Anti-inflammatory Alkaloids from the Stems of *Picrasma quassioides* Bennet

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During further chemical and biological investigations of *Picrasma quassioides* Bennet, four new bis-β-carboline alkaloids, quassidines E—H (1—4), and three new β-carboline alkaloids, canthin-16-one-14-butyric acid (5), 3-(1,1-dimethoxylmethyl)-β-carboline (6), and 6,12-dimethoxy-3-formyl-β-carboline (7), were isolated from its anti-inflammatory $CHCl_3$-soluble fraction. Structures of new compounds were elucidated and characterized by MS and NMR analysis. A plausible biogenetic pathway for quassidine E (1), the first bis-β-carboline alkaloid in which a canthin-6-one moiety and a β-carboline moiety were connected together by a single carbon–carbon bond from the nature, was proposed. Quassidines E—G (1—3) showed potent inhibitory activity on the production of nitric oxide (NO), tumor necrosis factor α (TNF-α), or interleukin 6 (IL-6) in mouse monocyte-macrophage RAW264.7 cells stimulated by lipopolysaccharide (LPS). Analysis of anti-inflammatory activity of all β-carboline and bis-β-carboline alkaloids from *P. quassioides* showed that the carbonyl groups or double carbon–carbon bonds at C-14 for β-carbolines and C-14′ for bis-β-carbolines were bioactive groups for their *in vitro* anti-inflammatory activity. Structure–activity relationship of these compounds on inhibitory activity of the three inflammatory cytokines was discussed.

Key words *Picrasma quassioides*; alkaloid; anti-inflammatory; bis-β-carboline; β-carboline

Chronic inflammation has been found to mediate a wide variety of diseases, including cardiovascular diseases, cancer, Alzheimer's disease, and autoimmune diseases.[1)] How is inflammation diagnosed and its biomarkers is not fully understood, however, the role of inflammatory cytokines, such as nitric oxide (NO), tumor necrosis factor α (TNF-α), and interleukin 6 (IL-6), have been linked with chronic inflammation.[2)] During our screening studies on NO inhibitors in traditional medicine, the $CHCl_3$-soluble fraction of 95% EtOH extract of *Picrasma quassioides* (Simaroubaceae) stems showed potent NO inhibitory activity.[3)] Previous chemical and biological investigations on the bioactive fraction have resulted in the isolation of four new bis-β-carbolines, quassidines A—D,[3)] and five new β-carbolines, together with 19 known alkaloids.[4,5)] Quassidine A was the first reported bis-β-carboline alkaloid possessing a novel cyclobutane unit.[3,6)] Our further investigation of the $CHCl_3$-soluble fraction led to the isolation and characterization of four new bis-β-carbolines, quassidines E—H (**1**—**4**), and three new β-carbolines, canthin-16-one-14-butyric acid (**5**), 3-(1,1-dimethoxylmethyl)-β-carboline (**6**), and 6,12-dimethoxy-3-formyl-β-carboline (**7**). Quassidine E (**1**) was a novel bis-β-carboline alkaloid in which a canthin-6-one moiety and a β-carboline moiety were connected together by a single carbon–carbon bond. In this paper, the isolation, structure elucidation, and *in vitro* anti-inflammatory activity of new compounds were reported. In addition, the plausible biogenetic pathway of quassidine E (**1**) was proposed and the structure–activity relationship of bis-β-carboline and β-carboline alkaloids was also discussed.

Results and Discussion

By a series of column chromatography using silica gel, Sephadex LH-20, and octadecylsilyl (ODS) in combination with reversed phase HPLC, the $CHCl_3$-soluble fraction of *P. quassioides* stems resulted in the purification of four new bis-β-carbolines (**1**—**4**) and three new β-carbolines (**5**—**7**), as shown in Fig. 1.

Quassidine E (**1**) was obtained as a yellowish powder and had a molecular formula of $C_{27}H_{18}N_4O_2$ established by the HR-ESI-MS ion peak at *m*/*z* 429.1356 $[M-H]^-$ (Calcd for $C_{27}H_{17}N_4O_2$, 429.1357). The UV spectrum of **1** displayed absorption bonds at 243, 282, 332, 367, and 383 nm, suggesting the presence of β-carboline chromophores.[7)] The absorption

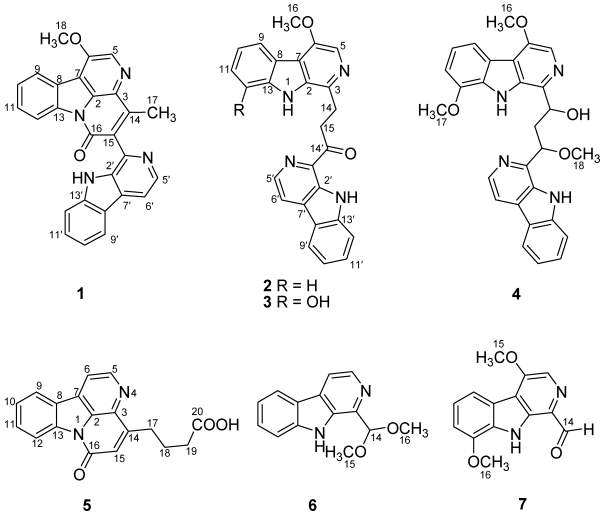

Fig. 1. Chemical Structures of **1**—**7** from *P. quassioides*

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Table 1. ^{1}H-NMR Spectroscopic Data (J in Hz) of Compounds **1**—**4** in DMSO-d_6

No.	**1**	**2**	**3**	**4**
1		11.71 (br s)	11.46 (br s)	11.03 (br s)
5	8.79 (s)	7.90 (s)	7.83 (s)	7.93 (s)
9	8.52 (d, 8.0)	8.19 (d, 8.0)	7.64 (d, 7.6)	7.77 (d, 7.8)
10	7.75 (td, 8.0,1.0)	7.24 (td, 8.0, 1.0)	7.03 (t, 7.6)	7.18 (t, 7.8)
11	7.62 (td, 8.0, 1.0)	7.51 (td, 8.0, 1.0)	6.93 (d, 7.6)	7.11 (d, 7.8)
12	8.27 (d, 8.0)	7.62 (d, 8.0)		
14		3.55 (t, 7.0)	3.56 (t, 7.0)	5.08 (m)
15		4.00 (t, 7.0)	3.91 (t, 7.0)	2.76 (m)
				2.63 (m)
1′	11.40 (br s)	11.88 (br s)	11.90 (br s)	11.40 (br s)
5′	8.48 (d, 5.2)	8.52 (d, 5.0)	8.53 (d, 5.0)	8.35 (d, 5.2)
6′	8.19 (d, 5.2)	8.43 (d, 5.0)	8.43 (d, 5.0)	8.08 (d, 5.2)
9′	8.29 (d, 8.0)	8.29 (d, 7.9)	8.28 (d, 8.0)	8.22 (d, 8.1)
10′	7.26 (td, 8.0, 1.0)	7.29 (td, 7.9, 1.0)	7.29 (td, 8.0, 1.0)	7.23 (td, 8.1, 1.0)
11′	7.52 (td, 8.0, 1.0)	7.57 (td, 7.9, 1.0)	7.58 (td, 8.0, 1.0)	7.54 (td, 8.1, 1.0)
12′	7.45 (d, 8.0)	7.78 (d, 7.9)	7.77 (d, 8.0)	7.69 (d, 8.1)
14′				5.00 (m)
16		4.04 (s)	4.01 (s)	4.06 (s)
17	2.36 (s)			4.03 (s)
18	4.33 (s)			3.18 (s)
12-OH			9.93 (s)	
14-OH				5.59 (d, 5.9)

Table 2. ^{13}C-NMR Spectroscopic Data of Compounds **1**—**4** in DMSO-d_6

No.	**1**	**2**	**3**	**4**
2	131.9	134.9	134.8	133.5
3	130.5	137.7	138.4	140.8
5	130.6	119.84	119.5	119.1
6	152.1	149.9	149.8	150.4
7	115.6	116.2	116.8	117.5
8	137.8	120.3	122.0	121.1
9	116.2	123.2	114.0	115.4
10	129.6	119.3	120.11	120.5
11	125.7	126.7	110.9	107.3
12	124.1	111.6	143.6	145.8
13	123.1	139.6	129.6	129.5
14	147.8	26.7	27.2	69.0
15	131.1	35.1	38.5	39.7
2′	134.8	133.9	134.0	140.7
3′	139.1	135.8	135.8	144.3
5′	137.9	137.3	137.4	137.0
6′	114.4	119.2	119.2	114.2
7′	128.0	130.9	130.9	120.5
8′	120.7	119.81	119.8	128.5
9′	121.7	121.7	121.7	121.4
10′	119.2	120.0	120.05	119.2
11′	128.1	128.7	128.8	128.0
12′	111.7	112.9	113.0	112.3
13′	140.4	141.8	141.8	133.3
14′		202.3	202.3	80.2
16	158.7	55.9	55.8	55.96
17	13.7			55.5
18	57.2			55.95

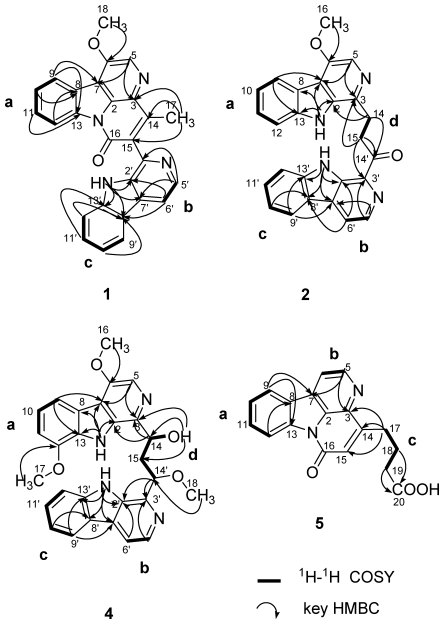

Fig. 2. Key Correlations and Partial Structures of **1**, **2**, **4** and **5**

bonds at 1665 and 1626 cm^{-1} in the IR spectrum of **1** indicated the presence of an α,β-unsaturated amide fragment. Analysis of the ^{1}H-NMR data in conjunction with the HSQC spectrum revealed the presence of one NH, one methoxyl, one methyl, eleven protonated sp^2 carbons (Table 1). The ^{13}C-NMR spectrum further showed the presence of one carbonyl, fourteen nonprotonated sp^2 carbons (including two oxygenated quaternary carbons) (Table 2). Although **1** was suggested to be related to bis-β-carboline alkaloids (such as quassidines A and B) by comparing the ^{1}H- and ^{13}C-NMR data,[3] the gross structure of **1** was quite different on the basis of a detailed analysis of 2D-NMR data including COSY, HSQC, HMBC spectra. Three partial structures **a**—**c** were deduced from COSY analysis of **1** (Fig. 2). HMBC correlations for H-5/C-3,7, H-9/C-7, H-10/C-8, and H-11/C-13 established partial structure of canthin-6-one moiety; the long-ranged correlations H_3-17/C-3,14,15 and H_3-18/C-6 determined the position of the methyl and methoxyl groups, respectively. Furthermore, HMBC correlations from H-5′ to C-7′ as well as correlations from H-6′ and H-10′ to C-8′ could connect two partial structural units **b** and **c**. The connection of β-carboline moiety was unambiguously established by

Table 3. ^{1}H- and ^{13}C-NMR Spectroscopic Data of Compounds **5**—**7**

No.	**5**[a]		**6**[b]		**7**[b]	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1			9.16 (br s)		10.13 (br s)	
2		131.3		133.5		136.4
3		135.8		140.2		131.8
5	8.84 (d, 4.9)	145.1	8.45 (d, 5.2)	138.2	8.26 (s)	123.2
6	8.32 (d, 4.9)	117.3	7.95 (d, 5.2)	114.9		154.9
7		129.5		130.1		119.0
8		124.2		121.1		121.0
9	8.37 (d, 8.0)	123.5	8.13 (d, 8.0)	121.6	7.88 (d, 8.0)	116.1
10	7.58 (td, 8.0, 1.0)	125.4	7.28 (td, 8.0, 1.0)	119.9	7.28 (t, 8.0)	121.8
11	7.75 (td, 8.0, 1.0)	130.7	7.56 (td, 8.0, 1.0)	128.6	7.03 (d, 8.0)	108.1
12	8.49 (d, 8.0)	116.1	7.54 (d, 8.0)	111.6		146.2
13		138.5		140.3		130.6
14		153.0	5.76 (s)	106.6	10.23 (s)	193.6
15	6.79 (s)	125.1	3.53 (s)	54.2	4.28 (s)	56.6
16		158.8	3.53 (s)	54.2	4.05 (s)	55.7
17	3.05 (t, 7.4)	28.5				
18	2.03 (quai, 7.4)	23.8				
19	2.35 (t, 7.4)	33.1				
20		174.1				

a) Measured in DMSO-d_6. *b*) Measured in $CDCl_3$.

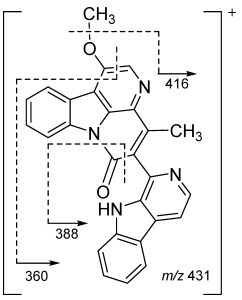

Fig. 3. Cleavage Pathway of Positive ESI-MS[4] Fragmentation of Quassidine E (**1**)

HMBC data: correlations H-5′/C-3′, H-6′/C-2′, and H-11′/C-13′ determined the position of the three quaternary carbons; cross peaks observed for NH/C-2′,7′,8′,13′ suggested the placement of the NH. Finally, the 6-methoxy-14-methylcanthin-16-one moiety was deduced to be connected to the β-carboline moiety *via* a single carbon–carbon bond between C-15 and C-3′. With all the information, the structure of **1** was determined as shown in Fig. 1. It was the first bis-β-carboline alkaloid obtained from the nature, in which the canthin-6-one moiety and the β-carboline moiety were connected together by a single carbon–carbon bond, named as quassidine E (**1**). The complete structure of **1** was further confirmed by its positive ESI-MS[4] analysis (Fig. 3).

Quassidine F (**2**) had a molecular formula of $C_{26}H_{20}N_4O_2$ established by the HR-ESI-MS ion peak at *m/z* 421.1681 $[M+H]^+$ (Calcd for $C_{26}H_{21}N_4O_2$, 421.1665). The UV spectrum of **2** showed absorption bonds at 244 and 285 nm. Four partial structures **a**—**d** deduced from COSY spectra were connected by key HMBC correlations as follows: (1) correlations for H-5/C-3,7, H-9/C-7,13, NH-1/C-2,7,8,13 and H_3-16/C-6 established one 6-methoxy-β-carboline moiety; (2) correlations for H-5′/C-3′,7′, H-6′/C-2′,8′, H-9′/C-7′,13′, and NH-1′/C-2′,7′,8′,13′ established the other β-carboline moiety; (3) correlations for H_2-14/C-2,3,14′ and H_2-15/C-3,3′,14′ determined the two β-carboline moieties were connected together by partial structure **d** and the carbonyl at C-14′ *via* the carbons C-3 and C-3′. Thus, the structure of **2** was determined as shown in Fig. 1, named as quassidine F.

Quassidine G (**3**) had a molecular formula of $C_{26}H_{20}N_4O_3$ established by the HR-ESI-MS ion peak at *m/z* 435.1480 $[M-H]^-$ (Calcd for $C_{26}H_{19}N_4O_3$, 435.1457), suggesting that **3** was a hydroxyl-derivative of **2**. Oxidization of C-12 aromatic methine was straightforward from ^{1}H- and ^{13}C-NMR data (δ_{OH} 9.93, δ_C 143.6) assigned as 12-hydroxylquassidine F, which was confirmed by the HMBC correlations of **3** from 12-OH to C-11, C-12, and C-13.

Quassidine H (**4**) had a molecular formula of $C_{28}H_{26}N_4O_4$ established by the HR-ESI-MS ion peak at *m/z* 483.2047 $[M+H]^+$ (Calcd for $C_{28}H_{27}N_4O_4$, 483.2032). The UV spectrum of **4** showed absorption bonds at 245 and 289 nm. The ^{1}H- and ^{13}C-NMR data of **4** were similar to those of **2**, and the main difference was the carbonyl C-14′, the methylene C-14, and the aromatic methine C-12 were correspondingly replaced by an oxygenated methine at δ_C 80.2, an oxygenated methine at δ_C 69.0, and an aromatic quaternary carbon at δ_C 145.8. The HMBC correlations for H_3-18/C-14′, 14-OH/C-14,3,15, and H_3-17/C-12 determined the placement of two methoxyl groups and the hydroxyl group (Fig. 2). Thus, the structure of **4** was assigned as shown in Fig. 1, named as quassidine H. The relative and absolute configuration of **4** remained to be determined in account of the scarcity of samples and the presence of 14′-OCH_3.

Compound **5** was obtained as a yellowish powder and had a molecular formula of $C_{18}H_{14}N_2O_3$ established by the HR-ESI-MS ion peak at *m/z* 305.0930 $[M-H]^-$ (Calcd for $C_{18}H_{13}N_2O_3$, 305.0926). The UV spectrum of **5** displayed absorption bonds at 243 and 281 nm, indicating the presence of a β-carboline chromophore. No NH was observed in the ^{1}H-NMR spectrum and 15 aromatic carbons were displayed in the ^{13}C-NMR spectrum, suggesting the presence of a canthin-6-one moiety. Three partial structures **a**—**c** were de-

duced from COSY analysis of **5** (Fig. 1). The HMBC correlations H_2-17/C-3,14,15 and H_2-19/C-20 determined the butyric acid moiety was located at position C-14. Thus, the structure of **5** was determined as canthin-16-one-14-butyric acid.

Compound **6** had a molecular formula of $C_{14}H_{14}N_2O_2$ which was established by the HR-ESI-MS ion peak at *m/z* 241.0976 $[M-H]^-$. It was induced to be a monomeric β-carboline by comparing the ^{1}H- and ^{13}C-NMR data of **6** to those of **2**. In the HMBC spectrum, H-14 showed correlations to C-2 and C-3, while H_3-15 and H_3-16 both showed correlations to C-14, which determined the dimethoxylmethyl group was located at C-3 position. Thus, the structure of **6** was finally determined as 3-(1,1-dimethoxylmethyl)-β-carboline, which might be an artifact arising from 3-formyl-β-carboline (**10**). The aldehyde group of **10** could carry out a nucleophilic addition reaction with CH_3OH, the solvent widely used in the experimental procedures, resulting in the production of **6**.

The molecular formula of **7** was established as $C_{14}H_{12}N_2O_3$ by its HR-ESI-MS. ^{1}H- and ^{13}C-NMR data of **7** were similar to those of **6** except for the formyl group (δ_H 10.23, δ_C 193.6) and two methoxyl groups (δ_C 55.7, δ_C 56.6). The HMBC correlations for H-14/C-2,3, H_3-15/C-6, and H_3-16/C-12 determined the formyl group and two methoxyl groups were attached at C-3, C-6, and C-12, respectively. Thus, the structure of **7** was determined as 6,12-dimethoxy-3-formyl-β-carboline, as shown in Fig. 1.

A plausible biogenetic pathway for quassidine E (**1**) was proposed as shown in Chart 1. The two precursors, picrasidine X (**1a**) and 3-acetyl-β-carboline (**1b**),[8,9] were known β-carboline alkaloids obtained from *P. quassioides*. Compound **1a** underwent decarboxylation and oxidation at C-15 to produce **1c**. When it lost a proton at CH_2-14, compound **1c** could convert to a carbanion, which attacked the carbonyl at C-14′ in **1b** to form **1d**. By intramolecular acylation and dehydration, compound **1d** was transformed to compound **1f**, which led to the final formation of qussidine E (**1**) following its oxidation and methylation at C-6.

The *in vitro* anti-inflammatory activity of seven new compounds (**1**—**7**) and 23 β-carbolines (**8**—**30**) was evaluated by monitoring the inhibition activity on the production of NO, TNF-α, or IL-6 in mouse monocyte-macrophage RAW 264.7 cells stimulated by lipopolysaccharide (LPS). As shown in Table 4, quassidine E (**1**), especially quassidines F (**2**) and G (**3**) showed potent inhibitory activity, whereas quassidines B—D[3] and H (**4**) showed potent toxicity on normal RAW 264.7 cells on the concentration of 100 μg/ml. The major difference of quassidines F (**2**) and G (**3**) with quassidines B—D and H (**4**) was the oxidation level of C-14′, ketone groups for the former and hydroxyl groups for the latter. Thus, the ketone groups at C-14′ might be the bioactive groups for quassidines F (**2**) and G (**3**), while the hydroxyl groups at C-14′ might be the toxic groups to normal RAW 264.7 cells for quassidines B—D and H (**4**) on test concentration of 100 μg/ml.

The similar conclusion was deduced by analyzing the inhibition activity of β-carbolines (**7**—**30**)[4,5] shown in Table 5. Compounds **7** and **10**—**15** showed much stronger inhibitory activity on NO, TNF-α, or IL-6 than other β-carbolines. The carbons C-14 of **7** and **10**—**15** were all sp^2 hybridization, such as aldehyde groups, ester groups, and double carbon–carbon bonds. As a consequence, the carbonyl groups or double carbon–carbon bonds at C-14 were crucial for *in vitro* anti-inflammatory activity of β-carbolines.

In this work, four new bis-β-carboline alkaloids, quassidines E—H (**1**—**4**), and three new β-carboline alkaloids (**5**—**7**) were isolated from the stems of *P. quassioides*. Their structures were fully elucidated by 2D-NMR analysis. A plausible biogenetic pathway for the novel quassidine E (**1**) was proposed. Analysis of the anti-inflammatory activity of bis-β-carboline and β-carboline alkaloids (**7**—**30**) suggested that the carbonyl groups or double carbon–carbon bonds at C-14 for β-carbolines and C-14′ for bis-β-carbolines were bioactive groups for these compounds.

Table 4. Inhibitory Effects of Bis-β-carbolines from *P. quassioides* on NO, TNF-α, and IL-6 Production in LPS-Stimulated RAW 264.7 Cells (n=4)

	Inhibition activity (IC_{50} μM)		
	NO	TNF-α	IL-6
Quassidine E (**1**)	20.51±1.67	25.64±2.32	45.35±3.29
Quassidine F (**2**)	9.85±0.76	>100	24.32±2.17
Quassidine G (**3**)	13.09±1.15	12.27±1.39	17.14±1.64
Quassidine H (**4**)	+++[b]	+++	+++
Hydrocortisone[a]	64.34±6.17	85.64±6.23	63.86±5.25

a) Hydrocortisone, positive control. *b*) Toxicity to normal RAW 264.7 cells on the concentration of 100 μg/ml.

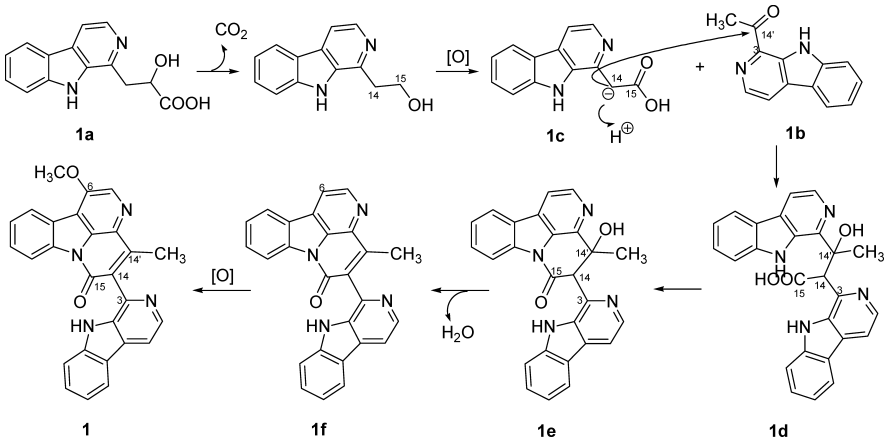

Chart 1. A Plausible Biogenetic Pathway for Quassidine E (**1**)

Table 5. Inhibitory Effects of β-Carbolines from *Picrasma quassioides* on NO, TNF-α, and IL-6 Production in LPS-Stimulated RAW 264.7 Cells (n=4)

	R_1	R_2 ([a]10-OH)	R_3	Inhibition activity (IC_{50} μM)		
				NO	TNF-α	IL-6
5	—	—	—	47.90±4.13	25.54±3.31	66.96±6.58
6	H	H	$CH(OCH_3)_2$	26.94±1.96	35.02±2.88	50.43±4.71
7	OCH_3	OCH_3	CHO	17.42±0.98	>100	>100
8	OCH_3	OH	$COOCH_3$	54.79±3.45	58.64±4.78	57.20±4.26
9	OH	H	$COOCH_3$	65.82±6.02	35.09±2.48	92.01±6.33
10	H	H	CHO	19.04±1.85	9.98±1.14	14.84±1.35
11	H	H	$COOCH_3$	7.68±0.83	29.23±2.08	3.87±0.58
12	H	H	$COOCH_2CH_3$	23.33±2.56	21.87±1.66	30.54±2.93
13	OCH_3		Vinyl	4.07±0.55	9.00±1.17	6.54±0.61
14	OCH_3	OCH_3	Vinyl	3.96±0.63	7.42±0.94	11.30±0.99
15	OCH_3	H	CHO	55.16±3.52	>100	13.17±1.11
16	OCH_3	OCH_3	CH_2CH_2OH	89.81±6.22	>100	>100
17	OCH_3	OCH_3	$CH(OH)CH_3$	+++[b]	+++	+++
18	OCH_3	OCH_3	$CH(OH)CH_2OH$	43.17±4.02	23.02±1.93	60.35±4.69
19	OCH_3	H	$CH(OCH_2CH_3)CH_2OH$	63.62±4.31	+++	+++
20	OCH_3	H	CH_2CH_2OH	77.85±0.83	>100	>100
21	OCH_3	H	$CH(OH)CH_2OH$	>100	>100	>100
22	H	H	CH_2OH	18.13±1.36	>100	33.40±0.46
23	OCH_3	H	CH_2CH_3	+++	>100	26.84±2.92
24	OCH_3	OCH_3	CH_2CH_3	+++	+++	+++
25	OCH_3	H	CH_3	>100	>100	>100
26	H	H	CH_2CH_2COOH	>100	>100	>100
27	H	H	$CH_2CH(OH)COOH$	>100	>100	>100
28	H	OH[a]	OH	>100	>100	>100
29	H	H	OH	74.87±6.88	32.39±3.06	41.65±3.94
30	H	H	OCH_3	63.46±7.21	64.41±5.23	>100
Hydrocortisone[a]				64.34±6.17	85.64±6.23	63.86±5.25

a) Hydrocortisone, positive control. *b*) Toxicity to normal RAW 264.7 cells on the concentration of 100 μg/ml.

Experimental

General Procedures Column chromatography was performed with silica gel (Haiyang; 200—300 mesh) and Sephadex LH-20 (Mitsubishi Kasei, Tokyo, Japan), HPLC purification was carried out on a Gilson apparatus equipped with a 306 pump and a UV/VIS-512 detector by using reversed-phase preparative column (21.2×250 mm, 5 μm, Welch XB-C18, v=8 ml/min). Optical rotations were measured on a Jasco P-1020 digital polartmeter (l=1 cm). UV spectra were recorded on a Jasco FTIR-400 spectrophotometer. 1D- and 2D-NMR spectra were acquired in DMSO-d_6 and $CDCl_3$ (chemical shifts were referenced to the solvent signals) on a Bruker Avance-400 operating at 400 MHz, using an inverse probe fitted with a gradient along the *Z*-axis. ESI-MS were recorded on a Finnigan LCQ Advantage MAX spectrometer, and HR-ESI-MS were measured on a Agilent 6210 LC/MSD TOF mass spectrometer.

Plant Material The stems of *P. quassioides* were collected from Guangxi Autonomy, China in June 2005, and authenticated at Guangzhou University of Traditional Chinese Medicine. A voucher specimen has been deposited at Institute of Traditional Chinese Medicine and Natural Products, Jinan University, Guangzhou (510632), China.

Extraction and Isolation The dried stems of *P. quassioides* (100 kg) were extracted with 95% ethanol, and the combined solvent extracts were concentrated under reduced pressure. The extract (200 g) was suspended in water and successively extracted with $CHCl_3$, EtOAc, and *n*-BuOH to yield $CHCl_3$-soluble fraction (128 g), EtOAc-soluble fraction (8.12 g), and *n*-BuOH fraction (20.8 g). The $CHCl_3$-soluble fraction was subjected to a silica gel column eluted with cyclohexane, cyclohexane/EtOAc, EtOAc, and MeOH to afford ten fractions (PQC1—PQC10). PQC3 (4.5 g, cyclohexane/EtOAc 50 : 50) was isolated by a silica gel chromatography column using gradient elution with $CHCl_3/CH_3OH$ (98 : 2—95 : 5) to yield four fractions. Purification of fraction PQC3-3 (1.1 g, $CHCl_3/CH_3OH$ 98 : 2) on a further silica gel chromatography column using an isocratic elution with $CHCl_3/CH_3OH$ 98 : 2 to afford compound **7** (26.3 mg). The fraction PQC7 (19.2 g, cyclohexane/EtOAc 50 : 50) was purified by silica gel chromatography column using gradient elution with $CHCl_3/CH_3OH$ (100 : 0—0 : 100) to afford seven fractions. The fourth fraction PQC7-4 (6.0 g, $CHCl_3/CH_3OH$ 98 : 2) was isolated on ODS-MPLC using a gradient elution with CH_3OH/H_2O (90 : 10—100 : 0) to yield ten subfractions, the eight subfraction PQC7-4a-8 (0.41 g) was purified on RP-HPLC (75% CH_3OH) to afford compounds **2** (28 mg) and **4** (5.4 mg). PQC7-5 (3.12 g, $CHCl_3/CH_3OH$ 94 : 6) was purified on RP-HPLC (75% CH_3OH) to yield compound **3** (10.2 mg), and PQC7-6 (0.51 g, $CHCl_3/CH_3OH$ 94 : 6) was purified on RP-HPLC (50% CH_3OH) to yield compound **6** (6.2 mg). PQC8 (6.8 g, cyclohexane/EtOAc 50 : 50) was isolated on silica gel chromatography column using a step gradient of $CHCl_3/CH_3OH$ to afford nine fractions, the third fraction PQC8-3 (0.37 g, $CHCl_3/CH_3OH$ 98 : 2) was purified on RP-HPLC (70% CH_3OH) to yield compound **1** (19.4 mg). In addition, the fourth fraction PQC8-4 (0.89 g, $CHCl_3/CH_3OH$ 98 : 2) was isolated on ODS-MPLC (70% CH_3OH) to afford PQC8-4-3 (0.24 g), which was further purified by Sphadex LH-20 ($CHCl_3/CH_3OH$ 50 : 50) to yield compound **5** (27.8 mg). The isolation procedures of compounds **8**—**30** were reported in our previous papers.[3,4]

Quassidine E (**1**): Yellowish powders; ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2; IR (KBr) ν_{max} 3320, 1665, 1626, 1604, 1492, 1339, 1317, 982, 742 cm^{-1}; UV ($CHCl_3$) λ_{max} (log ε) 243 (4.69), 282 (4.40), 332 (4.16), 367 (4.37), 382 (4.40) nm; HR-ESI-MS m/z 429.1356 $[M-H]^-$ (Calcd for $C_{27}H_{17}N_4O_2$, 429.1357); ESI-MS (positive) m/z 861.1 $[2M+H]^+$, 431.2 $[M+H]^+$; ESI-MS (negative) m/z 429.1 $[M-H]^-$; ESI-MS (positive) m/z 861 $[2M+H]^+$, 431 $[M+H]^+$; ESI-MS$^{2)}$ (positive) m/z 416 $[M+H-CH_3]^+$; ESI-MS$^{3)}$ (positive) m/z 388 $[M+H-CH_3-CO]^+$; ESI-MS$^{4)}$ (positive) m/z 360 $[M+H-CH_3-CO-CO]^+$.

Quassidine F (**2**): Yellowish needles; ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2; IR (KBr) ν_{max} 3160, 1673, 1624, 1592, 1516, 1493, 1455, 1428, 1347, 1253, 1119, 741 cm^{-1}; UV ($CHCl_3$) λ_{max} (log ε) 244 (4.66), 285 (4.36), 348 (3.98) nm; HR-ESI-MS m/z 421.1681 $[M+H]^+$ (Calcd for $C_{26}H_{21}N_4O_2$, 421.1665); ESI-MS (positive) m/z 421 $[M+H]^+$; ESI-MS (neg-

ative) *m/z* 419 $[M-H]^-$.

Quassidine G (**3**): Yellowish powders; ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2; IR (KBr) ν_{max} 3420, 2925, 1657, 1626, 1560, 1542, 1496, 1432, 1397, 1284, 1207, 746 cm^{-1}; UV ($CHCl_3$) λ_{max} (log ε) 244 (4.37), 288 (3.88), 349 (3.59) nm; HR-ESI-MS *m/z* 435.1480 $[M-H]^-$ (Calcd for $C_{26}H_{19}N_4O_3$, 435.1457); ESI-MS (positive) *m/z* 437 $[M+H]^+$; ESI-MS (negative) *m/z* 435 $[M-H]^-$.

Quassidine H (**4**): Yellowish powders; ^{1}H- and ^{13}C-NMR data, Tables 1 and 2; IR (KBr) ν_{max} 3423, 2929, 1630, 1579, 1505, 1426, 1319, 1077 cm^{-1}; UV ($CHCl_3$) λ_{max} (log ε) 245 (4.39), 289 (3.91), 348 (3.56) nm; HR-ESI-MS *m/z* 483.2047 $[M+H]^+$ (Calcd for $C_{28}H_{27}N_4O_4$, 483.2032); $[\alpha]_D^{25}$ 0 (c=0.5, $CHCl_3$); ESI-MS (positive) *m/z* 987.3 $[2M+Na]^+$, 483.2 $[M+H]^+$; ESI-MS (negative) *m/z* 481.5 $[M-H]^-$.

Canthin-16-one-14-butyric Acid (**5**): Yellowish powders; ^{1}H- and ^{13}C-NMR data, see Table 3; IR (KBr) ν_{max} 3433, 2935, 1660, 1634, 1594, 1481, 1452, 1422, 1272, 1125 cm^{-1}; UV ($CHCl_3$) λ_{max} (log ε) 243 (4.23), 281 (3.95) nm; IR (KBr) ν_{max} 3433, 2935, 1660, 1634, 1594, 1481, 1452, 1422, 1272, 1125 cm^{-1}; HR-ESI-MS *m/z* 305.0930 $[M-H]^-$ (Calcd for $C_{18}H_{13}N_2O_3$, 305.0926); ESI-MS (negative) *m/z* 305.2 $[M-H]^-$.

3-(1,1-Dimethoxylmethyl)-β-carboline (**6**): Yellowish powders; ^{1}H- and ^{13}C-NMR data, see Table 3; ESI-MS (positive) *m/z* 243 $[M+H]^+$; IR (KBr) ν_{max} 3447, 2932, 1627, 1495, 1430, 1320, 1273, 1127, 748 cm^{-1}; UV ($CHCl_3$) λ_{max} (log ε) 244 (4.69), 290 (4.50), 335 (3.95), 349 (3.96) nm; HR-ESI-MS *m/z* 241.0976 $[M-H]^-$ (Calcd for $C_{14}H_{13}N_2O_2$, 241.0983); ESI-MS (negative) *m/z* 241 $[M-H]^-$.

6,12-Dimethoxy-3-formyl-β-carboline (**7**): Yellowish powders; ^{1}H- and ^{13}C-NMR data, see Table 3; IR (KBr) ν_{max} 3309, 1671, 1578, 1491, 1416, 1344, 1306, 1259, 1060, 957, 724 cm^{-1}; UV ($CHCl_3$) λ_{max} (log ε) 244 (4.40), 284 (4.31), 376 (3.86) nm; HR-ESI-MS *m/z* 279.0733 $[M+Na]^+$ (Calcd for $C_{14}H_{12}N_2O_3Na$, 279.0746); ESI-MS (positive) *m/z* 279 $[M+Na]^+$, 257 $[M+H]^+$; ESI-MS (negative) *m/z* 255 $[M-H]^-$.

Bioassay for NO, TNF-α, and IL-6 Production Anti-inflammatory activity was evaluated according to the methods described in our previous paper.[3] Briefly, The RAW 264.7 cells were cultured in 96-well plates at the initial density of 5×10^5 cells/ml in RPMI 1640 medium (200 μl/well) for 1 h. Then the test compound dissolved in dimethyl sulfoxide (DMSO) at various concentrations was added (0.4 μl/well) and LPS (Sigma) was also added with a final concentration of 1 μg/ml. LPS groups only received LPS, and the control groups only received 0.4 μl DMSO per well. The Cells were cultured 24 h for NO analysis. NO was determined by measuring the amount of nitrite in the cell culture supernatant, using Griess reagent. 100 μl of the supernatant from incubates was mixed with an equal volume of Griess reagent, the absorbance at 540 nm were measured and the inhibitory rate was calculated.[10] Cytotoxicity was determined by the MTT (Sigma) colorimetric assay, after 24 h incubation with test compound. TNF-α and IL-6 were detected in the cell culture supernatant, using enzyme-linked immunosorbent assay kits, mouse TNF-α ELISA kit and mouse IL-6 ELISA kit (R & D), according to the manufacturer's recommendations, respectively.

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