 69.27; H, 8.10; N, 8.25.

4.1.5.3. (2*S*)-2-((*S*)-3,3-Dimethyl-1-oxo-10,10a-dihydroimidazo-[1,5-*b*]isoquinolin-2(1*H*,3*H*,5*H*)-yl)-3-methylpentanoic acid methylester (4c). Yield: 61%, colorless powder, mp 192–194 °C. $[\alpha]_D^{20}$ = −58.73 (c 0.25, $CHCl_3$); ESI/MS: 345 $[M+H]^+$; IR (KBr disk): 3448, 2968, 1728, 1683, 1448, 1423, 1384, 1261, 1215, 1201, 743 cm^{-1}. ^{1}H NMR (500 MHz, DMSO) δ_H = 7.19 (t, J = 7.4 Hz, 1H), 7.16 (d, J = 7.4 Hz, 1H), 7.13 (d, J = 7.4 Hz, 1H), 7.11 (t, J = 7.4 Hz, 1H), 4.01 (d, J = 15.0 Hz, 1H), 3.96 (d, J = 6.5 Hz, 1H), 3.71 (d, J = 15.0 Hz, 1H), 3.62 (s, 3H), 3.30 (dd, J = 5.0 Hz, J = 10.0 Hz, 1H), 2.92 (dd, J = 5.0 Hz, J = 15.0 Hz, 1H), 2.64 (dd, J = 10.0 Hz, J = 15.0 Hz, 1H), 2.22 (m, 2H), 1.69 (m, 1H), 1.39 (s, 3H), 1.22 (s, 3H), 0.96 (t, J = 6.5 Hz, 6H); ^{13}C NMR (125 MHz, DMSO) δ_C = 11.89, 16.02, 25.04, 26.53, 30.89, 31.08, 37.32, 46.29, 51.5, 55.60, 56.76, 78.53, 126.24, 126.38, 127.18, 129.20, 134.34, 135.35, 171.97, 173.40. Elemental Anal. Calcd for $C_{20}H_{28}N_2O_3$: C, 69.74; H, 8.19; N, 8.13. Found: C, 69.92; H, 8.00; N, 8.34.

4.1.5.4. (*S*)-2-((*S*)-3,3-Dimethyl-1-oxo-10,10a-dihydroimidazo-[1,5-*b*]isoquinolin-2(1*H*,3*H*,5*H*)-yl)propanoic acid methylester (4d). Yield: 51%, colorless powder, mp 201–203 °C. $[\alpha]_D^{20}$ = −38.96 (c 0.25, $CHCl_3$); ESI/MS: 303 $[M+H]^+$; IR (KBr disk): 3396, 2988, 2973, 1747, 1700, 1626, 1436, 1328, 1243, 1121, 748 cm^{-1}. ^{1}H NMR (500 MHz, DMSO) δ_H = 7.17 (t, J = 7.2 Hz, 1H), 7.14 (d, J = 7.2 Hz, 1H), 7.12 (d, J = 7.2 Hz, 1H), 7.10 (t, J = 7.2 Hz, 1H), 4.27 (q, J = 7.0 Hz, 1H), 3.98 (d, J = 14.0 Hz, 1H), 3.74 (d, J = 15.0 Hz, 1H), 3.62 (s, 3H), 3.22 (dd, J = 3.5 Hz, J = 10.5 Hz, 1H), 2.95 (d, J = 15.5 Hz, 1H), 2.68 (dd, J = 12.0 Hz, J = 14.5 Hz, 1H), 1.43 (d, J = 6.0 Hz, 3H), 1.41 (s, 3H), 1.277 (s, 3H); ^{13}C NMR (125 MHz, DMSO) δ_C = 16.85, 17.68, 25.43, 30.95, 33.82, 46.77, 49.02, 52.51, 56.76, 78.64, 126.27, 126.78, 127.22, 129.93, 133.76, 135.17, 170.77, 171.65. Elemental Anal. Calcd for $C_{17}H_{22}N_2O_3$: C, 67.53; H, 7.33; N, 9.26. Found: C, 67.31; H, 7.18; N, 9.58.

4.1.5.5. (*S*)-2-(3,3-Dimethyl-1-oxo-10,10a-dihydroimidazo[1,5-*b*]isoquinolin-2(1*H*,3*H*,5*H*)-yl)acetic acid methylester (4e). Yield: 52%, colorless powder, mp 188–190 °C. $[\alpha]_D^{20}$ = −54.20 (c 0.25, $CHCl_3$); ESI/MS: 289 $[M+H]^+$; IR (KBr disk): 3358, 2953, 2709, 1726, 1697, 1612, 1417, 1323, 1296, 1218, 976, 751 cm^{-1}. ^{1}H NMR (500 MHz,DMSO) δ_H = 7.17 (t, J = 7.3 Hz, 1H), 7.15 (d, J = 7.3 Hz, 1H), 7.12 (d, J = 7.3 Hz, 1H), 7.09 (t, J = 7.3 Hz, 1H), 4.09 (s, 2H), 3.98 (d, J = 14.0 Hz, 1H), 3.76 (s, 2H), 3.672 (s, 3H), 2.99 (dd, J = 3.5 Hz, J = 15.5 Hz, 1H), 2.70 (dd, J = 12.0 Hz, J = 15.0 Hz, 1H), 1.36 (s, 3H), 1.24 (s, 3H); ^{13}C NMR (125 MHz, DMSO) δ_C = 18.07, 24.93, 31.02, 33.82, 46.64, 52.36, 56.65, 78.08, 126.31, 126.61, 127.23, 129.93, 133.75, 135.20, 169.80, 171.51. Elemental Anal. Calcd for $C_{16}H_{20}N_2O_3$: C, 66.65; H, 6.99; N, 9.72. Found: C, 66.46; H, 6.85; N, 7.20.

4.1.5.6. (*S*)-2-((*S*)-3,3-Dimethyl-1-oxo-10,10a-dihydroimidazo-[1,5-*b*]isoquinolin-2(1*H*,3*H*,5*H*)-yl)-3-phenylpropanoic acid methylester (4f). Yield: 51%, colorless powder, mp 191–193 °C. $[\alpha]_D^{20}$ = −96.18 (c 0.25, $CHCl_3$); ESI/MS: 379 $[M+H]^+$; IR (KBr disk): 3423, 2923, 2774, 1745, 1698, 1457, 1434, 1417, 1372, 1245, 1220, 1177, 745, 703 cm^{-1}. ^{1}H NMR (500 MHz, DMSO) δ_H = 7.23 (t, J = 7.5 Hz, 2H), 7.18 (d, J = 7.5 Hz, 2H), 7.10 (t, J = 7.5 Hz, 1H), 7.13 (t, J = 7.4 Hz, 1H), 7.11 (d, J = 7.4 Hz, 1H), 7.09 (d, J = 7.4 Hz, 1H), 7.06 (t, J = 7.4 Hz, 1H), 4.36 (dd, J = 4.5 Hz, 1H), 3.82 (d, J = 14.0 Hz, 1H), 3.68 (s, 3H), 3.53 (t, J = 13.5 Hz, 1H), 3.32 (d, J = 4.5 Hz, 2H), 3.14 (dd, J = 4.0 Hz, J = 11.0 Hz, 1H), 2.94 (dd, J = 4.0 Hz, J = 16.0 Hz, 1H), 2.66 (dd, J = 11.0 Hz, J = 15.5 Hz, 1H), 1.26 (s, 3H), 0.93 (s, 3H); ^{13}C NMR (125 MHz, DMSO) δ /ppm = 17.75, 24.73, 30.86, 34.15, 46.67, 52.78, 56.46, 78.63, 126.24, 126.60, 127.09, 127.13, 128.75, 129.88, 130.24, 133.64, 135.03, 138.69, 170.87, 171.58. Elemental Anal. Calcd for $C_{23}H_{26}N_2O_3$: C, 72.99; H, 6.92; N, 7.40. Found: C, 72.80; H, 6.78; N, 7.21.

4.1.5.7. (*S*)-2-((*S*)-3,3-Dimethyl-1-oxo-10,10a-dihydroimidazo [1,5-*b*]isoquinolin-2(1*H*,3*H*,5*H*)-yl)-3-(4-hydroxyphenyl)propanoic acid methylester (4g). Yield: 53%, colorless powder, mp 204–207 °C. $[\alpha]_D^{20}$ = −134.50 (c 0.25, $CHCl_3$); ESI/MS: 395 $[M+H]^+$; IR (KBr disk): 3326, 2932, 2774, 1743, 1698, 1627, 1540, 1423, 1372, 1260, 1107, 747 cm^{-1}. ^{1}H NMR (500 MHz, DMSO) δ_H = 7.22 (d, J = 7.8 Hz, 2H), 7.19 (d, J = 7.8 Hz, 2H), 7.13 (t, J = 7.6 Hz, 1H), 7.10 (d, J = 7.6 Hz, 1H), 7.07 (d, J = 7.6 Hz, 1H), 7.05 (t, J = 7.6 Hz, 1H), 5.22 (s, 1H), 4.13 (dd, J = 6.5 Hz, J = 11.0 Hz, 1H), 3.89 (d, J = 15.5 Hz, 1H), 3.67 (s, 3H), 3.60 (t, J = 11.0 Hz, 1H), 3.42 (d, J = 16.0 Hz, 1H), 3.28 (dd, J = 6.5 Hz, J = 16.0 Hz, 1H), 3.23 (dd, J = 7.0 Hz, J = 7.5 Hz, 1H), 2.87 (dd, J = 7.0 Hz, J = 16.0 Hz, 1H), 2.64 (dd, J = 8.0 Hz, J = 15.5 Hz, 1H), 1.25 (s, 6H); ^{13}C NMR (125 MHz, DMSO) δ_C = 24.12, 24.87, 30.85, 33.67, 46.78, 52.9, 56.55, 57.53, 78.78, 115.26, 115.51, 126.56, 127.22, 129.44, 129.86, 130.11, 131.07, 131.54, 133.85, 135.22, 156.26, 171.223, 172.14. Elemental Anal. Calcd for $C_{23}H_{26}N_2O_4$: C, 70.03; H, 6.64; N, 7.10. Found: C, 69.81; H, 6.50; N, 7.33.

4.1.5.8. (*S*)-2-((*S*)-3,3-Dimethyl-1-oxo-10,10a-dihydroimidazo-[1,5-*b*]isoquinolin-2(1*H*,3*H*,5*H*)-yl)-3-(1*H*-indol-3-yl)propanoic acid methylester (4h). Yield: 51%, colorless powder, mp 174–176 °C. $[\alpha]_D^{20}$ = −127.80 (c 0.25, $CHCl_3$); ESI/MS: 418 $[M+H]^+$; IR (KBr disk): 3396, 2931, 2769, 1747, 1698, 1516, 1457, 1368, 1339, 1259, 1101, 743 cm^{-1}. ^{1}H NMR (500 MHz, DMSO) δ_H = 10.86 (s, 1H), 7.57 (d, J = 5.0 Hz, 1H), 7.53 (d, J = 5.0 Hz, 1H), 7.34 (d, J = 5.0 Hz, 1H), 7.30 (d, J = 5.0 Hz, 1H), 7.16 (t, J = 7.5 Hz, 1H), 7.13 (d, J = 7.5 Hz, 1H), 7.10 (d, J = 7.5 Hz, 1H), 7.07 (t, J = 7.5 Hz, 1H), 6.92 (s, 1H), 4.28 (dd, J = 4.5 Hz, J = 10.0 Hz, 1H), 3.74 (d, J = 12.5 Hz, 1H), 3.70 (s, 3H), 3.47 (dd, J = 10.0 Hz, J = 16.5 Hz, 2H), 3.46 (d, J = 14.0 Hz, 1H), 3.34 (m, 1H), 3.08 (dd, J = 4.0 Hz, J = 11.5 Hz, 1H), 2.96 (dd, J = 3.5 Hz, J = 15.5 Hz, 1H), 2.67 (dd, J = 11.0 Hz, J = 15.0 Hz, 1H), 1.22 (s, 3H), 0.81 (s, 3H); ^{13}C NMR (125 MHz, DMSO) δ_C = 17.63, 24.18, 24.85, 30.94, 46.68, 52.67, 56.09, 56.62, 78.65, 111.01, 111.93, 118.44, 118.93, 121.27, 124.82, 126.23, 126.59,126.74, 127.12, 128.08, 129.88, 133.68, 135.03, 136.47, 171.06, 171.29. Elemental Anal. Calcd for $C_{25}H_{27}N_3O_3$: C, 71.92; H, 6.52; N, 10.06. Found: C, 71.73; H, 6.36; N, 10.74.

4.1.5.9. (*S*)-2-((*S*)-3,3-Dimethyl-1-oxo-10,10a-dihydroimidazo [1,5-*b*]isoquinolin-2(1*H*,3*H*,5*H*)-yl)succinic acid dimethylester (4i). Yield: 60%, colorless powder, mp 134–136 °C. $[\alpha]_D^{20}$ = −131.12 (c 0.25, $CHCl_3$); ESI/MS: 361 $[M+H]^+$; IR (KBr disk): 3402, 2984, 2778, 1740, 1694, 1558, 1436, 1373, 1257, 1232, 1169, 756 cm^{-1}. ^{1}H NMR (500 MHz, DMSO) δ_H = 7.16 (t, J = 7.8 Hz, 1H), 7.14 (d, J = 7.8 Hz, 1H), 7.10 (d, J = 7.8 Hz, 1H), 7.07 (t, J = 7.8 Hz, 1H), 4.43 (t, J = 5.0 Hz, 1H), 3.97 (d, J = 15.0 Hz, 1H), 3.74 (d, J = 15.0 Hz, 1H), 3.65 (s, 3H), 3.64 (s, 3H), 3.33 (dd, J = 5.0 Hz, J = 15.0 Hz, 1H), 3.27 (dd, J = 10.0 Hz, J = 15.0 Hz, 1H), 2.93 (dd, J = 5.0 Hz, J = 15.0 Hz, 1H), 2.80 (dd, J = 5.0 Hz, J = 15.0 Hz, 1H), 2.64 (dd, J = 10.0 Hz, J = 15.0 Hz, 1H), 1.45 (s, 3H), 1.24 (s, 3H); ^{13}C NMR (125 MHz, DMSO) δ_C = 19.12, 24.93, 30.79, 34.81, 46.64, 50.51, 52.18, 53.06, 56.24, 78.72, 126.31, 126.63, 127.19, 129.91, 133.62, 135.07, 170.26, 171.38, 171.45. Elemental Anal. Calcd for $C_{19}H_{24}N_2O_5$: C, 63.32; H, 6.71; N, 7.77. Found: C, 63.53; H, 6.87; N, 7.56.

4.1.5.10. (*S*)-2-((*S*)-3,3-Dimethyl-1-oxo-10,10a-dihydroimidazo[1,5-*b*]isoquinolin-2(1*H*,3*H*,5*H*)-yl)pentanedioic acid dimethylester (4j). Yield: 56%, colorless powder, mp 181–182 °C. $[\alpha]_D^{20} = -140.20$ (*c* 0.25, $CHCl_3$); ESI/MS: 375 $[M+H]^+$; IR (KBr disk): 3423, 2988, 2769, 1745, 1684, 1423, 1357, 1268, 1173, 748 cm^{-1}. ^1H NMR (500 MHz, DMSO) $\delta_H = 7.24$ (t, *J* = 7.8 Hz, 1H), 7.22 (d, *J* = 7.8 Hz, 1H), 7.18 (d, *J* = 7.8 Hz, 1H), 7.12 (t, *J* = 7.8 Hz, 1H), 4.25 (m, 1H), 3.95 (d, *J* = 15.0 Hz, 1H), 3.72 (d, *J* = 15.0 Hz, 1H), 3.64 (s, 3H), 3.62 (s, 3H), 3.35 (dd, *J* = 5.0 Hz, *J* = 15.0 Hz, 1H), 3.29 (dd, *J* = 10.0 Hz, *J* = 15.0 Hz, 1H), 2.95 (dd, *J* = 5.0 Hz, *J* = 15.0 Hz, 1H), 2.83 (dd, *J* = 5.0 Hz, *J* = 15.0 Hz, 1H), 2.68 (dd, *J* = 10.0 Hz, *J* = 15.0 Hz, 1H), 1.38 (s, 3H), 2.34 (m, 1H), 2.21 (m, 1H), 1.29 (s, 3H); ^{13}C NMR (125 MHz, DMSO) $\delta_C = 24.22, 24.56, 24.84, 28.62, 29.33, 39.44, 55.25, 55.56, 59.81, 78.54, 125.98, 126.43, 126.67, 127.48, 131.33, 131.99, 168.54, 170.91, 171.48, 172.10$. Elemental Anal. Calcd for $C_{20}H_{26}N_2O_5$: C, 64.15; H, 7.00; N, 7.48. Found: C, 64.33; H, 6.85; N, 7.70.

4.1.5.11. (*S*)-6-(Benzyloxycarbonyl)-2-((*S*)-3,3-dimethyl-1-oxo-10,10a-dihydro-imidazo[1,5-*b*]isoquinolin-2(1*H*,3*H*,5*H*)-yl)hexanoic acid methylester (4k). Yield: 42%, colorless powder, mp 136–138 °C. $[\alpha]_D^{20} = -114.32$ (*c* 0.25, $CHCl_3$); ESI/MS: 494 $[M+H]^+$; IR (KBr disk): 3623, 3426, 2958, 2773, 1744, 1697, 1459, 1367, 1262, 1205, 1096, 750 cm^{-1}. ^1H NMR (500 MHz, DMSO) $\delta_H = 8.31$ (t, *J* = 9.0 Hz, 1H), 7.22 (t, *J* = 9.0 Hz, 1H), 7.20 (t, *J* = 9.0 Hz, 2H), 7.19 (t, *J* = 7.8 Hz, 2H), 7.18 (d, *J* = 7.8 Hz, 1H), 7.17 (d, *J* = 7.8 Hz, 1H), 7.10 (t, *J* = 7.8 Hz, 1H), 7.07 (t, *J* = 7.8 Hz, 1H), 5.10 (s, 2H), 4.12 (t, *J* = 6.0 Hz, 1H), 3.96 (d, *J* = 14.0 Hz, 1H), 3.74 (d, *J* = 14.5 Hz, 1H), 3.64 (s, 3H), 3.29 (dd, *J* = 10.0 Hz, *J* = 15.0 Hz, 1H), 3.21 (m, *J* = 6.0 Hz, 2H), 2.95 (dd, *J* = 5.0 Hz, *J* = 15.0 Hz, 1H), 2.68 (dd, *J* = 10.0 Hz, *J* = 15.0 Hz, 1H), 1.85 (m, *J* = 6.0 Hz, 2H), 1.65 (m, *J* = 6.0 Hz, 2H), 1.40 (s, 3H), 1.38 (s, 3H), 1.21 (m, *J* = 6.0 Hz, 2H); ^{13}C NMR (125 MHz, DMSO) $\delta_C = 21.40, 27.23, 27.15, 28.45, 29.12, 29.72, 41.95, 51.93, 53.56, 55.01, 59.21, 69.56, 126.25, 126.60, 127.22, 127.38, 128.54, 129.91, 133.38, 133.80, 135.19, 135.28, 136.52, 170.15, 172.04, 172.53$. Elemental Anal. Calcd for $C_{28}H_{35}N_3O_5$: C, 68.13; H, 7.15; N, 8.51. Found: C, 64.33; H, 6.85; N, 7.70.

4.1.5.12. (2*S*)-2-((*S*)-3,3-Dimethyl-1-oxo-10,10a-dihydroimidazo-[1,5-*b*]isoquinolin-2(1*H*,3*H*,5*H*)-yl)-3-hydroxybutanoic acid methylester (4l). Yield: 42%, colorless powder, mp 201–203 °C. $[\alpha]_D^{20} = -59.20$ (*c* 0.25, $CHCl_3$); ESI/MS: 333 $[M+H]^+$; IR (KBr disk): 3408, 2925, 2850, 1743, 1694, 1671, 1626, 1575, 1401, 1359, 1244, 1123, 747 cm^{-1}. ^1H NMR (500 MHz, DMSO) $\delta_H = 7.22$ (d, *J* = 7.8 Hz, 1H), 7.20 (d, *J* = 7.8 Hz, 1H), 7.18 (t, *J* = 7.8 Hz, 1H), 7.15 (t, *J* = 7.8 Hz, 1H), 4.28 (d, *J* = 7.0 Hz, 1H), 3.97 (d, *J* = 15.0 Hz, 1H), 3.73 (d, *J* = 15.0 Hz, 1H), 3.63 (s, 3H), 3.41 (m, 1H), 3.21 (dd, *J* = 4.0 Hz, *J* = 10.5 Hz, 1H), 2.97 (dd, *J* = 5.0 Hz, *J* = 15.5 Hz, 1H), 2.68 (dd, *J* = 11.0 Hz, *J* = 15.0 Hz, 1H), 1.39 (s, 3H), 1.277 (s, 3H), 1.21 (d, *J* = 7.0 Hz, 3H), 1.92 (s, 1H); ^{13}C NMR (125 MHz, DMSO) $\delta_C = 19.74, 24.84, 25.87, 33.86, 44.66, 47.86, 51.87, 55.66, 77.55, 126.67, 127.08, 127.96, 129.24, 132.91, 133.61, 174.11, 175.89$. Elemental Anal. Calcd for $C_{18}H_{24}N_2O_4$: C, 65.04; H, 7.28; N, 8.43. Found: C, 64.82; H, 7.12; N, 8.61.

4.1.5.13. (*S*)-4-Amino-2-((*S*)-3,3-dimethyl-1-oxo-10,10a-dihydroimidazo[1,5-*b*]-isoquinolin-2(1*H*,3*H*,5*H*)-yl)-4-oxobutanoic acid methylester (4m). Yield: 42%, colorless powder, mp 183–185 °C. $[\alpha]_D^{20} = -109.20$ (*c* 0.25, $CHCl_3$); ESI/MS: 346 $[M+H]^+$; IR (KBr disk): 3398, 2945, 2763, 1740, 1694, 1436, 1356, 1257, 1168, 751 cm^{-1}. ^1H NMR (500 MHz, DMSO) $\delta_H = 7.17$ (d, *J* = 7.8 Hz, 1H), 7.14 (d, *J* = 7.8 Hz, 1H), 7.12 (t, *J* = 7.8 Hz, 1H), 7.10 (t, *J* = 7.8 Hz, 1H), 6.01 (s, 2H), 4.43 (t, *J* = 7.0 Hz, 1H), 3.97 (d, *J* = 14.5 Hz, 1H), 3.65 (s, 3H), 3.34 (d, *J* = 7.0 Hz, 2H), 3.30 (dd, *J* = 8.0 Hz, *J* = 11.0 Hz, 1H), 2.93 (dd, *J* = 3.5 Hz, *J* = 15.5 Hz, 1H), 2.81 (dd, *J* = 5.5 Hz, *J* = 15.5 Hz, 1H), 2.65 (dd, *J* = 11.5 Hz, *J* = 15.5 Hz, 1H), 1.45 (s, 3H), 1.25 (s, 3H); ^{13}C NMR (125 MHz, DMSO) $\delta_C = 24.92, 25.04, 31.13, 34.18, 49.74, 51.96, 52.45, 58.86, 78.67, 126.54, 127.03, 127.23, 127.31, 134.00, 135.69, 172.40, 172.81, 173.86$. Elemental Anal. Calcd for $C_{18}H_{23}N_3O_4$: C, 62.59; H, 6.71; N, 12.17. Found: C, 64.82; H, 7.12; N, 8.61.

4.1.5.14. (*S*)-5-Amino-2-((*S*)-3,3-dimethyl-1-oxo-10,10a-dihydroimidazo[1,5-*b*]-isoquinolin-2(1*H*,3*H*,5*H*)-yl)-5-oxopentanoic acid methylester (4n). Yield: 42%, colorless powder, mp 159–161 °C. $[\alpha]_D^{20} = -140.20$ (*c* 0.25, $CHCl_3$); ESI/MS: 360 $[M+H]^+$; IR (KBr disk): 3356, 2955, 2748, 1740, 1684, 1436, 1364, 1236, 1174, 1123, 748 cm^{-1}. ^1H NMR (500 MHz, DMSO) $\delta_H = 7.18$ (d, *J* = 7.8 Hz, 1H), 7.16 (d, *J* = 7.8 Hz, 1H), 7.14 (t, *J* = 7.8 Hz, 1H), 7.12 (t, *J* = 7.8 Hz, 1H), 6.03 (s, 2H), 4.16 (t, *J* = 7.0 Hz, 1H), 3.96 (d, *J* = 14.5 Hz, 1H), 3.64 (s, 3H), 3.62 (s, 2H), 3.37 (dd, *J* = 4.0 Hz, *J* = 11.0 Hz, 1H), 2.92 (dd, *J* = 3.5 Hz, *J* = 15.5 Hz, 1H), 2.65 (dd, *J* = 11.0 Hz, *J* = 15.5 Hz, 1H), 2.48 (m, *J* = 7.0 Hz, 1H), 2.38 (m, *J* = 7.0 Hz, 2H), 1.379 (s, 3H), 1.291 (s, 3H); ^{13}C NMR (125 MHz, DMSO) $\delta_C = 24.07, 24.83, 25.81, 30.89, 33.80, 47.92, 51.20, 56.53, 56.77, 78.58, 124.67, 126.19, 126.56, 127.12, 133.73, 135.08, 163.79, 171.01, 171.56, 172.86$. Elemental Anal. Calcd for $C_{19}H_{25}N_3O_4$: C, 63.49; H, 7.01; N, 11.69. Found: C, 63.28; H, 7.17; N, 11.91.

4.1.5.15. (*S*)-2-((*S*)-3,3-Dimethyl-1-oxo-10,10a-dihydroimidazo[1,5-*b*]isoquinolin-2(1*H*,3*H*,5*H*)-yl)-6-(3-nitroguanidino)hexanoic acid methylester (4o). Yield: 43%, colorless powder, mp 186–188 °C. $[\alpha]_D^{20} = -147.13$ (*c* 0.25, $CHCl_3$); ESI/MS: 433 $[M+H]^+$; IR (KBr disk): 3356, 3264, 2933, 2772, 1742, 1674, 1516, 1456, 1434, 1360, 1260, 1233, 1190, 744 cm^{-1}. ^1H NMR (500 MHz, DMSO) $\delta_H = 9.12$ (s, 1H), 7.82 (s, 1H), 7.17 (d, *J* = 7.8 Hz, 1H), 7.14 (d, *J* = 7.8 Hz, 1H), 7.12 (t, *J* = 7.8 Hz, 1H), 7.10 (t, *J* = 7.8 Hz, 1H), 4.24 (dd, *J* = 4.0 Hz, *J* = 10.5 Hz, 1H), 3.830 (d, *J* = 14.0 Hz, 1H), 3.682 (s, 3H), 3.55 (d, *J* = 14.0 Hz, 1H), 3.46 (dd, *J* = 10.0 Hz, *J* = 15.0 Hz, 1H), 3.20 (dd, *J* = 5.0 Hz, *J* = 14.0 Hz, 1H), 3.14 (dd, *J* = 5.0 Hz, *J* = 10.0 Hz, 1H), 2.94 (dd, *J* = 4.0 Hz, *J* = 15.0 Hz, 1H), 2.68 (t, *J* = 4.0 Hz, 2H), 1.98 (d, *J* = 4.0 Hz, 2H), 1.58 (m, *J* = 4.0 Hz, 2H), 1.29 (s, 3H), 0.94 (s, 3H); ^{13}C NMR (125 MHz, DMSO) $\delta_C = 17.92, 24.21, 30.91, 33.83, 46.72, 52.59, 56.56, 56.91, 78.70, 115.52, 126.22, 126.59, 127.14, 127.23, 128.52, 128.69, 131.14, 133.70, 135.09, 135.24, 156.47, 170.96, 171.42$. Elemental Anal. Calcd for $C_{20}H_{28}N_6O_5$: C, 55.54; H, 6.53; N, 19.43. Found: C, 55.73; H, 6.68; N, 19.22.

4.1.5.16. (*S*)-2-((*S*)-3,3-Dimethyl-1-oxo-10,10a-dihydroimidazo[1,5-*b*]isoquinolin-2(1*H*,3*H*,5*H*)-yl)-4-(methylthio)butanoic acid methylester (4p). Yield: 37%, colorless powder, mp 174–176 °C. $[\alpha]_D^{20} = -89.13$ (*c* 0.25, $CHCl_3$); ESI/MS: 363 $[M+H]^+$; IR (KBr disk): 3422, 2997, 2787, 1733, 1705, 1456, 1418, 1350, 1268, 1240, 1148, 752 cm^{-1}. ^1H NMR (500 MHz, DMSO) $\delta_H = 7.16$ (d, *J* = 7.8 Hz, 1H), 7.13 (d, *J* = 7.8 Hz, 1H), 7.10 (t, *J* = 7.8 Hz, 1H), 7.08 (t, *J* = 7.8 Hz, 1H), 4.21 (t, *J* = 6.5 Hz, 1H), 3.96 (d, *J* = 14.5 Hz, 1H), 3.74 (d, *J* = 14.5 Hz, 1H), 3.65 (s, 3H), 3.36 (dd, *J* = 4.0 Hz, *J* = 11.0 Hz, 1H), 2.93 (dd, *J* = 3.5 Hz, *J* = 15.5 Hz, 1H), 2.66 (dd, *J* = 11.5 Hz, *J* = 15.5 Hz, 1H), 2.54 (d, *J* = 6.5 Hz, 2H), 2.32 (t, *J* = 6.5 Hz, 2H), 2.12 (s, 3H), 1.39 (s, 3H), 1.30 (s, 3H); ^{13}C NMR (125 MHz, DMSO) $\delta_C = 14.95, 19.40, 24.83, 29.14, 30.93, 46.78, 52.71, 52.90, 56.31, 78.67, 126.28, 126.62, 127.18, 129.92, 133.76, 135.15, 171.18, 171.31$. Elemental Anal. Calcd for $C_{19}H_{26}N_2O_3S$: C, 62.96; H, 7.23; N, 7.73. Found: C, 62.77; H, 7.38; N, 7.50.

4.1.5.17. (*R*)-2-((*S*)-3,3-Dimethyl-1-oxo-10,10a-dihydroimidazo-[1,5-*b*]isoquinolin-2(1*H*,3*H*,5*H*)-yl)-3-mercaptopropanoic acid methylester (4q). Yield: 40%, colorless powder, mp 146–148 °C. $[\alpha]_D^{20} = -89.13$ (*c* 0.25, $CHCl_3$); ESI/MS: 335 $[M+H]^+$; IR (KBr disk): 3356, 2987, 2787, 1740, 1673, 1418, 1350, 1289, 1240, 1176,

1101, 748 cm^{-1}. ^1H NMR (500 MHz, DMSO) δ_H = 7.16 (d, J = 7.7 Hz, 1H), 7.14 (d, J = 7.7 Hz, 1H), 7.11 (t, J = 7.7 Hz, 1H), 7.09 (t, J = 7.7 Hz, 1H), 4.36 (t, J = 7.0 Hz, 1H), 3.98 (d, J = 14.0 Hz, 1H), 3.76 (d, J = 13.5 Hz, 1H), 3.68 (s, 3H), 3.56 (dd, J = 6.5 Hz, J = 13.5 Hz, 1H), 3.33 (d, J = 7.0 Hz, 2H), 2.92 (dd, J = 3.5 Hz, J = 15.5 Hz, 1H), 2.65 (dd, J = 12.5 Hz, J = 15.5 Hz, 1H), 2.54 (s, 1H), 1.44 (s, 3H), 1.31 (s, 3H); ^{13}C NMR (125 MHz, DMSO) δ_C = 14.95, 19.40, 24.83, 29.14, 30.93, 46.78, 51.18, 52.71, 52.90, 56.31, 78.67, 126.28, 126.62, 127.18, 129.92, 133.76, 135.15, 171.18, 171.31. Elemental Anal. Calcd for $C_{17}H_{22}N_2O_3S$: C, 61.05; H, 6.63; N, 8.38. Found: C, 60.84; H, 6.47; N, 8.61.

4.1.6. General procedure for preparing 2-substituted (*S*)-2-(3,3-dimethyl-1-oxo-10,10a-dihydroimidazo[1,5-*b*]isoquinolin-2(1*H*,3*H*,5*H*)-yl)-acetic acids (5a–q)

At 0 °C to the solution of 1.45 mmol of 2-substituted (*S*)-2-(3,3-dimethyl-1-oxo-10,10a-dihydroimidazo[1,5-*b*]isoquinolin-2(1*H*,-3*H*,5*H*)-yl)-acetic acid methylesters (**4**) in 5 mL of methanol, 7 mL of 2 N NaOH was added to adjust pH 11. The reaction mixture was stirred at 0 °C for 3 h and TLC (CCl_3/CH_3OH, 5:1) indicated the complete disappearance of **4**. The reaction mixture was adjusted to pH 7 with aqueous solution of $KHSO_4$. The solution was evaporated under vacuum to remove methanol, adjusted to pH 2 with aqueous solution of $KHSO_4$, and extracted with ethyl acetate (30 mL × 3). The combined ethyl acetate was successively washed with saturated aqueous solution of NaCl (20 mL × 2) and dried with anhydrous Na_2SO_4. After filtration, the filtrate was evaporated to provide **5a–q**.

4.1.6.1. (*S*)-2-((*S*)-3,3-Dimethyl-1-oxo-10,10a-dihydroimidazo[1,5-*b*]isoquinolin-2(1*H*,3*H*,5*H*)-yl)-4-methylpentanoic acid (5a).

Yield: 88%, colorless powder, mp 188–190 °C. $[\alpha]_D^{20}$ = −41.23 (*c* 0.25, CH_3OH); ESI/MS: 331 $[M+H]^+$; IR (KBr disk): 2963, 1731, 1663, 1464, 1424, 1365, 1261, 1228, 1191, 745 cm^{-1}. ^1H NMR (300 MHz, DMSO) δ_H = 11.11 (s, 1H), 7.17 (d, J = 7.8 Hz, 1H), 7.15 (d, J = 7.8 Hz, 1H), 7.12 (t, J = 7.8 Hz, 1H), 7.08 (t, J = 7.8 Hz, 1H), 3.95 (d, J = 14.1 Hz, 1H), 3.88 (t, J = 6.9 Hz, 1H), 3.72 (d, J = 14.4 Hz, 1H), 3.31 (dd, J = 3.6 Hz, J = 10.8 Hz, 1H), 2.89 (dd, J = 3.6 Hz, J = 16.2 Hz, 1H), 2.65 (dd, J = 11.4 Hz, J = 15.6 Hz, 1H), 1.96 (t, J = 6.9 Hz, 2H), 1.69 (m, 1H), 1.40 (s, 3H), 1.19 (s, 3H), 0.92 (t, J = 6.0 Hz, 6H); ^{13}C NMR (75 MHz, DMSO) δ_C = 19.21, 21.92, 22.70, 23.25, 24.72, 30.97, 46.41, 55.72, 56.49, 78.76, 126.23, 126.34, 126.58, 129.24, 134.29, 135.22, 170.95, 172.78. Elemental Anal. Calcd for $C_{19}H_{26}N_2O_3$: C, 69.06; H, 7.93; N, 8.48. Found: C, 69.25; H, 7.79; N, 8.69.

4.1.6.2. (*S*)-2-((*S*)-3,3-Dimethyl-1-oxo-10,10a-dihydroimidazo-[1,5-*b*]isoquinolin-2(1*H*,3*H*,5*H*)-yl)-3-methylbutanoic acid (5b).

Yield: 92%, colorless powder, mp 174–176 °C. $[\alpha]_D^{20}$ = −8.89 (*c* 0.25, CH_3OH); ESI/MS: 317 $[M+H]^+$; IR (KBr disk): 2968, 1728, 1669, 1443, 1417, 1359, 1263, 1226, 1170, 743 cm^{-1}. ^1H NMR (300 MHz, DMSO) δ_H = 11.09 (s, 1H), 7.20 (d, J = 7.8 Hz, 1H), 7.19 (d, J = 7.8 Hz, 1H), 7.14 (t, J = 7.8 Hz, 1H), 7.10 (t, J = 7.8 Hz, 1H), 3.94 (d, J = 14.1 Hz, 1H), 3.99 (d, J = 6.9 Hz, 1H), 3.69 (d, J = 14.2 Hz, 1H), 3.30 (dd, J = 3.6 Hz, J = 11.2 Hz, 1H), 2.89 (dd, J = 3.7 Hz, J = 16.2 Hz, 1H), 2.65 (dd, J = 11.3 Hz, J = 16.1 Hz, 1H), 1.84 (m, 1H), 1.39 (s, 3H), 1.24 (s, 3H), 0.98 (t, J = 6.0 Hz, 6H); ^{13}C NMR (75 MHz, DMSO) δ_C = 21.12, 21.22, 22.49, 23.40, 28.07, 56.45, 56.76, 65.38, 81.74, 127.37, 127.50, 128.57, 128.69, 132.94, 133.34, 171.18, 171.77. Elemental Anal. Calcd for $C_{18}H_{24}N_2O_3$: C, 68.33; H, 7.65; N, 8.85. Found: C, 68.52; H, 7.81; N, 8.63.

4.1.6.3. (2*S*)-2-((*S*)-3,3-Dimethyl-1-oxo-10,10a-dihydroimidazo-[1,5-*b*]isoquinolin-2(1*H*,3*H*,5*H*)-yl)-3-methylpentanoic acid (5c).

Yield: 93%, colorless powder, mp 186–188 °C. $[\alpha]_D^{20}$ = −22.47 (*c* 0.25, CH_3OH); ESI/MS: 331 $[M+H]^+$; IR (KBr disk): 2965, 1735, 1682, 1456, 1428, 1386, 1259, 1217, 1191, 746 cm^{-1}. ^1H NMR (300 MHz, DMSO) δ_H = 11.07 (s, 1H), 7.21 (d, J = 7.8 Hz, 1H), 7.18 (d, J = 7.8 Hz, 1H), 7.15 (t, J = 7.8 Hz, 1H), 7.11 (t, J = 7.8 Hz, 1H), 3.94 (t, J = 6.5 Hz, 1H), 3.98 (d, J = 7.2 Hz, 1H), 3.72 (d, J = 14.2 Hz, 1H), 3.31 (dd, J = 3.7 Hz, J = 11.1 Hz, 1H), 2.92 (dd, J = 3.7 Hz, J = 16.1 Hz, 1H), 2.64 (dd, J = 11.4 Hz, J = 16.3 Hz, 1H), 2.22 (m, J = 6.5 Hz, 1H), 1.69 (m, J = 6.5 Hz, 2H), 1.39 (s, 3H), 1.21 (s, 3H), 0.96 (t, J = 6.5 Hz, 6H); ^{13}C NMR (75 MHz, DMSO) δ_C = 11.89, 16.02, 25.04, 26.53, 30.89, 31.08, 37.32, 46.29, 55.60, 56.76, 78.53, 126.24, 126.38, 127.18, 129.20, 134.34, 135.35, 171.97, 173.40. Elemental Anal. Calcd for $C_{19}H_{26}N_2O_3$: C, 69.06; H, 7.93; N, 8.48. Found: C, 69.27; H, 7.77; N, 8.70.

4.1.6.4. (*S*)-2-((*S*)-3,3-Dimethyl-1-oxo-10,10a-dihydroimidazo[1,5-*b*]isoquinolin-2(1*H*,3*H*,5*H*)-yl)propanoic acid (5d).

Yield: 92%, colorless powder, mp 197–199 °C. $[\alpha]_D^{20}$ = −45.06 (*c* 0.25, CH_3OH); ESI/MS: 289 $[M+H]^+$; IR (KBr disk): 2978, 1708, 1450, 1424, 1378, 1364, 1258, 1220, 739 cm^{-1}. ^1H NMR (300 MHz, DMSO) δ_H = 11.13 (s, 1H), 7.15 (d, J = 7.8 Hz, 1H), 7.13 (d, J = 7.8 Hz, 1H), 7.10 (t, J = 7.8 Hz, 1H), 7.08 (t, J = 7.8 Hz, 1H), 4.13 (q, J = 6.9 Hz, 1H), 3.95 (d, J = 14.4 Hz, 1H), 3.62 (d, J = 14.4 Hz, 1H), 3.29 (dd, J = 3.3 Hz, J = 11.1 Hz, 1H), 2.89 (dd, J = 3.3 Hz, J = 15.6 Hz, 1H), 2.59 (dd, J = 11.4 Hz, J = 15.6 Hz, 1H), 1.43 (d, J = 6.9 Hz, 3H), 1.40 (s, 3H), 1.26 (s, 3H); ^{13}C NMR (75 MHz, DMSO) δ_C = 15.68, 18.97, 24.94, 30.90, 46.69, 49.00, 56.68, 78.50, 126.25, 126.58, 127.20, 129.94, 133.84, 135.25, 170.24, 172.61. Elemental Anal. Calcd for $C_{16}H_{20}N_2O_3$: C, 66.65; H, 6.99; N, 9.72. Found: C, 66.44; H, 6.83; N, 9.94.

4.1.6.5. (*S*)-2-(3,3-Dimethyl-1-oxo-10,10a-dihydroimidazo[1,5-*b*]isoquinolin-2(1*H*,3*H*,5*H*)-yl)acetic acid (5e).

Yield: 86%, colorless powder, mp 186–188 °C. $[\alpha]_D^{20}$ = −127.63 (*c* 0.25, CH_3OH); ESI/MS: 275 $[M+H]^+$; IR (KBr disk): 2958, 1713, 1450, 1423, 1372, 1366, 1207, 744 cm^{-1}. ^1H NMR (300 MHz, DMSO) δ_H = 11.11 (s, 1H), 7.21 (d, J = 7.8 Hz, 1H), 7.19 (d, J = 7.8 Hz, 1H), 7.15 (t, J = 7.8 Hz, 1H), 7.12 (t, J = 7.8 Hz, 1H), 3.97 (d, J = 14.2 Hz, 1H), 3.93 (s, 2H), 3.74 (d, J = 14.1 Hz, 1H), 3.296 (dd, J = 3.6 Hz, J = 10.8 Hz, 1H), 2.978 (dd, J = 3.9 Hz, J = 15.6 Hz, 1H), 2.68 (dd, J = 11.7 Hz, J = 15.0 Hz, 1H), 1.35 (s, 3H), 1.22 (s, 3H); ^{13}C NMR (75 MHz, DMSO) δ_C = 25.05, 26.38, 31.06, 46.66, 56.74, 63.34, 77.99, 126.27, 126.87, 128.49, 129.92, 133.92, 135.26, 170.72, 171.28. Elemental Anal. Calcd for $C_{15}H_{18}N_2O_3$: C, 65.68; H, 6.61; N, 10.21. Found: C, 65.87; H, 6.78; N, 10.00.

4.1.6.6. (*S*)-2-((*S*)-3,3-Dimethyl-1-oxo-10,10a-dihydroimidazo-[1,5-*b*]isoquinolin-2(1*H*,3*H*,5*H*)-yl)-3-phenylpropanoic acid (5f).

Yield: 94%, colorless powder, mp 190–192 °C. $[\alpha]_D^{20}$ = −114.20 (*c* 0.25, CH_3OH); ESI/MS: 365 $[M+H]^+$; IR (KBr disk): 2978, 2956, 2774, 1744, 1698, 1457, 1433, 1417, 1372, 1245, 1219, 1170, 745, 703 cm^{-1}. ^1H NMR (500 MHz, DMSO) δ_H = 11.15 (s, 1H), 7.25 (t, J = 7.5 Hz, 2H), 7.22 (d, J = 7.5 Hz, 2H), 7.20 (t, J = 7.5 Hz, 1H), 7.18 (t, J = 7.4 Hz, 1H), 7.16 (d, J = 7.4 Hz, 1H), 7.13 (d, J = 7.4 Hz, 1H), 7.10 (t, J = 7.4 Hz, 1H), 4.18 (dd, J = 5.0 Hz, J = 10.0 Hz, 1H), 3.85 (d, J = 15.0 Hz, 1H), 3.59 (t, J = 10.0 Hz, 1H), 3.51 (d, J = 15.0 Hz, 1H), 3.28 (dd, J = 5.0 Hz, J = 15.0 Hz, 1H), 3.18 (dd, J = 5.0 Hz, J = 10.0 Hz, 1H), 2.88 (dd, J = 5.0 Hz, J = 15.0 Hz, 1H), 2.64 (dd, J = 10.0 Hz, J = 15.0 Hz, 1H), 1.23 (s, 3H), 0.82 (s, 3H); ^{13}C NMR (75 MHz, DMSO) δ_C = 24.67, 30.85, 34.15, 46.71, 56.68, 56.96, 78.57, 126.20, 126.58, 126.91, 127.13, 128.49, 128.68, 128.86, 129.89, 130.19, 130.60, 133.71, 135.09, 139.33, 171.30, 171.81. Elemental Anal. Calcd for $C_{22}H_{24}N_2O_3$: C, 72.50; H, 6.64; N, 7.69. Found: C, 72.69; H, 6.80; N, 7.47.

4.1.6.7. (*S*)-2-((*S*)-3,3-Dimethyl-1-oxo-10,10a-dihydroimidazo[1,5-*b*]isoquinolin-2(1*H*,3*H*,5*H*)-yl)-3-(4-hydroxyphenyl)propanoic acid (5g).

Yield: 92%, colorless powder, mp 196–198 °C. $[\alpha]_D^{20}$ = −27.07 (*c* 0.25, CH_3OH); ESI/MS: 381 $[M+H]^+$; IR (KBr disk):

3236, 2929, 2774, 1716, 1683, 1626, 1576, 1516, 1449, 1372, 1260, 1245, 745 cm^{-1}. ^1H NMR (300 MHz, DMSO) δ_H = 11.07 (s, 1H), 7.12 (d, *J* = 7.8 Hz, 2H), 7.10 (d, *J* = 7.8 Hz, 2H), 7.08 (t, *J* = 7.6 Hz, 1H), 7.07 (d, *J* = 7.6 Hz, 1H), 7.05 (d, *J* = 7.6 Hz, 1H), 7.03 (t, *J* = 7.6 Hz, 1H), 5.22 (s, 1H), 4.08 (dd, *J* = 6.6 Hz, *J* = 10.8 Hz, 1H), 3.84 (d, *J* = 15.6 Hz, 1H), 3.58 (t, *J* = 11.1 Hz, 1H), 3.41 (d, *J* = 15.9 Hz, 1H), 3.27 (dd, *J* = 6.6 Hz, *J* = 15.6 Hz, 1H), 3.21 (dd, *J* = 6.9 Hz, *J* = 7.5 Hz, 1H), 2.88 (dd, *J* = 6.9 Hz, *J* = 15.9 Hz, 1H), 2.64 (dd, *J* = 7.8 Hz, *J* = 15.3 Hz, 1H), 1.25 (s, 6H); ^{13}C NMR (75 MHz, DMSO) δ_C = 24.69, 24.92, 30.87, 33.77, 46.74, 56.40, 57.40, 78.64, 115.19, 115.39, 126.55, 127.14, 129.39, 129.88, 130.00, 131.03, 131.49, 133.76, 135.14, 156.21, 171.14, 171.99. Elemental Anal. Calcd for $C_{22}H_{24}N_2O_4$: C, 69.46; H, 6.36; N, 7.36. Found: C, 69.27; H, 6.20; N, 7.55.

4.1.6.8. (*S*)-2-((*S*)-3,3-Dimethyl-1-oxo-10,10a-dihydroimidazo[1,5-*b*]isoquinolin-2(1*H*,3*H*,5*H*)-yl)-3-(1H-indol-3-yl)propanoic acid (5h). Yield: 86%, colorless powder, mp 175–177 °C. $[\alpha]_D^{20}$ = −112.12 (*c* 0.25, CH_3OH); ESI/MS: 404 $[M+H]^+$; IR (KBr disk): 3256, 2931, 2769, 1716, 1686, 1516, 1456, 1367, 1339, 1259, 1242, 743 cm^{-1}. ^1H NMR (500 MHz, DMSO) δ_H = 11.90 (s, 1H), 10.91 (s, 1H), 7.56 (d, *J* = 5.0 Hz, 1H), 7.53 (d, *J* = 5.0 Hz, 1H), 7.34 (d, *J* = 5.0 Hz, 1H), 7.30 (d, *J* = 5.0 Hz, 1H), 7.16 (t, *J* = 5.0 Hz, 1H), 7.14 (d, *J* = 7.5 Hz, 1H), 7.11 (d, *J* = 7.5 Hz, 1H), 6.98 (t, *J* = 7.5 Hz, 1H), 4.12 (dd, *J* = 5.0 Hz, *J* = 10.0 Hz, 1H), 3.95 (dd, *J* = 6.6 Hz, *J* = 10.8 Hz, 1H), 3.79 (d, *J* = 10.0 Hz, 1H), 3.71 (m, 1H), 3.41 (d, *J* = 10.0 Hz, 1H), 3.25 (m, 1H), 3.05 (dd, *J* = 3.0 Hz, *J* = 10.0 Hz, 1H), 2.94 (dd, *J* = 3.0 Hz, *J* = 10.0 Hz, 1H), 2.67 (dd, *J* = 10.0 Hz, *J* = 15.0 Hz, 1H), 1.21 (s, 6H); ^{13}C NMR (125 MHz, DMSO) δ_C = 24.08, 24.79, 30.91, 33.81, 47.95, 56.45, 56.76, 65.38, 78.60, 111.47, 111.91, 118.43, 121.26, 124.68, 126.20, 126.57, 127.13, 128.10, 129.89, 133.73, 135.09, 136.47, 171.04, 172.07. Elemental Anal. Calcd for $C_{24}H_{25}N_3O_3$: C, 71.44; H, 6.25; N, 10.41. Found: C, 71.26; H, 6.10; N, 10.62.

4.1.6.9. (*S*)-2-((*S*)-3,3-Dimethyl-1-oxo-10,10a-dihydroimidazo[1,5-*b*]isoquinolin-2(1*H*,3*H*,5*H*)-yl)succinic acid (5i). Yield: 85%, colorless powder, mp 126–128 °C. $[\alpha]_D^{20}$ = −120.36 (*c* 0.25, CH_3OH); ESI/MS: 333 $[M+H]^+$; IR (KBr disk): 2984, 1733, 1683, 1558, 1404, 1206, 751 cm^{-1}. ^1H NMR (500 MHz, DMSO) δ_H = 7.17 (m, 4H), 4.42 (t, *J* = 5.0 Hz, 1H),3.96 (d, *J* = 15.0 Hz, 1H), 3.74 (d, *J* = 15.0 Hz, 1H), 3.32 (dd, *J* = 5.0 Hz, *J* = 15.0 Hz, 1H), 3.26 (dd, *J* = 10.0 Hz, *J* = 15.0 Hz, 1H), 2.94 (dd, *J* = 5.0 Hz, *J* = 15.0 Hz, 1H), 2.81 (dd, *J* = 5.0 Hz, *J* = 15.0 Hz, 1H), 2.63 (dd, *J* = 10.0 Hz, *J* = 15.0 Hz, 1H), 1.44 (s, 3H), 1.25 (s,3H); ^{13}C NMR (125 MHz, DMSO) δ_C = 19.25, 24.88, 30.57, 34.75, 46.45, 53.45, 56.75, 78.85, 126.28, 126.74, 127.21, 129.88, 133.48, 135.02, 170.22, 171.33, 171.47. Elemental Anal. Calcd for $C_{17}H_{20}N_2O_5$: C, 61.44; H, 6.07; N, 8.43. Found: C, 61.66; H, 6.22; N, 8.65.

4.1.6.10. (*S*)-2-((*S*)-3,3-Dimethyl-1-oxo-10,10a-dihydroimidazo[1,5-*b*]isoquinolin-2(1*H*,3*H*,5*H*)-yl)pentanedioic acid (5j). Yield: 87%, colorless powder, mp 172–175 °C. $[\alpha]_D^{20}$ = −74.20 (*c* 0.25, CH_3OH); ESI/MS: 347 $[M+H]^+$; IR (KBr disk): 2998, 1684, 1635, 1576, 1457, 750 cm^{-1}. ^1H NMR (500 MHz, DMSO) δ_H = 11.58 (s, 1H), 11.43 (s, 1H), 7.22 (t, *J* = 7.8 Hz, 1H), 7.20 (d, *J* = 7.8 Hz, 1H), 7.19 (d, *J* = 7.8 Hz, 1H), 7.15 (t, *J* = 7.8 Hz, 1H), 4.25 (m, 1H), 3.94 (d, *J* = 15.0 Hz, 1H), 3.72 (d, *J* = 15.0 Hz, 1H), 3.36 (dd, *J* = 5.0 Hz, *J* = 15.0 Hz, 1H), 3.28 (dd, *J* = 10.0 Hz, *J* = 15.0 Hz, 1H), 2.94 (dd, *J* = 5.0 Hz, *J* = 15.0 Hz, 1H), 2.83 (dd, *J* = 5.0 Hz, *J* = 15.0 Hz, 1H), 2.67 (dd, *J* = 10.0 Hz, *J* = 15.0 Hz, 1H), 2.33 (m, 1H), 2.22 (m, 1H), 1.37 (s, 3H), 1.28 (s, 3H); ^{13}C NMR (125 MHz, DMSO) δ_C = 24.21, 24.44, 24.78, 28.58, 29.36, 39.47, 55.52, 59.80, 78.50, 125.97, 126.42, 126.61, 127.55, 131.38, 131.87, 168.63, 170.93, 171.59, 172.33. Elemental Anal. Calcd for $C_{18}H_{22}N_2O_5$: C, 62.42; H, 6.40; N, 8.09. Found: C, 62.60; H, 6.56; N, 8.33.

4.1.6.11. (*S*)-6-Amino-2-((*S*)-3,3-dimethyl-1-oxo-10,10a-dihydroimidazo[1,5-*b*]-isoquinolin-2(1*H*,3*H*,5*H*)-yl)hexanoic acid (5k). Yield: 87%, colorless powder, mp 141–143 °C. $[\alpha]_D^{20}$ = – = −107.07 (*c* 0.25, CH_3OH); ESI/MS: 346 $[M+H]^+$; IR (KBr disk): 3648, 3326, 2930, 2774, 1735, 1627, 1596, 1558, 1423, 1205, 750, 574 cm^{-1}. ^1H NMR (500 MHz, DMSO) δ_H = 11.84 (s, 1H), 8.31 (t, *J* = 9.0 Hz, 2H), 7.16 (d, *J* = 7.8 Hz, 1H), 7.14 (d, *J* = 7.8 Hz, 1H), 7.12 (t, *J* = 7.8 Hz, 1H), 7.09 (t, *J* = 7.8 Hz, 1H), 4.12 (t, *J* = 6.0 Hz, 1H), 3.96 (d, *J* = 14.0 Hz, 1H), 3.74 (d, *J* = 14.5 Hz, 1H), 3.29 (dd, *J* = 10.0 Hz, *J* = 15.0 Hz, 1H), 3.22 (m, *J* = 6.0 Hz, 2H), 2.95 (dd, *J* = 5.0 Hz, *J* = 15.0 Hz, 1H), 2.68 (dd, *J* = 10.0 Hz, *J* = 15.0 Hz, 1H), 1.86 (m, *J* = 6.0 Hz, 2H), 1.65 (m, *J* = 6.0 Hz, 2H), 1.391 (s, 3H), 1.378 (s, 3H), 1.221 (m, *J* = 6.0 Hz, 2H); ^{13}C NMR (125 MHz, DMSO) δ_C = 21.28, 27.24, 27.18, 28.58, 29.25, 29.80, 41.97, 53.44, 55.11, 59.27, 69.58, 126.34, 127.38, 128.59, 133.45, 133.80, 136.52, 170.22, 172.57. Elemental Anal. Calcd for $C_{19}H_{27}N_3O_3$: C, 66.06; H, 7.88; N, 12.16. Found: C, 65.87; H, 7.73; N, 12.37.

4.1.6.12. (2*S*)-2-((*S*)-3,3-Dimethyl-1-oxo-10,10a-dihydroimidazo[1,5-*b*]isoquinolin-2(1*H*,3*H*,5*H*)-yl)-3-hydroxybutanoic acid (5l). Yield: 85%, colorless powder, mp 196–198 °C. $[\alpha]_D^{20}$ = −29.20 (*c* 0.25, CH_3OH); ESI/MS: 319 $[M+H]^+$; IR (KBr disk): 3328, 2928, 2850, 1671, 1626, 1575, 1401, 1244, 732, 599 cm^{-1}. ^1H NMR (500 MHz, DMSO) δ_H = 11.37 (s, 1H), 7.21 (d, *J* = 7.8 Hz, 1H), 7.19 (d, *J* = 7.8 Hz, 1H), 7.17 (t, *J* = 7.8 Hz, 1H), 7.13 (t, *J* = 7.8 Hz, 1H), 4.28 (d, *J* = 7.0 Hz, 1H), 3.96 (d, *J* = 15.0 Hz, 1H), 3.73 (d, *J* = 15.0 Hz, 1H), 3.42 (m, 1H), 3.22 (dd, *J* = 4.0 Hz, *J* = 10.5 Hz, 1H), 2.96 (dd, *J* = 5.0 Hz, *J* = 15.5 Hz, 1H), 2.69 (dd, *J* = 11.0 Hz, *J* = 15.0 Hz, 1H), 1.92 (s, 1H), 1.41 (s, 3H), 1.28 (s, 3H), 1.23 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, DMSO) δ_C = 16.78, 17.66, 25.48, 30.87, 33.85, 46.79, 49.11, 56.78, 78.62, 126.37, 126.68, 127.32, 129.98, 133.82, 135.26, 170.82, 171.57. Elemental Anal. Calcd for $C_{17}H_{22}N_2O_4$: C, 64.13; H, 6.97; N, 8.80. Found: C, 64.32; H, 6.81; N, 8.58.

4.1.6.13. (*S*)-4-Amino-2-((*S*)-3,3-dimethyl-1-oxo-10,10a-dihydroimidazo[1,5-*b*]-isoquinolin-2(1*H*,3*H*,5*H*)-yl)-4-oxobutanoic acid (5m). Yield: 88%, colorless powder, mp 192–194 °C. $[\alpha]_D^{20}$ = −169.10 (*c* 0.25, CH_3OH); ESI/MS: 332 $[M+H]^+$; IR (KBr disk): 2996, 1688, 1609, 1558, 1456, 1141, 750, 622 cm^{-1}. ^1H NMR (500 MHz, DMSO) δ_H = 11.25 (s, 1H), 7.17 (d, *J* = 7.5 Hz, 1H), 7.15 (d, *J* = 7.5 Hz, 1H), 7.13 (t, *J* = 7.5 Hz, 1H), 7.12 (t, *J* = 7.5 Hz, 1H), 6.03 (s, 2H), 4.43 (t, *J* = 7.0 Hz, 1H), 3.97 (d, *J* = 14.5 Hz, 1H), 3.74 (d, *J* = 14.0 Hz, 1H), 3.35 (m, *J* = 7.0 Hz, 1H), 3.28 (dd, *J* = 8.0 Hz, *J* = 11.0 Hz, 1H), 2.94 (dd, *J* = 3.5 Hz, *J* = 15.5 Hz, 1H), 2.81 (dd, *J* = 5.5 Hz, *J* = 15.5 Hz, 1H), 2.65 (dd, *J* = 11.5 Hz, *J* = 15.5 Hz, 1H), 1.46 (s, 3H), 1.27 (s, 3H); ^{13}C NMR (125 MHz, DMSO) δ_C = 24.97, 25.14, 31.21, 34.22, 49.84, 52.57, 58.98, 78.75, 126.59, 127.13, 127.33, 127.41, 134.11, 135.74, 172.54, 172.86, 173.76. Elemental Anal. Calcd for $C_{17}H_{21}N_3O_4$: C, 61.62; H, 6.39; N, 12.68. Found: C, 61.41; H, 6.22; N, 12.44.

4.1.6.14. (*S*)-5-Amino-2-((*S*)-3,3-dimethyl-1-oxo-10,10a-dihydroimidazo[1,5-*b*]-isoquinolin-2(1*H*,3*H*,5*H*)-yl)-5-oxopentanoic acid (5n). Yield: 89%, colorless powder, mp 156–158 °C. $[\alpha]_D^{20}$ = −24.25 (*c* 0.25, CH_3OH); ESI/MS: 346 $[M+H]^+$; IR (KBr disk): 2984, 1733, 1684, 1635, 1576, 1418, 1206, 746 cm^{-1}. ^1H NMR (500 MHz, DMSO) δ_H = 11.30 (s, 1H), 7.17 (d, *J* = 7.8 Hz, 1H), 7.15 (d, *J* = 7.8 Hz, 1H), 7.13 (t, *J* = 7.8 Hz, 1H), 7.10 (t, *J* = 7.8 Hz, 1H), 6.03 (s, 2H), 4.17 (t, *J* = 7.0 Hz, 1H), 3.96 (d, *J* = 14.5 Hz, 1H), 3.74 (d, *J* = 14.0 Hz, 1H), 3.64 (s, 2H), 3.37 (dd, *J* = 4.0 Hz, *J* = 11.0 Hz, 1H), 2.92 (dd, *J* = 3.5 Hz, *J* = 15.5 Hz, 1H), 2.65 (dd, *J* = 11.0 Hz, *J* = 15.5 Hz, 1H), 2.48 (m, *J* = 7.0 Hz, 1H), 2.38 (m, *J* = 7.0 Hz, 1H), 1.38 (s, 3H), 1.30 (s, 3H); ^{13}C NMR (125 MHz, DMSO) δ (ppm) = 24.14, 24.93, 25.71, 30.79, 33.82, 47.82, 56.43, 56.87, 78.48, 124.77, 126.29, 126.66, 127.22, 133.83, 135.18, 163.69, 171.14,

171.47, 172.88. Elemental Anal. Calcd for $C_{18}H_{23}N_3O_4$: C, 62.59; H, 6.71; N, 12.17. Found: C, 62.40; H, 6.55; N, 12.40.

4.1.6.15. (*S*)-2-((*S*)-3,3-Dimethyl-1-oxo-10,10a-dihydroimidazo-[1,5-*b*]isoquinolin-2(1*H*,3*H*,5*H*)-yl)-5-guanidinopentanoic acid (5o). Yield: 88%, colorless powder, mp 181–183 °C. $[\alpha]_D^{20}$ = −34.98 (*c* 0.25, CH_3OH); ESI/MS: 374 $[M+H]^+$; IR (KBr disk): 3358, 2933, 1683, 1614, 1516, 1449, 1373, 1338, 1246, 746 cm^{-1}. ^1H NMR (500 MHz, DMSO) δ_H = 11.51 (s, 1H), 9.13 (s, 2H), 7.80 (s, 1H), 7.17 (d, *J* = 7.8 Hz, 1H), 7.15 (d, *J* = 7.8 Hz, 1H), 7.13 (t, *J* = 7.8 Hz, 1H), 7.11 (t, *J* = 7.8 Hz, 1H), 4.24 (dd, *J* = 4.0 Hz, *J* = 10.5 Hz, 1H), 3.81 (d, *J* = 14.0 Hz, 1H), 3.53 (d, *J* = 14.5 Hz, 1H), 3.44 (dd, *J* = 10.0 Hz, *J* = 15.0 Hz, 1H), 3.21 (dd, *J* = 5.0 Hz, *J* = 14.0 Hz, 1H), 3.15 (dd, *J* = 5.0 Hz, *J* = 10.0 Hz, 1H), 2.95 (dd, *J* = 4.0 Hz, *J* = 15.0 Hz, 1H), 2.68 (dd, *J* = 11.0 Hz, *J* = 15.0 Hz, 2H), 1.99 (d, *J* = 4.0 Hz, 2H), 1.56 (m, *J* = 4.0 Hz, 2H), 1.27 (s, 3H), 0.82 (s, 3H); ^{13}C NMR (125 MHz, DMSO) δ_C = 17.94, 24.25, 30.94, 33.85, 46.77, 56.58, 56.99, 78.77, 126.23, 127.12, 128.45, 131.22, 133.79, 135.11, 156.45, 170.98, 171.41. Elemental Anal. Calcd for $C_{19}H_{27}N_5O_3$: C, 61.11; H, 7.29; N, 18.75. Found: C, 61.32; H, 7.45; N, 18.53.

4.1.6.16. (*S*)-2-((*S*)-3,3-Dimethyl-1-oxo-10,10a-dihydroimidazo [1,5-*b*]isoquinolin-2(1*H*,3*H*,5*H*)-yl)-4-(methylthio)butanoic acid (5p). Yield: 90%, colorless powder, mp 182–184 °C. $[\alpha]_D^{20}$ = −61.74 (*c* 0.25, CH_3OH); ESI/MS: 349 $[M++H]^+$; IR (KBr disk): 2998, 2916, 2580, 1723, 1688, 1654, 1437, 1368, 1268, 1245, 1191, 746 cm^{-1}. ^1H NMR (500 MHz, DMSO) δ_H = 11.61 (s, 1H), 7.17 (d, *J* = 7.8 Hz, 1H), 7.15 (d, *J* = 7.8 Hz, 1H), 7.12 (t, *J* = 7.8 Hz, 1H), 7.10 (t, *J* = 7.8 Hz, 1H), 4.21 (t, *J* = 6.5 Hz, 1H), 3.97 (d, *J* = 14.0 Hz, 1H), 3.74 (d, *J* = 14.5 Hz, 1H), 3.36 (dd, *J* = 4.0 Hz, *J* = 11.0 Hz, 1H), 2.92 (dd, *J* = 3.5 Hz, *J* = 15.5 Hz, 1H), 2.66 (dd, *J* = 11.0 Hz, *J* = 15.5 Hz, 1H), 2.54 (d, *J* = 6.5 Hz, 2H), 2.30 (t, *J* = 6.5 Hz, 2H), 2.10 (s, 3H), 1.38 (s, 3H), 1.31 (s, 3H); ^{13}C NMR (125 MHz, DMSO) δ_C = 14.91, 19.47, 24.74, 29.22, 30.87, 46.82, 52.89, 56.35, 78.69, 126.34, 126.572, 127.24, 129.98, 133.82, 135.24, 171.21, 171.35. Elemental Anal. Calcd for $C_{18}H_{24}N_2O_3S$: C, 62.04; H, 6.94; N, 8.04. Found: C, 61.85; H, 6.78; N, 8.27.

4.1.6.17. (*R*)-2-((*S*)-3,3-Dimethyl-1-oxo-10,10a-dihydroimidazo [1,5-*b*]isoquinolin-2(1*H*,3*H*,5*H*)-yl)-3-mercaptopropanoic acid (5q). Yield: 90%, colorless powder, mp 152–154 °C. $[\alpha]_D^{20}$ = −21.35 (*c* 0.25, CH_3OH); ESI/MS: 321 $[M+H]^+$; IR (KBr disk): 2986, 2503, 1713, 1676, 1417, 1289, 1211, 1101, 746 cm^{-1}. ^1H NMR (500 MHz, DMSO) δ_H = 11.28 (s, 1H), 7.17 (d, *J* = 7.7 Hz, 1H), 7.15 (d, *J* = 7.7 Hz, 1H), 7.13 (t, *J* = 7.7 Hz, 1H), 7.11 (t, *J* = 7.7 Hz, 1H), 4.39 (t, *J* = 7.0 Hz, 1H), 3.97 (d, *J* = 14.0 Hz, 1H), 3.77 (d, *J* = 13.5 Hz, 1H), 3.55 (dd, *J* = 6.5 Hz, *J* = 13.5 Hz, 1H), 3.34 (d, *J* = 7.0 Hz, 2H), 2.93 (dd, *J* = 4.0 Hz, *J* = 15.5 Hz, 1H), 2.66 (dd, *J* = 12.5 Hz, *J* = 15.5 Hz, 1H), 2.54 (s, 1H), 1.44 (s, 3H), 1.31 (s, 3H); ^{13}C NMR (125 MHz, DMSO) δ_C = 14.85, 19.43, 24.86, 29.24, 30.83, 46.85, 52.68, 52.82, 56.41, 78.72, 126.38, 126.52, 127.28, 129.82, 133.81, 135.24, 171.26, 171.41. Elemental Anal. Calcd for $C_{16}H_{20}N_2O_3S$: C, 59.98; H, 6.29; N, 8.74. Found: C, 59.77; H, 6.14; N, 8.51.

4.2. Bioassays

4.2.1. Vasodilation assay

A constant temperature trough (CS501, Chongqing YinHe experimental apparatus Ltd of China) was used to insure the buffer warmed; a tension transducer (Hang JZ101, Beidian Xinghang machine and equipment Ltd) and a two-channel physiological recorder (LMS-2B, Chengdu apparatus manufacturer) were used to evaluate the vasodilative effect. The Male Wistar rats weighing 250–300 g (purchased from Animal Center of Capital Medical University) were used. Immediately after decapitation, rat aortic strips were stripped and put in a perfusion bath with 15 mL warmed (37 °C), oxygenated (95%O_2/5%CO_2) Kreb's solution (pH 7.4). The aortic strip was connected to a tension transducer, and the relaxation contraction curve of muscles was registered. Administration of 59 μM NE induced hypertonic contraction of the vessel strip. As the contraction reaches its maximum, NE was washed out, and the vessel strip was stabilized for 30 min. After renewal of the solution, 59 μM NE was added. When the hypertonic contraction value of the aortic strip reached the peak, 15 μL ethanol (negative control) or 15 μL solution of **5a–q** in ethanol was administrated to observe their vasodilation.

4.2.2. 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 (six 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 **5a–q** (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–q** 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 **5a–q** (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 (**5a–q**) 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–q**)]/(A_m of NS) × 100%. A_m% of NS is the value of platelet aggregation induced by PAF, ADP, AA and TH without **5a–q** and are 52.30 ± 1.78%, 50.16 ± 3.65%, 49.62 ± 2.90% and 61.20 ± 2.97%, respectively. The concentration versus inhibition rate curve is plotted to determine the IC_{50} values with GWBASIC.EXE program.

4.2.3. In vivo anti-thrombotic assay of intravenously injection of 5a–q in rat model

The assessments described here were performed based on a protocol reviewed and approved by the ethics committee of Capital Medical University. The committee assures the welfare of the animals was maintained in accordance to the requirements of the animal welfare act and according to the guide for care and use of laboratory animals. Aspirin and **5a–q** 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 anesthetized with pentobarbital sodium (80.0 mg/kg, ip) and the right carotid artery and left jugular vein were separated. A weighed 6 cm thread was inserted into the middle of a polyethylene tube. The polyethylene tube was filled with

heparin sodium (50 IU/mL in NS) and one end was inserted into the left jugular vein. From the other end of the polyethylene tube, heparin sodium was injected as anticoagulant, then NS or **5a–q** was injected, and this end was inserted into the right carotid artery. Blood was allowed to flow from the right carotid artery to the left jugular vein, through the polyethylene tube for 15 min. The thread was removed to obtain the weight of the wet thrombus.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.12.005.

References and notes

1. Chaireti, R.; Jennersjo, C.; Lindahl, T. L. *Thromb. Res.* **2009**, *124*, 178.
2. Lopez, J. A.; Chen, J. *Thromb. Res.* **2009**, *123*, S30.
3. Arias-Salgado, E. G.; Larrucea, S.; Butta, N.; Fernandez, D.; Garcia-Munoz, S.; Parrilla, R.; Ayuso, M. S. *Thromb. Res.* **2008**, *122*, 640.
4. Surin, W. R.; Barthwal, M. K.; Dikshit, M. *Thromb. Res.* **2008**, *122*, 786.
5. Gibbins, J. M. *Atherosclerosis* **2009**, *206*, 1.
6. Skinner, M. P. *Heart Lung Circ.* **2007**, *16*, 176.
7. Lane, J. W.; Estevez, A.; Mortara, K.; Callan, O.; Spencer, J. R.; Williams, R. M. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3180.
8. Gonzalez, J. F.; de la Cuesta, E.; Avendano, C. *Bioorg. Med. Chem.* **2007**, *15*, 112.
9. Klopfenstein, S. R.; Evdokimov, A. G.; Colson, A. O.; Fairweather, N. T.; Neuman, J. J.; Maier, M. B.; Gray, J. L.; Gerwe, G. S.; Stake, G. E.; Howard, B. W.; Farmer, J. A.; Pokross, M. E.; Downs, T. R.; Kasibhatla, B.; Peters, K. G. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1574.
10. Li, J. J.; Wang, H.; Qu, F.; Musial, C.; Tino, J. A.; Robl, J. A.; Slusarchyk, D.; Golla, R.; Seethala, R.; Dickinson, K.; Giupponi, L.; Grover, G.; Sleph, P.; Flynn, N.; Murphy, B. J.; Gordon, D.; Kung, M.; Stoffel, R. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1799.
11. Xiao, A.; Zhang, Z.; An, L.; Xiang, Y. *J. Mol. Model.* **2008**, *14*, 149.
12. Okuda, K.; Kotake, Y.; Ohta, S. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2853.
13. Pyo, M. K.; Lee, D. H.; Kim, D. H.; Lee, J. H.; Moon, J. C.; Chang, K. C.; Yun-Choi, H. S. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4110.
14. Leoncini, G.; Signorello, M. G.; Grossi, G. C.; Di Braccio, M. *Thromb. Res.* **1997**, *87*, 483.
15. Shams, G.; Romstedt, K. J.; Lust, L. A.; Clark, M. T.; Miller, D. D.; Feller, D. R. *Gen. Pharmacol.* **1997**, *28*, 323.
16. Pyo, M. K.; Kim, J. M.; Jin, J. L.; Chang, K. C.; Lee, D. H.; Yun-Choi, H. S. *Thromb. Res.* **2007**, *120*, 81.
17. Yun-Choi, H. S.; Pyo, M. K.; Park, K. M.; Chang, K. C.; Lee, D. H. *Thromb. Res.* **2001**, *104*, 249.
18. Yang, J.; Hua, W. Y.; Wang, F. X.; Wang, Z. Y.; Wang, X. *Bioorg. Med. Chem.* **2004**, *12*, 6547.
19. Saha, S. L.; Roche, V. F.; Pendola, K.; Kearley, M.; Lei, L.; Romstedt, K. J.; Herdman, M.; Shams, G.; Kaisare, V.; Feller, D. R. *Bioorg. Med. Chem.* **2002**, *10*, 2779.
20. Malovichko, O. L.; Petrus, A. S.; Krysko, A. A.; Kabanova, T. A.; Andronati, S. A.; Karaseva, T. L.; Kiriyak, A. V. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5294.
21. Zhang, X.; Wang, W.; Cheng, S.; Zhao, M.; Zheng, M.; Chang, H. W.; Wu, J.; Peng, S. *Bioorg. Med. Chem.* **2010**, *18*, 1536.
22. Cheng, S.; Zhang, X.; Wang, W.; Zhao, M.; Zheng, M.; Chang, H. W.; Wu, J.; Peng, S. *Eur. J. Med. Chem.* **2009**, *44*, 4904.
23. Zheng, M.; Zhang, X.; Zhao, M.; Chang, H. W.; Wang, W.; Wang, Y.; Peng, S. *Bioorg. Med. Chem.* **2008**, *16*, 9574.
24. Liu, J.; Zhao, M.; Cui, G.; Zhang, X.; Wang, J.; Peng, S. *J. Med. Chem.* **2008**, *51*, 4715.