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Pyrolo[1,2:4,5]-1,4-dioxopyrazino[1,2:1,6]pyrido[3,4-*b*]indoles: A Group of Urokinase Inhibitors, their Synthesis, and Stereochemistry-Dependent Activity

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Antifibrinolytic agents are required during complex surgeries to decrease bleeding; their pro-thrombotic potency and efficacy in causing hemostasis has attracted much attention. To discover new inhibitors of urokinase with high selectivity for antifibrinolytic effects over pro-thrombotic effects, the 12-position of (5a*S*,12*S*,14a*S*)- and (5a*S*,12*R*,14a*S*)-5,14-dioxo-1,2,3,5,5a,6,11,12,14,14a-decahydro-5*H*,14*H*-pyrolo[1,2:4,5]pyrazino[1,2:1,6]pyrido[3,4-*b*]indoles were modified with L-Ala, L-Asp, L-Phe, L-Trp, L-Lys, L-Ser, Gly, and L-Leu to provide 16 (5a*S*,12*S*,14a*S*) and (5a*S*,12*R*,14a*S*) derivatives. In a murine bleeding model, the (5a*S*,12*S*,14a*S*) derivatives containing L-Ala, L-Asp, L-Phe, and L-Trp induced blood coagulation for the treated mice; they also stimulated thrombus formation in a rat thrombosis model, but the other derivatives inhibited thrombosis. The most potent compound, the L-Asp derivative, showed a good therapeutic window: the minimum effective dose for coagulation was <1 nmol kg^{-1}, whereas at 10 nmol kg^{-1}, no pro-thrombotic effect was observed. This type of coagulation action was correlated with a mechanism of urokinase inhibition, and these results could lead to the discovery of novel urokinase inhibitors.

Introduction

Antifibrinolytic agents have been widely and successfully used to decrease blood loss caused by injuries as well as complex surgeries.[1–3] Antifibrinolytic agents in current clinical use include polypeptides such as aprotinin (Trasylol, Bayer)[4,5] and amino acids such as tranexamic acid and ε-aminocaproic acid (EACA, Figure 1).[6–8] These agents have different mechanisms of action and cannot be substituted with each other.[6,9] Due to the close relationship between the coagulation and fibrinolysis pathways, it is reasonable that antifibrinolytic agents were generally thought to prompt thrombus formation.[9,10] Aprotinin, an inhibitor of both kallikrein and plasmin, has been proven to inhibit thrombus formation in vivo, while tranexamic acid, the competitive inhibitor of plasminogen activation, dose-dependently increases thrombus formation under the same conditions.[11] Although small molecules like tranexamic acid and EACA have elicited effects similar to those of aprotinin in some clinical studies, the potential increased risk of thrombus formation should be highly concerning.[12–14] Nonetheless, the significant advantages of small molecules, such as low cost and oral bioavailability, have attracted the same amount of interest in their use as is the case for larger polypeptide agents.[15–17]

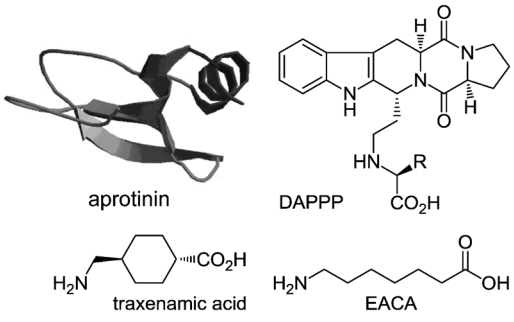

Figure 1. Structures of the polypeptide aprotinin (from PDB ID: 3LDJ), the oligopeptide analogue series (5a*S*,12*S*,14a*S*)-5,14-dioxo-12-(2-[amino acid]-*N*-ylethyl-1-yl)-1,2,3,5,5a,6,11,12,14,14a-decahydro-5*H*,14*H*-pyrolo-[1,2:4,5]pyrazino[1,2:1,6]pyrido[3,4-*b*]indoles (DAPPP), and amino acids tranexamic acid and ε-aminocaproic acid (EACA).

We recently reported a novel antifibrinolytic small molecule CIPPC (**5d**, the 12*S* isomer discussed below), which can be considered an oligopeptide analogue.[18] It was discovered from a combined study of molecular modeling and compound library screening. CIPPC was shown to decrease rat tail bleeding times in vivo (minimal effective dose: 1 nmol kg^{-1}) and to inhibit the action of urokinase plasminogen activator (u-PA; also known as urokinase, or UK) in vitro. However, it also increased thrombus formation in vivo (minimal effective dose: 10 nmol kg^{-1}) in a dose-dependent manner. Cardiovascular events such as deep-vein thrombosis, myocardial infarction, pulmonary embolism, and stroke are serious health hazards, while intravascular thrombosis is known to be among the most prominent causes of morbidity and mortality.[19] There-

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fore, the pro-coagulant agents that lack pro-thrombotic activity are needed.

To increase the antifibrinolytic activity and to minimize the adverse effects of CIPPC, we designed a series of CIPPC analogues (DAPPP group, Figure 1) by changing the configuration at C12 and by incorporating various functional groups on an amino acid side chain. In the current compound series, nonpolar (methyl and 2-methylpropyl) and polar (hydroxymethyl) groups, acidic (carboxymethyl) and basic (4-aminobutyl-1-yl) groups, and an aromatic (benzyl) group were used as substitutes of the original indole-3-yl-methyl group on CIPPC. By changing the configuration at C12, two sets of diastereomers, C12*S* analogues (compounds **5a**–**h**) and C12*R* analogues (compounds **5′a**–**h**) were generated. The eight pairs of diastereomers (including CIPPC and its C12 diastereomer) were tested on a time-resolved tail-bleeding model in mice and an arteriovenous shunt thrombosis model in rats to identify their antifibrinolytic efficacy and thrombotic effects. Further observations with a thrombus clot lysis assay and a thrombolytic assay showed that compound **5c** inhibits u-PA-mediated thrombolysis in vitro and in vivo.

Results and Discussion

Chemistry

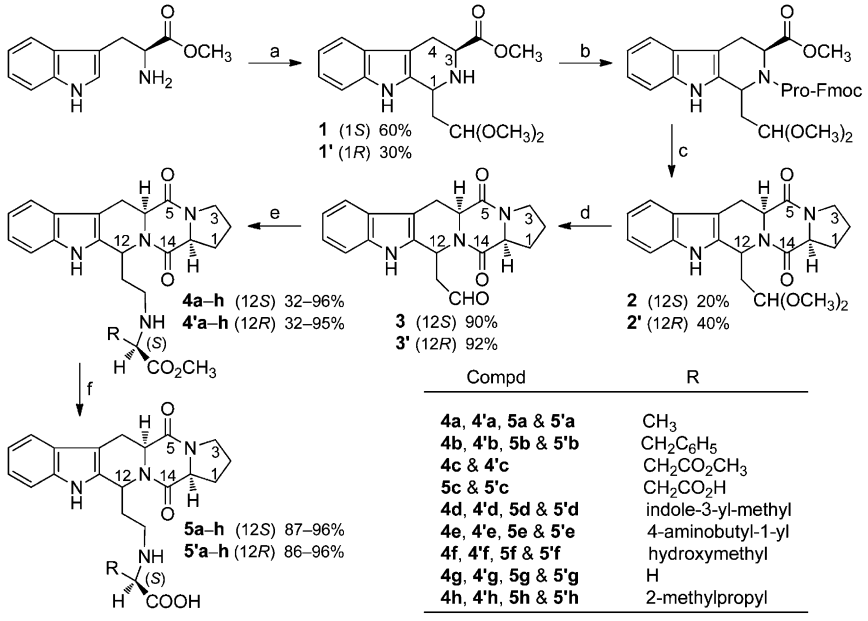
A six-step reaction sequence illustrated in Scheme 1 was used to synthesize the (5a*S*,12*S*,14a*S*) isomers **5a**–**h** and the (5a*S*,12*R*,14a*S*) isomers **5′a**–**h** of DAPPP. Pictet–Spengler condensation of L-Trp-OCH_3 and 1,1,3,3-tetramethoxypropane provided the (1*S*,3*S*) isomer (**1**, 60% yield) and (1*R*,3*S*) isomer (**1′**, 30% yield) of 1-(2,2-dimethoxyethyl)-β-carboline-3-carboxylic acid methyl ester. The configuration of the C1 atom of **1** and **1′** was assigned by nuclear Overhauser effect (NOE) spectra. In these tests compound **1** exhibited a positive NOE signal between 1-OCH_3 and 3-OCH_3, whereas **1′** exhibited positive NOE signals between 1-H and 3-OCH_3 as well as 3-H and 1-OCH_3. Therefore, the C1 atoms of **1** and **1′** were assigned the *S* and *R* configurations, respectively. In the presence of thionyl chloride, **1** was treated successively with Fmoc-L-Pro and diisopropylamine to effect an in situ cyclization and to provide cyclodipeptide analogue **2** (20% yield). Similarly, **1′** was converted into **2′** (40% yield). The configuration at C1 of **2** and **2′** was confirmed by NOE experiments; compound **2** exhibited no positive NOE signal between the methoxy group and 5a-H, whereas **2′** exhibited a positive NOE signal between the 5a-H and the methoxy group. Therefore, the C1 configurations for **2** and **2′** are respectively *S* and *R*.

para-Toluenesulfonic acid mediated hydrolysis of **2** provided aldehyde **3** in 90% yield. Reductive alkylation of amino acid methyl ester and **3** provided **4a**–**h** (32–96% yield). The hydrolysis of **4a**–**h** provided (5a*S*,12*S*,14a*S*)-DAPPP (**5a**–**h**, 87–96% yield). Similarly, *para*-toluenesulfonic acid promoted hydrolysis of **2′** provided the (5a*S*,12*R*,14a*S*) isomer of **3** (**3′**, 92% yield); reductive alkylation of amino acid methyl ester and **3′** provided **4′a**–**h** (32–95% yield), and the hydrolysis of **4′a**–**h** provided (5a*S*,12*R*,14a*S*)-DAPPP (**5′a**–**h**, 86–96% yield).

Biological evaluation of DAPPP derivatives

In vivo evaluation of coagulation in a tail bleeding assay

The in vivo coagulation activities of **5a**–**h** and **5′a**–**h** were determined by monitoring the tail bleeding times of compound-treated mice. In this assay, ICR mice were fed normal saline (NS, negative control), aspirin (165 μmol kg^{-1}, positive control), or each of **5a**–**h** and **5′a**–**h** (0.1 μmol kg^{-1}). Thirty minutes after administration, mice were placed in a tube holder with tails protruding, and a 2-mm cut was made on the tail. Flowing blood was gently wiped away with a tissue every 30 s until bleeding ceased, and the observed bleeding time was recorded (Table 1). The tail bleeding times of mice receiving 0.1 μmol kg^{-1} of each of **5a**–**d** are significantly shorter than those of the mice receiving NS, suggesting that **5a**–**d** can induce blood coagulation. On the other hand, the tail bleeding times of mice receiving 0.1 μmol kg^{-1} of each of **5e**–**h** and **5′a**–**h** are equal to those of the mice receiving NS, suggest-



Compd	R
4a, **4'a**, **5a** & **5'a**	CH_3
4b, **4'b**, **5b** & **5'b**	$CH_2C_6H_5$
4c & **4'c**	$CH_2CO_2CH_3$
5c & **5'c**	CH_2CO_2H
4d, **4'd**, **5d** & **5'd**	indole-3-yl-methyl
4e, **4'e**, **5e** & **5'e**	4-aminobutyl-1-yl
4f, **4'f**, **5f** & **5'f**	hydroxymethyl
4g, **4'g**, **5g** & **5'g**	H
4h, **4'h**, **5h** & **5'h**	2-methylpropyl

Scheme 1. Synthesis of (5a*S*,12*S*,14a*S*)-DAPPP (**5a**–**h**) and (5a*S*,12*R*,14a*S*)-DAPPP (**5′a**–**h**): a) 1,1,3,3-tetramethoxypropane, HCl (5 M), CH_3OH, 45 °C, 48 h; b) Fmoc-L-Pro-OH, $SOCl_2$, reflux, 5 h, Et_2O, then CH_2Cl_2, **1**, 0 °C, 0.5 h; c) $(iPr)_2NH$, RT, 24 h; d) acetone, *p*-TsOH, 45 °C, 1 h; e) Et_3N, AA-OCH_3, Na_2SO_4, RT, 3 h, then KBH_4, CH_3OH, 1 h; f) NaOH (2 M), RT, 2 h.

Table 1. In vivo evaluation of coagulation effects of **5a–h** and **5′a–h** in mice using a tail bleeding assay.[a]

Compd	Time [s]	Compd	Time [s]
NS	280.9 ± 25.5	Aspirin	313.1 ± 29.7[c]
5a	179.7 ± 29.5[b]	**5′a**	276.7 ± 17.6
5b	171.9 ± 28.2[b]	**5′b**	269.1 ± 30.1
5c	154.9 ± 28.9[b]	**5′c**	269.9 ± 30.8
5d	182.7 ± 29.9[b]	**5′d**	274.0 ± 30.3
5e	277.8 ± 27.9	**5′e**	275.8 ± 27.6
5f	266.8 ± 29.0	**5′f**	272.3 ± 30.6
5g	267.3 ± 30.1	**5g**	274.7 ± 27.3
5h	269.0 ± 28.5	**5h**	277.1 ± 25.9

[a] Data represent the mean ± SD tail bleeding time ($n = 10$); NS = normal saline (vehicle); doses used: aspirin: 165 μmol kg^{-1}, **5a–h** and **5′a–h**: 0.1 μmol kg^{-1}; [b] Compared with the vehicle control (NS), $p < 0.01$; [c] Compared with the vehicle control (NS), $p < 0.05$.

ing that **5e–h** and **5′a–h** cannot induce blood coagulation. Among **5a–d** and **5′a–d**, only the former induced blood coagulation, indicating that the blood coagulation activity of DAPPPs depend on the configuration at C12. Among **5a–h**, only **5a–d** induced blood coagulation, indicating that the blood coagulation capacity of (5a*S*,12*S*,14a*S*)-DAPPP is influenced by the side chain of the amino acid moiety.

Dose-dependent blood coagulation in mice treated with 5c

To observe the dose-dependent effects of **5a–d** in the in vivo tail bleeding time assay, the most potent compound **5c** was selected as a representative, and oral doses of 100, 10, and 1 nmol kg^{-1} were used. Aspirin (165 μmol kg^{-1}) was used as positive control, NS was used as negative control, and tail bleeding times were recorded and compared with those of **5′c**, **5d**, and **5′d**. Figure 2 shows that not only the tail bleeding times of mice orally administered compound **5c** at 100, 10, and 1 nmol kg^{-1} are significantly shorter than those of the mice that received NS, but also that the tail bleeding times of mice orally administered **5c** steadily decreased with increasing dose. This means that **5c** dose-dependently inhibits bleeding in treated mice, and even at a dose of 1 nmol kg^{-1}, **5c** still elicits blood coagulation.

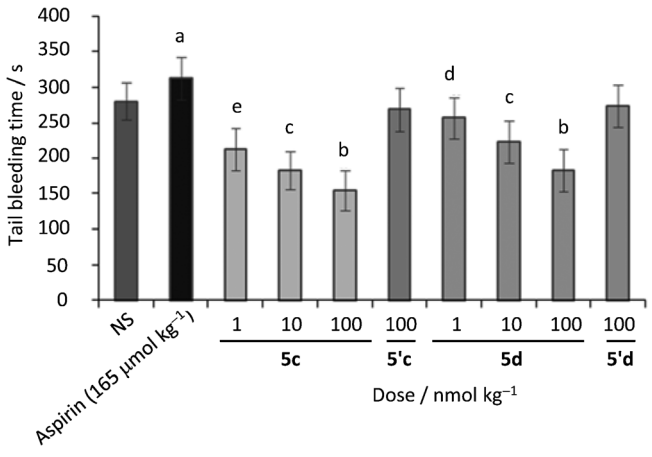


Figure 2. Tail bleeding time assay with mice orally administered **5c**, **5′c**, **5d**, **5′d**. Values represent the mean ± SD, NS = vehicle, $n = 12$. a) Compared with NS $p < 0.05$, with 100, 10, and 1 nmol kg^{-1} **5c** and **5d** $p < 0.01$; b) compared with NS and 1 nmol kg^{-1} **5c** or **5d** $p < 0.01$, with 10 nmol kg^{-1} **5c** or **5d** $p < 0.05$; c) compared with NS $p < 0.01$, with 1 nmol kg **5c** or **5d** $p < 0.05$; d) compared with NS $p < 0.05$; e) compared with NS $p < 0.01$.

In vivo pro-thrombotic or antithrombotic effects

Pro-thrombotic and antithrombotic activities were determined by monitoring the weight of the thrombus formed inside the carotid artery of treated rats. In this thrombosis assay, Wistar rats were fed NS, aspirin (165 μmol kg^{-1}) as positive control, or each of **5a–h** and **5′a–h** at 0.1 μmol kg^{-1}, and the thrombus weights were recorded. Table 2 shows that the thrombus weight of rats orally administered **5a–d** are significantly higher than those of mice that received NS. This indicates that compounds **5a–d** stimulate thrombus formation in rats. Table 2 also shows that the thrombus weight of the rats orally administered 0.1 μmol kg^{-1} of each of **5e–h** and **5′a–h** are significantly lower than those of rats that received NS. This indicates that **5e–h** and **5′a–h** inhibit thrombus formation. The fact that **5a–d** and **5′a–d** have opposing actions suggests that the configuration at C12 of DAPPP is critical for its antithrombotic and pro-thrombotic activities on treated rats. Interestingly, the most potent compound **5c** in tail bleeding time assay is not the most potent pro-thrombotic agent in the thrombosis assay; it induces a mild pro-thrombotic effect at a dose of 0.1 μmol kg^{-1}. This suggests that antifibrinolytic therapy with **5c** within a certain dose range might not induce thrombosis.

Table 2. In vivo evaluation of the pro-thrombotic and antithrombotic activities of **5a–h** and **5′a–h** in rats.[a]

Compd	Weight [mg]	Compd	Weight [mg]
NS	26.22 ± 2.03[b]	Aspirin	13.22 ± 1.67
5a	31.33 ± 2.04[c]	**5′a**	23.49 ± 2.01
5b	34.14 ± 2.03[c]	**5′b**	23.79 ± 2.08
5c	29.08 ± 1.34[c]	**5′c**	22.24 ± 2.06
5d	34.84 ± 2.84[c]	**5′d**	25.71 ± 1.12
5e	18.22 ± 1.93[c]	**5′e**	16.14 ± 1.31
5f	24.62 ± 1.83[c]	**5′f**	16.33 ± 1.42
5g	17.54 ± 1.61[c]	**5g**	15.44 ± 1.81
5h	24.20 ± 1.41[c]	**5h**	19.20 ± 1.70

[a] Data represent the mean ± SD weight of wet thrombus ($n = 12$); NS = normal saline (vehicle); doses used: aspirin: 165 μmol kg^{-1}, **5a–h** and **5′a–h**: 0.1 μmol kg^{-1}. [b] Compared with **5a–h** and **5′a–h**, $p < 0.01$; [c] Compared with the corresponding **5′** derivative, $p < 0.01$.

Dose–effect relationship of 5c for thrombogenesis

To clarify the therapeutic window of **5c**, its dose–effect relationship for pro-thrombotic activity was determined. In this in vivo thrombogenesis assay, rats were orally administered **5c** at 1 μmol kg^{-1} and 100 and 10 nmol kg^{-1}, or aspirin at 165 μmol kg^{-1} as positive control, or NS alone. The thrombus weight of the rats is shown in Figure 3. The results show that

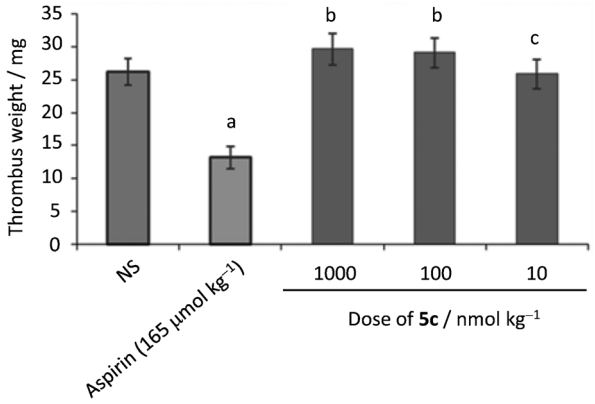


Figure 3. Thrombus weights from rats orally administered **5c** at 1000, 100, and 10 nmol kg^{-1}. Values represent the mean ±SD, $n=12$. a) Compared with NS, as well as 1000, 100, and 10 nmol kg^{-1} **5c** $p<0.01$; b) compared with NS and 10 nmol kg^{-1} **5c** $p<0.01$; c) compared with NS $p>0.05$.

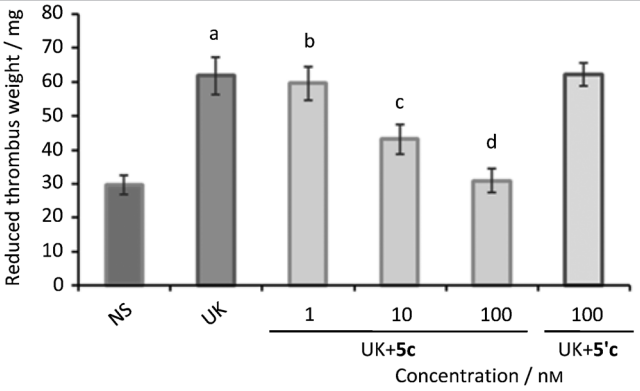


Figure 4. In vitro thrombolytic activity of UK with and without **5c**. Values represent the mean ±SD, NS = vehicle, [UK] = 100 IU mL^{-1}, $n=8$. a) Compared with NS, as well as UK plus 10 and 100 nM **5c** $p<0.01$, with UK plus 1 nM **5c** and UK plus 100 nM **5′c** $p>0.05$; b) compared with NS, as well as UK plus 100 and 10 nM **5c** $p<0.01$; c) compared with NS and UK plus 100 nM **5c** $p<0.01$; d) compared with NS $p>0.05$.

the thrombus weight of the rats given 1 μmol kg^{-1} **5c** is equal to those from rats administered **5c** at 100 nmol kg^{-1}, suggesting that doses above 100 nmol kg^{-1} have no greater effect on thrombosis activity. Thrombus weights of rats given **5c** at 10 nmol kg^{-1} are equal to those of rats given NS, suggesting that **5c** has no thrombosis activity at this dose. Therefore, **5c** is a weak thrombosis agent, and a safe dose against thrombus formation would be no greater than 10 nmol kg^{-1}.

Effect of 5c on the in vitro thrombus clot lysis activity of urokinase

To identify the antagonism of **5a–d** against urokinase (UK), the in vitro thrombus clot lysis activity of UK (final concentration: 100 IU mL^{-1}) was compared with those of UK (100 IU mL^{-1}) plus the representative **5c** (final concentration: 100, 10, or 1 nM). The decrease in thrombus weight was used to represent activity. Figure 4 shows that the decrease in thrombus weight elicited by UK alone is significantly greater than those from UK plus **5c** at 100, 10, and 1 nM. This means that **5c** antagonizes the in vitro lysis activity of UK. Figure 4 also indicates that the decreases in thrombus weights of UK plus **5c** steadily diminish with increasing concentrations of **5c**. This means that **5c** antagonizes the in vitro lysis activity of UK in a concentration-dependent manner. Figure 4 further indicates that the decreased thrombus weight effected by UK plus 100 nM **5c** is effectively equal to that of NS. This means that the in vitro lysis activity of UK can be completely antagonized by **5c** at 100 nM.

Effect of 5c on the in vivo thrombolytic activity of urokinase

To confirm the efficacy of **5a–d** as in vivo inhibitors of UK, the in vivo thrombolytic activity of UK was compared with those of UK plus the representative **5c** concentrations. The decrease in thrombus weights were used as an indicator of this activity. Figure 5 shows that the decreased thrombus weight from rats given UK at 20 000 IU kg^{-1} is significantly greater than those from rats administered the same amount of UK and pre-fed with **5c** at 100, 10, and 1 nmol kg^{-1}. This means that **5c** antagonizes UK-induced thrombolysis in vivo. Figure 5 also shows that the antagonism of **5c** steadily increases with the increase in dose. This means that **5c** dose-dependently antagonizes the in vivo thrombolytic activity of UK. The decreased thrombus weight from rats given UK at 20 000 IU kg^{-1}, pre-fed with **5c** at 100 nmol kg^{-1} is equal to that from rats given NS. This means that **5c** at 100 nmol kg^{-1} completely antagonizes the in vivo thrombolytic action of UK. Figure 5 further indicates that the decreased thrombus weight of rats administered UK (20 000 IU kg^{-1}) pre-fed with 100 nmol kg^{-1} **5′c** is equal to that of the rats given UK alone. This means that **5′c** (100 nmol kg^{-1}) does not antagonize the in vivo thrombolytic action of UK.

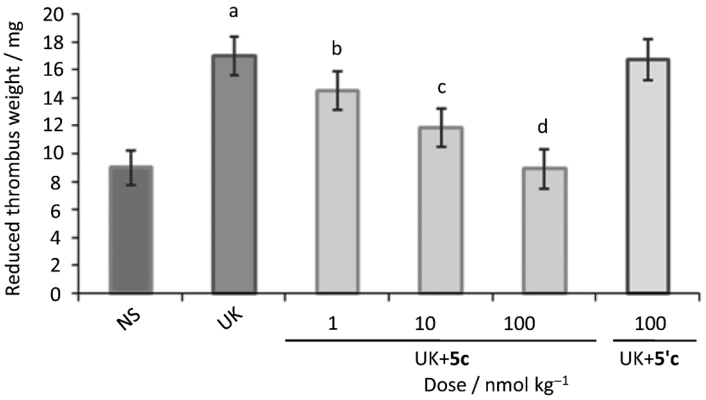


Figure 5. Effect of **5c** on the in vivo thrombolytic activity of UK. Decreased thrombus weight values represent the mean ±SD, NS = vehicle, [UK] = 20 000 IU kg^{-1}, $n=12$. a) Compared with NS as well as UK plus 1, 10, and 100 nM **5c** $p<0.01$, with UK plus 100 nM **5′c** $p>0.05$; b) compared with NS, as well as UK plus 10 and 100 nM **5c** $p<0.01$; c) compared with NS, as well as UK plus 100 nM **5c** $p<0.01$; d) compared with NS $p>0.05$.

Effect of 5c on the interaction of urokinase and plasminogen

It is well documented that both tissue-type plasminogen activator (t-PA) and u-PA (UK) catalyze the conversion of zymogen plasminogen to plasmin and play a central role in the fibrinolytic processes. Whereas the primary role of t-PA is the dissolution of blood clots in vessels, u-PA mediates cell-related proteolysis.[20,21] Our previous work suggested that the antithrombotic activity of pyrazino-pyridoindole-1,4-diones is a platelet-mediated process.[22] In this context, the effect of compound **5c** on the interaction between UK and plasminogen was monitored by electrophoresis, and the results are shown in Figure 6. SDS-PAGE of plasminogen alone (PLG, 5 μL, final concentration: 0.1 U mL^{-1}) plus NS (10 μL) gives two bands of PLG. In electrophoresis of PLG (5 μL, 0.1 U mL^{-1}) plus UK (5 μL, final concentration: 100 U mL^{-1}) and NS (5 μL), these PLG bands disappeared; PLG is completely degraded by UK. Electrophoresis of PLG (5 μL, 0.1 U mL^{-1}) plus UK (5 μL, 100 U mL^{-1}) and **5c** (5 μL, 2500 ng μL^{-1}) again gives two PLG bands, indicating the action of UK is completely antagonized by **5c**. In electrophoresis of PLG (5 μL, 0.1 U mL^{-1}) plus UK (5 μL, 100 U mL^{-1}) and **5c** (5 μL) in a series of final concentrations (2500, 1250, 250, 125, 25, 12.5, 2.5, 1.25, 0.25, 0.05, and 0.01 ng μL^{-1}), the intensity of the two PLG bands steadily decreases with decreasing **5c** concentration. Therefore, **5c** concentration-dependently antagonizes the action of UK.

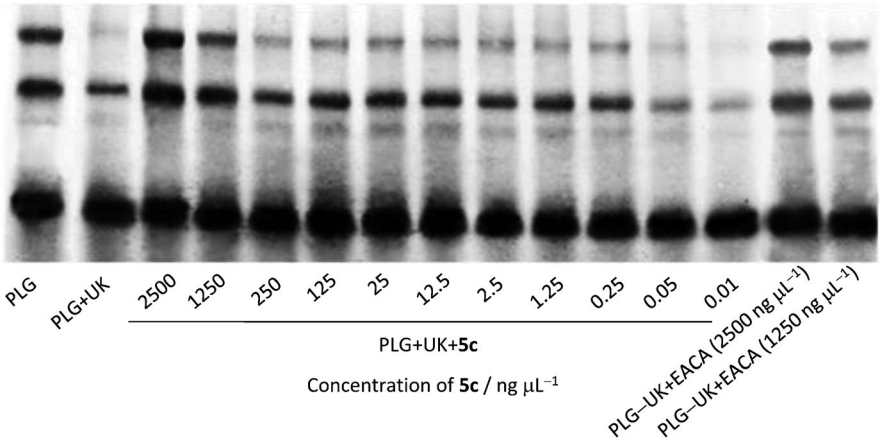


Figure 6. SDS-PAGE of plasminogen (PLG, 5 μL, final concentration 0.1 U mL^{-1}) plus NS (10 μL); PLG (5 μL, 0.1 U mL^{-1}) plus UK (5 μL, final concentration 100 U mL^{-1}) and NS (5 μL); PLG (5 μL, 0.1 U mL^{-1}) plus UK (5 μL, 100 U mL^{-1}) and 5 μL **5c** (in a series of final concentrations as indicated); PLG (5 μL, 0.1 U mL^{-1}) plus UK (5 μL, 100 U mL^{-1}) and EACA (5 μL, final concentration 2500 ng μL^{-1}); PLG (5 μL, 0.1 U mL^{-1}) plus UK (5 μL, 100 U mL^{-1}) and EACA (5 μL, 1250 ng μL^{-1}).

Conclusions

As increasing attention has been given to current small-molecular antifibrinolytic agents in current clinical use, such as tranexamic acid, which carry a significant risk of prompting thrombus formation, we carried out our investigation of new u-PA inhibitors. In summary, we designed and synthesized a group of pyrolo[1,2:4,5]-1,4-dioxopyrazino[1,2:1,6]pyrido[3,4-*b*]indoles (DAPPP), and compounds **5a–d** showed strong hemostatic activities in a mouse tail bleeding assay, with the most potent compound, **5c**, demonstrating dose-dependent effects. The coagulation potency of DAPPP depends on its configuration at C12 and the substituent group. Compounds **5a–d** show pro-thrombotic effects in a rat thrombosis assay; however, **5c** showed very mild activity; this indicates that **5c** has a good therapeutic window for antifibrinolytic therapy. Specifically, the minimal effective dose of **5c** for hemostatic activity is <1 nmol kg^{-1}, whereas the safe dose of **5c** against thrombus formation is 10 nmol kg^{-1}. Further investigation of **5c** in vitro and in vivo shows that inhibition of UK is involved in this coagulation process, and the SDS-PAGE assays supplied direct evidence that **5c** inhibits the function of u-PA on plasminogen in vitro.

Experimental Section

Chemistry

General: All chemicals were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA) and were purified when necessary. Column chromatography was performed using silica gel (200–300 mesh, Qingdao Haiyang Chemical Co., Qingdao, P. R. China). Purity of intermediates (>95%) and products (>97%) were determined by TLC analysis (Merck silica gel plates 60 F_{254}, 0.25 mm layer thickness) and HPLC analysis (Hewlett Packard HP Agilent 1050 system equipped with a Waters C_{18} column 4.6×150 mm). Melting points (mp) were determined in capillary tubes on an electrothermal SM/XMP apparatus and are uncorrected. Mass spectrometry (MS) was performed on a Micromass Quattro micro TM API bench-top mass spectrometer (Waters Co.) using electrospray ionization (ESI). Infrared spectra (IR) were run on a Shimadzu 8010M spectrophotometer, using the KBr disk method. ^{1}H NMR (500 Hz) and ^{13}C NMR (125 Hz) spectra were recorded on a Bruker Advance 500 spectrometer in $CDCl_3$ with TMS as an internal standard, and chemical shifts (δ) are expressed in parts per million (ppm). Optical rotations were determined with a Jasco P-1020 polarimeter.

1-(2,2-Dimethoxyethyl)-1,2,3,4-tetrahydrocarboline-3-carboxylic acid methyl ester (1 and 1′): A suspension of L-tryptophan methyl ester (5.0 g, 24.5 mmol) and 1,1,3,3-tetramethoxypropane (6.0 mL, 23.6 mmol) in CH_3OH (50 mL) was adjusted to pH 1–2 with HCl (5 M) and stirred at 45 °C for 48 h. After evaporation of the solvent in vacuo, the residue was diluted with water (50 mL) and extracted with EtOAc (3×30 mL). The combined organic extracts were washed successively with 10% aq Na_2CO_3 (3×30 mL) and saturated aq NaCl (2×30 mL), then dried over with anhyd Na_2SO_4, filtered and concentrated in vacuo. Purifi-

cation by column chromatography ($CHCl_3/CH_3OH$, 30:1) gave **1** (3.2 g, 60%) and **1′** (1.6 g, 30%).

(1*S*,3*S*)-1-(2,2-Dimethoxyethyl)-1,2,3,4-tetrahydrocarboline-3-carboxylic acid methyl ester (1): ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 8.50$ (s, 1H), 7.48 (d, $J = 7.9$ Hz, 1H), 7.34 (d, $J = 7.9$ Hz, 1H), 7.15 (m, $J = 7.1$ Hz, 2H), 4.66 (t, $J = 6.9$ Hz, 1H), 4.38 (t, $J = 6.9$ Hz, 1H), 4.14 (t, $J = 4.0$ Hz, 1H), 3.94 (dd, $J = 6.9$, 2.9 Hz,1H), 3.74 (s, 3H), 3.47 (s, 3H), 3.40 (s, 3H), 3.13 (m, $J = 2.1$ Hz, 1H), 2.96 (m, $J = 2.1$ Hz, 1H), 2.41 (s, 1H), 2.32 (t, $J = 7.1$ Hz, 1H), 2.22 ppm (t, $J = 4.0$ Hz, 1H); MS (ESI+): *m/z*: 319 $[M+H]^+$.

(1*R*,3*S*)-1-(2,2-Dimethoxyethyl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole-3-carboxylic acid methyl ester (1′): ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 8.80$ (s, 1H), 7.49 (d, $J = 7.2$ Hz, 1H), 7.34 (d, $J = 7.8$ Hz, 1H), 7.15 (m, $J = 7.1$ Hz, 2H), 4.66 (q, $J = 3.2$ Hz, 1H), 4.31 (m, $J = 3.2$ Hz, 1H), 3.94 (s, 3H), 3.82 (dd, $J = 7.0$, 3.9 Hz,1H), 3.51 (s, 3H), 3.43 (s, 3H), 3.13 (m, $J = 2.1$ Hz, 1H), 2.84 (m, $J = 2.1$ Hz, 1H), 2.22 (t, $J = 7.1$ Hz, 1H), 2.12 (t, $J = 4.0$ Hz, 1H), 1.96 ppm (s, 1H); MS (ESI+): *m/z*: 319 $[M+H]^+$.

(5a*S*,12*S*,14a*S*)-5,14-Dioxo-12-(2,2-dimethoxyethyl)-1,2,3,5,5a,6,11,12,14,14a-decahydro-5*H*,14*H*-pyrolo[1,-2:4,5]pyrazino-[1,2:1,6]pyrido[3,4-*b*]indole (2): Fmoc-L-Pro (5.0 g, 14.8 mmol) was treated dropwise with $SOCl_2$ (50 mL), and the reaction mixture was held at reflux for 5 h. The mixture was concentrated in vacuo to remove excess $SOCl_2$. The residue was treated with Et_2O (30 mL) to provide Fmoc-protected prolinyl chloride (colorless powder), which was treated with **1** (3.2 g, 10 mmol) in CH_2Cl_2 (50 mL) at 0 °C and the mixture was stirred for 0.5 h. The reaction mixture was adjusted to pH 9 with $(i\text{Pr})_2\text{NH}$ and stirred at RT for 24 h. The reaction mixture was concentrated in vacuo, and the residue was purified on column chromatography ($CHCl_3/CH_3OH$, 40:1) to give **2** (2.61 g, 40%): mp: 180–183 °C; $[\alpha]_D^{20} = +76$ ($c = 0.35$, $CHCl_3/CH_3OH$, 1:1); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 10.14$ (s, 1H), 8.67 (s, 1H), 7.50 (t, $J = 7.5$ Hz, 1H), 7.34 (t, $J = 7.5$ Hz, 1H), 7.22 (d, $J = 7.5$ Hz, 1H), 7.09 (d, $J = 7.5$ Hz, 1H), 5.82 (t, $J = 6.0$ Hz, 1H), 4.76 (t, $J = 6.0$ Hz, 1H), 4.41 (t, $J = 4.5$ Hz, 1H), 4.14 (t, $J = 4.0$ Hz, 1H), 3.92 (m, 1H), 3.64 (dd, $J = 4.5$, 15.0 Hz, 1H), 3.46 (s, 3H), 3.40 (s, 3H), 2.86 (dd, $J = 11.1$, 16.0 Hz, 1H), 2.51 (m, 2H), 1.90–2.20 ppm (m, 5H); IR (KBr): $\tilde{\nu} = 3346$, 2933, 2832, 1683, 1645, 1462, 1335, 744 cm^{-1}; MS (ESI+): *m/z*: 384 $[M+H]^+$; Anal. calcd for $C_{21}H_{25}N_3O_4$: C 65.78, H 6.57, N 10.96, found: C 66.02, H 6.74, N 10.75.

(5a*S*,12*R*,14a*S*)-5,14-Dioxo-12-(2,2-dimethoxyethyl)-1,2,3,5,5a,6,11,12,14,14a-decahydro-5*H*,14*H*-pyrolo[1,-2:4,5]pyrazino-[1,2:1,6]pyrido[3,4-*b*]indole (2′): Fmoc-L-Pro (5.0 g, 14.8 mmol) and **1′** (3.2 g, 10 mmol) were reacted using the procedure as for preparing **2** to give compound **2′** (1.30 g, 20%): mp: 205–207 °C; $[\alpha]_D^{20} = +76$ ($c = 0.33$, $CHCl_3/CH_3OH$, 1:1); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 10.17$ (s, 1H), 8.65 (s, 1H), 7.52 (t, $J = 7.6$ Hz, 1H), 7.36 (t, $J = 7.6$ Hz, 1H), 7.23 (d, $J = 7.6$ Hz, 1H), 7.11 (d, $J = 7.6$ Hz, 1H), 5.80 (t, $J = 6.2$ Hz, 1H), 4.70 (t, $J = 6.2$ Hz, 1H), 4.32 (t, $J = 4.6$ Hz, 1H), 4.10 (t, $J = 4.2$ Hz, 1H), 3.89 (m, 1H), 3.86 (s, 3H), 3.75 (s, 3H), 3.64 (dd, $J = 4.6$, 15.1 Hz, 1H), 2.84 (dd, $J = 11.0$, 15.8 Hz, 1H), 2.54 (m, 2H), 1.92–2.26 ppm (m, 5H); IR (KBr): $\tilde{\nu} = 3348$, 2937, 2829, 1688, 1640, 1467, 1330, 749 cm^{-1}; MS (ESI+): *m/z*: 384 $[M+H]^+$; Anal. calcd for $C_{21}H_{25}N_3O_4$: C 65.78, H 6.57, N 10.96, found: C 66.02, H 6.74, N 10.75.

(5a*S*,12*S*,14a*S*)-5,14-Dioxo-12-cabonylmethyl-1,2,3,5,5a,6,11,12,14,14a-decahydro-5*H*,14*H*-pyrolo[1,2:4,5]pyrazino[1,2:1,6]pyrido-[3,4-*b*]indole (3): A solution of **2** (200 mg, 0.52 mmol) in acetone (15 mL) was treated with *p*-TsOH (20 mg) and then stirred at 45 °C for 1 h, until TLC ($CHCl_3/CH_3OH$, 20:1) indicated complete disappearance of **2**. The reaction mixture was treated with Et_3N (0.5 mL), concentrated in vacuo to remove acetone, and purified by column chromatography ($CHCl_3/CH_3OH$, 40:1). Recrystallization from acetone gave **3** (158 mg, 90%): mp: 133–135 °C; $[\alpha]_D^{20} = +176$ ($c = 0.71$, $CHCl_3/CH_3OH$, 1:1); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 10.22$ (s, 1H), 9.86 (s, 1H), 7.52 (t, $J = 7.6$ Hz, 1H), 7.35 (t, $J = 7.6$ Hz, 1H), 7.25 (d, $J = 7.6$ Hz, 1H), 7.06 (d, $J = 7.6$ Hz, 1H), 5.83 (t, $J = 6.4$ Hz, 1H), 5.12 (t, $J = 4.1$ Hz, 1H), 4.77 (t, $J = 6.1$ Hz, 1H), 4.42 (t, $J = 4.6$ Hz, 1H), 3.90 (m, 1H), 3.65 (dd, $J = 4.6$, 14.7 Hz, 1H), 2.88 (dd, $J = 11.0$, 15.8 Hz, 1H), 2.52 (m, 2H), 1.92–2.21 ppm (m, 4H); IR (KBr): $\tilde{\nu} = 3340$, 2937, 2835, 1726, 1684, 1643, 1460, 1332, 743 cm^{-1}; MS (ESI+): *m/z*: 338 $[M+H]^+$; Anal. calcd for $C_{19}H_{19}N_3O_3$: C 67.64, H 5.68, N 12.46, found: C 67.85, H 5.53, N 12.69.

(5a*S*,12*R*,14a*S*)-5,14-Dioxo-12-cabonylmethyl-1,2,3,5,5a,6,11,12,14,14a-decahydro-5*H*,14*H*-pyrolo[1,2:4,5]pyrazino[1,2:1,6]pyrido-[3,4-*b*]indole (3′): Compound **2′** (200 mg, 0.52 mmol) was reacted using the same procedure described for the preparation of **2** to give **3′** (162 mg, 92%): mp: 133–135 °C; $[\alpha]_D^{20} = +176$ ($c = 0.71$, $CHCl_3/CH_3OH$, 1:1); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 10.26$ (s, 1H), 9.83 (s, 1H), 7.54 (t, $J = 7.5$ Hz, 1H), 7.33 (t, $J = 7.5$ Hz, 1H), 7.27 (d, $J = 7.5$ Hz, 1H), 7.09 (d, $J = 7.5$ Hz, 1H), 5.80 (t, $J = 6.2$ Hz, 1H), 5.10 (t, $J = 4.3$ Hz, 1H), 4.70 (t, $J = 6.0$ Hz, 1H), 4.35 (t, $J = 4.3$ Hz, 1H), 3.95 (m, 1H), 3.60 (dd, $J = 4.3$, 14.0 Hz, 1H), 2.82 (dd, $J = 11.2$, 15.4 Hz, 1H), 2.55 (m, 2H), 1.90–2.23 ppm (m, 4H); IR (KBr): $\tilde{\nu} = 3344$, 2932, 2830, 1730, 1680, 1647, 1463, 1335, 748 cm^{-1}; MS (ESI+): *m/z*: 338 $[M+H]^+$; Anal. calcd for $C_{19}H_{19}N_3O_3$: C 67.64, H 5.68, N 12.46, found: C 67.85, H 5.53, N 12.69.

(5a*S*,12*S*/*R*,14a*S*)-5,14-Dioxo-12-(2-amino-acid-methyl ester-*N*-ylethyl-1-yl)-1,2,3,5,-5a,6,11,12,14,14a-decahydro-5*H*,14*H*-pyrolo-[1,2:4,5]pyrazino[1,2:1,6]pyrido-[3,4-*b*]indoles (4a–h and 4′a–h): A solution of **3** or **3′** (0.52 mmol) in $CHCl_3$ (10 mL) was treated with the appropriate amino acid methyl ester (0.52 mmol), Et_3N (0.5 mL) and anhyd Na_2SO_4 (3 g). The reaction mixture was stirred at RT for 3 h, then mixed with a solution of KBH_4 (100 mg, 1.85 mmol) in CH_3OH (5 mL), and stirred for another 1 h, until TLC ($CHCl_3/CH_3OH$, 20:1) indicated complete disappearance of the starting material (**3** or **3′**). The reaction mixture was concentrated in vacuo, and the residue was mixed with $CHCl_3$ (10 mL) and deionized water (10 mL). The two-phase solution was treated with HCl (1 mL, 5 M) to decompose excess KBH_4, and adjusted to pH 9 with aq NH_3 (5 M). The $CHCl_3$ phase was separated, and the aqueous phase was extracted with $CHCl_3$ (3×5 mL). The combined organic phases were washed with saturated aq NaCl (20 mL), dried over with anhyd Na_2SO_4, filtered and concentrated in vacuo. Purification by column chromatography ($CHCl_3/CH_3OH$, 30:1) gave **4a–h** or **4′a–h**.

(5a*S*,12*S*,14a*S*)-5,14-Dioxo-12-(2-alanine-methyl ester-*N*-ylethyl-1-yl)-1,2,3,5,5a,6,11,12,14,14a-decahydro-5*H*,14*H*-pyrolo-[1,2:4,5]pyrazino[1,2:1,6]pyrido[3,4-*b*]indole (4a): Yield: 212 mg (96%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 10.55$ (s, 1H), 7.45 (d, $J = 7.9$ Hz, 1H), 7.38 (d, $J = 7.9$ Hz, 1H), 7.11 (t, $J = 7.4$ Hz, 1H), 6.99 (t, $J = 7.4$ Hz, 1H), 5.74 (d, $J = 10.0$ Hz, 1H), 4.31 (m, 2H), 3.67 (s, 3H), 3.62 (q, $J = 8.4$ Hz, 1H), 3.46 (dt, $J = 2.4$ Hz, 10.1 Hz, 1H), 3.21 (q, $J = 6.5$ Hz, 1H), 3.14 (dd, $J = 15.0$, 3.92 Hz, 1H), 2.96 (m, 2H), 2.79 (s, 1H), 2.38 (s, 1H), 2.07 (m, 1H), 1.99 (m, 2H), 1.85 (s, 3H), 1.30 ppm (d, $J = 6.9$ Hz, 3H); IR (KBr): $\tilde{\nu} = 3345$, 2936, 2834, 1733, 1685, 1642, 1465, 1336, 1295, 748 cm^{-1}; MS (ESI+): *m/z*: 425 $[M+H]^+$.

(5a*S*,12*S*,14a*S*)-5,14-Dioxo-12-(2-aspartic-acid-dimethyl ester-*N*-ylethyl-1-yl)-1,2,3,5,5a,6,11,12,14,14a-decahydro-5*H*,14*H*-pyrolo-[1,2:4,5]pyrazino[1,2:1,6]pyrido[3,4-*b*]indole (4b): Yield: 227 mg (88%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 10.71$ (s, 1H), 7.47 (d, $J = 7.9$ Hz, 1H), 7.38 (d, $J = 8.0$ Hz, 1H), 7.06 (t, $J = 7.0$ Hz, 1H), 7.03 (t, $J = 7.2$ Hz, 1H), 5.69 (d, $J = 7.7$ Hz, 1H), 4.34 (m, 2H), 3.67 (s, 3H),

3.65 (s, 3H), 3.62 (m, 2H), 3.46 (t, J=9.5 Hz, 2H), 3.14 (dd, J=4.1, 15.0 Hz, 2H), 2.96 (m, 2H), 2.68 (dd, J=8.8, 16.0 Hz, 1H), 2.28 (m, 2H), 2.11 (m, 1H), 1.93 ppm (m, 4H); IR (KBr): $\tilde{\nu}$=3343, 2930, 2835, 1731, 1679, 1642, 1465, 1330, 1294, 741 cm^{-1}; MS (ESI+): m/z: 483 $[M+H]^+$.

(5a*S*,12*S*,14a*S*)-5,14-Dioxo-12-(2-phenylalanine-methyl ester-*N*-ylethyl-1-yl)-1,2,3,5,5a,6,11,12,14,14a-decahydro-5*H*,14*H*-pyrolo-[1,2:4,5]pyrazino[1,2:1,6]pyrido[3,4-*b*]indole (4c): Yield: 247 mg (95%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=10.63 (s, 1H), 7.49 (d, J=7.8 Hz, 1H), 7.41 (d, J=8.0 Hz, 1H), 7.35 (m, 5H), 7.16 (t, J=7.6 Hz, 1H), 7.06 (t, J=7.6 Hz, 1H), 5.64 (dd, J=3.3, 11.0 Hz, 1H), 4.39 (m, 1H), 4.33 (dd, J=3.4, 11.2 Hz, 1H), 3.65 (s, 3H), 3.63 (m, 3H), 3.15 (m, 3H), 2.97 (m, 2H), 2.39 (m, 1H), 2.26 (m, 1H), 2.17 (m, 1H), 1.97 (m, 1H), 1.89 (m, 2H), 1.28 ppm (m, 2H); IR (KBr): $\tilde{\nu}$=3344, 2935, 2834, 1731, 1683, 1643, 1466, 1337, 1295, 740 cm^{-1}; MS (ESI+): m/z: 501 $[M+H]^+$.

(5a*S*,12*S*,14a*S*)-5,14-Dioxo-12-(2-tryptophan-methyl ester-*N*-ylethyl-1-yl)-1,2,3,5,5a,6,11,12,14,14a-decahydro-5*H*,14*H*-pyrolo-[1,2:4,5]pyrazino[1,2:1,6]pyrido[3,4-*b*]indole (4d): Yield: 263 mg (94%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=10.86 (s, 1H), 10.53 (s, 1H), 7.64 (d, J=7.9 Hz, 1H), 7.49 (d, J=8.0 Hz, 1H), 7.35 (m, 2H), 7.27 (s, 1H), 7.09 (m, 2H), 6.99 (m, 2H), 5.69 (d, J=8.1 Hz, 1H), 4.31 (m, 2H), 3.67 (s, 3H), 3.61 (m, 2H), 3.44 (m, 1H), 3.28 (dd, J=14.8, 5.5 Hz, 1H), 3.12 (m, 2H), 2.95 (m, 2H), 2.64 (m, 1H), 2.23 (m, 2H), 2.08 (m, 1H), 1.93 ppm (m, 4H); IR (KBr): $\tilde{\nu}$=3346, 2935, 2837, 1730, 1678, 1644, 1459, 1337, 1295, 742 cm^{-1}; MS (ESI+): m/z: 540 $[M+H]^+$.

(5a*S*,12*S*,14a*S*)-5,14-Dioxo-12-(2-lysine-methyl ester-*N*-ylethyl-1-yl)-1,2,3,5,5a,6,11,12,14,14a-decahydro-5*H*,14*H*-pyrolo-[1,2:4,5]pyrazino[1,2:1,6]pyrido[3,4-*b*]indole (4e): Yield: 80 mg (32%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=10.62 (s, 1H), 7.49 (d, J=7.6 Hz, 1H), 7.39 (d, J=7.6 Hz, 1H), 7.15 (t, J=7.6 Hz, 2H), 7.06 (t, J=7.4 Hz, 1H), 5.77 (dd, J=3.2, 10.8 Hz, 1H), 4.39 (m, 1H), 4.36 (dd, J=3.8, 11.0 Hz, 1H), 3.68 (s, 3H), 3.64 (m, 2H), 3.15 (m, 2H), 2.95 (m, 2H), 2.35 (m, 2H), 2.31 (m, 2H), 2.21 (m, 3H), 1.97 (m, 2H), 1.85 (m, 2H), 1.66 (m, 2H), 1.57 (m, 2H), 1.29 ppm (m, 2H); IR (KBr): $\tilde{\nu}$=3347, 2928, 2836, 1731, 1686, 1640, 1458, 1337, 1294, 741 cm^{-1}; MS (ESI+): m/z: 482 $[M+H]^+$.

(5a*S*,12*S*,14a*S*)-5,14-Dioxo-12-(2-serine-methyl ester-*N*-ylethyl-1-yl)-1,2,3,5,5a,6,11,12,14,14a-decahydro-5*H*,14*H*-pyrolo-[1,2:4,5]pyrazino[1,2:1,6]pyrido[3,4-*b*]indole (4f): Yield: 213 mg (93%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=10.60 (s, 1H), 7.47 (d, J=7.9 Hz, 1H), 7.39 (d, J=8.0 Hz, 1H), 7.12 (t, J=7.7 Hz, 1H), 6.99 (t, J=7.7 Hz, 1H), 5.76 (dd, J=3.3, 10.0 Hz, 1H), 4.37 (m, 2H), 3.75 (m, 1H), 3.66 (m, 2H), 3.65 (s, 3H), 3.48 (m, 1H), 3.15 (m, 2H), 2.98 (m, 1H), 2.79 (s, 1H), 2.25 (m, 2H), 2.00 (m, 1H), 1.94 (m, 1H), 1.89 ppm (m, 5H); IR (KBr): $\tilde{\nu}$=3344, 2936, 2833, 1732, 1685, 1641, 1465, 1337, 1293, 746 cm^{-1}; MS (ESI+): m/z: 441 $[M+H]^+$.

(5a*S*,12*S*,14a*S*)-5,14-Dioxo-12-(2-glycine-methyl ester-*N*-ylethyl-1-yl)-1,2,3,5,5a,6,11,12,14,14a-decahydro-5*H*,14*H*-pyrolo-[1,2:4,5]pyrazino[1,2:1,6]pyrido[3,4-*b*]indole (4g): Yield: 192 mg (90%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=10.58 (s, 1H), 7.47 (d, J=7.9 Hz, 1H), 7.42 (d, J=8.0 Hz, 1H), 7.12 (t, J=7.4 Hz, 1H), 6.99 (t, J=7.4 Hz, 1H), 5.82 (dd, J=4.4, 9.3 Hz, 1H), 4.36 (m, 1H), 3.67 (s, 3H), 3.66 (q, J=14.4 Hz, 1H), 3.46 (m, 1H), 3.25 (m, 2H), 3.14 (dd, J=4.2, 14.6 Hz, 1H), 2.98 (m, 1H), 2.82 (m, 2H), 2.29 (m, 2H), 2.11 (m, 1H), 1.95 ppm (m, 5H); IR (KBr): $\tilde{\nu}$=3344, 2935, 2834, 1731, 1677, 1642, 1458, 1337, 1293, 741 cm^{-1}; MS (ESI+): m/z: 411 $[M+H]^+$.

(5a*S*,12*S*,14a*S*)-5,14-Dioxo-12-(2-leucine-methyl ester-*N*-ylethyl-1-yl)-1,2,3,5,5a,6,11,12,14,14a-decahydro-5*H*,14*H*-pyrolo-[1,2:4,5]pyrazino[1,2:1,6]pyrido[3,4-*b*]indole (4h): Yield: 228 mg (94%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=10.49 (s, 1H), 7.48 (d, J=7.7 Hz, 1H), 7.41 (d, J=7.8 Hz, 1H), 7.14 (t, J=7.2 Hz, 1H), 7.06 (t, J=7.4 Hz, 1H), 5.69 (dd, J=11.1, 2.6 Hz, 1H), 4.38 (t, J=5.2 Hz, 1H), 4.36 (dd, J=12.0, 8.0 Hz, 1H), 3.66 (s, 3H), 3.64 (m, 2H), 3.48 (dt, J=2.8, 7.0 Hz, 1H), 3.36 (t, J=6.6 Hz, 1H), 3.15 (dd, J=14.8, 4.3 Hz, 1H), 2.98 (t, J=11.7 Hz, 1H), 2.85 (t, J=9.5 Hz, 1H), 2.39 (s, 1H), 2.33 (m, 2H), 2.21 (dq, J=10.4, 2.6 Hz, 1H), 1.95 (m, 4H), 1.58 (m, 2H), 0.94 ppm (dd, J=12.0, 6.2 Hz, 6H); IR (KBr): $\tilde{\nu}$=3346, 2934, 2835, 1731, 1685, 1640, 1465, 1337, 1293, 740 cm^{-1}; MS (ESI+): m/z: 467 $[M+H]^+$.

(5a*S*,12*R*,14a*S*)-5,14-Dioxo-12-(2-alanine-methyl ester-*N*-ylethyl-1-yl)-1,2,3,5,5a,6,11,12,14,14a-decahydro-5*H*,14*H*-pyrolo-[1,2:4,5]pyrazino[1,2:1,6]pyrido[3,4-*b*]indole (4′a): Yield: 207 mg (94%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=10.50 (s, 1H), 7.43 (d, J=7.8 Hz, 1H), 7.37 (d, J=7.8 Hz, 1H), 7.12 (t, J=7.4 Hz, 1H), 6.97 (t, J=7.4 Hz, 1H), 5.82 (d, J=10.1 Hz, 1H), 4.39 (m, 2H), 3.69 (q, J=8.2 Hz, 1H), 3.65 (s, 3H), 3.51 (dt, J=2.6, 9.8 Hz, 1H), 3.28 (q, J=6.6 Hz, 1H), 3.14 (dd, J=3.9, 15.0 Hz, 1H), 2.87 (m, 2H), 2.70 (s, 1H), 2.31 (s, 1H), 1.98 (m, 1H), 1.94 (m, 2H), 1.80 (s, 3H), 1.27 ppm (d, J=6.7 Hz, 3H); IR (KBr): $\tilde{\nu}$=3343, 2934, 2831, 1730, 1681, 1645, 1462, 1332, 1297, 743 cm^{-1}; MS (ESI+): m/z: 425 $[M+H]^+$.

(5a*S*,12*R*,14a*S*)-5,14-Dioxo-12-(2-aspartic-acid-dimethyl ester-*N*-ylethyl-1-yl)-1,2,3,5,5a,6,11,12,14,14a-decahydro-5*H*,14*H*-pyrolo-[1,2:4,5]pyrazino[1,2:1,6]pyrido[3,4-*b*]indole (4′b): Yield: 224 mg (87%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=10.62 (s, 1H), 7.48 (d, J=7.7 Hz, 1H), 7.36 (d, J=8.0 Hz, 1H), 7.09 (t, J=7.1 Hz, 1H), 7.05 (t, J=7.1 Hz, 1H), 5.73 (d, J=7.5 Hz, 1H), 4.39 (m, 2H), 3.69 (s, 3H), 3.67 (m, 2H), 3.65 (s, 3H), 3.50 (t, J=9.4 Hz, 2H), 3.14 (dd, J=4.2, 15.0 Hz, 2H), 2.82 (m, 2H), 2.63 (dd, J=8.6, 15.5 Hz, 1H), 2.22 (m, 2H), 2.00 (m, 1H), 1.84 ppm (m, 4H); IR (KBr): $\tilde{\nu}$=3340, 2934, 2831, 1735, 1676, 1640, 1462, 1334, 1291, 743 cm^{-1}; MS (ESI+): m/z: 497 $[M+H]^+$.

(5a*S*,12*R*,14a*S*)-5,14-Dioxo-12-(2-phenylalanine-methyl ester-*N*-ylethyl-1-yl)-1,2,3,5,5a,6,11,12,14,14a-decahydro-5*H*,14*H*-pyrolo-[1,2:4,5]pyrazino[1,2:1,6]pyrido[3,4-*b*]indole (4′c): Yield: 242 mg (93%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=10.55 (s, 1H), 7.51 (d, J=7.9 Hz, 1H), 7.43 (d, J=8.1 Hz, 1H), 7.41 (m, 5H), 7.21 (t, J=7.7 Hz, 1H), 7.11 (t, J=7.7 Hz, 1H), 5.69 (dd, J=3.5, 10.7 Hz, 1H), 4.44 (m, 1H), 4.38 (dd, J=3.5, 10.7 Hz, 1H), 3.64 (s, 3H), 3.62 (m, 3H), 3.15 (m, 3H), 2.35 (m, 2H), 2.30 (m, 1H), 2.21 (m, 1H), 2.11 (m, 1H), 1.90 (m, 1H), 1.85 (m, 2H), 1.24 ppm (m, 2H); IR (KBr): $\tilde{\nu}$=3342, 2933, 2837, 1735, 1680, 1646, 1462, 1332, 1291, 743 cm^{-1}; MS (ESI+): m/z: 501 $[M+H]^+$.

(5a*S*,12*R*,14a*S*)-5,14-Dioxo-12-(2-tryptophan-methyl ester-*N*-ylethyl-1-yl)-1,2,3,5,5a,6,11,12,14,14a-decahydro-5*H*,14*H*-pyrolo-[1,2:4,5]pyrazino[1,2:1,6]pyrido[3,4-*b*]indole (4′d): Yield: 261 mg (93%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=10.54 (s, 1H), 10.50 (s, 1H), 7.66 (d, J=7.7 Hz, 1H), 7.53 (d, J=8.1 Hz, 1H), 7.39 (m, 2H), 7.30 (s, 1H), 7.14 (m, 2H), 7.00 (m, 2H), 5.76 (d, J=8.0 Hz, 1H), 4.38 (m, 2H), 3.69 (s, 3H), 3.64 (m, 2H), 3.48 (m, 1H), 3.31 (dd, J=5.1, 14.0 Hz, 1H), 3.12 (m, 2H), 2.27 (m, 2H), 2.17 (m, 1H), 2.04 (m, 2H), 1.99 (m, 1H), 1.90 ppm (m, 4H); IR (KBr): $\tilde{\nu}$=3342, 2933, 2834, 1735, 1673, 1640, 1462, 1334, 1291, 740 cm^{-1}; MS (ESI+): m/z: 540 $[M+H]^+$.

(5a*S*,12*R*,14a*S*)-5,14-Dioxo-12-(2-lysine-methyl ester-*N*-ylethyl-1-yl)-1,2,3,5,5a,6,11,12,14,14a-decahydro-5*H*,14*H*-pyrolo-[1,2:4,5]pyrazino[1,2:1,6]pyrido[3,4-*b*]indole (4′e): Yield: 82 mg (32%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=10.66 (s, 1H), 7.47 (d, J=

7.5 Hz, 1H), 7.36 (d, J=7.5 Hz, 1H), 7.14 (t, J=7.5 Hz, 2H), 7.04 (t, J=7.2 Hz, 1H), 5.82 (dd, J=3.4, 10.6 Hz, 1H), 4.42 (m, 1H), 4.40 (dd, J=3.6, 11.2 Hz, 1H), 3.65 (s, 3H), 3.62 (m, 2H), 3.15 (m, 2H), 2.70 (m, 2H), 2.31 (m, 2H), 2.27 (m, 2H), 2.16 (m, 3H), 1.92 (m, 2H), 1.80 (m, 2H), 1.62 (m, 2H), 1.53 (m, 2H), 1.25 ppm (m, 2H); IR (KBr): $\tilde{\nu}$=3347, 2934, 2835, 1731, 1686, 1640, 1452, 1335, 1293, 742 cm^{-1}; MS (ESI+): m/z: 482 $[M+H]^+$.

(5a*S*,12*R*,14a*S*)-5,14-Dioxo-12-(2-serine-methyl ester-*N*-ylethyl-1-yl)-1,2,3,5,5a,6,11,12,14,14a-decahydro-5*H*,14*H*-pyrolo-[1,2:4,5]pyrazino[1,2:1,6]pyrido[3,4-*b*]indole (4′f): Yield: 218 mg (95%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=10.62 (s, 1H), 7.49 (d, J=7.7 Hz, 1H), 7.36 (d, J=8.1 Hz, 1H), 7.10 (t, J=7.8 Hz, 1H), 6.96 (t, J=7.6 Hz, 1H), 5.79 (dd, J=3.5, 10.1 Hz, 1H), 4.42 (m, 2H), 3.81 (m, 1H), 3.69 (m, 2H), 3.66 (s, 3H), 3.53 (m, 1H), 3.15 (m, 2H), 2.87 (m, 1H), 2.63 (s, 1H), 2.20 (m, 2H), 1.96 (m, 1H), 1.90 (m, 1H), 1.83 ppm (m, 5H); IR (KBr): $\tilde{\nu}$=3343, 2930, 2835, 1731, 1686, 1641, 1464, 1335, 1291, 740 cm^{-1}; MS (ESI+): m/z: 441 $[M+H]^+$.

(5a*S*,12*R*,14a*S*)-5,14-Dioxo-12-(2-glycine-methyl ester-*N*-ylethyl-1-yl)-1,2,3,5,5a,6,11,12,14,14a-decahydro-5*H*,14*H*-pyrolo-[1,2:4,5]pyrazino[1,2:1,6]pyrido[3,4-*b*]indole (4′g): Yield: 196 mg (92%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=10.60 (s, 1H), 7.50 (d, J=7.8 Hz, 1H), 7.45 (d, J=7.9 Hz, 1H), 7.15 (t, J=7.3 Hz, 1H), 6.94 (t, J=7.3 Hz, 1H), 5.86 (dd, J=4.3, 9.1 Hz, 1H), 4.42 (m, 1H), 3.66 (s, 3H), 3.62 (q, J=14.1 Hz, 1H), 3.49 (m, 1H), 3.31 (m, 2H), 3.14 (dd, J=4.3, 14.2 Hz, 1H), 2.87 (m, 1H), 2.70 (m, 2H), 2.21 (m, 2H), 2.02 (m, 1H), 1.90 ppm (m, 5H); IR (KBr): $\tilde{\nu}$=3342, 2934, 2832, 1732, 1665, 1640, 1461, 1335, 1298, 741 cm^{-1}; MS (ESI+): m/z: 411 $[M+H]^+$.

(5a*S*,12*R*,14a*S*)-5,14-Dioxo-12-(2-leucine-methyl ester-*N*-ylethyl-1-yl)-1,2,3,5,5a,6,11,12,14,14a-decahydro-5*H*,14*H*-pyrolo-[1,2:4,5]pyrazino[1,2:1,6]pyrido[3,4-*b*]indole (4′h): Yield: 221 mg (91%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=10.52 (s, 1H), 7.51 (d, J=7.8 Hz, 1H), 7.46 (d, J=7.9 Hz, 1H), 7.17 (t, J=7.3 Hz, 1H), 7.10 (t, J=7.5 Hz, 1H), 5.72 (dd, J=11.0, 2.4 Hz, 1H), 4.43 (t, J=5.3 Hz, 1H), 4.39 (dd, J=12.1, 7.9 Hz, 1H), 3.67 (s, 3H), 3.65 (m, 2H), 3.51 (dt, J=7.1, 2.9 Hz, 1H), 3.42 (t, J=6.4 Hz, 1H), 3.15 (dd, J=14.5, 4.4 Hz, 1H), 2.86 (t, J=11.4 Hz, 1H), 2.72 (t, J=9.2 Hz, 1H), 2.33 (s, 1H), 2.27 (m, 2H), 2.19 (dq, J=10.0, 2.4 Hz, 1H), 1.90 (m, 4H), 1.51 (m, 2H), 0.95 ppm (dd, J=11.8, 6.0 Hz, 6H); IR (KBr): $\tilde{\nu}$=3340, 2934, 2833, 1738, 1684, 1640, 1465, 1330, 1291, 740 cm^{-1}; MS (ESI+): m/z: 467 $[M+H]^+$.

(5a*S*,12*S*/*R*,14a*S*)-5,14-Dioxo-12-(2-amino-acid-*N*-ylethyl-1-yl)-1,2,3,5,5a,6,11,12,14,14a-decahydro-5*H*,14*H*-pyrolo-[1,2:4,5]pyrazino[1,2:1,6]pyrido[3,4-*b*]indoles (5a–h and 5′a–h): A solution of NaOH (300 mg, 7.5 mmol) in CH_3OH (5 mL) at 0 °C was treated with **4a–h** or **4′a–h** (0.5 mmol). The reaction mixture was stirred at RT for 2 h, until TLC ($CHCl_3$/CH_3OH, 20:1) indicated complete disappearance of **4a–h** or **4′a–h**. The reaction mixture was adjusted to pH 3 with HCl (2 M) and then concentrated in vacuo. The residue was purified by column chromatography ($CHCl_3$/CH_3OH, 30:1) to give **5a–h** or **5′a–h**.

(5a*S*,12*S*,14a*S*)-5,14-Dioxo-12-(2-alanine-*N*-ylethyl-1-yl)-1,2,3,5,5a,6,11,12,14,14a-decahydro-5*H*,14*H*-pyrolo-[1,2:4,5]pyrazino[1,2:1,6]pyrido[3,4-*b*]indole (5a): Yield: 195 mg (95%): mp: 231–235 °C; $[\alpha]_D^{20}$ = +109 (c=0.35, $CHCl_3$/CH_3OH, 1:1); ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=11.81 (s, 1H), 10.65 (s, 1H), 7.43 (d, J=7.8 Hz, 1H), 7.35 (d, J=8 Hz, 1H), 7.07 (t, J=7.2 Hz, 1H), 6.98 (t, J=7.3 Hz, 1H), 5.72 (d, J=10.3 Hz, 1H), 4.31 (m, 2H), 3.61 (q, J=8.6 Hz, 1H), 3.44 (dt, J=10.1, 2.2 Hz, 1H), 3.23 (q, J=6.3 Hz, 1H), 3.11 (dd, J=15.1, 3.96 Hz, 1H), 2.95 (m, 2H), 2.77 (s, 1H), 2.37 (s, 1H), 2.05 (m, 1H), 1.97 (m, 2H), 1.84 (s, 3H), 1.28 ppm (d, J=6.9 Hz, 3H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ=174.0, 167.2, 164.4, 136.5, 133.9, 126.5, 121.6, 119.1, 118.2, 111.9, 106.0, 58.4, 57.8, 55.4, 47.7, 45.4, 43.5, 31.1, 29.9, 25.8, 21.9, 17.4 ppm; IR (KBr): $\tilde{\nu}$=3346, 3133, 3001, 2932, 2835, 2712, 1682, 1641, 1462, 1331, 741 cm^{-1}; MS (ESI+): m/z: 411 $[M+H]^+$; Anal. calcd for $C_{22}H_{26}N_4O_4$: C 64.37, H 6.38, N 13.65, found: C 64.16, H 6.22, N 13.43.

(5a*S*,12*S*,14a*S*)-5,14-Dioxo-12-(2-aspartic-acid-*N*-ylethyl-1-yl)-1,2,3,5,5a,6,11,12,14,14a-decahydro-5*H*,14*H*-pyrolo-[1,2:4,5]pyrazino[1,2:1,6]pyrido[3,4-*b*]indole (5b): Yield: 227 mg (96%): mp: 218–222 °C; $[\alpha]_D^{20}$ = +77 (c=0.47, $CHCl_3$/CH_3OH, 1:1); ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=11.2 (s, 2H), 10.69 (s, 1H), 7.45 (d, J=7.8 Hz, 1H), 7.36 (d, J=8.1 Hz, 1H), 7.09 (t, J=7.2 Hz, 1H), 7.00 (t, J=7.4 Hz, 1H), 5.67 (d, J=7.9 Hz, 1H), 4.32 (m, 2H), 3.65 (m, 2H), 3.44 (t, J=9.7 Hz, 2H), 3.11 (dd, J=15.2, 4 Hz, 2H), 2.98 (m, 1H), 2.69 (dd, J=16.3, 9.1 Hz, 1H), 2.26 (m, 2H), 2.08 (m, 2H), 1.91 ppm (m, 4H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ=172.7, 171.1, 167.3, 164.4, 136.4, 133.5, 126.4, 121.8, 119.2, 118.3, 111.9, 106.2, 79.6, 58.4, 57.5, 55.4, 47.6, 46.0, 45.5, 43.7, 36.0, 29.8, 21.9 ppm; MS (ESI+): m/z: 455 $[M+H]^+$; Anal. calcd for $C_{23}H_{26}N_4O_6$: C 60.78, H 5.77, N 12.33, found: C 60.96, H 5.92, N 12.55.

(5a*S*,12*S*,14a*S*)-5,14-Dioxo-12-(2-phenylalanine-*N*-ylethyl-1-yl)-1,2,3,5,5a,6,11,12,14,14a-decahydro-5*H*,14*H*-pyrolo-[1,2:4,5]pyrazino[1,2:1,6]pyrido[3,4-*b*]indole (5c): Yield: 231 mg (95%): mp: 186–189 °C; $[\alpha]_D^{20}$ = +75 (c=0.53, $CHCl_3$/CH_3OH, 1:1); ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=11.06 (s, 1H), 10.67 (s, 1H), 7.47 (d, J=7.8 Hz, 1H), 7.38 (d, J=8.1 Hz, 1H), 7.31 (m, 4H), 7.11 (t, J=7.5 Hz, 2H), 7.02 (t, J=7.7 Hz, 1H), 5.63 (dd, J=11.2, 3.1 Hz, 1H), 4.37 (m, 1H), 4.30 (dd, J=11.5, 3.6 Hz, 1H), 3.61 (m, 3H), 3.12 (m, 3H), 2.98 (m, 2H), 2.37 (m, 1H), 2.28 (m, 1H), 2.18 (m, 1H), 1.99 (m, 1H), 1.88 (m, 2H), 1.25 ppm (m, 2H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ=170.1, 167.5, 164.2, 136.4, 135.1, 133.0, 129.9, 129.0, 128.0, 126.5, 122.0, 119.4, 118.5, 111.8, 106.6, 60.5, 58.4, 55.4, 47.3, 45.4, 44.0, 35.5, 30.1, 29.8, 29.5 ppm; MS (ESI+): m/z: 487 $[M+H]^+$; Anal. calcd for $C_{28}H_{30}N_4O_4$: C 69.12, H 6.21, N 11.51, found: C 69.34, H 6.05, N 11.28.

(5a*S*,12*S*,14a*S*)-5,14-Dioxo-12-(2-tryptophan-*N*-ylethyl-1-yl)-1,2,3,5,5a,6,11,12,14,14a-decahydro-5*H*,14*H*-pyrolo-[1,2:4,5]pyrazino[1,2:1,6]pyrido[3,4-*b*]indole (5d): Yield: 250 mg (95%): mp: 230–235 °C; $[\alpha]_D^{20}$ = +62 (c=0.39, $CHCl_3$/CH_3OH, 1:1); ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=11.55 (s, 1H), 10.59 (s, 1H), 7.60 (d, J=7.9 Hz, 1H), 7.49 (d, J=8.1 Hz, 1H), 7.31 (m, 2H), 7.24 (s, 1H), 7.04 (m, 2H), 6.97 (m, 2H), 5.66 (d, J=8.3 Hz, 1H), 4.27 (m, 2H), 3.57 (m, 2H), 3.41 (m, 1H), 3.23 (dd, J=15.1, 5.3 Hz, 1H), 3.08 (m, 2H), 2.93 (m, 2H), 2.69 (m, 1H), 2.27 (m, 2H), 2.01 (m, 1H), 1.88 ppm (m, 5H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ=185.6, 167.2, 164.4, 136.7, 136.4, 133.6, 127.8, 126.4, 124.4, 121.8, 121.4, 119.3, 119.0, 118.8, 118.3, 111.8, 110.0, 106.1, 62.9, 58.4, 55.3, 47.7, 45.4, 44.4, 31.3, 29.9, 27.3, 25.8, 22.8, 22.0 ppm; MS (ESI+): m/z: 526 $[M+H]^+$; Anal. calcd for $C_{30}H_{31}N_5O_4$: C 68.55, H 5.94, N 13.32, found: C 68.32, H 5.79, N 13.08.

(5a*S*,12*S*,14a*S*)-5,14-Dioxo-12-(2-lysine-*N*-ylethyl-1-yl)-1,2,3,5,5a,6,11,12,14,14a-decahydro-5*H*,14*H*-pyrolo-[1,2:4,5]pyrazino[1,2:1,6]pyrido[3,4-*b*]indole (5e): Yield: 110 mg (90%): mp: 220–225 °C; $[\alpha]_D^{20}$ = +36 (c=0.39, $CHCl_3$/CH_3OH, 1:1); ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=11.03 (s, 1H), 10.59 (s, 1H), 7.46 (d, J=7.5 Hz, 1H), 7.34 (d, J=7.5 Hz, 1H), 7.10 (t, J=7.5 Hz, 1H), 7.09 (t, J=7.6 Hz, 1H), 5.73 (dd, J=11.1, 3.0 Hz, 1H), 4.37 (m, 1H), 4.31 (dd, J=11.5, 3.6 Hz, 1H), 3.12 (m, 2H), 2.98 (m, 2H), 2.53 (m, 2H), 2.37 (m, 2H), 2.28 (m, 2H), 2.18 (m, 2H), 2.12 (s, 2H), 1.99 (m, 2H), 1.88 (m, 2H), 1.64 (m, 2H), 1.55 (m, 2H), 1.25 ppm (m, 2H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ=175.0, 169.3, 164.6, 136.4, 133.2,

126.1, 121.0, 118.7, 118.0, 111.4, 106.3, 58.6, 57.1, 55.2, 47.2, 45.9, 43.0, 42.5, 34.8, 31.6, 31.0, 29.2, 25.1, 22.4, 21.3, 17.1 ppm; MS (ESI+): *m/z*: 468 $[M+H]^+$; Anal. calcd for $C_{25}H_{33}N_5O_4$: C 64.22, H 7.11, N 14.98, found: C 64.01, H 7.02, N 14.77.

(5a*S*,12*S*,14a*S*)-5,14-Dioxo-12-(2-serine-*N*-ylethyl-1-yl)-1,2,3,5,5a,6,11,12,14,14a-decahydro-5*H*,14*H*-pyrolo-[1,2:4,5]pyrazino[1,2:1,6]pyrido[3,4-*b*]indole (5 f): Yield: 186 mg (87%): mp: 230–235 °C; $[\alpha]_D^{20}=+83$ ($c=0.29$, $CHCl_3/CH_3OH$, 1:1); ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=11.67$ (s, 1H), 10.61 (s, 1H), 7.43 (d, $J=7.8$ Hz, 1H), 7.35 (d, $J=8.1$ Hz, 1H), 7.07 (t, $J=7.6$ Hz, 1H), 6.98 (t, $J=7.5$ Hz, 1H), 5.71 (dd, $J=10.2$, 3.5 Hz, 1H), 4.32 (m, 1H), 3.71 (m, 1H), 3.61 (m, 2H), 3.44 (m, 1H), 3.12 (m, 2H), 2.95 (m, 1H), 2.75 (s, 1H), 2.28 (m, 2H), 2.09 (s, 1H), 2.04 (m, 1H), 1.97 (m, 1H), 1.85 (m, 5H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta=174.1$, 167.1, 164.4, 136.5, 134.0, 126.5, 121.6, 119.1, 118.2, 111.9, 105.9, 64.5, 61.2, 58.4, 55.4, 47.8, 45.9, 44.6, 31.3, 29.8, 25.8, 23.0 ppm; MS (ESI+): *m/z*: 427 $[M+H]^+$; Anal. calcd for $C_{22}H_{26}N_4O_5$: C 61.96, H 6.15, N 13.14, found: C 62.19, H 6.00, N 12.92.

(5a*S*,12*S*,14a*S*)-5,14-Dioxo-12-(2-glycine-*N*-ylethyl-1-yl)-1,2,3,5,5a,6,11,12,14,14a-decahydro-5*H*,14*H*-pyrolo-[1,2:4,5]pyrazino[1,2:1,6]pyrido[3,4-*b*]indole (5 g): Yield: 176 mg (89%): mp: 233–237 °C; $[\alpha]_D^{20}=+59$ ($c=0.37$, $CHCl_3/CH_3OH$, 1:1); ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=11.85$ (s, 1H), 10.59 (s, 1H), 7.43 (d, $J=7.8$ Hz, 1H), 7.37 (d, $J=8.1$ Hz, 1H), 7.07 (t, $J=7.2$ Hz, 1H), 6.98 (t, $J=7.2$ Hz, 1H), 5.78 (dd, $J=9.9$, 4.3 Hz, 1H), 4.32 (m, 1H), 3.62 (q, $J=14.8$ Hz, 1H), 3.43 (m, 1H), 3.21 (m, 2H), 3.10 (dd, $J=15.1$, 4.0 Hz, 1H), 2.95 (m, 1H), 2.85 (m, 2H), 2.26 (m, 2H), 2.05 (m, 1H), 1.91 ppm (m, 5H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta=173.2$, 167.0, 164.4, 136.6, 133.9, 126.5, 121.5, 119.0, 118.1, 111.9, 106.0, 58.4, 55.4, 51.6, 47.6, 45.3, 31.5, 29.8, 25.9, 22.6, 21.9 ppm; MS (ESI+): *m/z*: 397 $[M+H]^+$; Anal. calcd for $C_{21}H_{24}N_4O_4$: C 63.62, H 6.10, N 14.13, found: C 63.41, H 5.93, N 13.90.

(5a*S*,12*S*,14a*S*)-5,14-Dioxo-12-(2-leucine-*N*-ylethyl-1-yl)-1,2,3,5,5a,6,11,12,14,14a-decahydro-5*H*,14*H*-pyrolo-[1,2:4,5]pyrazino[1,2:1,6]pyrido[3,4-*b*]indole (5 h): Yield: 199 mg (88%): mp: 127–130 °C; $[\alpha]_D^{20}=+83$ ($c=0.36$, $CHCl_3/CH_3OH$, 1:1); ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=11.40$ (s, 1H), 10.60 (s, 1H), 7.44 (d, $J=7.8$ Hz, 1H), 7.35 (d, $J=8.1$ Hz, 1H), 7.09 (t, $J=7.3$ Hz, 1H), 7.00 (t, $J=7.5$ Hz, 1H), 5.66 (dd, $J=11.0$, 2.8 Hz, 1H), 4.34 (t, $J=5.0$ Hz, 1H), 4.31 (dd, $J=12.5$, 8.2 Hz, 1H), 3.60 (m, 1H), 3.44 (dt, $J=7.2$, 2.6 Hz, 1H), 3.32 (t, $J=6.9$ Hz, 1H), 3.11 (dd, $J=15.1$, 4.1 Hz, 1H), 2.96 (t, $J=12.1$ Hz, 1H), 2.82 (t, $J=9.9$ Hz, 1H), 2.35 (s, 1H), 2.29 (m, 1H), 2.18 (dq, $J=10.7$, 2.8 Hz, 1H), 1.90 (m, 4H), 1.76 (m, 2H), 1.53 (m, 2H), 0.90 ppm (dd, $J=12.4$ Hz, 6.5 Hz, 6H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta=171.9$, 167.3, 164.3, 136.4, 133.6, 126.4, 121.8, 119.2, 118.3, 111.8, 106.2, 60.7, 58.4, 58.1, 55.4, 47.6, 45.7, 45.4, 43.9, 30.9, 29.8, 25.7, 24.9, 23.3, 22.7 ppm; MS (ESI+): *m/z*: 453 $[M+H]^+$; Anal. calcd for $C_{25}H_{32}N_4O_4$: C 66.35, H 7.13, N 12.38, found: C 66.54, H 7.29, N 12.60.

(5a*S*,12*R*,14a*S*)-5,14-Dioxo-12-(2-alanine-*N*-ylethyl-1-yl)-1,2,3,5,5a,6,11,12,14,14a-decahydro-5*H*,14*H*-pyrolo-[1,2:4,5]pyrazino[1,2:1,6]pyrido[3,4-*b*]indole (5′a): Yield: 195 mg (95%): mp: 224–228 °C; $[\alpha]_D^{20}=-67$ ($c=0.45$, $CHCl_3/CH_3OH$, 1:1); ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=12.00$ (s, 1H), 10.62 (s, 1H), 7.43 (d, $J=7.8$ Hz, 1H), 7.37 (d, $J=8$ Hz, 1H), 7.07 (t, $J=7.6$ Hz, 1H), 6.98 (t, $J=7.6$ Hz, 1H), 5.83 (d, $J=10.3$ Hz, 1H), 4.34 (dd, $J=11.7$, 4.3 Hz, 2H), 3.64 (q, $J=6.3$ Hz, 2H), 3.43 (dt, $J=2.6$ Hz, 9.0 Hz, 1H), 3.26 (q, $J=6.6$ Hz, 1H), 3.12 (dd, $J=15.0$, 3.8 Hz, 1H), 2.96 (t, $J=12.6$ Hz, 1H), 2.88 (s, 1H), 2.28 (t, $J=5.2$ Hz, 1H), 2.08 (s, 1H), 1.97 (m, 1H), 1.87 (s, 4H), 1.30 ppm (d, $J=6.9$ Hz, 3H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta=173.6$, 167.3, 164.3, 136.6, 133.8, 126.4, 121.5, 119.0, 118.2, 111.9, 105.9, 80.0, 58.4, 55.4, 47.5, 46.0, 45.4, 29.9, 25.9, 22.8, 21.8, 16.7 ppm; MS (ESI+): *m/z*: 411 $[M+H]^+$; Anal. calcd for $C_{22}H_{26}N_4O_4$: C 64.37, H 6.38, N 13.65, found: C 64.55, H 6.52, N 13.42.

(5a*S*,12*R*,14a*S*)-5,14-Dioxo-12-(2-aspartic-acid-*N*-ylethyl-1-yl)-1,2,3,5,5a,6,11,12,14,14a-decahydro-5*H*,14*H*-pyrolo-[1,2:4,5]pyrazino[1,2:1,6]pyrido[3,4-*b*]indole (5′b): Yield: 222 mg (95%): mp: 218–222 °C; $[\alpha]_D^{20}=-75$ ($c=0.56$, $CHCl_3/CH_3OH$, 1:1); ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=11.6$ (s, 1H), 7.42 (d, $J=7.9$ Hz, 1H), 7.35 (d, $J=8.2$ Hz, 1H), 7.06 (t, $J=7.0$ Hz, 1H), 6.97 (t, $J=7.4$ Hz, 1H), 5.78 (s, 1H), 4.32 (m, 2H), 3.62 (q, $J=10.4$ Hz, 2H), 3.43 (t, $J=8.8$ Hz, 2H), 3.09 (dd, $J=15.2$, 4 Hz, 2H), 2.94 (m, 1H), 2.70 (m, 1H), 2.26 (m, 2H), 2.08 (m, 2H), 1.91 ppm (m, 4H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta=174.5$, 167.1, 164.5, 136.5, 134.1, 126.5, 121.5, 118.7, 118.1, 111.8, 105.9, 79.7, 58.2, 57.3, 55.1, 47.8, 46.1, 45.4, 29.8, 25.8, 23.8, 21.5 ppm; MS (ESI+): *m/z*: 469 $[M+H]^+$; Anal. calcd for $C_{23}H_{26}N_4O_6$: C 60.78, H 5.77, N 12.33, found: C 60.99, H 5.91, N 12.10.

(5a*S*,12*R*,14a*S*)-5,14-Dioxo-12-(2-phenylalanine-*N*-ylethyl-1-yl)-1,2,3,5,5a,6,11,12,14,14a-decahydro-5*H*,14*H*-pyrolo-[1,2:4,5]pyrazino[1,2:1,6]pyrido[3,4-*b*]indole (5′c): Yield: 232 mg (96%): mp: 180–183 °C; $[\alpha]_D^{20}=-24$ ($c=0.33$, $CHCl_3/CH_3OH$, 1:1); ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=11.32$ (s, 1H), 7.44 (d, $J=7.8$ Hz, 1H), 7.33 (d, $J=8.1$ Hz, 1H), 7.27 (m, 3H), 7.20 (m, 1H), 7.09 (t, $J=7.5$ Hz, 2H), 6.99 (t, $J=7.5$ Hz, 1H), 5.67 (dd, $J=10.5$, 2.9 Hz, 1H), 4.30 (m, 2H), 3.60 (m, 2H), 3.43 (m, 2H), 3.09 (m, 2H), 2.97 (m, 2H), 2.83 (m, 1H), 2.75 (m, 1H), 2.26 (m, 2H), 2.04 (m, 1H), 1.92 (m, 2H), 1.85 ppm (m, 2H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta=171.8$, 167.2, 164.3, 138.0, 136.4, 133.6, 129.7, 128.7, 126.9, 126.5, 121.7, 119.2, 118.3, 111.8, 106.2, 62.8, 58.4, 55.4, 47.6, 45.4, 44.1, 37.4, 31.2, 29.9, 25.8, 21.9, 21.5 ppm; MS (ESI+): *m/z*: 487 $[M+H]^+$; Anal. calcd for $C_{28}H_{30}N_4O_4$: C 69.12, H 6.21, N 11.51, found: C 69.30, H 6.06, N 11.73.

(5a*S*,12*R*,14a*S*)-5,14-Dioxo-12-(2-tryptophan-*N*-ylethyl-1-yl)-1,2,3,5,5a,6,11,12,14,14a-decahydro-5*H*,14*H*-pyrolo-[1,2:4,5]pyrazino[1,2:1,6]pyrido[3,4-*b*]indole (5′d): Yield: 250 mg (93%): mp: 148–152 °C; $[\alpha]_D^{20}=-50$ ($c=0.52$, $CHCl_3/CH_3OH$, 1:1); ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=11.51$ (s, 1H), 10.61 (s, 1H), 7.60 (d, $J=7.9$ Hz, 1H), 7.44 (d, $J=8.1$ Hz, 1H), 7.33 (dd, $J=3.7$ Hz, 8.1 Hz, 2H), 7.26 (d, $J=1.8$ Hz, 1H), 7.07 (m, 2H), 6.99 (t, $J=7.6$ Hz, 1H), 6.95 (t, $J=7.6$ Hz, 1H), 5.70 (dd, $J=10.6$, 3.2 Hz, 1H), 4.31 (m, 2H), 3.69 (t, $J=6.1$ Hz, 1H), 3.58 (m, 1H), 3.41 (m, 1H), 3.25 (dd, $J=15.3$, 5.6 Hz, 1H), 3.17 (dd, $J=15.2$, 6.8 Hz, 1H), 3.10 (dd, $J=15.1$, 4.0 Hz, 1H), 2.94 (m, 2H), 2.86 (m, 1H), 2.32 (m, 1H), 2.23 (m, 1H), 2.07 (m, 1H), 1.92 (s, 3H), 1.81 ppm (m, 2H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta=172.4$, 167.3, 164.3, 136.7, 136.4, 133.6, 127.8, 126.5, 124.4, 121.7, 121.4, 119.2, 118.9, 118.7, 118.3, 111.8, 109.7, 106.2, 62.2, 58.4, 55.4, 47.6, 45.4, 44.1, 29.8, 27.3, 25.8 ppm; MS (ESI+): *m/z*: 526 $[M+H]^+$; Anal. calcd for $C_{30}H_{31}N_5O_4$: C 68.55, H 5.94, N 13.32, found: C 68.34, H 5.77, N 13.10.

(5a*S*,12*R*,14a*S*)-5,14-Dioxo-12-(2-lysine-*N*-ylethyl-1-yl)-1,2,3,5,5a,6,11,12,14,14a-decahydro-5*H*,14*H*-pyrolo-[1,2:4,5]pyrazino[1,2:1,6]pyrido[3,4-*b*]indole (5′e): Yield: 112 mg (90%): mp: 150–154 °C; $[\alpha]_D^{20}=-79$ ($c=0.33$, $CHCl_3/CH_3OH$, 1:1); ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=10.97$ (s, 1H), 10.55 (s, 1H), 7.43 (d, $J=7.5$ Hz, 1H), 7.31 (d, $J=7.5$ Hz, 1H), 7.12 (t, $J=7.5$ Hz, 1H), 7.07 (t, $J=7.6$ Hz, 1H), 5.72 (dd, $J=11.1$, 3.0 Hz, 1H), 4.38 (m, 1H), 4.34 (dd, $J=11.5$, 3.6 Hz, 1H), 3.11 (m, 2H), 2.96 (m, 2H), 2.40 (m, 2H), 2.30 (m, 2H), 2.26 (m, 2H), 2.14 (m, 2H), 2.01 (s, 2H), 1.98 (m, 2H), 1.87 (m, 2H), 1.62 (m, 2H), 1.53 (m, 2H), 1.24 ppm (m, 2H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta=175.2$, 169.0, 164.3, 136.1, 133.0,

126.6, 121.3, 118.5, 118.4, 111.2, 106.0, 58.3, 57.6, 55.0, 49.9, 47.8, 43.2, 42.9, 34.3, 31.3, 31.1, 29.5, 25.4, 22.1, 21.0, 17.6 ppm; MS (ESI+): *m/z*: 468 $[M+H]^+$; Anal. calcd for $C_{25}H_{33}N_5O_4$: C 64.22, H 7.11, N 14.98, found: C 64.04, H 7.26, N 15.21.

(5a*S*,12*R*,14a*S*)-5,14-Dioxo-12-(2-serine-*N*-ylethyl-1-yl)-1,2,3,5,5a,6,11,12,14,14a-decahydro-5*H*,14*H*-pyrolo-[1,2:4,5]pyrazino[1,2:1,6]pyrido[3,4-*b*]indole (5′f): Yield: 183 mg (86%): mp: 224–230 °C; $[\alpha]_D^{20}=-67$ ($c=0.36$, $CHCl_3/CH_3OH$, 1:1); ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=11.87$ (s, 1H), 10.60 (s, 1H), 7.42 (d, $J=7.8$ Hz, 1H), 7.39 (d, $J=8.1$ Hz, 1H), 7.06 (t, $J=7.1$ Hz, 1H), 6.97 (t, $J=7.5$ Hz, 1H), 5.84 (dd, $J=10.2$, 3.6 Hz, 1H), 4.33 (m, 1H), 3.69 (dd, $J=11.0$, 4.8 Hz, 1H), 3.61 (m, 2H), 3.44 (m, 1H), 3.12 (m, 2H), 2.95 (m, 1H), 2.82 (m, 1H), 2.28 (m, 2H), 2.07 (s, 1H), 2.03 (m, 1H), 1.97 (m, 1H), 1.87 pp, (m, 5H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta=173.7$, 167.1, 164.4, 136.5, 133.9, 126.4, 121.5, 119.0, 118.2, 112.0, 105.9, 64.7, 61.7, 58.4, 55.4, 47.5, 45.4, 44.5, 31.5, 29.8, 25.9, 22.8 ppm; MS (ESI+): *m/z*: 427 $[M+H]^+$; Anal. calcd for $C_{22}H_{26}N_4O_5$: C 61.96, H 6.15, N 13.14, found: C 61.75, H 6.31, N 12.91.

(5a*S*,12*R*,14a*S*)-5,14-Dioxo-12-(2-glycine-*N*-ylethyl-1-yl)-1,2,3,5,5a,6,11,12,14,14a-decahydro-5*H*,14*H*-pyrolo-[1,2:4,5]pyrazino[1,2:1,6]pyrido[3,4-*b*]indole (5′g): Yield: 174 mg (88%): mp: 223–227 °C; $[\alpha]_D^{20}=-72$ ($c=0.36$, $CHCl_3/CH_3OH$, 1:1); ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=11.82$ (s, 1H), 10.62 (s, 1H), 7.41 (d, $J=7.8$ Hz, 1H), 7.36 (d, $J=8.0$ Hz, 1H), 7.06 (t, $J=7.2$ Hz, 1H), 6.99 (t, $J=7.3$ Hz, 1H), 5.74 (dd, $J=9.9$, 4.3 Hz, 1H), 4.33 (m, 1H), 3.62 (q, $J=8.6$ Hz, 1H), 3.45 (m, 1H), 3.23 (m, 2H), 3.12 (dd, $J=15.1$, 4.0 Hz, 1H), 2.94 (m, 1H), 2.87 (m, 2H), 2.27 (m, 2H), 2.05 (m, 1H), 1.90 ppm (m, 5H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta=173.0$, 167.2, 164.7, 136.4, 133.8, 126.6, 121.4, 119.1, 118.2, 111.7, 106.3, 58.5, 55.2, 51.7, 47.5, 45.2, 31.4, 29.9, 25.7, 22.8, 21.7 ppm; MS (ESI+): *m/z*: 397 $[M+H]^+$; Anal. calcd for $C_{21}H_{24}N_4O_4$: C 63.62, H 6.10, N 14.13, found: C 63.82, H 6.26, N 14.35.

(5a*S*,12*R*,14a*S*)-5,14-Dioxo-12-(2-leucine-*N*-ylethyl-1-yl)-1,2,3,5,5a,6,11,12,14,14a-decahydro-5*H*,14*H*-pyrolo-[1,2:4,5]pyrazino[1,2:1,6]pyrido[3,4-*b*]indole (5′h): Yield: 201 mg (89%): mp: 188–191 °C; $[\alpha]_D^{20}=-85$ ($c=0.52$, $CHCl_3/CH_3OH$, 1:1); ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=11.4$ (d, 1H), 10.62 (s, 1H), 7.45 (d, $J=7.8$ Hz, 1H), 7.35 (d, $J=8.1$ Hz, 1H), 7.09 (t, $J=7.3$ Hz, 1H), 7.00 (t, $J=7.4$ Hz, 1H), 5.74 (d, $J=9.3$ Hz, 1H), 4.34 (m, 1H), 3.60 (q, $J=8.6$ Hz, 2H), 3.43 (dt, $J=8.2$, 2.2 Hz, 1H), 3.28 (q, $J=6.5$ Hz, 1H), 3.12 (dd, $J=15.2$, 3.9 Hz, 1H), 2.96 (t, $J=17.1$ Hz, 1H), 2.83 (t, $J=9.7$ Hz, 1H), 2.34 (s, 1H), 2.26 (m, 1H), 2.11 (s, 1H), 1.90 (m, 4H), 1.71 (m, 2H), 1.52 (m, 2H), 0.90 ppm (dd, $J=12.5$ Hz, 6.5 Hz, 6H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta=173.0$, 171.7, 167.6, 164.4, 136.5, 133.1, 126.4, 121.9, 119.3, 118.3, 111.9, 106.3, 60.9, 58.4, 55.4, 47.5, 46.0, 45.5, 43.7, 30.2, 29.8, 25.8, 24.8, 23.0, 19.0 ppm; MS (ESI+): *m/z*: 453 $[M+H]^+$; Anal. calcd for $C_{25}H_{32}N_4O_4$: C 66.35, H 7.13, N 12.38, found: C 66.56, H 7.00, N 12.15.

Biology

General: Biological data were analyzed using an ANOVA test, and $p<0.05$ was considered statistically significant.

Animals: Male imprinting control region (ICR) mice weighing 28–31 g and male Wister rats weighing 250–300 g, purchased from the Experimental Animal Center at Peking University Health Science Center, were maintained at 21 °C with a natural day/night cycle in a conventional animal colony. The assessments described herein were performed according to a protocol reviewed and approved by the Ethics Committee of Capital Medical University. The committee assures the welfare of animals in research is maintained in accordance with the requirements of the Animal Welfare Act and according to the committee's guide for the care and use of laboratory animals.

*In vivo tail bleeding time assay of oral **5a–h** and **5′a–h**:* Male ICR mice were housed in a 12 h light/12 h dark cycle at 21±2 °C for one day before operation. Each of them was administered (oral gavage) 0.6 mL of normal saline (NS) containing each of **5a–h** and **5′a–h** (0.1 μmol kg^{-1}), or 0.6 mL NS containing aspirin (165 μmol kg^{-1}) or 0.6 mL NS alone. Thirty minutes after administration, mice were placed in a tube holder with tail protruding, and a 2 mm cut was made on the tail. Flowing blood was gently wiped away with a tissue every 30 s until bleeding ceased, and the observed bleeding time was recorded.

*In vivo thrombogenesis assay of oral **5a–h** and **5′a–h**:* Male Wistar rats were fed 0.1 μmol kg^{-1} of each of **5a–h** and **5′a–h** in NS or aspirin (165 μmol kg^{-1}) in NS (0.6 mL) or NS alone, and then the rats were anesthetized with sodium pentobarbital (80.0 mg kg^{-1}, i.p.). Thirty minutes later the right carotid artery and left jugular vein were separated. A weighed 6 cm thread was inserted into a polyethylene tube, which was filled with heparin sodium (50 IU mL^{-1} in NS) and one end was inserted into the left jugular vein, while the other was inserted into the right carotid artery. Blood flowed from the right carotid artery to the left jugular vein through the polyethylene tube for 15 min. The thread was removed, and the weight of the wet thrombus was recorded.

*In vitro thrombus clot lysis assay of UK with and without **5c**:* The cylindrical thrombus prepared from rat blood was carefully removed from the cylindrical thrombus-forming tube and suspended into an incubation bottle filled with 8 mL distilled water for 1 h. The thrombus was then removed from the bottle, weighed precisely to record its initial weight, and suspended in another incubation bottle filled with 8 mL NS or a solution of UK (100 IU mL^{-1}, Sigma, UK) in 8 mL NS or UK (100 IU mL^{-1}) plus **5c** (100, 10, or 1 nM) in 8 mL NS. The bottle was incubated at 37 °C and agitated on a rocking-bed (70 rpm) for 1 h, and the thrombus was precisely weighed to record its final weight. Subtracting the final weight from the initial weight, the decreased weight of the thrombus was obtained and used to represent the in vitro thrombus lysis potency.

*In vivo thrombolytic assay of UK on rats pretreated with oral **5c**:* Male Wistar rats were fed with three doses (100, 10, and 1 nmol kg^{-1}) of **5c** in NS or NS (0.6 mL) alone. The rats were then anesthetized with sodium pentobarbital (80.0 mg kg^{-1}, i.p.). Thirty minutes later the right carotid artery and left jugular vein were separated. The cylindrical thrombus-supporting helix (15 circles, pitch 1.2 mm, diameter 1.0 mm) was placed into the cylindrical thrombus-forming tube, and this tube was then immediately filled with arterial blood (0.2 mL) from the right carotid artery. After 15 min the cylindrical thrombus was carefully removed from the thrombus-forming tube, weighed precisely to record its initial weight, and then inserted into a polyethylene tube. The tube was filled with heparin sodium (50 IU mL^{-1} in NS), and one of the ends was inserted into the left jugular vein. Heparin sodium was injected via the other end of the polyethylene tube as the anticoagulant, followed by injection of a solution of UK (3 mL, 20000 IU kg^{-1}) in NS. The blood was circulated through the polyethylene tube for 90 min, after which the cylindrical thrombus was carefully taken out of the polyethylene tube, and its final weight was recorded precisely. Subtracting the final weight from the initial weight, the decreased weight of the thrombus was obtained and used to represent the in vivo thrombolytic potency.

*Effect of **5c** on electrophoresis of UK and plasminogen*: SDS-PAGE is a general technique for detecting the action of urokinase on plasminogen (PLG), and was carried out in a vertical slab gel unit DYY-6C. The separation gel (sodium dodecyl sulfate, GE healthcare, USA) was 10 cm high, 1.5 mm thick, and had a total polyacrylamide (T) concentration of 12%, with a cross-linking component (C; bisacrylamide) of 4%, and contained 10% glycerol (GE healthcare, USA). A stacking gel of ~1.2 cm height with 4% T and 3% C was used. A solution of PLG (5 μL, final concentration 0.1 U mL^{-1}, Sigma, USA) and NS (10 μL) or a solution of PLG (5 μL, final concentration 0.1 U mL^{-1}) and UK (5 μL, final concentration 100 U mL^{-1}) plus 5 μL NS or a solution of PLG (5 μL, final concentration 0.1 U mL^{-1}) and UK (5 μL, final concentration 100 U mL^{-1}) plus 5 μL **5c** (in a series of final concentrations ranging from 0.01 to 2500 ng μL^{-1}) was incubated at 37 °C for 30 min. The separation of these solutions was performed at a constant current of ~100 mA, starting at 90 V (increasing to ~120 V at the end of the run), and lasting for 2 h until the bromophenol blue (as a tracer, Sigma, USA) band had reached the bottom of the gel. Gels were stained in methanol/acetic acid/water (45:10:45) with coomassie brilliant blue R250 (0.1%, Amresco, USA) for 4 h. The gels were kept in methanol/acetic acid/water (10:10:80) for ~14 h for destaining.

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