Anti-*Helicobacter pylori* and Thrombin Inhibitory Components from Chinese Dragon's Blood, *Dracaena cochinchinensis*

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Chemical studies on the constituents of *Dracaena cochinchinensis* led to the discovery of eight new flavonoid derivatives (**1**–**8**) along with 14 known compounds (**9**–**22**). The identification and structural elucidation of these isolates were based on spectral analyses. All isolates were tested for antibacterial activities against *Helicobacter pylori* (ATCC43504) and thrombin inhibitory effects. As a result, new flavonoid derivatives **6** and **7** and (2*S*)-4′,7-dihydroxy-8-methylflavan (**11**) were found to be most efficacious against *H. pylori* (ATCC43504) with MIC values of 29.5, 29.5, and 31.3 μM, respectively, and the seven new flavonoid derivatives (**1**–**7**) and one known biflavonoid (**9**) were observed to exhibit moderate thrombin inhibitory activity.

Dragon's blood, a dark red resinous substance derived from various plants involved in five different genuses including *Daemonorops* (Palmaceae), *Calamus* (Palmaceae), *Dracaena* (Agavaceae), *Pterocarpus* (Leguminosae), and *Croton* (Euphorbiaceae),[1] has been used for the treatment of wounds, leucorrhea, fractures, diarrhea, piles, and peptic ulcers for a long time.[2] The red resin of *Dracaena cochinchinensis* (Lour.) S.C. Chen (Agavaceae), believed to be the original source of Chinese dragon's blood, has been widely used in traditional Chinese medicine for promoting blood circulation and treating traumatic and visceral hemorrhages.[2] Previous phytochemical studies on the genus *Dracaena* resulted in the identification of flavonoids[3–5] and steroids.[6–10] Flavonoids from the genus *Dracaena* were reported to exhibit antiestrogenic,[11,12] antioxidative,[11–13] and bacteriostatic activities,[14] and steroids were reported with antiproliferative effects.[15–17] As part of an ongoing search for new bioactive natural products from traditional Chinese medicine, a systematical chemical investigation has been undertaken on the stems of *D. cochinchinensis* (Lour.) S.C. Chen. Here we report the isolation and structural elucidation of eight new flavonoid derivatives characterized as cochinchinenenes A–D (**1**–**4**), (2*R*)-8-methylsocotrin-4′-ol (**5**), cochinchinenins B and C (**6**, **7**), and cochinchinenone (**8**), along with 14 known compounds (**9**–**22**). All isolates were tested for antibacterial activities against *Helicobacter pylori* (ATCC43504) and thrombin inhibitory effects.

Results and Discussion

Powdered, air-dried stems of *D. cochinchinensis* (5 kg) was extracted with 95% EtOH at room temperature (3 × 72 h). After removing the solvent, the aqueous residue was partitioned in sequence with $CHCl_3$ and *n*-BuOH, yielding $CHCl_3$ and *n*-BuOH extracts. The two fractions were subjected to a series of chromatographic steps to afford eight new flavonoid derivatives (**1**–**8**) and 14 known compounds (**9**–**22**).

Compound **1**, isolated as a pale yellow solid, showed an accurate $[M + Na]^+$ ion at *m/z* 549.2251 in the HRESIMS, corresponding to the empirical molecular formula $C_{33}H_{34}O_6$. It displayed intense IR absorptions indicative of the presence of hydroxy groups (3403 cm^{-1}) and aromatic rings (1598, 1510, and 1452 cm^{-1}). The UV spectrum exhibited absorption at λ_{max} 328 nm, which was assignable to a stilbene core. The ^1H NMR spectrum of **1** (Table 1) demonstrated the existence of two sets of protons of a 1,4-disubstituted aromatic ring [δ 7.35 and 6.75 (both 2H, d, 8.8), and δ 7.19 and 6.71 (both 2H, d, 9.0)]; three aromatic protons of a 1,2,4-trisubstituted benzene ring [δ 6.24 (1H, dd, 8.1, 2.1), 6.33 (1H, d, 2.1), and 6.74 (1H, d, 8.1)]; two overlapping aromatic singlets [δ 6.69 (2H, s)]; two typical *trans*-alkene protons [δ 6.89 and 7.02 (1H, d, 16.2, each)]; characteristic aliphatic protons [δ 2.40 (2H, m) and 2.30 (2H, m)]; one methine proton at δ 4.56 (1H, t, 7.7); and four methoxy proton resonances [δ 3.70 (6H, s) and 3.74 (6H, s)] in its structure. The ^{13}C NMR spectrum of **1** (Table 2) indicated 33 carbon resonances separated by DEPT experiments into 11 quaternary sp^2 carbons with six linked to an oxygen atom; 16 tertiary carbons comprising 15 sp^2 carbons and one sp^3 carbon; two secondary sp^3 carbons; and four methoxy carbons. The complete assignment of the protonated carbons was made using the HSQC spectrum, while analysis of the HMBC and ROESY spectra of **1** led to the definition of a stilbene moiety and a deoxotetrahydrochalcone residue in the structure of **1** (Figure 2). The linkage of the stilbene moiety and the deoxotetrahydrochalcone residue was established between C-4 (δ 122.6) and C-γ (δ 40.7) by the ^1H–^{13}C long-range correlation between H-γ (δ 4.56) and C-3/5 (δ 160.6), which was corroborated by the NOE correlations between 3-OCH_3/5-OCH_3 (δ 3.74) and H-2‴/6‴ (δ 7.19) and H-γ (δ 4.56). The dominant fragment peaks at *m/z* 270 and 256 [cleavage of the C(γ)–C(4) bond] in the EIMS of **1** (Figure 3) were also in agreement with such a linkage. Therefore, compound **1** was characterized as the new 1-[4-(3,5-dimethoxy-4′-hydroxystilbenyl)]-1-(4-methoxyphenyl)-3-(2-methoxy-4-hydroxyphenyl)propane and assigned the trivial name cochinchinenene A.

Compound **2** was obtained as a colorless gum. The high-resolution ESIMS exhibited a pseudomolecular ion peak at *m/z* 535.2086 $[M + Na]^+$, in accordance with the molecular formula $C_{32}H_{32}O_6$. The UV absorption at 328 nm was characteristic of a stilbene skeleton.[18] Its IR spectrum displayed the presence of aromatic rings (1606, 1510, and 1466 cm^{-1}) and hydroxy functions (3406 cm^{-1}). The ^1H NMR spectrum of **2** (Table 1) disclosed 15 aromatic and olefinic protons at δ 7.21–6.21, together with five aliphatic protons [δ 2.36 (2H, m), 2.45 (2H, m), and 4.56 (1H, m)] and three methoxy resonances [δ 3.75 (3H, s), 3.71 (3H, s), and 3.62 (3H, s)]. The ^{13}C NMR spectrum of **2** (Table 2) indicated 32 carbons comprising 11 quaternary carbons, 16 tertiary carbons, two

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Table 1. ^{1}H NMR Data of Compounds **1–8** (400 MHz; **1–4** in methanol-d_4, **5–7** in acetone-d_6, and **8** in DMSO-d_6)

no.	1	2	3	4	5	6	7	8
2	6.69 s				4.98 dd (6.6, 7.9)			
3					2.17 m	6.59 s	6.46 s	5.38 s
					1.82 m			
4		6.33 d (1.8)	6.34 d (2.4)	6.27 d (2.5)	2.92 m			
					2.72 m			
5					6.86 s			5.38 s
6	6.69 s	6.56 d (1.8)	6.55 d (2.4)	6.48 d (2.5)		7.17 s	7.13 s	
11(-CH_3)					2.14 s			
2-OCH_3						3.83 s	3.71 s	3.70 s
3-OCH_3	3.74 s		3.67 s					
4-OCH_3						3.78 s		
5-OCH_3	3.74 s	3.75 s						
6-OCH_3								3.70 s
2′, 6′	7.35 d (8.8)	7.13 d (8.3)	7.12 d (8.0)	7.13 d (8.5)	7.30 d (8.2)	7.89 d (8.7)	7.89 d (8.5)	7.70 d (8.6)
3′, 5′	6.75 d (8.8)	6.72 d (8.3)	6.72 d (8.0)	6.73 d (8.5)	6.84 d (8.2)	6.90 d (8.7)	6.90 d (8.5)	6.70 d (8.6)
α	6.89 d (16.2)	6.92 d (15.9)	6.92 d (16.4)	6.94 d (16.0)		3.14 m	3.12 m	2.51 m
β	7.02 d (16.2)	6.67 d (15.9)	6.66 d (16.4)	6.64 d (16.0)		2.91 m	2.90 m	2.19 m
3″	6.33 d (2.1)	6.32 d (2.5)	6.32 d (2.4)	6.33 d (2.4)	6.43 d (2.5)	6.42 d (2.1)	6.42 d (2.4)	
5″	6.24 dd (8.1, 2.1)	6.21 dd (8.4, 2.5)	6.21 dd (8.1, 2.4)	6.22 dd (7.9, 2.4)	6.35 dd (8.1, 2.5)	6.32 dd (7.9, 2.1)	6.32 dd (8.1, 2.4)	
6″	6.74 d (8.1)	6.75 d (8.4)	6.71 d (8.1)	6.77 d (7.9)	6.88 d (8.1)	6.85 d (7.9)	6.87 d (8.1)	
2‴-OCH_3	3.70 s	3.62 s	3.62 s	3.65 s	3.76 s	3.74 s	3.72 s	
2‴, 6‴	7.19 d (9.0)	7.21 d (8.2)	7.05 d (8.6)	7.15 d (8.6)	7.18 d (8.6)	7.08 d (8.5)	7.22 d (8.4)	
3‴, 5‴	6.71 d (9.0)	6.78 d (8.2)	6.64 d (8.6)	6.68 d (8.6)	6.75 d (8.6)	6.70 d (8.5)	6.78 d (8.4)	
4‴-OCH_3	3.70 s	3.71 s					3.70 s	
α′	2.30 m	2.36 m	2.36 m	2.35 m	2.20 m	2.15 m	2.19 m	
β′	2.40 m	2.45 m	2.42 m	2.45 m	2.50 m	2.42 m	2.45 m	
γ	4.56 t (7.7)	4.56 m	4.56 m	4.53 m	4.32 t (7.7)	4.22 t (8.0)	4.29 t (7.8)	

Table 2. ^{13}C NMR Data of Compounds **1–8** (100 MHz; **1–4** in methanol-d_4, **5–7** in acetone-d_6, and **8** in DMSO-d_6)

no.	1	2	3	4	5	6	7	8
1	139.2 s	141.2 s	141.1 s	141.3 s		121.3 s	120.6 s	71.6 s
2	104.2 d	123.8 s	124.8 s	123.1 s	77.7 d	156.9 s	156.6 s	171.7 s
3	160.6 s	158.3 s	161.2 s	158.0 s	30.7 t	96.2 d	99.3 d	100.6 d
4	122.6 s	102.1 d	100.2 d	103.4 d	25.5 t	157.0 s	154.3 s	186.2 s
5	160.6 s	160.2 s	157.8 s	157.3 s	125.3 d	125.8 s	123.7 s	100.6 d
6	104.2 d	104.5 d	106.9 d	106.3 d	125.2 s	129.2 d	129.4 d	171.7 s
7					151.6 s			
8					111.9 s			
9					152.0 s			
10					113.4 s			
11 (-CH_3)					8.7 q			
2-OCH_3						55.5 q	55.1 q	56.0 q
3-OCH_3	56.6 q		56.4 q					
4-OCH_3						55.8 q		
5-OCH_3	56.6 q	55.8 q						
6-OCH_3								56.0 q
1′	131.0 s	131.2 s	131.3 s	131.5 s	134.0 s	130.0 s	130.0 s	127.9 s
2′,6′	129.3 d	129.0 d	129.2 d	129.1 d	127.7 d	130.9 d	130.9 d	130.3 d
3′,5′	117.0 d	116.7 d	117.0 d	116.9 d	115.5 d	115.7 d	115.6 d	115.2 d
4′	158.8 s	158.4 s	158.7 s	158.5 s	157.3 s	162.2 s	162.2 s	162.2 s
α	127.7 d	127.3 d	127.1 d	127.5 d		39.1 t	39.2 t	32.1 t
β	129.5 d	130.8 d	131.7 d	130.3 d		25.8 t	25.9 t	31.4 t
C=O						198.1 s	198.2 s	196.5 s
1″	123.9 s	123.5 s	123.5 s	123.9 s	122.1 s	122.0 s	122.0 s	
2″	160.2 s	160.1 s	160.3 s	160.2 s	158.9 s	158.9 s	158.9 s	
3″	100.2 d	100.1 d	100.2 d	100.2 d	99.3 d	99.3 d	99.3 d	
4″	158.0 s	157.9 s	158.1 s	158.0 s	157.2 s	157.3 s	157.3 s	
5″	107.9 d	107.9 d	108.0 d	108.0 d	106.9 d	107.0 d	107.0 d	
6″	131.6 d	131.5 d	131.7 d	131.7 d	130.3 d	130.5 d	130.4 d	
2″-OCH_3	56.1 q	55.9 q	56.1 q	56.1 q	55.1 q	55.1 q	55.0 q	
1‴	139.4 s	139.3 s	138.2 s	138.6 s	137.2 s	137.3 s	138.7 s	
2‴,6‴	130.5 d	130.1 d	130.2 d	130.3 d	129.5 d	129.4 d	129.4 d	
3‴,5‴	114.4 d	114.6 d	116.0 d	116.0 d	115.3 d	115.3 d	113.8 d	
4‴	159.2 s	159.1 s	156.2 s	156.2 s	155.8 s	155.7 s	158.2 s	
4‴-OCH_3	56.1 q	56.0 q					55.2 q	
α′	34.5 t	34.9 t	35.0 t	35.3 t	36.6 t	36.5 t	36.4 t	
β′	30.2 t	29.6 t	29.7 t	29.8 t	28.8 t	28.9 t	28.8 t	
γ	40.7 d	41.3 d	41.0 d	41.3 d	42.9 d	42.3 d	42.4 d	

secondary carbons, and three methoxy carbons. Examination of the ^{1}H and ^{13}C NMR data of **2** revealed that **2** was similar to **1** except that two *meta*-coupled doublets at δ 6.33 and 6.56 (1H, d, 1.8, each) could be observed in **2**, in place of the symmetrical aromatic proton pattern at δ 6.69 (2H, s)] in **1**, suggesting also a stilbene moiety and a deoxotetrahydrochalcone unit in the structure of **2**. The complete assignment of the protonated carbon resonances was made using the HSQC spectrum, while analysis of the HMBC and

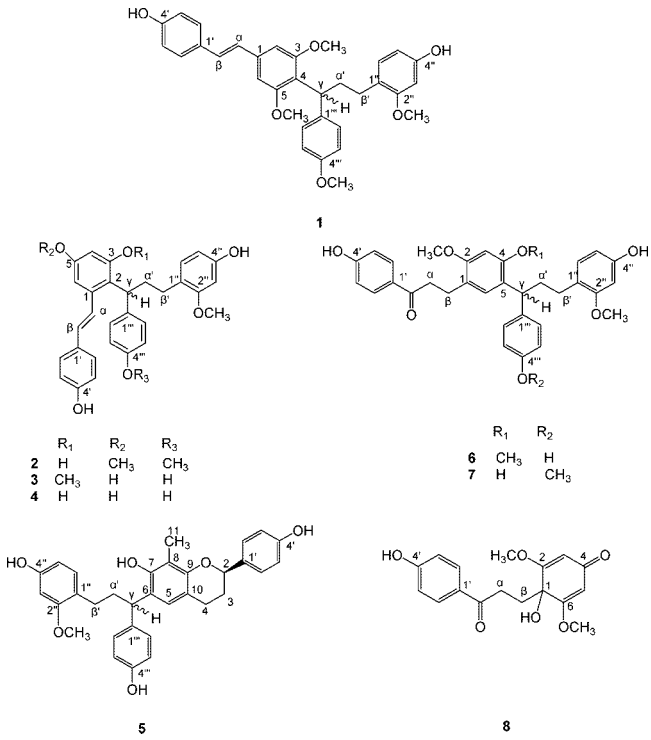

Figure 1. Structures of compounds **1**–**8**.

ROESY spectra of **2** confirmed the existence of a stilbene moiety and a deoxotetrahydrochalcone unit in the structure of **2** (Figure 2). HMBC correlations between the olefinic proton at δ 6.92 (H-α) and the carbons at δ 123.8 (C-2) and 104.5 (C-6), between the aromatic proton at δ 6.56 (H-6) and the carbons at δ 102.1 (C-4) and 123.8 (C-2), and between the proton at δ 6.33 (H-4) and the carbons at δ 104.5 (C-6) and 123.8 (C-2), respectively, gave evidence of the positions of C-2, C-4, and C-6 on the aromatic ring, indicating the position of the methoxy group at C-3 or C-5. Observed HMBC correlation between the methoxy group at δ 3.75 and the carbon resonance at δ 160.2 (C-5), together with the strong NOE cross-peaks detected between the methoxy group at δ 3.75 and H-4 (δ 6.33) and H-6 (δ 6.56), located the methoxy group at C-5. Other HMBC correlations for the deoxotetrahydrochalcone moiety were similar to those observed in compound **1**, suggesting the position of the methoxy groups at C-4‴ and C-2″ and the hydroxy group at C-4″. The linkage of the stilbene and deoxotetrahydrochalcone moieties was further established as between C-2 (δ 123.8) and C-γ (δ 41.3) by the HMBC correlation between H-α' (δ 2.36) and C-2 (δ 123.8), which was consistent with the significant fragment peaks at *m/z* 270 and 242 in its EIMS spectrum (Figure 3). The 4‴-OCH$_3$ (δ 3.71) was proved by the HMBC correlations between the mehoxy protons and C-4‴, as well as NOE cross-peaks between the methoxy protons and H-3‴/H-5‴. Similarly, the 2″-position of the methoxy group at δ 3.62 was provided by HMBC correlations at H-β' and 2″-OCH$_3$ to C-2″, which was confirmed by NOE cross-peaks between 2″-OCH$_3$ and H-3″. Thus, the structure of **2** was confirmed to be the new 1-[2-(5-methoxy-3,4′-dihydroxystilbenyl)]-1-(4-methoxyphenyl)-3-(2-methoxy-4-hydroxyphenyl)propane, assigned the trivial name cochinchinenene B.

Compounds **3** and **4** had molecular formulas of $C_{31}H_{30}O_6$, and $C_{30}H_{28}O_6$, respectively, as deduced from HRESIMS and NMR analyses. The NMR data (Tables 1 and 2) revealed that compounds **3** and **4** possessed an identical structural skeleton made up of one stilbene and one deoxotetrahydrochalcone moiety, similar to that of **2**. The striking differences in the ^{1}H NMR and ^{13}C NMR spectra of **2**–**4** mainly comprised the number of methoxy groups. In the HMBC spectrum of **3**, ^{13}C–^{1}H long-range correlation between H-6 (δ 6.55) and carbons at δ 127.1 (C-α), 157.8 (C-5), and 124.8 (C-2) and between the methoxy group at δ 3.67 as well as the proton at δ 6.34 (H-4) and the carbon at δ 161.2 (C-3) gave evidence of the hydroxy group at C-5 and the methoxy group at C-3, which was further confirmed by NOE cross-peaks between the methoxy group at δ 3.67 and H-4. Likewise, in the HMBC spectrum of **4**, ^{13}C–^{1}H long-range correlations were found between H-6 at δ 6.48 and carbons at δ 127.5 (C-α), 157.3 (C-5), and 123.1 (C-2) and between H-4 at δ 6.27 and carbons at δ 158.0 (C-3), 157.3 (C-5), and 123.1 (C-2), which revealed two hydroxy groups at C-3 and C-5. Observed ion peaks at *m/z* 256 and 242 in the EIMS spectrum of **3** and at *m/z* 256 and 228 in the EIMS spectrum of **4** also corresponded to the structures of compounds **3** and **4**, respectively. Thus, the structures of **3** and **4**, named cochinchinenenes C and D, were identified to be 1-[2-(3-methoxy-4′,5-dihydroxystilbenyl)]-1-(4-hydroxyphenyl)-3-(2-methoxy-4-hydroxyphenyl)propane (**3**) and 1-[2-(3,4′,5-trihydroxystilbenyl)]-1-(4-hydroxyphenyl)-3-(2-methoxy-4-hydroxyphenyl)propane (**4**), respectively.

This is the first report of a novel class of flavonoid derivatives consisting of a stilbene moiety and a deoxotetrahydrochalcone residue connected by a C–C bond from natural sources as represented by compounds **1**–**4**.

Compound **5**, obtained as a pale yellow, amorphous powder, had the empirical molecular formula $C_{32}H_{32}O_6$, as deduced from HRESIMS and NMR analysis. Its IR spectrum showed the absorptions of hydroxy groups (3386 cm^{-1}) and aromatic rings (1598, 1510, and 1469 cm^{-1}). The ^{1}H NMR spectrum of **5** showed two sets of protons of 1,4-disubstituted aromatic ring [δ 7.30 and 6.84 (both 2H, d, 8.2) and δ 7.18 and 6.75 (both 2H, d, 8.6)]; three aromatic protons of a 1,2,4-trisubstituted benzene ring [δ 6.35 (1H, dd, 8.1, 2.5), 6.43 (1H, d, 2.5), and 6.88 (1H, d, 8.1)]; one aromatic singlet [δ 6.86 (1H, s)]; aliphatic protons [δ 2.92 and 2.72 (both 1H, m), 2.17 and 1.82 (both 1H, m)]; one methine proton [δ 4.32 (1H, t, 7.7)]; one methyl singlet [δ 2.14 (3H, s)]; and one methoxy group [δ 3.76 (3H, s)] (Table 1). The presence of one typical oxymethine proton of a flavan skeleton at δ 4.98 (1H, dd, 6.6, 7.9) and characteristic aliphatic protons at δ 2.20 and 2.50 (both 2H, m) due to a deoxotetrahydrochalcone moiety indicated a biflavonoid structure consisting of flavan and deoxotetrahydrochalcone moieties for **5**. The ^{13}C NMR spectrum of **5** (Table 2) displayed 32 carbon resonances, comprising 12 quaternary carbons, 14 tertiary carbons, four aliphatic secondary carbons, one methoxy carbon, and one methyl carbon. The ^{1}H NMR and ^{13}C NMR data of **5** corresponded closely to those of socotrin-4′-ol, a biflavonoid obtained from *Dracaena cinnabari*,[19] except that its H-8 was replaced by a methyl group [δ 2.14 (3H, s)] in **5**, which was supported by HMBC correlations as shown in Figure 2. In addition, a strong NOE effect between 2″-OCH$_3$ (δ 3.76) and H-3″, in combination with HMBC correlations observed at 2″-OCH$_3$/C-2″ and at H-β'/C-2″, proved the 2″-position of the methoxy group in its structure. Compound **5** displayed a positive Cotton effect at λ_{max} 278 nm and a negative Cotton effect at λ_{max} 246 nm, a result similar to that reported for (2*R*)-flavan-7-ol,[20] and hence the *R* configuration was defined for C-2 of **5**. Compound **5** was thus characterized as (2*R*)-2-(4-hydroxyphenyl)-6-[1-(4-hydroxyphenyl)-3-(4-hydroxy-2- methoxyphenyl)propyl]-8-methylchroman-7-ol and assigned the trivial name (2*R*)-8-methylsocotrin-4′-ol.

Compounds **6** and **7** had identical molecular formulas, $C_{33}H_{34}O_7$, as deduced from HRESIMS and NMR analyses. Comparison of the NMR data of **6** and **7** with those of known biflavonoid derivatives previously reported from *Dracaena* plants revealed that the structures of **6** and **7** resembled that of cinnabarone, a biflavonoid isolated from *D. cinnabari*,[21] except for the existence of three methoxy groups in both **6** and **7**, instead of only one methoxy group in cinnabarone. The three methoxy groups were deduced to be located at C-2, C-4, and C-2″ in **6** by ^{1}H–^{13}C long-range correlations at H$_2$-β/C-2, 2-OCH$_3$/C-2, H-γ/C-4, 4-OCH$_3$/C-4, H$_2$-β'/C-2″, and 2″-OCH$_3$/C-2″ in its HMBC spectrum, and the results were further confirmed by NOE

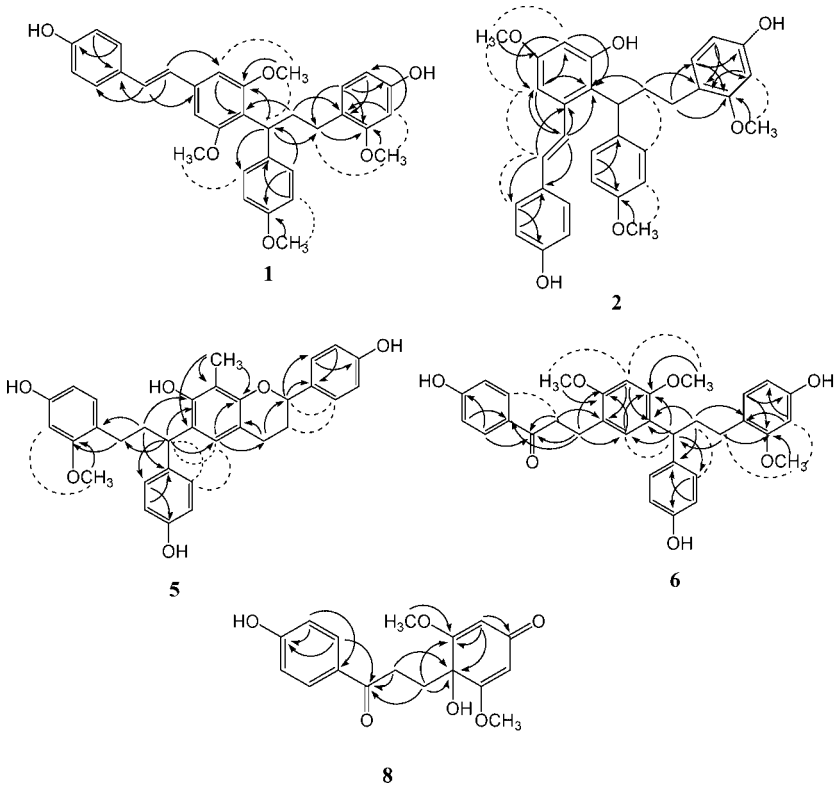

Figure 2. Main ^{1}H–^{13}C long-range correlation (^{1}H⟶^{13}C) and NOE correlation (---) in the HMBC and ROESY spectra of compounds **1**, **2**, **5**, **6**, and **8**.

correlations between 2-OCH_3 and H-3, 4-OCH_3 and H-3, and 2″-OCH_3 and H-3″, respectively. Likewise, the three methoxy groups in **7** were located at C-2, C-2″, and C-4‴ by observation of the HMBC cross-peaks at H_2-β/C-2, 2-OCH_3/C-2, 4‴-OCH_3/C-4‴, H_2-β'/C-2″, and 2″-OCH_3/C-2″ as well as NOE correlations between 2-OCH_3 and H-3, 4‴-OCH_3 and H-3‴ (H-5‴), and 2″-OCH_3 and H-3″, respectively. Therefore, the structures of **6** and **7**, given the trivial names cochinchinenins B and C, were established as 1-[5-(2,4-dimethoxy-4′-hydroxydihydrochalconyl)]-1-(4-hydroxyphenyl)-3-(2-methoxy-4-hydroxyphenyl)propane and 1-[5-(2-methoxy-4,4′-dihydroxydihydrochalconyl)]-1-(4-methoxyphenyl)-3-(2-methoxy-4-hydroxyphenyl)propane, respectively.

Compound **8** was obtained as a pale red gum with the molecular formula $C_{17}H_{18}O_6$, deduced from HRESIMS and NMR analysis. The IR spectrum of **8** showed the presence of hydroxy groups (3357 and 3297 cm^{-1}), conjugated carbonyl functions (1655 and 1654 cm^{-1}), and aromatic rings (1606, 1585, 1517, and 1459 cm^{-1}). The ^{1}H NMR spectrum of **8** revealed four protons of one 1,4-disubstituted benzene ring [δ 7.70 and 6.70 (2H each, d, 8.6)], two overlapping olefinic proton singlets [δ 5.38 (2H, s)], typical aliphatic protons [δ 2.51 and 2.19 (2H each, m)], and two methoxy groups [δ 3.70 (6H, s)] (Table 1). The ^{13}C NMR spectrum of **8** displayed 17 carbon resonances separated by DEPT into seven quaternary carbons including two carbonyls at δ 196.5 and 186.2, four quaternary sp^2 carbons and one quaternary sp^3 carbon linked to an oxygen atom, six tertiary sp^2 carbons, two aliphatic secondary sp^3 carbons, and two methoxy carbons (Table 2). The complete assignments of the protonated carbon resonances were made using the HSQC spectrum of **8**, while analysis of the HMBC data led to the definition of the structure of **8** (Figure 1), in which, ^{1}H–^{13}C long-range correlations were observed at H-3 (H-5)/C-1, C-2 (C-6), C-4; 2-OCH_3 (6-OCH_3)/C-2 (C-6); H-2′ (H-6′)/C=O, C-4′; H-3′ (H-5′)/C-1′, C-4′; H-α/C=O, C-1, C-β; and H-β/C=O, C-2 (C-6), C-1, C-α. The EIMS of **8** displayed the base peak at *m/z* 121 [cleavage of the carbonyl–C(α) bond, ketone α-cleavage], and further diagnostic fragments were detected at *m/z* 170 and 148 [cleavage of the C(1)–C(β) bond, allyl carbon α-cleavage]. These fragment ions were in agreement with the structure of **8**. Therefore, compound **8** was determined to be the new 4-hydroxy-4-[3-(4-hydroxyphenyl)-3-oxopropyl]-3,5-dimethoxycyclohexa-2,5-dienone and assigned the trivial name cochinchinenone.

In addition to the eight new flavonoid derivatives (**1**–**8**), 14 known compounds were also isolated and characterized by comparison with literature data as 1-[5-(2-methoxy-4,4′-dihydroxydihydrochalconyl)]-1-(4-hydroxyphenyl)-3-(2-methoxy-4-hydroxyphenyl)propane (**9**),[22] (2*R*)-4′-hydroxy-7-methoxy-8-methylflavan (**10**),[23] (2*S*)-4′,7-dihydroxy-8-methylflavan (**11**),[24] 7-hydroxy-3-(4-hydroxybenzyl)-8-methoxychroman (**12**),[25] (2*S*)-4′,5-dihydroxy-7-methoxy-8-methylflavan (**13**),[26] 4,4′,6-trihydroxy-2-methoxydihydrochalcone (**14**),[27] 4,4′-dihydroxy-2′-methoxychalcone (**15**),[28] (–)-7-hydroxy-4′-methoxyflavan (**16**),[29] 4,4′-dihydroxy-2-methoxydihydrochalcone (**17**),[30] *trans*-3,5-dihydroxy-4′-methoxystilbene (**18**),[31] 4′-hydroxy-2,4-dimethoxydihydrochalcone (**19**),[32] *trans*-3,4′,5-trihydroxystilbene (**20**),[33] 2,4,4′-trihydroxydihydrochalcone (**21**),[34] and 4,4′-dihydroxy-2,6-dimethoxydihydrochalcone (**22**).[27]

According to the literature, the extract of *D. cochinchinensis* has been used for the treatment of peptic ulcers and fractures and for promoting blood circulation. In order to find possible biologically active components toward the ailments, two available relevant bioassays, i.e., anti-*Helicobacter pylori* and thrombin inhibition tests, were performed on the isolated compounds from *D. cochinchinensis*.

Helicobacter pylori, a spiral Gram-negative bacterium, is strongly associated with human gastritis, peptic ulcer, and gastric cancer.[35–37] Although a variety of drugs with susceptibility for *H. pylori*, such as antibiotics (e.g., amoxicillin), bactericidal agents (e.g., bismuth salt), and antiprotozoal compounds (e.g., metronidazole), are effective in the clinic, their adverse effects and acquired resistance have been problematic in eradication efforts.[38–40] Discovery and development of alternative anti-*H. pylori* therapeutics are therefore of great importance. Dragon's blood is a traditional remedy well known for the treatment of infected wounds and peptic ulcers,[2]

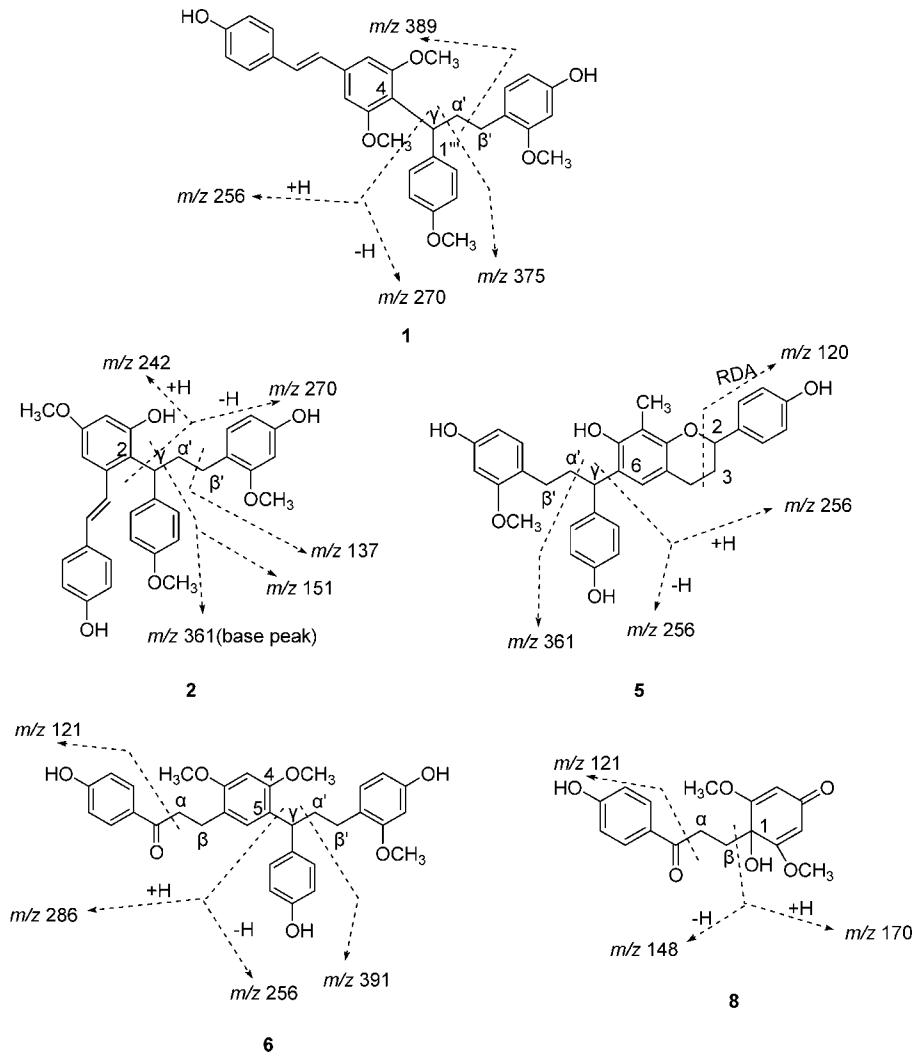

Figure 3. Main fragment ions in the EIMS of compounds **1**, **2**, **5**, **6**, and **8**.

and its antimicrobial activities have been described.[41–43] In this study, the growth inhibitory effects of all isolates against *H. pylori* (ATCC43504) were evaluated. As a result, the two new flavonoid derivatives **6** and **7**, and (2*S*)-4′,7-dihydroxy-8-methylflavan (**11**), were found to be most efficacious against *H. pylori* (ATCC43504) with MIC values of 29.5, 29.5, and 31.3 μM, respectively. Compared to amoxicillin, these compounds exhibited inhibitory effects on the growth of a sensitive strain of *H. pylori* (ATCC43504) at much higher concentrations. However, it should be noted that these compounds were effective in suppressing this metronidazole-resistant strain of *H. pylori*, with comparable MIC values to the synthetic agents NE-2001 and TG44.[44,45]

The effect of these compounds on blood coagulation was assessed in a thrombin inhibition assay. Thrombin is a trypsin-like serine proteinase and acts in the blood coagulation cascade by catalyzing the conversion of fibrinogen to fibrin and by converting factor XIII to factor XIIIa, which then cross-links the fibrin clot. Thrombin also activates upstream zymogen factors V, VIII, and XI, which further accelerate the clotting cascade by thrombin synthesis.[46] At present, clinical treatment of thrombosis involves the administration of heparin and its low molecular weight derivatives or oral anticoagulants of the dicumarol type, which all indirectly inhibit thrombin. These drugs have limitations with regard to their efficacy and low therapeutic index, leading to the need for extensive monitoring. Thrombin has been under intense investigation for over a decade with the aim of identifying potent novel inhibitors that might be useful as anticoagulation agents.[47,48] Flavonoids are natural antioxidants acting on membrane phospholipids, nucleic acids, and proteins. Recently, scientific interest has been focused on the ways this class of polyphenols acts as specific ligands/effectors toward several important macromolecules. For instance, polyphenols have been shown to play an important role in the inhibition of a thrombin-like enzyme, elastase.[49] In this study, thrombin inhibitory activities of flavonoid derivatives (**1**–**7** and **9**) were demonstrable, although the effects were moderate (Table 4 and Figure 4). To study their selectivity, the effects of these compounds on another serine protease, dipeptidyl peptidase IV (DPP-IV), were measured, but no obvious inhibition was observed at concentrations up to 20 mg/mL (Table 4). Pending significant improvement in their potencies, these leads may have the potential to be developed as new therapeutic agents for thrombosis and stroke.

In a recent Japanese patent, compound **9** was reported to be a granulation tissue formation enhancer, which might be relevant to the wound-healing effect of *D. cochinchinensis*.[22]

Experimental Section

General Experimental Procedures. Optical rotations were measured with Perkin-Elmer 241MC instrument. CD spectra were recorded on a JASCO J-810 spectrometer. UV spectra were recorded with a Beckman DU-7 spectrometer. IR spectra were recorded using a Perkin-Elmer 577 spectrometer. HRESIMS data were obtained on a Mariner spectrometer. LREIMS measurements were made with a Finnigan MAT 95 instrument. NMR experiments were run on a Bruker AM 400 spectrometer with TMS as internal standard. Preparative HPLC was carried out using a Varian SD-1 instrument equipped with a Merck NW25 C_{18} column (10 μm, 20 mm × 250 mm) and ProStar 320 UV/vis detector. Column chromatographic separations were carried out

Table 3. Growth Inhibitory Effects of Compounds **1–22** against *Helicobacter pylori* (ATCC43504).

compound	MIC (μM)
1	60.8
2	62.5
3	64.3
4	66.1
5	62.5
6	29.5
7	29.5
8	201.3
1-[5-(2-methoxy-4,4′-dihydroxydihydrochalconyl)]-1-(4-hydroxyphenyl)-3-(2-methoxy-4-hydroxyphenyl)propane (**9**)	60.6
(2*R*)-4′-hydroxy-7-methoxy-8-methylflavan (**10**)	59.3
(2S)-4′,7-dihydroxy-8-methylflavan (**11**)	31.3
7-hydroxy-3-(4-hydroxybenzyl)-8-methoxychroman (**12**)	55.9
(2S)-4′,5-dihydroxy-7-methoxy-8-methylflavan (**13**)	55.9
4,4′,6-trihydroxy-2-methoxydihydrochalcone (**14**)	111.1
4,4′-dihydroxy-2′-methoxychalcone (**15**)	118.5
(–)-7-hydroxy-4′-methoxyflavan (**16**)	250.0
4,4′-dihydroxy-2-methoxydihydrochalcone (**17**)	235.3
trans-3,5-dihydroxy-4′-methoxystilbene (**18**)	264.5
4′-hydroxy-2,4-dimethoxydihydrochalcone (**19**)	223.8
trans-3,4′,5-trihydroxystilbene (**20**)	280.7
2,4,4′-trihydroxydihydrochalcone (**21**)	248.1
4,4′-dihydroxy-2, 6-dimethoxydihydrochalcone (**22**)	211.9
amoxycillin[a]	0.08
metronidazole[a]	747.7

[a] Amoxycillin and metronidazole were used as positive controls.

Table 4. Thrombin and DPP-IV Inhibitory Activities of Compounds **1–7** and **9**

compound	thrombin IC_{50} (μM)	DPP-IV IC_{50} (μM)
1	>9.5	>36.9
2	17.8	>39.1
3	26.7	>40.2
4	>41.3	>41.3
5	21.5	>39.1
6	12.3	>36.9
7	>9.2	>36.9
1-[5-(2-methoxy-4,4′-dihydroxydihydrochalconyl)]-1-(4-hydroxyphenyl)-3-(2-methoxy-4-hydroxyphenyl) propane (**9**)	26.3	>36.9
argatroban[a]	5.4 nM	N/A

[a] Argatroban was used as a positive control. N/A, not applicable.

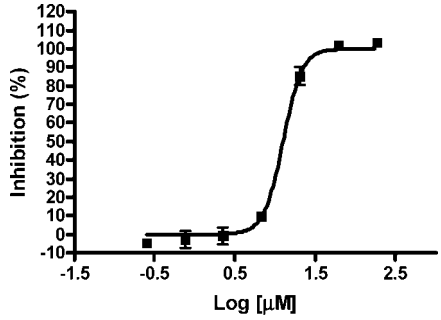

Figure 4. Dose–response characteristics of compound **6** against thrombin activity.

using silica gel H60 (300–400 mesh), zcx-II (100–200 mesh) (Qingdao Haiyang Chemical Group Corporation, Qingdao, People's Republic of China), ODS (40–63 μm) (Merck), and Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden) as packing materials. Lobar column chromatography was carried out using Lichroprep Si60 (40–63 μm) (Merck, Germany) equipped with a LabAlliance Series I pump (LabAlliance Co.). HSGF254 Si gel TLC plates (Yantai Chemical Industrial Institute, Yantai, People's Republic of China) and RP-18 WF_{254} TLC plates (Merck) were used for analytical TLC.

Plant Material and Biochemical Reagents. The stems of *D. cochinchinensis* (Lour.) S. C. Chen (Agavaceae) were collected in Ningming County, Guangxi autonomous region, People's Republic of China, in January 2006, and identified by Professor Jingui Shen of Shanghai Institute of Materia Medica, Chinese Academy of Sciences. A voucher specimen (No. 20070522) is deposited in the Herbarium of Shanghai Institute of Materia Medica, Chinese Academy of Sciences. The following biochemical agents were purchased: amoxicillin and metronidazole from Sigma-Aldrich Chemical Co. (St. Louis, MO), Mueller-Hinton agar from Oxoid Ltd. (Basingstoke, UK), lysed sheep blood from Shanghai Kunbo Trading Co., Ltd. (Shanghai, China), and Benzoyl-FVR-AMC from Calbiochem (UK). Human thrombin was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (NICPBP, China), Benzoyl-FVR-AMC was from Calbiochem (UK), and recombinant human DPP-IV and Ala-Pro-AMC were expressed and purified at our laboratory.

Extraction and Isolation. Powdered, air-dried stems of *D. cochinchinensis* (Lour.) S. C. Chen (5.0 kg) were percolated with 95% EtOH at room temperature (3 × 72 h). The solvents were evaporated *in vacuo*, and the residue was suspended in H_2O and then partitioned with $CHCl_3$ and *n*-BuOH (1 L × 3 each), successively, to give a $CHCl_3$ extract (1.0 kg) and an *n*-BuOH extract (300.0 g). The $CHCl_3$ extract (1.0 kg) was subjected to Si gel CC eluting with a gradient of petroleum ether and acetone (10:1, 5:1, 3:1, 2:1, 1:1, 0:1, each 1.5 L), and five fractions (F_1–F_5) were obtained. F_2 (29.2 g) was further separated by ODS CC eluting with a gradient of MeOH–H_2O (1:1, 7:3, 9:1, and 1:0) and followed by preparative HPLC using a gradient of MeOH–H_2O (70% to 100% over 70 min, 10 mL/min) to afford (2*R*)-4′-hydroxy-7-methoxy-8-methylflavan (**10**) (181.6 mg) and (–)-7-hydroxy-4′-methoxyflavan (**16**) (318.8 mg). F_3 (28.8 g) was separated into three subfractions (F_{31}–F_{33}) by ODS CC eluting with a gradient of MeOH–H_2O (1:1, 7:3, 9:1, and 1:0). F_{32} (18.1 g) was submitted to Lobar column chromatography and then separated by PTLC, to give (2*S*)-4′,7-dihydroxy-8-methylflavan (**11**) (74.3 mg), 7-hydroxy-3-(4-hydroxybenzyl)-8-methoxychroman (**12**) (37.4 mg), (2*S*)-4′,5-dihydroxy-7-methoxy-8-methylflavan (**13**) (16.2 mg), 2,4′-dihydroxy-4-methoxydihydrochalcone (**17**) (55.2 mg), *trans*-3,5-dihydroxy-4′-methoxystilbene (**18**) (19.0 mg), and 4′-hydroxy-2,4- dimethoxydihydrochalcone (**19**) (82.8 mg). F_4 (30.0 g) was separated into two subfractions (F_{41}, F_{42}) by ODS CC eluting with a gradient of MeOH–H_2O (2:3, 1:1, 7:3, 9:1, and 1:0). F_{41} (7.3 g) was chromatographed over preparative HPLC using a gradient of MeOH–H_2O (45% to 65% over 80 min, 10 mL/min) and followed by PTLC [$CHCl_3$–MeOH (8:1) to yield **8** (12.6 mg), 4,4′,6-trihydroxy-2-methoxydihydrochalcone (**14**) (18.9 mg), 4,4′-dihydroxy-2′-methoxychalcone (**15**) (10.1 mg), *trans*-3,4′,5-trihydroxystilbene (**20**) (33.1 mg), 2,4,4′-trihydroxydihydrochalcone (**21**) (45.5 mg), and 4,4′-dihydroxy-2,6-dimethoxydihydrochalcone (**22**) (15.0 mg). F_{42} (20.2 g) was subjected to CC over silica gel zcx-II (100–200 mesh) using a gradient of $CHCl_3$–MeOH (20:1, 10:1, 8:1, 6:1, 4:1, and 0:1) as eluent to yield subfractions F_{421}–F_{426}. F_{421} (1.5 g) was separated by preparative HPLC using a gradient of 70% to 100% MeOH in H_2O over 70 min, followed by PTLC [$CHCl_3$–MeOH (15:1)], and then passed through a Sephadex LH-20 column with EtOH as eluent, to afford **1** (20.1 mg). F_{422} (1.2 g) was separated by preparative HPLC using a gradient of 60% to 80% MeOH in H_2O over 70 min, followed by PTLC [$CHCl_3$–MeOH (10:1)], and then passed through a Sephadex LH-20 column with EtOH as eluent to give **2** (5.0 mg) and **7** (12.1 mg). Compounds **5** (130.2 mg) and **6** (15.1 mg) were obtained from F_{423} (3.0 g) using the above method. Purification of F_{425} (1.0 g) and F_{426} (0.9 g) by HPLC (using a gradient of 60% to 80% MeOH in H_2O over 70 min) and a Sephadex LH-20 column eluted with EtOH resulted in the purification of **3** (11.3 mg) and **9** (122.1 mg). Compound **4** (80.6 mg) was obtained from F_5 (20.5 g) by CC eluting with $CHCl_3$–MeOH (10:1) and then repeated PHPLC (using a gradient of 45% to 65% MeOH in H_2O over 80 min).

Susceptibility Testing. Amoxicillin and metronidazole were commercially available (Sigma-Aldrich, St. Louis, MO). Amoxicillin and the test compounds were dissolved in DMSO and metronidazole in H_2O. The stock solutions were serially diluted in sterile H_2O to give final concentrations on the day of use. The MICs for the standard *H. pylori* strain ATCC43504 were determined by an agar dilution method. Briefly, Mueller-Hinton agar (Oxoid, Basingstoke, UK) plates (10 mL/each) were prepared containing 7% lysed sheep blood (Shanghai Kunbo Trading Co., Ltd., China) and 2-fold serial dilutions of the test compounds. They were inoculated with 5 μL of each bacterial

suspension (10^7 cfu/mL) using a multipoint inoculator (Sakuma, Tokyo, Japan) and incubated at 37°C for 3 days under a microaerobic atmosphere in an incubator consisting of 5% O_2, 10% CO_2, and 85% N_2 with 98% humidity (Napco Co., Winchester, VA). Antibiotic-free plates and plates with corresponding dilutions of DMSO were used as negative controls to ensure bacteria viability and no contaminants in the inoculums.

Thrombin Assay. Compounds were incubated with human thrombin (NICPBP, China) and 20 μM fluorogenic substrate, Benzoyl-FVR-AMC (Calbiochem, UK), in 50 mM Tris pH 8.0, 150 mM NaCl, 2.5 mM $CaCl_2$, and 2% DMSO in 96-well plates. The cleavage of the substrate was followed by monitoring the change in fluorescence at 460 nm (excitation at 355 nm) for 15 min at room temperature on an EnVision (Perkin-Elmer) plate reader. Initial reaction rates were measured, and IC_{50}'s were calculated from replicate curves using GraphPad Prism software (San Diego, CA); standard errors were within statistically acceptable limits. Positive control was provided by argatroban, which is well described in the thrombin literature.[50]

DPP-IV Assay. Recombinant human DPP-IV activity was assayed in 100 mM Hepes, pH 7.5, with 0.01 nM DPP-IV, 10 μM Ala-Pro-AMC, and 2% DMSO in black 96-well plates. The measurement and data process were similar to the thrombin assay. Positive control was provided by P32/98.[51]

Cochinchinenene A (1): pale yellow solid; $[\alpha]_D^{20}$ +3.0 (*c* 0.08, CH_3OH); UV (MeOH) λ_{max} (log ε) 328 (2.65) nm; IR (KBr) ν_{max} 3404, 2933, 1598, 1510, 1452, 1243, 1120, 1035, 954, 833 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 526 [M^+] (10), 389 (1), 375 (35), 270 (40), 256 (10), 121 (100); HRESIMS *m/z* 549.2251 [M + Na]$^+$ (calcd for $C_{33}H_{34}O_6Na$, 549.2253).

Cochinchinenene B (2): colorless gum; $[\alpha]_D^{20}$ +1.0 (*c* 0.16, CH_3OH); UV (MeOH) λ_{max} (log ε) 328 (2.60) nm; IR (KBr) ν_{max} 3406, 2933, 1606, 1510, 1466, 1245, 1120, 1036, 955, 833 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 512 [M^+] (10), 361 (100), 270 (30), 242 (30), 151 (30), 137 (60); HRESIMS *m/z* 535.2086 [M + Na]$^+$ (calcd for $C_{32}H_{32}O_6Na$, 535.2097).

Cochinchinenene C (3): colorless gum; $[\alpha]_D^{20}$ +6.0 (*c* 0.18, CH_3OH); UV (MeOH) λ_{max} (log ε) 328 (2.58) nm; IR (KBr) ν_{max} 3385, 2933, 1598, 1512, 1456, 1238, 1195, 1036, 955, 835 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 498 [M^+] (1), 347 (5), 256 (10), 242 (100); HRESIMS *m/z* 521.1956 [M + Na]$^+$ (calcd for $C_{31}H_{30}O_6Na$, 521.1940).

Cochinchinenene D (4): white, amorphous powder; $[\alpha]_D^{20}$ +2.0 (*c* 0.15, CH_3OH); UV (MeOH) λ_{max} (log ε) 328 (2.63) nm; IR (KBr) ν_{max} 3415, 2933, 1606, 1510, 1452, 1238, 1172, 1012, 955, 835 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 484 [M^+] (1), 333 (5), 256 (35), 228 (100), 137 (25); HRESIMS *m/z* 507.1793 [M + Na]$^+$ (calcd for $C_{30}H_{28}O_6Na$, 507.1784).

(2*R*)-8-Methylsocotrin-4′-ol (5): pale yellow, amorphous powder; $[\alpha]_D^{20}$ +1.0 (*c* 0.10, CH_3OH); UV (MeOH) λ_{max} (log ε) 281 (2.21) nm; IR (KBr) ν_{max} 3386, 2935, 1614, 1510, 1469, 1236, 1114, 1035, 953, 831 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 512 [M^+] (5), 361 (10), 256 (50), 137 (50), 120 (50), 166 (100); HRESIMS *m/z* 535.2097 [M + Na]$^+$ (calcd for $C_{32}H_{32}O_6Na$, 535.2097). CD (c 5.1×10^{-3} M, MeOH) λ ($\Delta\varepsilon$) 246 (−2.54), 278 (+2.40) nm.

Cochinchinenin B (6): colorless gum; $[\alpha]_D^{20}$ +4.0 (*c* 0.14, CH_3OH); UV (MeOH) λ_{max} (log ε) 281 (2.48) nm; IR (KBr) ν_{max} 3412, 2933, 1648, 1600, 1510, 1454, 1288, 1205, 1168, 1112, 1035, 953, 833 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 542 [M^+] (10), 391 (45), 286 (95), 256 (45), 181 (100), 137 (45), 121 (60); HRESIMS *m/z* 565.2200 [M + Na]$^+$ (calcd for $C_{33}H_{34}O_7Na$, 565.2202).

Cochinchinenin C (7): colorless gum; $[\alpha]_D^{20}$ +3.0 (*c* 0.19, CH_3OH); UV (MeOH) λ_{max} (log ε) 280 (2.47) nm; IR (KBr) ν_{max} 3396, 2935, 1654, 1600, 1510, 1452, 1284, 1245, 1201, 1168, 1114, 1035, 953, 833 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 542 [M^+] (5), 391 (30), 272 (10), 270 (15), 167 (100), 137 (40), 121 (45); HRESIMS *m/z* 565.2214 [M + Na]$^+$ (calcd for $C_{33}H_{34}O_7Na$, 565.2202).

Cochinchinenone (8): pale red gum; UV (MeOH) λ_{max} (log ε) 224 (2.66), 243 (2.73), 281 (2.68) nm; IR (KBr) ν_{max} 3357, 2942, 1655, 1606, 1585, 1459, 1365, 1242, 1215, 1083, 843 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 318 [M^+] (5), 170 (35), 148 (20), 121 (100); HRESIMS *m/z* 341.1002 [M + Na]$^+$ (calcd for $C_{17}H_{18}O_6Na$, 341.1001).

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