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Chemical and microbial semi-synthesis of tetrahydroprotoberberines as inhibitors on tissue factor procoagulant activity

Hai-Xia Ge [a,c,†], Jian Zhang [a,†], Ling Chen [b], Jun-Ping Kou [b,*], Bo-Yang Yu [a,*]

[a] State Key Laboratory of Natural Medicines, China Pharmaceutical University, 24# Tongjia Xiang Street, Nanjing 210009, China
[b] Department of Complex Prescription of Traditional Chinese Medicine, China Pharmaceutical University, 639# Longmian Avenue, Nanjing 211198, China
[c] Department of Pharmacy, Huzhou Teachers College, Huzhou 313000, China

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ABSTRACT

To discover new inhibitors on tissue factor procoagulant activity, 21 tetrahydroprotoberberines were screened on the model of human THP-1 cells stimulated by lipopolysaccharide. Among these tetrahydroprotoberberines, several unique compounds were synthesized through microbial transformation: compound **6** (*l*-corydalmine) was obtained through regio-selective demethylation by *Streptomyces griseus* ATCC 13273, whereas compounds**4a, 4b, 5h**, and **5i** were microbial glycosylation products by *Glliocladium deliquescens* NRRL1086. The bioassay results showed that compounds **3** (tetrahydroberberine), **10** (tetrahydroberberrubine), and **5f** (cinnamyl ester of **5**) and **5i** (glycosidic product of **5**), exhibited the most potential effects, with IC_{50} values of 8.35, 6.75, 3.75, and 8.79 nM, respectively. The preliminary structure and activity relationship analysis revealed that the 2,3-methylenedioxy group of the A ring was essential for the strong inhibitory effects, and the *R* configuration of the chiral center C-14 showed higher activity than *S*-form products. The formation of fatty acid or aromatic acid esters of compound **5**, except the cinnamyl esters, would weaken its effects. It is also interesting to note that the glycosylation of tetrahydroprotoberberines will maintain and even enhance the inhibitory effects. Because of the importance of glycochemistry in new drug discovery and development, this deserves further exploration and may provide some guide on the semi-synthesis of tetrahydroprotoberberines as tissue factor pathway inhibitors. Our findings also provide some potential leading compounds for tissue factor-related diseases, such as cancer and cardiovascular diseases.

1. Introduction

Tissue factor (TF), a 47-kD membrane-bound glycoprotein and a member of the cytokine-receptor superfamily, is the primary initiator of the coagulation cascade in both hemostasis and thrombosis.[1] Elevated levels of TF are observed in patients with cardiovascular risk factors, such as hypertension, diabetes, dyslipidemia, and smoking, as well as in those with acute coronary syndromes.[2] TF may indeed be involved in the pathogenesis of atherosclerosis by promoting thrombus formation. Thus, inhibition of TF procoagulant activity or induction appears to be an attractive target for the treatment of cardiovascular diseases.[3] Furthermore, TF upregulation resulting from its enhanced exposure to clotting factor FVII/FVIIa often manifests not only a hypercoagulable state but also an inflammatory one.[4] Advanced cancer is associated with a hypercoagulable state, and TF expression by cancer cells has received widespread attention because of its significant contribution to the pathogenesis of cancer progression and metastasis.[5] Therefore, pharmaceutical intervention in the TF pathway could possibly provide novel treatment strategies for cardiovascular disease, septicemia, and tumor. Screening for new TF pathway inhibitors is thus becoming one of the hot points in the field of drug development and correlative basic research.[6] To explore new TF pathway inhibitors, we optimized one convenient and high-throughput chromogenic assay method by using a commercial human prothrombin complex instead of purified coagulation factors.[7] This method was tested on simvastatin and curcumin, which showed IC_{50} values of 0.1–1 μM, which fits well with the reported results on previous models.[8–10]

Tetrahydroprotoberberines (THPB), a class of abundant, naturally occurring tetracyclic alkaloids that contain an isoquinoline skeleton with substituent methoxyl or hydroxyl groups on the two benzene rings and a stereogenic center at C-14, have been demonstrated to possess various biological activities. These include analgesic activity; anticonvulsant, anti-anxiety, antidepressant, and antipsychotic activity in the central nervous system; anti-

* Corresponding author. Tel.: +86 25 83271321; fax: +86 25 83313080 (B.-Y.Y.); tel.: +86 25 86185157; fax: +86 25 86185158 (J.-P.K.).
E-mail addresses: junpingkou@163.com (J.-P. Kou), boyangyu59@163.com (B.-Y. Yu).
† These authors contributed equally to the work.

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arrhythmic, antihemorrhagic, and hypotensive activities in the cardiovascular system; and other activities.[11] In the screening test, we found that some protoberberines showed an inhibitory effect on TF procoagulant activity in human monocytes stimulated by lipopolysaccharide (LPS).[12] In this study, we prepared a series of THPB derivatives by chemical and microbial semi-synthesis to further evaluate their applications as TF pathway inhibitors. Because biocatalysis has become one of the promising methods for the structural modification of natural products, we demonstrate several unique microbial transformations. Compound **6** (*l*-corydalmine) was synthesized through regio-selective demethylation by *Streptomyces griseus* ATCC 13273, and compounds **4a**, **4b**, **5h**, and **5i** were microbial glycosylation products by *Gliocladium deliquescens* NRRL1086. Their inhibitory effects on TF procoagulant activity in human monocytes stimulated by LPS were assayed, and the preliminary structure–activity relationship was also investigated. This study should provide a better understanding of TF pathway inhibitor discovery and the multi-activity of THPBs for further clinical application.

2. Results and discussion

2.1. Synthesis of THPB analogues 1–10 and their inhibitory effects on TF procoagulant activity

2.1.1. Synthesis of THPB analogues 1–10

Compounds **1** (*l*-tetrahydropalmatine) and **8** (*l*-stepholidine, *l*-SPD) were obtained commercially, and compound **2** was the racemic mixture of compound **1**. Compounds **2** and **3** were obtained by palmatine and berberine hydrochloride reduction reaction with $NaBH_4$, respectively,[13] and both has a rotation value of 0.

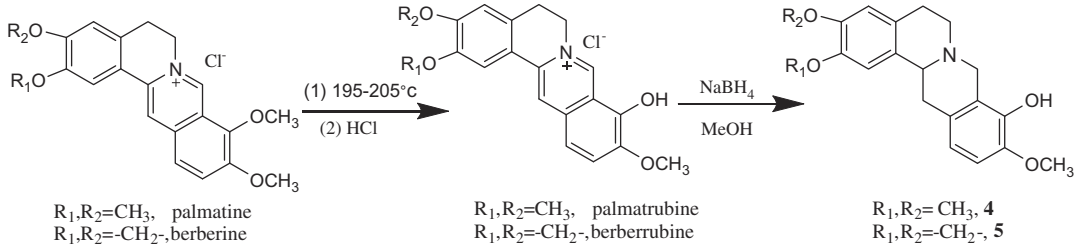
Compounds **4** and **5** were synthesized as in Scheme 1, in which the chloride salt of palmatine and berberine undergoes pyrolysis monodemethylation[14] to yield a red compound, palmatrubine, and berberrubine, reducing them to **4** and **5**. Compound **7** was synthesized from berberine hydrochloride reacted with phloroglucinol in H_2SO_4 (60%) at 90–95 °C and reduction reaction (Scheme 2).[13,15] Compound **6** (*l*-corydalmine, *l*-CDL) was prepared by bioconversion of *Streptomyces griseus* ATCC 13273 from *l*-tetrahydropalmatine (Scheme 3). Compounds **9** and **10**, a pair of enantiomers of compound **5**, were obtained through the classical chemical resolution by using (+)-di-*p*-toluoyl-L-tartaric acid (DTTA) in 95% ethanol (Scheme 4),[16] and their absolute configuration was confirmed through the chiral column HPLC and CD spectrum according to the literature.[17]

2.1.2. Inhibitory effects of THPB analogues 1–10 on TF procoagulant activity

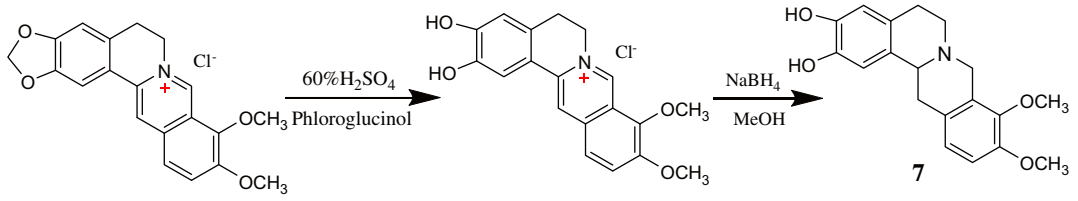
The THPB analogues (**1–10**) were screened for their inhibitory effect on TF procoagulant activity in human THP-1 cells stimulated by LPS. Table 1 shows the results. Most of the compounds exhibited higher activity than the positive control curcumin. Compounds **3**, **5**, and **10** even showed IC_{50} values of 8.35, 12.17, and 6.75 nM, respectively, indicating that THPBs were one type of effective TF pathway inhibitors. After a careful analysis of the chemical structures of the related compounds, we found that the substitute groups of the A ring may play an essential role in the TF inhibitory effects. The compounds bearing the methylenedioxy group at C-2 and C-3 showed the highest activities. The substitution group of two free hydroxyl groups or the methyloxy may decrease the effects; in particular, the compound with the substitution of ortho-dihydroxyl groups in ring A exhibited the lowest activity with an IC_{50} value of 968.90 nM, which was almost a hundredfold higher compared with compounds **3** or **10**. In our experiment, the substitution groups on the D ring seemed to have less impact on THPB analogues' inhibitory effect.

Another interesting aspect of the results was that the stereochemistry of the chiral center of C-14 may also influence the effects. In our tested compounds, there was one pair of isomers: compound **9**, bearing the *S*-form configuration of C-14, and compound **10**, which was the *R*-form. Compound **10** showed higher activity than compound **9**, with the IC_{50} values of 6.75 and 36.41 nM, respectively, indicating a higher activity of *R*-form compounds compared with the corresponding *S*-form compounds. Such results could be also deduced from the results of compounds **1** and **2**. Compound **1** was the *S*-form, which exhibited an IC_{50} value of 22.78 nM, while compound **2**, as the racemic mixtures, had a much lower inhibitory effect. In the structure and activity research on THPBs on some other bioassay models, the *S*-form compounds usually had stronger activity than the *R*-form.[18] We may hypothesize from this that the inner interactions between the small molecular and the amino acid moieties of the target protein may be much different from the previous research, which deserves further exploration.

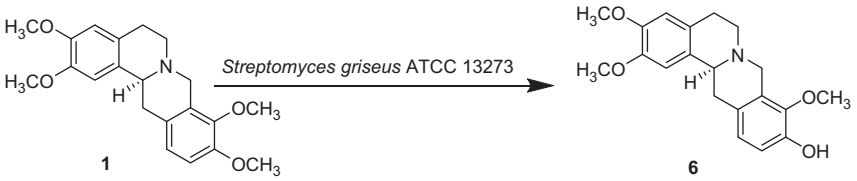
Among all the tested compounds, compound **5** was the most attractive, not only because of its strong activity but also because of the free hydroxyl group substituted on C-9, which can provide an active site for further esterification or glycosylation. Thus, we synthesized a series of derivatives of compound **5** through chemical and microbial methods.



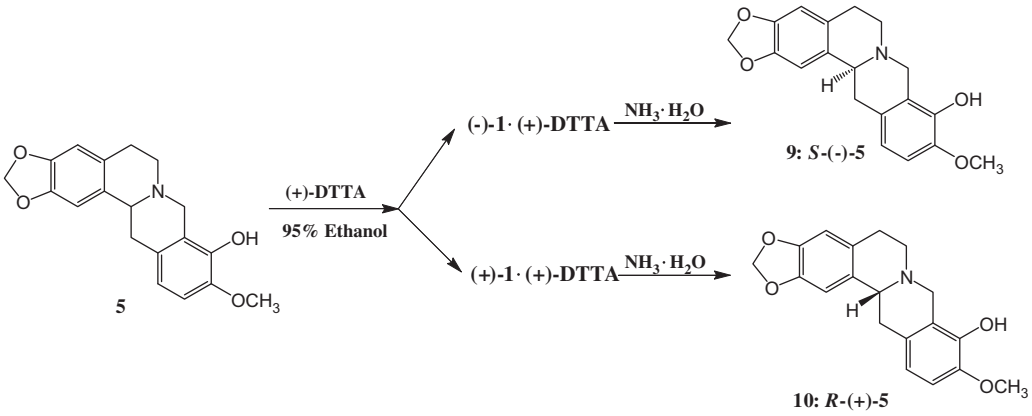
Scheme 1. Synthetic routes of compounds **4** and **5**.



Scheme 2. Synthetic routes of substrate **7**.



Scheme 3. Microbial semi-synthesis of compound **6** by *Streptomyces griseus* ATCC13273



Scheme 4. The resolution routes of compound **5**.

2.2. Synthesis of derivatives of compound 5 and their effects on TF procoagulant activity

2.2.1. Synthesis of ester derivatives of compound 5

Compound **5a** was synthesized according to the general acetylation reaction procedure by acetic anhydride and compound **5**. Compounds **5b–5g** were synthesized by acylate reaction of the corresponding acyl chloride and compound **5** (Scheme 5). The structures of compounds **5a**–**5g** were confirmed with MS, ^{13}C NMR, and ^{1}H NMR.

2.2.2. Inhibitory effects of the ester derivatives 5a–5g on TF procoagulant activity

In the ester derivatives **5a**–**5g**, different acyloxy substituents on C-9 affected the TF inhibitory activity greatly. The straight chain fatty acid esters of compounds **5** and **5a**–**5c** showed less activity, and their IC_{50} values declined considerably from 63.06 to 1501 nM along with the chain extension from C2–C4. Moreover, compounds **5d** and **5e**, which bear aromatic acyloxy substituents on C-9, also displayed much weaker activity than compound **5**. It was amazing that although compound **5f**, which was the cinnamoyl ester of **5**, showed the highest inhibitory activity with the an IC_{50} value of 3.75, compound **5g**, which was the acetylferuloyl ester of **5**, exhibited lower activity with an the IC_{50} value of 201.93 nM. In succeeding experiments we investigated the activity of cinnamic acid and ferulic acid. Both of them showed no significant inhibitory effects (IC_{50} values higher than 10 μM). The structure differences between **5f** and **5g** were the acyl moieties, so the subunit of the substituents on the benzene ring may have a great influence on TF inhibitory effect.

2.3. Synthesis of glycosylation products of compounds 4 and 5 and their effects on TF procoagulant activity

2.3.1. Synthesis of compounds 4a, 4b, 5h, and 5i by biotransformation pathway

Because of the importance of glycochemistry in drug discovery,[19,20] we synthesized the glycosidic products **5h** and **5i** based on our previous discovery.[21] *Gliocladium deliquescens* NRRL1086 was one of the most used strain in our research; it contained astonishing glycosylation ability on natural products.[21,22] Thus, compounds **4**–**10** were employed in the screening test, and compound **4** could also be transformed to **4a** and **4b** (Scheme 6).

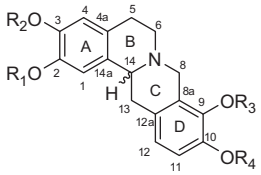
The ESI-MS of compounds **4a** and **4b** showed the same quasi-molecular ion at *m*/*z* 504.3, indicating a 162 amu mass increase from that of **4**. It was suggested that a hexose moiety was introduced, as their ^{13}C NMR spectra all showed the presence of six new signals at δ 103, 74, 76, 69, 77, and 61 ppm, which are characteristic of β-D-glucose. A coupling constant of 7.5 Hz for H-1′ indicated that the stereochemistry of the glycosidic linkage at C-1′ of D-glucose is β. This deduction was supported by the long-range coupling between H-1′ and C-9 in their HMBC experiment. All the ^{13}C NMR and ^{1}H NMR data of compounds **4a** and **4b** were carefully and unambiguously assigned by extensive 2D-NMR techniques (DEPT, HMBC, HSQC). The data revealed that the two compounds were the glycosidic products of **4**, and there was only one hydroxyl group in the skeleton of compound **4**. Further CD spectra of compound **4a** exhibited negative Cotton effects, whereas compound **4b** showed positive Cotton effects of around 210 nM. When the CD spectra of substrate **4** were compared, the *S*-form substrate of **4** showed negative Cotton effects, whereas the *R*-type isomer showed positive Cotton effects of around 210 nM.[17] It can be deduced that the chiral carbon of the aglycone part of compound **4a** is of *S* configuration, whereas compound **4b** is of *R* configuration. This conclusion was further confirmed by their optical rotation values, in which compounds **4a** and **4b** showed $[\alpha]_D^{24}$ −221.3 (*c* 0.501, pyridine) and $[\alpha]_D^{24}$ 235.1 (*c* 0.520, pyridine), respectively.

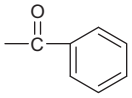
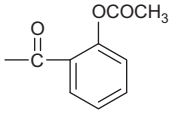
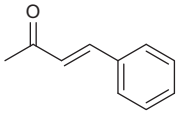
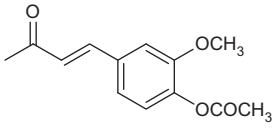
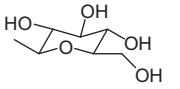
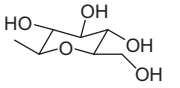
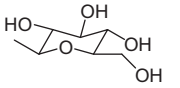
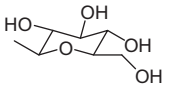
Compounds **5h** and **5i** were the glycosidic products of the racemic substrate **5**. Similar to compounds **4a** and **4b**, they were also a pair of disasteoisomers, and their structures were elucidated unambiguously based on the MS, ^{13}C NMR, ^{1}H NMR, DEPT, HMBC, HSQC, and CD.[21]

2.3.2. Inhibitory effects of the compounds 4a, 4b, 5h, and 5i on TF procoagulant activity

The IC_{50} values of the TF inhibitory activity of compounds **4a**, **4b**, **5h**, and **5i** were 16.80, 12.61, 38.59, and 8.79 nM, respectively. These four compounds are two pairs of enantiomerical isomers.

Table 1
Structures of compounds and their IC_{50} values on TF procoagulant activity

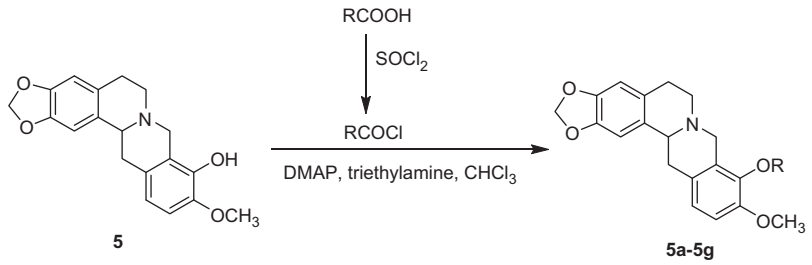


No.	C-14	R_1	R_2	R_3	R_4	IC_{50} (nM)
1	–	CH_3	CH_3	CH_3	CH_3	22.78 ± 3.73
2	±	CH_3	CH_3	CH_3	CH_3	14.98 ± 2.18
3	±	$-CH_2-$		CH_3	CH_3	8.35 ± 0.63
4	±	CH_3	CH_3	H	CH_3	187.26 ± 52.93
5	±	$-CH_2-$		H	CH_3	12.17 ± 1.24
6	–	CH_3	CH_3	CH_3	H	152.43 ± 17.86
7	±	H	H	CH_3	CH_3	968.90 ± 161.65
8	–	H	CH_3	CH_3	H	104.58 ± 15.62
9	–	$-CH_2-$		H	CH_3	36.41 ± 0.83
10	+	$-CH_2-$		H	CH_3	6.75 ± 0.38
5a	±	$-CH_2-$		$COCH_3$	CH_3	63.06 ± 3.79
5b	±	$-CH_2-$		$COCH_2CH_3$	CH_3	118.80 ± 31.97
5c	±	$-CH_2-$		$CO(CH_2)_4CH_3$	CH_3	1501.43 ± 98.06
5d	±	$-CH_2-$			CH_3	376.52 ± 29.69
5e	±	$-CH_2-$			CH_3	555.37 ± 63.21
5f	±	$-CH_2-$			CH_3	3.75 ± 0.34
5g	±	$-CH_2-$			CH_3	201.93 ± 14.73
5h	–	$-CH_2-$			CH_3	38.59 ± 6.56
5i	+	$-CH_2-$			CH_3	8.79 ± 0.72
4a	–	CH_3	CH_3		CH_3	16.80 ± 1.97
4b	+	CH_3	CH_3		CH_3	12.61 ± 1.41
Curcumin						199.63 ± 16.52

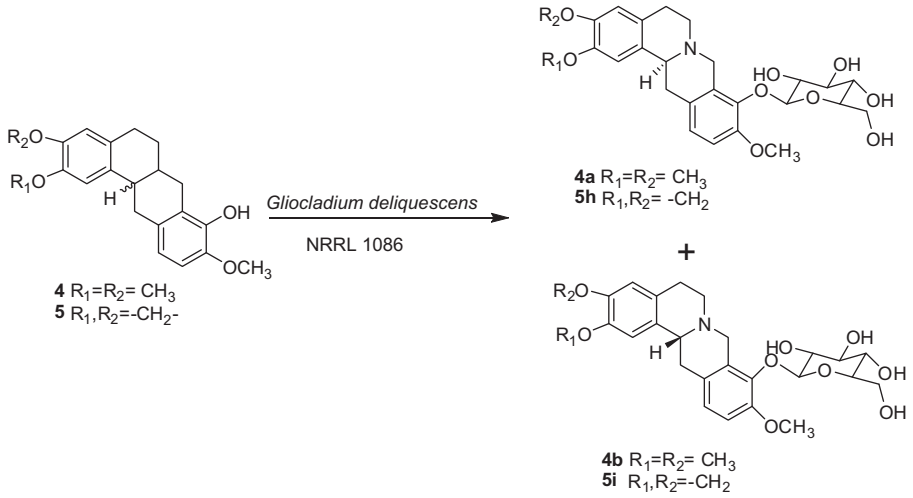
The *R*-form products also showed higher activity than the *S*-form. Compared with the IC_{50} value of compound **4**, the glycosidic products showed much enhanced activity. On the contrary, where compound **5** was concerned, the activity of glycosidic products exhibited no significant improvement. Glycosylated secondary metabolites continue to serve as an important source for drug discovery. A significant number of glycosylated small molecules, such as heparin, amikacin, and cytarabine, have been shown to be clinically useful for the treatment of a variety of diseases, including bacterial and fungal infections, cancer, and other human diseases.[20–23] As the first synthesized glycosidic THPBs, their in vivo bioassays are now under investigation.

3. Conclusions

In summary, we prepared a series of THPB analogues **1**–**10** and derivatives (**5a–5i, 4a, 4b**) through a combination of chemical and microbial means, and examined their inhibitory effects on TF procoagulant activity in human THP-1 monocytic cells stimulated by LPS. Among them, several unique compounds were first synthesized through microbial transformation. Compound **5** (*l*-corydalmine) was obtained through regio-selective demethylation by *Streptomyces griseus* ATCC 13273. Compounds **4a, 4b, 5h**, and **5i** were microbial glycosylation products that are enantio-selective by *Gliocladium deliquescens* NRRL1086 and exhibited enhanced or



Scheme 5. Synthetic routes of esters derivatives of compound **5**.



Scheme 6. Biotransformation of compounds **4** and **5** by *Gliocladium deliquescens* NRRL 1086.

similar TF inhibitory activities with their aglycone. We found that the configuration of C-14 would play an important role in their inhibitory activity and that *R*-form compounds showed stronger effects than the *S*-form. Compound **5f**, with cinnamoyl group in C-9, showed the most attractive activity among all the compounds. Due to the multifunctional role of TF in many physiopathologic processes, these potent THPB compounds would be promising lead compounds in the discovery of new cardiovascular and anticarcinogenic drugs, and this information will provide a better understanding of the chemical and biological research of THPBs.

4. Experiments

4.1. General experimental methods

Thin-layer chromatography (TLC) was performed on silica gel GF254 plates of 0.5 mm of thickness for analysis. Layers were air dried and activated at 110 °C for 0.5 h before use. NMR spectra were recorded on a Bruker Avance 500 spectrometer with tetramethylsilane as the internal standard, and chemical shifts were expressed in δ (parts per million). ESIMS experiments were performed on an Agilent 1100 Series MSD Trap mass spectrometer. CD spectra were recorded on a JASCO J-810 spectropolarimeter. Optical rotations were obtained using a JASCO P-1020 digital polarimeter. *Streptomyces griseus* ATCC 13273 and *Gliocladium deliquescens* NRRL 1086 was obtained from a courtesy of Professor J.P.N. Rosazza of University of Iowa, USA.

Cultures were grown by a two-stage procedure in 30 mL of potato medium (PD) held in a 150 mL Erlenmeyer flask. The PD medium was prepared as follows: 200 g of peeled potatoes were cut into pieces, boiled in water for 20 min, and filtered. Twenty grams of glucose, 3 g of KH_2PO_4, and 1.5 g of $MgSO_4{\cdot}7H_2O$ were added into the filtrate and diluted with distilled water to 1 L before being autoclaved at 121 °C for 15 min before use.

4.2. Preparation and spectral properties of some compounds

4.2.1. (±)-2,3,10-Trimethoxy-9-hydroxy-5,6,13,14-tetrahydro-8*H*-dibenzo[*a*,*g*]quinolizine (4)

Palmatine hydrochloride (5 g, 0.013 mol) was heated at 190 °C in a dry over under vacuum (20–30 mmHg) for 15 min. The crude product was recrystallized to provide 2.70 g (62%) of palmatrubine; A mixture of palmatrubine (2.70 g, 0.0078 mol) and 65 mL EtOH/HCl (95:5) was stirred at room temperature for 1 h, and then the solid were filtered and washed with EtOH two times to supply palmatrubine chloride 3.18 g (94%); A mixture of palmatrubine chloride (2.74 g, 0.0074 mol) and MeOH (70 mL) was refluxed for 20 min to dissolve, then $NaBH_4$ (1.40 g, 0.037 mol) was added in small portions with stirring at room temperature. Two hours later, the resulting solids were filtered, washed with MeOH to crude which recrystallized from EtOAc, to provide **4** 0.94 g (32%).

White crystals. ESI-MS *m/z*: 342.2 $[M+H]^+$. ^1H NMR ($CDCl_3$, 500 MHz) δ: 2.68 (m, 2H, 6-H, 13-H), 2.86 (m, 1H, 5-H), 3.22 (m, 3H, 13-H, 6-H, 5-H), 3.54 (d, J = 15.0 Hz, 1H, 8-H), 3.58 (m, 1H, 14-H), 3.87 (s, 6H, $-OCH_3$), 3.89 (s, 3H, $-OCH_3$), 4.25 (d, J = 15.0 Hz, 1H, 8-H), 5.68 (s, 1H, –OH), 6.68 (d, J = 8.0 Hz, 1H, 12-H), 6.74 (d, J = 8.0 Hz, 1H, 11-H), 6.62 (s, 1H, 4-H), 6.74 (s, 1H, 1-H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 108.70 (C1), 147.54 (C2), 147.48 (C3), 109.00 (C4), 121.29 (C4a), 29.31 (C5), 51.46 (C6), 53.51 (C8), 126.88 (C8a), 141.56 (C9), 144.06 (C10), 111.42 (C11), 119.28 (C12), 128.08 (C12a), 36.41 (C13), 59.30 (C14), 129.81 (C14a), 56.17 (2-OCH_3), 56.11 (3-OCH_3), 55.87 (10-OCH_3).

4.2.2. (±)-5,6,13,14-Tetrahydro-9-hydroxy-10-methoxy-2,3-(methylenedioxy)-8*H*-dibenzo[*a*,*g*]quinolizine (5)

Preparation and purification of **5** was carried out according to the procedure of compound **4**, starting from berberine hydrochloride (5 g, 0.0123 mol). Yield: 2.02 g (62%).

White crystal. ESI-MS *m/z*: 326.2 $[M+H]^+$. ^1H NMR ($CDCl_3$, 500 MHz) δ: 2.63 (m, 2H, 13-H, 6-H), 2.82 (m, 1H, 5-H), 3.16 (m, 3H, 13-H, 6-H, 5-H), 3.51 (d, J = 16.0 Hz, 1H, 8-H), 3.52 (m, 1H, 14-H), 3.86 (s, 3H, $-OCH_3$), 4.23 (d, J = 16.0 Hz, 1H, 8-H), 5.67 (s, 1H, 9-OH), 5.91 (d,d, J = 1.5 Hz, 2H, $-OCH_2O-$), 6.59 (s, 1H, 4-H), 6.66 (d, J = 8.5 Hz, 1H, 12-H), 6.73 (d, J = 8.5 Hz, 1H, 11-H), 6.73 (s, 1H, 1-H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 105.59 (C1), 144.07 (C2), 145.93 (C3), 108.44 (C4), 121.30 (C4a), 29.67 (C5), 51.34 (C6), 53.47 (C8), 127.93 (C8a), 141.57 (C9), 146.15 (C10), 109.02 (C11), 119.32 (C12), 128.10 (C12a), 36.57 (C13), 59.62 (C14), 131.02 (C14a), 100.76 ($-OCH_2O-$), 56.20 (10-OCH_3).

4.2.3. (−)-2,3,9-Trimethoxy-10-hydroxy-5,6,13,14-tetrahydro-8*H*-dibenzo[*a*,*g*]quinolizine (6)[24]

Using 24-h-old stage II cultures of *Streptomyces griseus* ATCC 13273, a total of 500 mg of **1** was distributed evenly among fifty 150 mL Erlenmeyer flasks. Substrate-containing cultures were incubated for 120 h, then filtrated and extracted with equal amount of EtOAc. The organic solvent layer was removed, evaporated to dryness. The extract was subjected to silica gel column chromatography eluted with petroleum ether/acetone (9:1, v/v) to afford the product **6** 0.173 g (38%).

White powders. ESI-MS *m/z*: 342.2$[M+H]^+$. ^1H NMR ($CDCl_3$, 500 MHz) δ:2.65 (m, 2H, 6-H, 13-H), 2.82 (m, 1H, 5-H), 3.16 (m, 1H, 5-H), 3.23 (m, 2H, 13-H, 6-H), 3.56 (m, 2H, 14-H, 8-H), 3.77, 3.85, 3.88 (s, 9H, 2,3,9-OCH_3), 4.22 (d, J = 15.0 Hz, 1H, 8-H), 6.23 (brs, 1H, –OH), 6.61 (s, 1H, 4-H), 6.70 (d, J = 8.0 Hz, 1H, 12-H), 6.72 (s, 1H, 1-H), 6.75 (d, J = 8.0 Hz, 1H, 11-H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 108.63 (C1), 147.55 (C2), 147.48 (C3), 111.39 (C4), 126.55 (C4a), 28.86 (C5), 51.46 (C6), 53.77 (C8), 126.96 (C8a), 143.52 (C9), 146.54 (C10), 114.66 (C11), 124.59 (C12), 127.74 (C12a), 35.97 (C13), 60.37 (C14), 129.53 (C14a), 55.80 (2-OCH_3), 56.05 (3-OCH_3), 59.38 (9-OCH_3). The optical rotations was $[\alpha]_D^{20}$ −380 (c 0.1, $CHCl_3$).

4.2.4. (±)-9,10-Dimethoxy-2,3-dihydroxy-5,6,13,14-tetrahydro-8*H*-dibenzo[*a*,*g*]quinolizine (7)

Berberine hydrochloride (5 g, 0.013 mol) and phloroglucin (5 g, 0.04 mol) were mixed with 60% H_2SO_4 (40 mL) and stirred at 95 °C in an oil bath for 20 min. The mixture was cooled, concentrated, and resuspended in H_2O/acetone (1:1), and then the intermediate was purified by silica gel chromatography ($CHCl_3$/MeOH, 15:1) to obtain 2.18 g (44%). A mixture of the intermediate (2.18 g, 0.006 mol) and MeOH (45 mL) was refluxed for 20 min to dissolve, then $NaBH_4$ (1.13 g, 0.03 mol) was added in small portions with stirring at room temperature. Two hours later, the resulting solids were filtered, washed with MeOH and purified by silica gel chromatography ($CHCl_3$/MeOH, 25:1) to obtain **7** 1.05 g (53%).

Pale yellow powders. ESI-MS *m/z*: 328.2 $[M+H]^+$. ^1H NMR (DMSO-d_6, 500 MHz) δ: 2.79 (m, 1H, 6-H), 3.04 (m, 1H, 13-H), 3.23 (m, 1H, 5-H), 3.41 (m, 2H, 6-H, 5-H), 3.58 (m, 1H, 13-H), 3.77 (m, 1H, 8-H), 3.79 (s, 3H, $-OCH_3$), 3.82 (s, 3H, $-OCH_3$), 4.35 (m, 1H, 14-H), 4.59 (m, 1H, 8-H), 6.60 (s, 1H, 4-H), 6.79 (s, 1H, 1-H), 7.04 (d, J = 8.5 Hz, 1H, 12-H), 7.07 (d, J = 8.5 Hz, 1H, 11-H), 8.99 (s, 1H, –OH), 9.28 (s, 1H, –OH). ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 109.05 (C1), 144.39 (C2), 144.12 (C3), 109.36 (C4), 114.91 (C4a), 28.89 (C5), 51.06 (C6), 53.23 (C8), 123.65 (C8a), 139.72 (C9), 143.58 (C10), 111.29 (C11), 112.40 (C12), 124.56 (C12a), 35.84 (C13), 59.52 (C14), 127.57 (C14a), 58.56 ($-OCH_3$), 55.71 ($-OCH_3$).

4.2.5. (±)-5,6,13,14-Tetrahydro-9-acetoxy-10-methoxy-2,3-(methylenedioxy)-8*H*-dibenzo[*a*,*g*]quinolizine (5a)

Compound **5** (2 g, 0.006 mol) was added to $CHCl_3$ (30 mL) and stirred for dissolving at room temperature, acetic anhydride (2.8 mL, 0.03 mol), 2 mL triethylamine and slight 4-dimethylamiopryidine were added to the solution. The mixture was stirred for 3 h at room temperature, then washed three times by water, saturated $NaHCO_3$ solution and saturated NaCl solution, respectively. The $CHCl_3$ phase was dried over sodium sulfate and evaporated to crude which recrystallized from EtOAC to obtain **5a** 1.86 g (82%).

Light yellow crystal. ESI-MS *m/z*: 368.2 $[M+H]^+$. ^1H NMR ($CDCl_3$, 500 MHz) δ: 2.33 (s, 3H, $COCH_3$), 2.62 (m, 2H, 13-H, 6-H), 2.83 (m, 1H, 5-H), 3.11 (m, 2H, 6-H, 5-H), 3.20 (m, 1H, 13-H), 3.45 (d, J = 16.0 Hz, 1H, 8-H), 3.56 (m, 1H, 14-H), 3.80 (s, 3H, $-OCH_3$), 4.01 (d, J = 16.0 Hz, 1H, 8-H), 5.90, 5.91 (d, d, J = 1.5 Hz, 2H, $-OCH_2O-$), 6.58 (s, 1H, 4-H), 6.71 (s, 1H, 1-H), 6.82 (d, J = 8.5 Hz, 1H, 12-H), 6.99 (d, J = 8.5 Hz, 1H, 11-H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 105.56 (C1), 146.00 (C2), 146.19 (C3), 108.41 (C4), 127.71 (C4a), 29.56 (C5), 51.12 (C6), 53.44 (C8), 127.65 (C8a), 136.19 (C9), 149.01 (C10), 110.59 (C11), 126.44 (C12), 128.25 (C12a), 36.19 (C13), 59.33 (C14), 130.71 (C14a), 100.78 ($-OCH_2O-$), 56.07 (10-OCH_3), 168.58(–CO–), 20.43 ($-CH_3$).

4.2.6. (±)-5,6,13,14-Tetrahydro-9-propionyloxy-10-methoxy-2,3-(methylenedioxy)-8*H*-dibenzo[*a*,*g*]quinolizine (5b)

Preparation and purification of **5b** was carried out according to the general procedure, starting from compound **5** and propionyl chloride. Yield: 87%.

Light yellow powders. ESI-MS *m/z*: 382.2 $[M+H]^+$. ^1H NMR ($CDCl_3$, 500 MHz) δ: 1.34 (t, 3H, J = 7.5 Hz, $-CH_3$), 2.66 (m, 4H, 6-H, 13-H, $-CH_2-$), 2.86 (m, 2H, 5-H), 3.15 (m, 2H, 5-H, 6-H), 3.26, 3.23 (dd, 1H, J = 16.0 Hz, 3.5 Hz, 13-H), 3.48 (d, 1H, J = 16.0 Hz, 8-H), 3.61 (m, 1H, 14-H), 3.83 (s, 3H, $-OCH_3$), 4.04 (d, 1H, J = 16.0 Hz, 8-H), 5.94, 5.95 (d, d, 2H, J = 1.5 Hz, $-OCH_2O-$), 6.61 (s, 1H, 4-H), 6.75 (s, 1H, 1-H), 6.86 (d, 1H, J = 8.0 Hz, 12-H), 7.03 (d, 1H, J = 8.5 Hz, 11-H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 105.59 (C1), 149.08 (C2), 146.19 (C3), 108.41 (C4), 127.66 (C4a), 29.59 (C5), 51.14 (C6), 53.45 (C8), 127.74 (C8a), 136.27 (C9), 146.0 (C10), 110.63 (C11), 126.31 (C12), 128.30 (C12a), 36.21 (C13), 59.34 (C14), 130.79 (C14a), 100.78 ($-OCH_2O-$), 56.11 (10-OCH_3), 172.05 (–CO–), 27.29 ($-CH_2-$), 9.35 ($-CH_3$).

4.2.7. (±)-5,6,13,14-Tetrahydro-9-hexanoyloxy-10-methoxy-2,3-(methylenedioxy)-8*H*-dibenzo[*a*,*g*]quinolizine (5c)

Preparation and purification of **5c** was carried out according to the general procedure, starting from compound **5** and hexanoyl chloride. Yield: 83%.

White powders. ESI-MS *m/z*: 424.3$[M+H]^+$. ^1H NMR ($CDCl_3$, 500 MHz) δ: 0.94 (t, 3H, J = 7.0 Hz, 6′-CH_3), 1.42 (m, 4H, 5′,4′-CH_2CH_2-), 1.79 (m, 2H, 3′-CH_2-), 2.60 (t, 2H, J = 7.5 Hz, 2′-CH_2-), 2.64 (m, 2H, 6-H, 13-H), 2.83 (m, 1H, 5-H), 3.10 (m, 2H, 5-H, 6-H), 3.20, 3.23 (dd, 1H, J = 16.0 Hz, 3.5 Hz, 13-H), 3.45 (d, 1H, J = 16.0 Hz, 8-H), 3.57 (m, 1H, 14-H), 3.79 (s, 3H, $-OCH_3$), 4.0 (d, 1H, J = 16.0 Hz, 8-H), 5.90, 5.91 (d, d, 2H, J = 1.5 Hz, $-OCH_2O-$), 6.58 (s, 1H, 4-H), 6.71 (s, 1H, 1-H), 6.82 (d, 1H, J = 8.5 Hz, 12-H), 6.99 (d, 1H, J = 8.5 Hz, 11-H),. ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 105.59 (C1), 149.08 (C2), 146.21 (C3), 108.42 (C4), 127.66 (C4a), 29.60 (C5), 51.14 (C6), 53.54 (C8), 127.74 (C8a), 136.29 (C9), 146.0 (C10), 110.63 (C11), 126.31 (C12), 128.30 (C12a), 36.22 (C13), 59.34 (C14), 130.80 (C14a), 100.79 ($-OCH_2O-$), 56.06 (10-OCH_3), 171.37 (–CO–), 33.95 (2′-CH_2-), 31.32 (3′-CH_2-), 24.86 (4′-CH_2-), 22.36 (5′-CH_2-), 13.97 (6′-CH_3).

4.2.8. (±)-5,6,13,14-Tetrahydro-9-benzoyloxy-10-methoxy-2,3-(methylenedioxy)-8*H*-dibenzo[*a*,*g*]quinolizine (5d)

Preparation of **5d** was carried out according to the general procedure, starting from compound **5** and benzoyl chloride and purification by silica gel column chromatography. Yield: 76%.

White powders. ESI-MS *m/z*: 430.2 $[M+H]^+$. ^1H NMR ($CDCl_3$, 500 MHz) δ: 2.58(m, 2H, 6-H, 13-H), 2.85(m, 1H, 5-H), 3.07(m, 2H, 6-H, 5-H), 3.27, 3.24 (dd, 1H, J = 16.0, 3.5 Hz, 13-H), 3.50 (d, 1H, J = 15.0 Hz, 8-H), 3.58 (m, 1H, 14-H), 3.79 (s, 3H, $-OCH_3$), 4.07 (d, 1H, J = 15.0 Hz, 8-H), 5.91, 5.90 (d, d, 2H, J = 1.5 Hz, $-OCH_2O-$), 6.57(s, 1H, 4-H), 6.73 (s, 1H, 1-H), 6.87 (d, 1H, J = 8.5 Hz, 12-H), 7.05 (d, 1H, J = 8.5 Hz, 11-H), 7.53 (m, 2H, 3′-ArH, 5′-ArH), 7.65(m, 1H, 4′-ArH), 8.24(m, 2H, 2′-ArH, 6′-ArH). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 105.60 (C1), 149.29 (C2), 146.21 (C3), 108.43 (C4), 26.52 (C4a), 29.61 (C5), 51.19 (C6), 53.57 (C8), 127.77 (C8a), 136.32 (C9), 146.00 (C10), 110.77 (C11), 128.58 (C12), 127.81 (C12a), 36.32 (C13), 59.44 (C14), 130.37 (C14a), 100.79 ($-OCH_2O-$), 56.17 ($-OCH_3$), 164.19 (–CO–), 130.82 (Ar-C1′), 133.51(Ar-C4′), 129.36 (Ar-C6′, Ar-C2′), 128.59 (Ar-C5′, Ar-C3′).

4.2.9. (±)-5,6,13,14-Tetrahydro-9-(2-acetoxy)benzoyloxy-10-methoxy-2,3-(methylenedioxy)-8*H*-dibenzo[*a*,*g*]quinolizine (5e)

Preparation of **5e** was carried out according to the general procedure, starting from compound **5** and *o*-acetylsalicylryl chloride and purification by silica gel column chromatography. Yield: 56%.

White powders. ESI-MS *m/z*: 488.2 $[M+H]^+$. ^1H NMR ($CDCl_3$, 500 MHz) δ: 2.29 (s, 3H, -$OCOCH_3$), 2.63(m, 2H, 6-H, 13-H), 3.0(m, 3H, 5-CH_2, 6-H), 3.29(m, 1H, 13-H), 3.52(m, 2H, 14-H, 8-H), 3.79 (s, 3H, $-OCH_3$), 4.03 (m, 1H, 8-H), 5.92(s, 2H, $-OCH_2O-$), 6.58 (s, 1H, 4-H), 6.72 (s, 1H, 1-H), 6.88 (d, 1H, J = 8.5 Hz, 12-H), 7.05 (d, 1H, J = 8.5 Hz, 11-H), 7.18 (dd, 1H, J = 8.0 Hz, J = 1.5 Hz, 3′-ArH), 7.40 (m, 1H, 4′-ArH), 7.65 (m, 1H, 5′-ArH), 8.27 (dd, 1H, J = 8.0 Hz, J = 1.5 Hz, 6′-ArH). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 105.56 (C1), 149.17 (C2), 146.20 (C3), 108.44 (C4), 127.79 (C4a), 29.71 (C5), 51.17 (C6), 53.48 (C8), 127.86 (C8a), 136.06 (C9), 145.99 (C10), 110.78 (C11), 126.68 (C12), 128.68 (C12a), 36.33 (C13), 59.43 (C14), 130.79 (C14a), 100.79 ($-OCH_2O-$), 56.15 ($-OCH_3$), 132.40 (Ar-C1′), 151.33 (Ar-C2′), 122.59 (Ar-C3′), 134.41 (Ar-C4′), 124.05 (Ar-C5′), 126.15(Ar-C6′), 161.60 ($-COCH_3$), 169.59 (–CO-Ar), 21.07 ($-CH_3$).

4.2.10. (±)-5,6,13,14-Tetrahydro-9-(3-phenyl)acryloxy)-10-methoxy-2,3-(methylenedioxy)-8*H*-dibenzo[*a*,*g*]quinolizine (5f)

Preparation and purification of **5f** was carried out according to the general procedure, starting from compound **5** and cinnamoyl chloride and purification by silica gel column chromatography. Yield: 62%.

Light yellow powders. ESI-MS *m/z*: 456.2 $[M+H]^+$. ^1H NMR ($CDCl_3$, 500 MHz) δ: 2.62 (m, 2H, 6-H, 13-H), 2.86 (m, 1H, 5-H), 3.10 (br, 2H, 5-H, 6-H), 3.24 (dd, 1H, J = 16.0 Hz, 3.0 Hz, 13-H), 3.49 (d, 1H, J = 15.0 Hz, 8-H), 3.58(br, 1H, 14-H), 3.81 (s, 3H, $-OCH_3$), 4.06 (d, 1H, J = 15.0 Hz, 8-H), 5.91 (s, 2H, $-OCH_2O-$), 6.57 (s, 1H, 4-H), 6.70 (d, 1H, J = 16.0 Hz, –CH═), 6.72 (s, 1H, 1-H), 6.86 (d, 1H, J = 8.0 Hz, 12-H), 7.03 (d, 1H, J = 8.0 Hz, 11-H), 7.42 (m, 3H, 3″-ArH, 4″-ArH, 5″-ArH,), 7.60 (m, 2H, 2″-ArH, 6″-ArH), 7.90(d, 1H, J = 16.0 Hz, ═CH-Ar). 13C NMR ($CDCl_3$, 125 MHz) δ: 105.59 (C1), 149.21 (C2), 146.70 (C3), 108.43 (C4), 127.73 (C4a), 29.61 (C5), 51.18 (C6), 53.57 (C8), 127.80 (C8a), 136.16 (C9), 146.20 (C10), 110.67 (C11), 126.48 (C12), 128.55 (C12a), 36.29 (C13), 59.41 (C14), 130.81 (C14a), 100.79 ($-OCH_2O-$), 56.16 ($-OCH_3$), 164.50 (–CO–), 116.82 (–C═), 146.0 (═C-Ar), 134.30 (Ar-C1″), 130.68 (Ar-C4″), 129.0 (Ar-C3″, Ar-C5″), 128.36 (Ar-C2″, Ar-C6″).

4.2.11. (±)-5,6,13,14-Tetrahydro-9-[3-(3-methoxyl-4-acetoxy)phenyl]acryloxy-10-methoxy-2,3-(methylenedioxy)-8*H*-dibenzo[*a*,*g*]quinolizine (5g)

Preparation and purification of **5g** was carried out according to the general procedure, starting from compound **5** and acetylferuloyl chloride and purification by silica gel column chromatography. Yield: 65%.

White powders. ESI-MS *m/z*: 544.3 $[M+H]^+$. ^1H NMR ($CDCl_3$, 500 MHz) δ: 2.33 (s, 3H, $-COCH_3$), 2.62 (m, 2H, 6-H, 13-H), 2.85 (m, 1H, 5-H), 3.11 (m, 2H, 5-H, 6-H), 3.24 (dd, 1H, J = 16.0 Hz, 3.5 Hz, 13-H), 3.48 (d, 1H, J = 16.0 Hz, 8-H), 3.58 (m, 1H, 14-H), 3.82 (s, 3H, $-OCH_3$), 3.89 (s, 3H, $-OCH_3$), 4.05 (d, 1H, J = 16.0 Hz, 8-H), 5.91, 5.92 (d,d, 2H, J = 1.5 Hz, $-OCH_2O-$), 6.58 (s, 1H, 4-H), 6.65 (d, 1H, J = 16.0 Hz, 2′-CH═), 6.73 (s, 1H, 1-H), 6.86 (d, 1H, J = 8.5 Hz, 12-H), 7.03 (d, 1H, J = 8.5 Hz, 3″-ArH), 7.09 (d, 1H, J = 8.5 Hz, 11-H), 7.20 (d, 1H, J = 8.5 Hz, 2″-ArH), 7.20 (s, 1H, 6″-ArH), 7.85 (d, 1H, J = 16.0 Hz, 3′═CH-Ar). 13C NMR ($CDCl_3$, 125 MHz) δ: 105.59 (C1), 149.18 (C2), 146.21 (C3), 108.44 (C4), 127.76 (C4a), 29.61 (C5), 51.20 (C6), 53.57 (C8), 127.80 (C8a), 136.14 (C9), 146.01 (C10), 110.67 (C11), 126.53 (C12), 128.55 (C12a), 36.30 (C13), 59.42 (C14), 130.80 (C14a), 100.80 ($-OCH_2O-$), 56.01 ($-OCH_3$), 56.16 ($-OCH_3$), 168.67 (–CO–), 164.36 (–CO–Me), 121.62 (–C═), 141.88 (═C-Ar), 133.22 (Ar-C1″), 117.03 (Ar-C2″), 123.39 (Ar-C3″), 145.95 (Ar-C4″), 151.57 (Ar-C5″), 111.49 (Ar-C6″), 20.65 ($-CH_3$).

4.2.12. (−)-2,3,10-Trimethoxy-9-β-D-glucosyl-5,6,13,14-tetrahydro-8*H*-dibenzo[*a*,*g*]quinolizine (4a)[23]

Using 24-h-old stage II cultures of *G. deliquescens* NRRL1086, a total of 500 mg of **4** was distributed evenly among fifty 150 mL Erlenmeyer flasks. Substrate-containing cultures were incubated for 120 h, then filtrated and extracted with equal amount of EtOAc. The organic solvent layer was removed, evaporated to dryness. The extract was subjected to silica gel column chromatography eluted with chloroform/methanol (30:1, v/v) to afford the product **4a** 311 mg (43%).

Yellow powders. HR-ESI-MS *m/z*: 504.2229 $[M+H]^+$. ^1H NMR (DMSO-d_6, 500 MHz) δ: 2.45 (m, 1H, 6-H), 2.56 (m, 1H, 13-H), 2.61 (m, 1H, 5-H), 2.91 (m, 1H, 5-H), 3.07 (m, 1H, 6-H), 3.08 (m, 1H, 5′-H), 3.20 (m, 1H, 4′-H), 3.23 (m, 1H, 2′-H), 3.24 (m, 1H, 3′-H), 3.34 (m, 1H, 13-H), 3.37 (d, 1H, J = 16.0 Hz, 8-H), 3.39 (m, 1H, 14-H), 3.46 (m, 1H, 6′-H), 3.62 (m, 1H, 6′-H), 3.73 (s, 3H, $-OCH_3$), 3.74 (s, 3H, $-OCH_3$), 3.76 (s, 3H, $-OCH_3$), 4.33 (d, 1H, J = 16.0 Hz, 8-H), 4.35 (t, 1H, J = 5.5 Hz, 6′-OH), 4.93 (d, 1H, J = 7.5 Hz, 1′-H), 4.93 (s, 1H, 4′-OH), 4.99 (s, 1H, 3′-OH), 4.99 (s, 1H, 2′-OH), 6.68 (s, 1H, 4-H), 6.86 (s, 1H, 1-H), 6.88 (d, 1H, J = 11.0 Hz, 12-H), 6.89 (d, 1H, J = 11.0 Hz, 11-H). ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 109.49 (C1), 149.06 (C2), 147.14 (C3), 111.74 (C4), 126.33 (C4a), 28.57 (C5), 50.51 (C6), 53.90 (C8), 127.97 (C8a), 141.48 (C9), 147.13 (C10), 111.93 (C11), 123.88 (C12), 129.29 (C12a), 35.57 (C13), 58.59 (C14), 129.79 (C14a), 102.92 (C1′), 74.25 (C2′), 76.48 (C3′), 69.79 (C4′),77.10 (C5′), 60.82 (C6′), 55.39 (10-OCH_3), 55.71 (3-OCH_3), 56.34 (2-OCH_3).

4.2.13. (+)-2,3,10-Trimethoxy-9-β-D-glucosyl-5,6,13,14-tetrahydro-8*H*-dibenzo[*a*,*g*]quinolizine (4b)

Preparation and purification of **4b** was carried out according to the procedure of compound **4a**, Yield: 16%.

Yellow powders. HR-ESI-MS *m/z*: 504.2231 $[M+H]^+$. ^1H NMR (DMSO-d_6, 500 MHz) δ: 2.45 (m, 1H, 6-H), 2.56 (m, 1H, 13-H), 2.60 (m, 1H, 5-H), 2.91 (m, 1H, 5-H), 3.04 (m, 1H, 6-H), 3.06 (m, 1H, 5′-H), 3.15 (m, 1H, 4′-H), 3.20 (m, 1H, 2′-H), 3.22 (m, 1H, 3′-H), 3.34 (m, 1H, 13-H), 3.42 (m, 1H, 14-H), 3.45 (m, 1H, 6′-H), 3.51(d, 1H, J = 16.0 Hz, 8-H), 3.63 (m, 1H,6′-H), 3.73 (s, 3H, $-OCH_3$), 3.74 (s, 3H, $-OCH_3$), 3.76 (s, 3H, $-OCH_3$), 4.13 (d, 1H, J = 16.0 Hz, 8-H), 4.30 (s, 1H, 6′-OH), 4.78 (d, J = 7.5 Hz, 1H, 1′-H),

4.92 (s, 1H, 4′-OH), 4.98 (s, 1H, 3′-OH), 4.98 (s, 1H, 2′-OH), 6.68 (s, 1H, 4-H), 6.86 (s, 1H, 1-H), 6.88 (d, 1H, *J* = 9.0 Hz, 12-H), 6.89 (d, 1H, *J* = 9.0 Hz, 11-H). ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 109.44 (C1), 148.88 (C2), 147.14 (C3), 111.72 (C4), 126.35 (C4a), 28.56 (C5), 50.66 (C6), 53.97 (C8), 127.86 (C8a), 141.65 (C9), 147.11 (C10), 111.74 (C11), 123.90 (C12), 129.71 (C12a), 35.54 (C13), 58.59 (C14), 129.85 (C14a), 103.47 (C1′), 74.17 (C2′), 76.50 (C3′), 69.95 (C4′), 77.05 (C5′), 61.01 (C6′), 55.38 (10-OCH_3), 55.71 (3-OCH_3), 56.28 (2-OCH_3).

4.2.14. (−)-5,6,13,14-Tetrahydro-9-β-D-glucosyl-10-methoxy-2,3-(methylenedioxy)-8*H*-dibenzo[*a*,*g*]quinolizine (5h)

Using 24-h-old stage II cultures of *G. deliquescens* NRRL1086, a total of 500 mg of **5** was distributed evenly among fifty 150 mL Erlenmeyer flasks. Substrate-containing cultures were incubated for 120 h, then filtrated and extracted with equal amount of EtOAc. The organic solvent layer was removed, evaporated to dryness. The extract was subjected to silica gel column chromatography eluted with chloroform/methanol (30:1, v/v) to afford the product **5h** 323 mg (44%).

White powders. HR-ESI-MS *m/z*: 488.1915 $[M+H]^+$. ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 2.46 (m, 1H, 6-H), 2.54 (m, 1H, 13-H), 2.60 (m, 1H, 5-H), 2.89 (m, 1H,5-H), 3.06 (m, 1H, 6-H), 3.07 (m, 1H, 5′-H), 3.18 (m, 1H, 4′-H), 3.22 (m, 1H, 2′-H), 3.23 (m, 1H, 3′-H), 3.26 (m, 1H, 13-H), 3.36 (m, 1H, 14-H), 3.37 (d, 1H, *J* = 16.0 Hz, 8-H), 3.45 (m, 1H, 6′-H), 3.62 (m, 1H, 6′-H), 3.76 (s, 3H, –OCH_3), 4.32 (d, 1H, *J* = 16.0 Hz, 8-H), 4.33 (t, 1H, *J* = 5.5 Hz, 6′-OH), 4.92 (d, 1H, *J* = 5.0 Hz, 4′-OH), 4.92 (d, 1H, *J* = 7.5 Hz, 1′-H), 4.95 (d, 1H, *J* = 4.0 Hz, 3′-OH), 4.99 (d, 1H, *J* = 4.0 Hz, 2′-OH), 5.94, 5.93 (d, d, *J* = 1.0 Hz, 2H, –OCH_2O–), 6.66 (s, 1H, 4-H), 6.85 (d, 1H, *J* = 8.0 Hz, 12-H), 6.89 (s, 1H, 1-H), 6.89 (d, 1H, *J* = 8.0 Hz, 11-H). ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 105.66 (C1), 145.35 (C2), 145.62 (C3), 108.00 (C4), 127.42 (C4a), 29.00 (C5), 50.29 (C6), 53.79 (C8), 127.83 (C8a), 141.49 (C9), 149.10 (C10), 111.96 (C11), 123.94 (C12), 129.19 (C12a), 35.61 (C13), 58.82 (C14), 130.97 (C14a), 102.95 (C1′), 74.23 (C2′), 76.48 (C3′), 69.80 (C4′), 77.07 (C5′), 60.84 (C6′), 100.44 (–OCH_2O–), 56.36 (10-OCH_3).

4.2.15. (+)-5,6,13,14-Tetrahydro-9-β-D-glucosyl-10-methoxy-2,3-(methylenedi oxy)-8*H*-dibenzo[*a*,*g*]quinolizine (5i)

Preparation and purification of **5i** was carried out according to the procedure of compound **5h**. Yield: 5%.

White powders. HR-ESI-MS *m/z*: 488.1917 $[M+H]^+$. ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 2.46 (m, 1H, 6-H), 2.53 (m, 1H, 13-H), 2.59 (m, 1H, 5-H), 2.89 (m, 1H, 5-H), 3.05 (m, 1H, 6-H), 3.06 (m, 1H, 5′-H), 3.14 (m, 1H, 4′-H), 3.20 (m, 1H, 2′-H), 3.21 (m, 1H, 3′-H), 3.28 (m, 1H, 13-H), 3.40 (m, 1H, 14-H), 3.44 (m, 1H, 6′-H), 3.50 (d, 1H, *J* = 16.0 Hz, 8-H), 3.62 (m, 1H, 6′-H), 3.76 (s, 3H, –OCH_3), 4.12 (d, 1H, *J* = 16.0 Hz, 8-H), 4.29 (t, 1H, *J* = 5.5 Hz, 6′-OH), 4.78 (d, 1H, *J* = 7.5 Hz, 1′-H), 4.87 (d, 1H, *J* = 5.0 Hz, 4′-OH), 4.94 (d, 1H, *J* = 4.5 Hz, 3′-OH), 4.96 (d, 1H, *J* = 4.0 Hz, 2′-OH), 5.95, 5.94 (d, d, 2H, *J* = 1.0 Hz, –OCH_2O–), 6.66 (s, 1H, 4-H), 6.85 (d, 1H, *J* = 8.5 Hz, 12-H), 6.89 (s, 1H, 1-H), 6.89 (d, 1H, *J* = 8.5 Hz, 11-H). ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 105.59 (C1), 145.31 (C2), 145.60 (C3), 107.97 (C4), 127.42 (C4a), 28.98 (C5), 50.45 (C6), 53.86 (C8), 127.74 (C8a), 141.65 (C9), 148.89 (C10), 111.76 (C11), 123.94 (C12), 129.59 (C12a), 35.60 (C13), 58.84 (C14), 131.01 (C14a), 103.49 (C1′), 74.16 (C2′), 76.50 (C3′), 69.94 (C4′), 77.06 (C5′), 60.98 (C6′), 100.43 (–OCH_2O–), 56.30 (10-OCH_3).

4.3. The measurement of TF procoagulant activity

4.3.1. Cell culture

The human monocytic cell line (THP-1) cells were obtained from the cell bank of type culture collection of Chinese Academy of Sciences (Shanghai Institute of Cell Biology, Chinese Academy of Sciences), and cultured in RPMI-1640 medium with 10% heat-inactivated fetal calf serum at 37 °C in 5% CO_2.

4.3.2. Induction of TF and drug treatment

According to our previous reports,[7,25] THP-1 cells (1×10^6 cells/mL) were initially incubated with control vehicle (DMSO, 0.1% V/V) or various compounds at indicated concentrations (0.01–1 μM) for 1 h in a 96-well plate, and then stimulated with 500 ng/mL LPS for 5 h to induce TF activity. At the end of incubation, cells were sedimented by centrifugation and resuspended in RPMI-1640. The cell suspension was frozen at −20 °C until TF activity measurement. The cell lysates were frozen and thawed three times before they were used in the assay. Cell lysates (45 μL) were incubated with a reagent mixture (5 μL, pH 7.3) containing 10 g/L prothrombin complex and 100 mM $CaCl_2$ in a 96-well plate at 37 °C for 15 min. Then, 50 μL of factor Xa chromogenic substrate (0.5 mM) containing 100 mM EDTA (pH 8.4) was added, and the absorbance was measured at 405 nM with a microplate reader (Sunrise, TECAN Austria GmbH, Grodig, Austria). The IC_{50} of each compound on TF procoagulatant activity was calculated.

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