JOURNAL OF NATURAL PRODUCTS

Article

pubs.acs.org/jnp

Bioactive Quassinoids from the Seeds of *Brucea javanica*

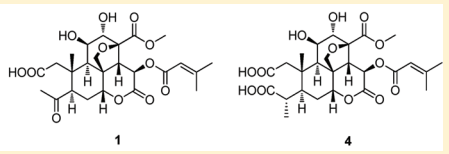
Jun-Hong Liu,[†,‡] Nan Zhao,[†,‡] Gui-Jie Zhang,[†] Shi-Shan Yu,*[,†] Li-Jun Wu,[‡] Jing Qu,[†] Shuang-Gang Ma,[†] Xiao-Guang Chen,[†] Tian-Qi Zhang,[†] Jian Bai,[†] Hui Chen,[†] Zhen-Feng Fang,[†] Fen Zhao,[†] and Wei-Bin Tang[†]

[†]State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, NO.1 Xian Nong Tan Street, Beijing 100050, People's Republic of China

[‡]School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, NO.103 Wen Hua Road, Shenyang 110016, People's Republic of China

Supporting Information

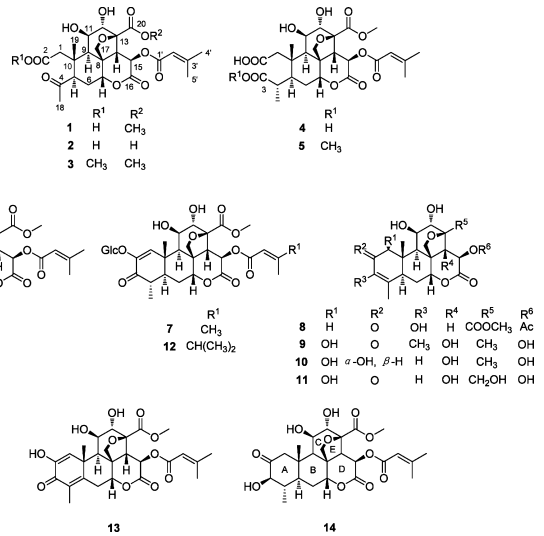
ABSTRACT: Six new quassinoids (**1**–**6**) and eight known compounds of this type (**7**–**14**) were isolated from the seeds of *Brucea javanica*. Their structures were elucidated by analysis of their spectroscopic data and from chemical evidence. Compounds **1**–**5** were found to be unusual quassinoids with a 2,3-seco A ring. The configurations at C-4 in **4** and **5** were determined by a difference circular dichroism method. In in vitro bioassays, **8** and **10** showed inhibitory activities for nitric oxide production in LPS-activated macrophages, with IC_{50} values of 1.9 and 5.0 μM, respectively, while compounds **6**, **8**–**11**, **13**, and **14** exhibited cytotoxicity against five human tumor cell lines (HCT-8, HepG2, BGC-823, A549, and SKVO3), having IC_{50} values in the range 0.12–9.3 μM.



Brucea javanica (L.) Merr. (Simaroubaceae) is an evergreen shrub distributed widely in southeast Asia and northern Australia. In southeast Asia, all parts of *B. javanica* are employed as an antimalarial treatment, and the seeds of this plant are used for the alleviation of dysentery and skin conditions such as warts and corns.[1−5] This plant is known to be an abundant source of quassinoids,[6−10] which have a wide spectrum of biological activities, such as potential antiamebic, anti-HIV, antimalarial, antitubercular, antitumor, cancer chemopreventive, and cytotoxic activities.[11−19] As part of a continued program aimed at the discovery of bioactive compounds from medicinal plants, the seeds of *B. javanica* were investigated. Six new quassinoid derivatives, bruceanic acids E and F (**1** and **2**), bruceanic acid E methyl ester (**3**), javanic acids A and B (**4** and **5**), and javanicolide H (**6**), along with eight known compounds, bruceoside A (**7**),[20] bruceines B (**8**),[21] D (**9**),[22] E (**10**),[22] and H (**11**),[22] bruceantinoside A (**12**),[23] dehydrobrusatol (**13**),[24] and javanicolide E (**14**),[25] were isolated. These compounds were examined for their bioactivities including their potential anti-inflammatory effects and cytotoxicity against five cultured human tumor cell lines (HCT-8, HepG2, BGC-823, A549, and SKVO3). Details of the isolation, structure elucidation, and biological activities of these metabolites are reported herein.

RESULTS AND DISCUSSION

The ethyl acetate-soluble part of the ethanol extract of the seeds was fractionated by column chromatography and purified by preparative HPLC to afford six new (**1**–**6**) and eight known (**7**–**14**) quassinoids. The structures of compounds **1**–**6** were determined by spectroscopic data interpretation.



Bruceanic acid E (**1**) was obtained as an amorphous powder. Its molecular formula was established as $C_{25}H_{32}O_{12}$ from the $[M + H]^+$ ion peak at m/z 525.1960 (calcd for $C_{25}H_{33}O_{12}$, 525.1967) in the HRESIMS. Its ^{1}H NMR and ^{13}C NMR spectra were similar to those of burceanic acid A,[26] with a 2,3-seco A ring, except for signals corresponding to the side chain located at C-15, as seen in Table 1. The ^{1}H NMR spectrum showed signals ascribable to two tertiary methyls (one carbonyl-bearing) (δ_H 1.90 and 2.26), two olefinic methyls

Received: November 21, 2011
Published: April 16, 2012

Table 1. NMR Spectroscopic Data (500 MHz, C_5D_5N) for Bruceanic Acids E (1) and F (2) and Bruceanic Acid E Methyl Ester (3)

	bruceanic acid E (1)[a]		bruceanic acid F (2)[a]		bruceanic acid E methyl ester (3)[a]	
position	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1a	3.41 d (16.5)	42.6	3.45, d (16.0)	42.6	3.59[c]	41.4
1b	2.98 d (16.5)		2.98, d (16.5)		3.10, d (13.5)	
2		174.7		174.7		172.8
4		210.4		210.6		210.1
5	4.24, br d (12.0)	49.8	4.27, br d (11.0)	49.8	3.04, d (4.5)	52.5
6α	2.32[c]	29.8	2.33[c]	29.8	2.32[c]	28.9
6β	2.18[c]		2.18[c]		2.50, br d (15.5)	
7	5.07, br s	83.0	5.07, br s	83.2	4.95[c]	82.9
8		46.5		46.5		46.4
9	3.54, br s	36.3	3.58, br s	36.5	3.59[c]	35.3
10		40.4		40.4		39.0
11	5.23, d (3.5)	73.7	5.29[c]	73.8	4.82, d (4.0)	73.6
12	5.14[c]	76.5	5.36[c]	77.1	5.10[c]	75.9
13		82.9		82.9		82.9
14	[b]	49.9	[b]	49.9	[b]	48.8
15	[b]	68.5	[b]	68.7	[b]	69.1
16		168.1		168.4		167.3
17	3.93, d (6.5) 5.13[c]	73.5	4.00, br s 5.20[c]	73.6	3.92, br s 5.14[c]	74.0
18	2.26, s	31.7	2.27, s	31.8	2.28, s	29.0
19	1.90, s	19.9	1.92, s	19.9	1.77, s	23.6
20		171.1		174.7		171.2
OCH_3-2					3.54, s	51.2
OCH_3-20	3.69, s	52.3			3.70, br s	52.3
1′		165.5		165.8		165.7
2′	5.80, s	116.0	5.75, s	116.4	5.75, s	116.0
3′		158.4		157.6		158.4
4′	2.13, s	20.1	2.08, s	20.0	2.09, s	20.1
5′	1.62, s	27.0	1.42, s	26.8	1.59, s	27.0

[a]Data assignments are based on HSQC and HMBC experiments. [b]Not assigned. [c]Overlapping signal.

(δ_H 2.13 and 1.62), one oxygen-bearing methyl (δ_H 3.69), and one olefinic proton (δ_H 5.80) (Table 1) (Figure S6, Supporting Information). Analysis of the ^{13}C NMR and DEPT spectra revealed that **1** possesses an acetyl group (δ_C 210.4 and 31.7), four carbonyl carbons (δ_C 174.7, 171.1, 168.1, and 165.5), three aliphatic quaternary carbons (δ_C 83.0, 46.5, and 40.4), seven aliphatic tertiary carbons (δ_C 82.9, 76.5, 73.7, 68.5, 49.9, 49.8, and 36.3), three aliphatic secondary carbons (δ_C 73.5, 42.6, and 29.8), an oxygen-bearing methyl carbon (δ_C 52.3), two olefinic methyl carbons (δ_C 20.1 and 27.0), and an aliphatic methyl carbon (δ_C 19.8). The quaternary carbon at δ_C 83.0 and the tertiary carbon at δ_C 82.9 were both found to be attached to an oxygen atom and were assigned to C-13 and C-7, respectively. The secondary carbon at δ_C 73.5 was assigned to C-17, attached to an oxygen bridge (Figures S7 and S8, Supporting Information). On the basis of the ^1H NMR, ^{13}C NMR, HSQC, and HMBC spectra, the C-15 side chain was confirmed to be a senecioyl moiety [δ_H 2.13 (H_3-4′), 1.62 (H_3-5′), and 5.80 (H-2′) and δ_C 165.5 (C-1′), 116.0 (C-2′), 158.4 (C-3′), 20.1 (C-4′), and 27.0 (C-5′)] (Figure 1), showing correlations

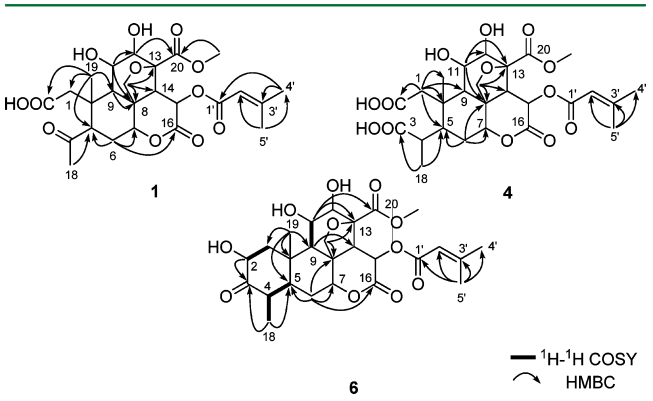


Figure 1. Selected $^1H-^1H$ COSY and HMBC correlations of **1**, **4**, and **6**.

of OCH_3-20/H-2′, H_3-4′, and H_3-5′ in the NOESY spectrum (Figure 2).[27] The HSQC and HMBC spectra revealed the

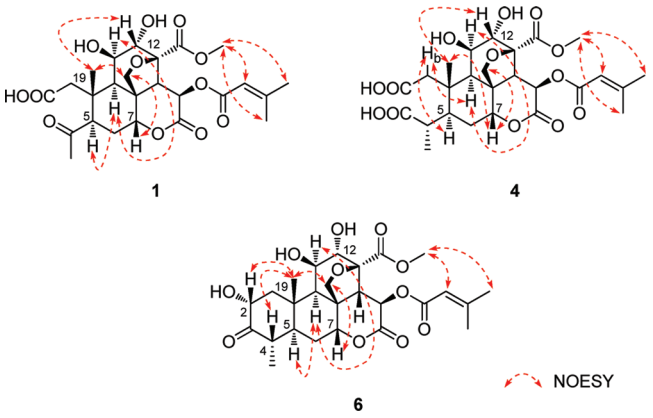


Figure 2. Key NOESY correlations of **1**, **4**, and **6**.

presence of a carboxymethyl group [δ_C 174.7 (C-2) and 42.6 (C-1)] attached to C-10 with an α-orientation, as demonstrated by a NOESY correlation between H_3-19/H-17a (Figure 2). The methyl signal of H_3-18 showed a HMBC correlation with C-5, indicating a linkage at this position (Figure 1). A correlation between H-5 and H-9 in the NOESY experiment indicated that H-5 is α-oriented (Figure 2). The correlations of H-9/H-11, H_3-19/H-12, and H-17b/H-7 in the NOESY spectrum confirmed that the orientations of the ring B, C, D, and E substituents were the same as dehydrobrusatol (Figure 2) (Figures S9–S11, Supporting Information).[9] Thus, the structure of **1** was established as shown.

Bruceanic acid F (**2**) was obtained as an amorphous powder. Its molecular formula was determined to be $C_{24}H_{30}O_{12}$ from the $[M + H]^+$ ion peak at m/z 511.1821 (calcd for $C_{24}H_{31}O_{12}$, 511.1810) in the HRESIMS. The ^1H NMR and ^{13}C NMR signals of **2** were nearly identical to those of **1** and confirmed the presence of an acetyl moiety [δ_C 210.6 (C-4) and 31.8 (C-18) and δ_H 2.27 (H_3-18)] and a carboxymethyl group [δ_C 174.7 (C-2) and 42.6 (C-1) and δ_H 3.45, 2.98 (H_2-1)]. The linkage positions of the acetyl and carboxymethyl groups were determined from the HMBC correlations. The HMBC correlations from H_3-18 to C-4 and C-5 and from H_2-1 to C-

dx.doi.org/10.1021/np200920c | J. Nat. Prod. 2012, 75, 683–688

2, C-5, C-9, C-10, and C-19 indicated the acetyl and carboxymethyl groups to be located at C-5 and C-10, respectively. However, there was an absence of any resonance for an oxygen-bearing methyl moiety [δ_H 3.69 and δ_C 52.3] in **2** corresponding to the ester methyl located at C-20 in **1** (Table 1) (Figures S14−S17, Supporting Information). The ROESY spectrum indicated that the functional group configuration of **2** is identical to that of **1** (Figure S18, Supporting Information). Therefore, the structure of **2** was characterized as shown.

Bruceanic acid E methyl ester (**3**) was obtained as an amorphous powder. The molecular formula was determined to be $C_{26}H_{34}O_{12}$ from the $[M + H]^+$ ion peak at m/z 539.2126 (calcd for $C_{26}H_{35}O_{12}$, 539.2123) in the HRESIMS. Its ^{1}H NMR and ^{13}C NMR spectra were very similar to those of **1** except for the resonances of an additional ester methyl group (δ_H 3.54 and δ_C 51.2) (Table 1) (Figures S21−S23, Supporting Information). The HSQC and HMBC spectra revealed the ester methyl group to be connected to C-2 (δ_C 172.8) (Figures S24, S25, Supporting Information). On analysis of the NOESY spectrum, the correlations of H_3-19/H-17 and H-12, H-1b/H-5 and H-9, H-9/H-11, and H-7/H-17 indicated the configuration of **3** to be identical to that of **1** (Figure S26, Supporting Information). Therefore, the structure of **3** was defined as shown.

Javanic acid A (**4**) was obtained as an amorphous powder. Its molecular formula was determined to be $C_{26}H_{34}O_{13}$ from the $[M + H]^+$ ion peak at m/z 555.2092 (calcd for $C_{26}H_{35}O_{13}$, 555.2072) in the HRESIMS. The ^{1}H NMR spectrum showed resonances ascribable to three tertiary methyls (δ_H 1.56, 1.76, and 2.11), a secondary methyl (δ_H 1.36), an oxygen-bearing methyl group (δ_H 3.65), and an olefinic proton (δ_H 5.77) (Figure S29, Supporting Information). The ^{1}H NMR and ^{13}C NMR spectra of **4** were very similar to those of **1** except for the resonances ascribable to the substituted group at C-5 (Table 2). This substituent was confirmed to be a 1-carboxyethyl group based on the carbon signals at δ_C 179.5, 37.7, and 15.4 in the ^{13}C NMR spectrum, as well as the ^{1}H−^{1}H COSY correlations of H-18/H-4 and H-4/H-5. In the HMBC spectrum, the correlations (Figure 1) from H_3-18 to C-3, C-5 and from H-4 to C-3, C-5, C-6, C-18 further demonstrated the presence of a 1-carboxyethyl group at C-5, and the correlations from H_2-1 to C-2, C-5, C-9, and C-10 indicated that a carboxymethyl group [δ_C 174.5 (C-2) and 41.7 (C-1)] was attached to C-10 (Figure 1). The HMBC correlations from the isolated methyl H_3-5′ to C-1′, C-2′, C-3′, and C-4′ and key NOESY data including correlations of OCH_3-20/H-2′, H_3-4′, and H_3-5′ supported the connectivity of the senecioyl moiety in the same location as **1** (Figures S31, S32, Supporting Information).[27] The configurations of rings B, C, D, and E substituents were the same as those of **1**, as demonstrated by the correlations between H-9/H-11, H_3-19/H-12, and H-17b/H-7 in the NOESY spectrum (Figure 2). In addition, the correlations of H-5/H-1b, H-5/H-9, and H_3-19/H-7 implied that the 1-carboxyethyl group at C-5 and the carboxymethyl moiety at C-10 are β-oriented and α-oriented, respectively (Figure S33, Supporting Information).

The configuration of C-4 in **4** was elucidated on the basis of the Cotton effect at about 225 nm in methanol in the difference circular dichroism (DIF CD) spectrum.[28−30] The Cotton effect near 225 nm is generally assumed to correspond to the n → π^* transition of α-alkyl acid and esters, which can be used as a diagnostic CD absorption to determine the absolute configuration of C-4 in **4**.[28,29] However, **4** possesses many chiral carbons connected to different chromophores. The CD curve of **4** reflects the overall chiralities. The overlapping of additional acid and ester chromophores around 225 nm in the CD spectrum made it difficult to evaluate the sign of the Cotton effect generated from the substituent group at C-5. Since **4** and **1** have the same structure except for their functional groups at C-5, both were considered to adopt approximately the same conformation, including the population of possible rotamers. Therefore, subtraction of the CD curve of **1** from that of **4** would be expected to give a DIF CD spectrum, attributable to the Cotton effects of one positive 1-carboxyethyl moiety in **4** and one negative acetyl moiety in **1**.[30] The sign of the Cotton effect around 225 nm in the DIF CD spectrum was related only to the C-4 chirality of the 1-carboxyethyl moiety, while the Cotton effect at 280 nm corresponded to the acetyl group located at C-5 in **1**. Thus, this simple and reliable method would be expected to give a significant and reduced circular dichroism spectrum attributable to the 1-carboxyethyl moiety. Therefore, the absolute configuration of the C-4 in **4** could be proposed by means of the empirical rule for α-alkyl acids and

Table 2. NMR Spectroscopic Data (500 MHz, C_5D_5N) for Javanic Acids A (4) and B (5) and Javanicolide H (6)

	javanic acid A (**4**)[a]		javanic acid B (**5**)[a]		javanicolide H (**6**)[a]	
position	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1(a/α)	3.44, d (16.5)	41.7	3.36, d (16.5)	41.6	3.15, dd (11.5, 6.5)	48.0
1(b/β)	3.11, d (16.0)		2.91, d (16.5)		1.73[c]	
2		174.5		174.1	4.72, dd (12.0, 7.0)	72.3
3		179.5		177.4		212.1
4	3.72[c]	37.7	3.12, m	37.8	2.50, m	42.1
5	3.29, m	38.2	3.47, m	37.6	1.89[c]	48.3
6α	2.41, d (14.5)	28.9	2.25, m	28.9	2.03, m	30.8
6β	1.84, m		1.80, m		1.66[c]	
7	5.09[c]	83.4	4.99, br s	83.4	4.98, br s	83.7
8		46.4		46.4		46.4
9	3.87[c]	35.9	3.73[c]	36.3	2.33, m	43.0
10		42.1		42.0		38.5
11	5.30[c]	74.4	5.21, d (4.5)	74.4	4.86, d (4.0)	73.7
12	5.25[c]	76.7	5.15, br s	76.7	5.05, br s	75.9
13		82.9		82.9		82.7
14	[b]	48.6	[b]	49.0	[b]	51.2
15	[b]	69.4	[b]	68.7	[b]	68.4
16		168.1		168.2		168.2
17	3.91[c]		3.90, d (6.5)		3.92, d (6.0)	
	5.09[c]	73.5	5.04, d (7.0)	73.6	5.16, d (7.5)	74.0
18	1.36, d (6.5)	15.4	1.20, d (7.5)	14.8	1.09, d (6.0)	11.4
19	1.76, s	19.8	1.67, s	19.6	1.85, s	16.3
20		171.2		171.2		171.4
OCH_3-3			3.53, s	51.5		
OCH_3-20	3.65[c]	52.3	3.70, s	52.3	3.74, s	52.3
1′		166.0		168.2		165.2
2′	5.77, s	116.0	5.79, s	116.1	5.81, s	115.9
3′		158.5		158.2		158.4
4′	2.11, s	20.0	2.11, s	20.1	2.10, s	20.1
5′	1.56, s	26.9	1.61, s	27.0	1.62, s	27.0

[a]Data assignments are based on HSQC and HMBC experiments. [b]Not assigned. [c]Overlapping signal.

dx.doi.org/10.1021/np200920c | J. Nat. Prod. 2012, 75, 683−688

esters by correlating the α-alkyl acid subunit with the sign of the Cotton effect around 225 nm. This allows the correlation of the configuration at C-4 with the sign of the Cotton effect near 225 nm: the *S*-configuration gives a negative Cotton effect and the *R*-configuration gives a positive effect.[28,29] Accordingly, the negative Cotton effect at 228 nm in the DIF CD spectrum of **4** (Figures S1, S2, Supporting Information) permitted assignment of a 4*S* absolute configuration (Figures S4, S5, Supporting Information). The structure of **4** was elucidated as shown.

Javanic acid B (**5**) was obtained as an amorphous powder. Its molecular formula was determined to be $C_{27}H_{36}O_{13}$ from the $[M + H]^+$ ion peak at *m/z* 569.2239 (calcd for $C_{27}H_{37}O_{13}$, 569.2229) in the HRESIMS. The ^{1}H NMR and ^{13}C NMR spectra of **5** and **4** were similar (Table 2), suggesting that **5** and **4** have the same carbon framework (Figures S36, S37, Supporting Information). The only difference found between these two compounds was in the resonances of an additional ester methyl group (δ_H 3.53 and 51.5) in **5** (Table 2). The location of the ester methyl group was elucidated by interpretation of the HMBC data. The HMBC correlation from the ester methyl protons (δ_H 3.53) to C-3 (δ_C 177.4) indicated that the ester methyl group is attached to C-3 (Figures S38, S39, Supporting Information). Analysis of the NOESY spectrum and the negative Cotton effect at 225 nm in the DIF CD spectrum of **5** indicated the absolute configuration of this quassinoid to be identical with that of **4** (Figures S9, S1, and S3, Supporting Information). Thus, the structure of **5** was determined as shown.

Javanicolide H (**6**) was obtained as an amorphous powder. Its molecular formula was determined to be $C_{26}H_{34}O_{11}$ from the $[M + Na]^+$ ion peak at *m/z* 545.2006 (calcd for $C_{26}H_{34}NaO_{11}$, 545.1993) in the HRESIMS. Its ^{1}H NMR spectrum showed resonances for a secondary methyl (δ_H 1.09), a tertiary methyl (δ_H 1.85), two olefinic methyls (δ_H 1.62 and 2.10), an oxygen-bearing methyl group (δ_H 3.74), and an olefinic proton (δ_H 5.81) (Table 2), which indicated that **6** is a quassinoid with a picrasane skeleton.[27] The ^{1}H NMR and ^{13}C NMR data of **6** exhibited a close similarity to those of javanicolide E[25] except for the location of the ketocarbonyl in ring A (Figures S43, S44, Supporting Information). The HMBC correlations from H-1a, H-2, and H_3-18 to the ketocarbonyl carbon (δ_C 212.1) and a key ^{1}H–^{1}H COSY correlation of H_2-1 with H-2 suggested the ketocarbonyl to be located at C-3 (Figure 1) in **6** (Figures S45–S47, Supporting Information). In contrast, in the case of javanicolide E, an AB coupling system of the two protons of C-1 indicated the ketocarbonyl to be located at C-2. The relative configuration of H-2 was designated with the β-orientation due to the observation of correlations of H_3-19/H-2, H-4/H-17a, H-17b/H-7, H-9/H-5, and H-9/H-11 in the NOESY spectrum (Figure 2) (Figure S48, Supporting Information). Thus, the structure of **6** was elucidated as shown.

All compounds isolated were subjected to an examination of their propensity to inhibit NO production in rat polymorphonuclear leukocytes induced by LPS.[31] Compounds **8** and **10** displayed significant inhibitory effects on NO production, and their IC_{50} values were 1.9 and 5.0 μM, respectively, and were compared to dexamethasone (IC_{50} 0.0023 μM).

Quassinoids **1**–**14** were examined for their cytotoxic activities against five human cancer cell lines (HCT-8, HepG2, BGC-823, A549, and SKOV3), with paclitaxel as the positive control (Table 3).[32] Compounds **8**, **9**, and **11** exhibited cytotoxicity against all of the human cancer cell lines in which they were evaluated, whereas compounds **6** and **14** showed selective cytotoxicity against the BGC-823, A549, and SKOV3 cell lines. Compound **10** exhibited selective cytotoxicity against the HCT-8, HepG2, BGC-823, and SKOV3 cell lines, and compound **13** against the HepG2, BGC-823, A549, and SKOV3 cell lines. Compounds **1**–**5**, **7**, and **12** were inactive (>10 μM) in this assay.

Table 3. Cytotoxic Activity of Compounds 1–14 by the MTT Method

	IC_{50} (μM)[a]				
sample	HCT-8	HepG2	BGC-823	A549	SKOV3
6	>10	>10	0.52	0.95	0.23
8	2.0	0.81	0.81	1.3	0.12
9	2.0	1.2	1.2	3.9	0.76
10	6.7	2.9	2.2	>10	2.2
11	1.3	2.8	2.1	9.3	0.33
13	>10	3.3	4.8	7.3	2.5
14	>10	>10	1.43	6.36	1.49
paclicaxel[b]	0.051	0.0044	0.0033	0.016	<0.0001

[a]Compounds **1**–**5**, **7**, and **12** were inactive against all cell lines tested (IC_{50} >10 μM). [b]Positive control.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured with a JASCO P-2000 polarimeter. UV spectra were taken with a JASCO V-650 spectrophotometer. CD spectra were measured on a JASCO J-815 CD spectrometer. IR spectra were recorded on a Nicolet 5700 FT-IR microscope instrument (FT-IR microscope transmission). NMR spectra were recorded on an INOVA-500 MHz spectrometer in pyridine-d_5. HRESIMS were measured using an Agilent Technologies 6250 Accurate Mass Q-TOF LC/MS spectrometer. Preparative HPLC was carried out on a Shimadzu LC-6AD instrument with a SPD-10A detector using a YMC-Pack ODS-A column (250 × 20 mm, 5 μm). Analytical HPLC was measured on an Agilent 1100 Series instrument with a DAD detector using a YMC column (RP-18 4.6 × 100 mm). Column chromatography was performed using polyamide (60–100 mesh, Jiangsu Linjiang Chemical Reagents Factory, Taizhou, People's Republic of China), Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden), ODS (50 μm, Merck), and silica gel (200–300 mesh, Qingdao Marine Chemical Factory, Qingdao, People's Republic of China). TLC was carried out with GF254 plates (Qingdao Marine Chemical Factory). Spots were visualized by spraying with 10% H_2SO_4 acid in EtOH followed by heating.

Plant Material. The seeds of *B. javanica* were collected from Qinzhou City, Guangxi Province, People's Republic of China, in April 2009, and identified by Prof. Lin Ma (Department of Natural Products Chemistry, Institute of Materia Medica, Chinese Academy of Medical Sciences). A voucher specimen (ID-S-2361) has been deposited in the Herbarium of the Department of Medicinal Plants, Institute of Materia Medica, Chinese Academy of Medical Sciences, People's Republic of China.

Extraction and Isolation. The air-dried and milled seeds (47 kg) were soaked in petroleum ether (3 × 250 L, 24 h) to give defatted seeds, which were then extracted with 95% ethanol (3 × 250 L, 3 h) under reflux. After removal of the solvent under vacuum, the viscous concentrate (5 kg) obtained was suspended in H_2O and then partitioned with petroleum ether, EtOAc, and *n*-BuOH consecutively. The extracts were evaporated under vacuum to afford petroleum ether-soluble (1300 g), EtOAc-soluble (600 g), and *n*-BuOH-soluble (2000 g) extracts. The EtOAc-soluble extract (600 g) was loaded on a silica gel column (4 kg, 100–200 mesh) and eluted sequentially with $CHCl_3$ containing increasing amounts of MeOH (1:0, 50:1, 30:1, 20:1, 10:1, 5:1, and 0:1), to yield seven fractions (1–7).

dx.doi.org/10.1021/np200920c | J. Nat. Prod. 2012, 75, 683–688

Fraction 2 (245 g) afforded three subfractions (2A–1C) after being fractionated by polyamide (60–100 mesh) column chromatography. Fraction 2A (100 g) was subjected to preparative reversed-phase MPLC and eluted with a gradient of MeOH–H_2O (0:1 to 1:1, then 1:0), to afford three subfractions (2A1–2A3). Fraction 2A1 (20 g) was separated using MPLC (ODS column), eluted with a gradient of 0–45% MeOH in H_2O, to yield 18 subfractions (2A1A–2A1R). Fraction 2A1K (5 g) was crystallized from MeOH to afford **8** (3 g). Fraction 2A1L (380 mg) was further chromatographed by preparative reversed-phase HPLC [MeCN–H_2O (32:68)] to afford **1** (13 mg), **2** (2 mg), and **3** (3 mg). Separation of fraction 2A1O (800 mg) by Sephadex LH-20, eluted with MeOH–H_2O (60:40), and preparative reversed-phase HPLC with the mobile phase MeCN–H_2O (28:78) yielded **4** (7 mg), **5** (8 mg), **6** (7 mg), and **14** (3 mg). Compound **13** (27 mg) was obtained from fraction 2A1Q (800 mg) using preparative reversed-phase HPLC [mobile phase: MeCN–H_2O (28:78)]. Fraction 3 (15 g) was crystallized from MeOH to afford **9** (3 g).

Fraction 4 was eluted sequentially with $CHCl_3$ containing increasing amounts of MeOH (30:1, 25:1, 20:1, and 10:1) over silica gel (500 g), to yield four subfractions (4A–4D). Subfraction 4C (1.2 g) was separated further using a Sephadex LH-20 column, eluted with MeOH–H_2O (40:60), followed by preparative reversed-phase HPLC using MeCN–H_2O (30:70), to afford **12** (24 mg).

Fraction 6 (90 g) was subjected to reversed-phase MPLC and eluted with a mixture of H_2O and MeOH, starting with 0% MeOH to 100% MeOH in 10% increments, to afford three subfractions (6A–6C). Repeated silica gel column chromatography of fraction 6A, eluted with $CHCl_3$–MeOH (15:1), yielded five subfractions (6A1–6A5). Separation of fraction 6A2 (130 mg) by preparative reversed-phase HPLC using MeCN–H_2O (27:77) gave **11** (25 mg). Fraction 6A3 (8 g) crystallized from MeOH to afford crystals of **7** (1.6 g) and a mother liquor. This mother liquor (fraction 6A3m) was chromatographed further over Sephadex LH-20, eluted with H_2O, to remove pigments. The column was eluted isocratically with MeOH–H_2O (30:70) to yield five subfractions (6A3m1–6A3m5). Fraction 6A3m4 (102 mg) was purified by preparative reversed-phase HPLC, using MeCN–H_2O (25:75), to afford **10** (60 mg).

*Bruceanic acid E (**1**):* amorphous powder; $[\alpha]_D^{20}$ +79.3 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 220 (4.28) nm; CD (MeOH) 205 ($\Delta\varepsilon$ 3.02), 225 ($\Delta\varepsilon$ 6.30), 263 ($\Delta\varepsilon$ 0.34), 284 ($\Delta\varepsilon$ 0.58) nm; IR ν_{max} 3497, 2956, 1731, 1648 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m*/*z* 525.1960 $[M + H]^+$ (calcd for $C_{25}H_{33}O_{12}$, 525.1967).

*Bruceanic acid F (**2**):* amorphous powder, $[\alpha]_D^{20}$ +41.1 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 219 (3.94) nm; IR ν_{max} 3439, 2961, 1712, 1646 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m*/*z* 511.1821 $[M + H]^+$ (calcd for $C_{24}H_{31}O_{12}$, 511.1810).

*Bruceanic acid E methyl ester (**3**):* amorphous powder; $[\alpha]_D^{20}$ +47.0 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 219 (4.08) nm; IR ν_{max} 3474, 2950, 1723, 1643 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m*/*z* 539.2126 $[M + H]^+$ (calcd for $C_{26}H_{35}O_{12}$, 539.2123).

*Javanic acid A (**4**):* amorphous powder; $[\alpha]_D^{20}$ +115.7 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 220 (4.28) nm; CD (MeOH) 207 ($\Delta\varepsilon$ 2.90), 223.5 ($\Delta\varepsilon$ 5.48), 266.5 ($\Delta\varepsilon$ −0.03), 281 ($\Delta\varepsilon$ 0.09) nm; DIF CD (MeOH) 227.5 ($\Delta\varepsilon$ −0.80) nm; IR ν_{max} 3487, 2956, 1725, 1648 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; HRESIMS *m*/*z* 555.2092 $[M + H]^+$ (calcd for $C_{26}H_{35}O_{13}$, 555.2072).

*Javanic acid B (**5**):* amorphous powder; $[\alpha]_D^{20}$ +70.5 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 220 (4.17) nm; CD (MeOH) 224 ($\Delta\varepsilon$ 3.33) nm; DIF CD (MeOH) 225 ($\Delta\varepsilon$ −2.90) nm; IR ν_{max} 3500, 2955, 1730, 1648 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; HRESIMS *m*/*z* 569.2239 $[M + H]^+$ (calcd for $C_{27}H_{37}O_{13}$, 569.2229).

*Javanicolide H (**6**):* amorphous powder; $[\alpha]_D^{20}$ +63.4 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 220 (4.12) nm; IR ν_{max} 3466, 2953, 1730, 1646 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; HRESIMS *m*/*z* 545.2006 $[M + Na]^+$ (calcd for $C_{26}H_{34}NaO_{11}$, 545.1993).

Determination of Absolute Configuration of C-4 in Compounds 4 and 5 by DIF CD Spectroscopy. Following a previous literature report,[28] solutions of **1** (0.2 mg), **4** (0.2 mg), and **5** (0.2 mg) in methanol (1 mL) were each subjected to CD measurement. Then, the CD spectra of **1**, **4**, and **5** were each transformed to a molar-CD spectrum, and the DIF CD spectra of **4** and **5** (subtraction of the CD curve of compound **1** from those of **4** and **5**) were obtained from the molar-CD spectrum. The observed sign of the diagnostic band at 228 nm in the DIF CD spectrum was correlated to the absolute configuration of C-4 in **4**.[29,30] To analyze **5**, the observed sign of the diagnostic band at 225 nm in the DIF CD spectrum was correlated to the absolute configuration of C-4 in **5**.[29,30]

Inhibitory Effects on Nitric Oxide Production in LPS-Activated Macrophages. The procedure used for nitric oxide determination was based on the Griess reaction.[31] A 100 mL amount of culture supernatant or sodium nitrite standard (5.2–103.6 μm) was mixed with an equal volume of Griess reagent [a mixture of 0.1% *N*-(1-naphthyl)ethylenediamine dihydrochloride and 1% sulfanilamide in 5% phosphoric acid; the two parts being mixed together within 1 h of preparation], using a 96-well plate. After 20 min at room temperature, the absorbance at 540 nm was measured by a microtitration plate reader. Dexamethasone was used as the positive control.

Cytotoxicity Assays. The EtOH extract and isolates were tested for their cytotoxicity against HCT-8 (human colon cancer), HepG2 (human hepatoma cancer), BGC-823 (human gastric cancer), A549 (human lung epithelia cancer), and SKOV3 (human ovarian cancer) cancer cell lines, using an established colorimetric MTT assay protocol.[32] Paclitaxel was used as the positive control.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

HRESIMS, IR, 1D and 2D NMR spectra of compounds **1**–**6**, CD spectra of compounds **1**, **4**, and **5**, DIF CD spectra of compounds **4** and **5** (subtraction of the CD curve of compound **1** from **4** and **5**), 1D NMR spectra of known compounds **7**–**14**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +86-10-63165324. Fax: +86-10-63017757. E-mail: yushishan@imm.ac.cn.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This project was supported by the Natural Science Foundation of China (No. 21132009) and the National Science and Technology Project of China (No. 2012ZX09301002-002). We are grateful to the Department of Instrumental Analysis, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College for measuring the spectroscopic data.

■ REFERENCES

(1) Lin, L. Z.; Zhang, J. L.; Xu, R. S. *Acta Chim. Sin.* **1982**, *40*, 73–78.
(2) O'Neill, M. J.; Bray, D. H.; Boardman, P.; Chan, K. L.; Phillipson, J. D. *J. Nat. Prod.* **1987**, *50*, 42–48.
(3) Yoshikawa, M.; Harada, E.; Shunji, A.; Yamahara, J.; Murakami, N.; Shibuya, H.; Kitagawa, I. *Chem. Pharm. Bull.* **1993**, *41*, 2101–2105.
(4) Kitagawa, I.; Mahmud, T.; Simanjuntak, P.; Hori, K.; Uji, T.; Shibuya, H. *Chem. Pharm. Bull.* **1994**, *42*, 1416–1421.
(5) China Pharmacopoeia Committee. *Chinese Pharmacopoeia*; Chemical Industry Press: Beijing, 2000; Part 1, pp 208–209.
(6) Yoshimura, S.; Sakaki, T.; Ishibashi, M.; Tsuyuki, T.; Takahashi, T.; Matsushita, K.; Honda, T. *Chem. Pharm. Bull.* **1984**, *32*, 4698–4701.
(7) Sakaki, T.; Yoshimura, S.; Ishibashi, M.; Tsuyuki, T.; Takahashi, T.; Honda, T.; Nakanishi, T. *Chem. Pharm. Bull.* **1984**, *32*, 4702–4705.

(8) Sakaki, T.; Yoshimura, S.; Ishibashi, M.; Tsuyuki, T.; Takahashi, T.; Honda, T.; Nakanishi, T. *Bull. Chem. Soc. Jpn.* **1985**, *58*, 2680−2686.
(9) Ohnishi, S.; Fukamiya, N.; Okano, M. *J. Nat. Prod.* **1995**, *58*, 1032−1038.
(10) Kim, I. H.; Suzuki, R.; Hitotsuyanagi, Y.; Takeya, K. *Tetrahedron* **2003**, *59*, 9985−9989.
(11) Okano, M.; Fukamiya, N.; Tagahara, K.; Cosentino, M.; Lee, T. T. Y.; Morris-Natschke, S.; Lee, K. H. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 701−706.
(12) Morre, D. J.; Zeichhardt, H.; Maxeiner, H. G.; Grunert, H. P.; Sawitzky, D.; Grieco, P. *Life Sci.* **1998**, *62*, 213−219.
(13) Itokawa, H.; Takeya, K.; Hitotsuyanagi, Y.; Morita, H. *J. Biochem. Mol. Biol. Biophys.* **2000**, *4*, 213−222.
(14) Tamura, S.; Fukamiya, N.; Okano, M.; Tokuda, H.; Aoi, W.; Mukainaka, T.; Nishino, H.; Tagahara, K.; Koike, K. *Cancer Lett.* **2002**, *185*, 47−51.
(15) Murakami, C.; Fukamiya, N.; Tamura, S.; Okano, M.; Bastow, K. F.; Tokuda, H.; Mukainaka, T.; Nishino, H.; Lee, K. H. *Bioorg. Med. Chem.* **2004**, *12*, 4963−4968.
(16) Guo, Z.; Vangapandu, S.; Sindelar, R. W.; Walker, L. A.; Sindelar, R. D. *Curr. Med. Chem.* **2005**, *12*, 173−190.
(17) Subeki, M. H.; Takahashi, K.; Nabeta, K.; Yamasaki, M.; Maede, Y.; Katakura, K. *J. Nat. Prod.* **2007**, *70*, 1654−1657.
(18) Elkhateeb, A.; Yamasaki, M.; Maede, Y.; Katakura, K.; Nabeta, K.; Matsuura, H. *Nat. Prod. Commun.* **2008**, *3*, 145−148.
(19) Wright, C. W.; O'Neill, M. J.; Phillipson, J. D.; Warhurst, D. C. *Antimicrob. Agents Chemother.* **1988**, *32*, 1725−1729.
(20) Lee, K. H.; Imakura, Y.; Huang, H. C. *J. Chem. Soc., Chem. Commun.* **1977**, 69−70.
(21) O'Neill, M. J.; Bray, D. H.; Boardman, P.; Chan, K. L.; Phillipson, J. D.; Warhurst, D. C.; Peters, W. *J. Nat. Prod.* **1987**, *50*, 41−42.
(22) Xie, J. X.; Ji, Z. *Yaoxue Xuebao* **1981**, *16*, 53−55.
(23) Rahman, S.; Fukamiya, N.; Tokuda, H.; Nishino, H.; Tagahara, K.; Lee, K. H.; Okano, M. *Bull. Chem. Soc. Jpn.* **1999**, *72*, 751−756.
(24) Sim, K. Y.; Sims, J. J.; Geissman, T. A. *J. Org. Chem.* **1968**, *33*, 429−431.
(25) Yan, X. H.; Chen, J.; Di, Y. T.; Fang, X.; Dong, J. H.; Sang, P.; Wang, Y. H.; He, H. P.; Zhang, Z. K.; Hao, X. J. *J. Agric. Food Chem.* **2010**, *58*, 1572−1577.
(26) Toyota, T.; Fukamiya, N.; Okano, M. *J. Nat. Prod.* **1990**, *53*, 1526−1532.
(27) Kim, I. H.; Takashima, S.; Hitotsuyanagi, Y.; Hasuda, T.; Takeya, K. *J. Nat. Prod.* **2004**, *67*, 863−868.
(28) Fredga, A.; Jennings, J. P.; Klyne, W.; Scopes, P. M.; Sjöberg, B.; Sjöberg, S. *J. Chem. Soc.* **1965**, 3928−3933.
(29) Gottarelli, G.; Scopes, P. M. *J. Chem. Soc. (C)* **1967**, 1370−1373.
(30) Mori, Y.; Kohchi, Y.; Suzuki, M.; Furukawa, H. *J. Am. Chem. Soc.* **1992**, *114*, 3557−3559.
(31) Green, L. C.; Wagner, D. A.; Glogowski, J.; Skipper, P. L.; Wishnok, J. S.; Tannenbaum, S. R. *Anal. Biochem.* **1982**, *126*, 131−138.
(32) Ma, S. G.; Tang, W. Z.; Liu, Y. X.; Hu, Y. C.; Yu, S. S.; Zhang, Y.; Chen, X. G.; Qu, J.; Ren, J. H.; Liu, Y. B.; Xu, S.; Liu, J.; Liu, Y. Y.; Li, Y.; Lü, H. N.; Wu, X. F. *Phytochemistry* **2011**, *72*, 115−125.