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Bioactivity-guided isolation of cytotoxic constituents of *Brucea javanica* collected in Vietnam

Li Pan [a], Young-Won Chin [a], Hee-Byung Chai [a], Tran Ngoc Ninh [b], Djaja Djendoel Soejarto [c], A. Douglas Kinghorn [a,*]

[a] Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, 500 West 12th Avenue, Columbus, OH 43210, USA
[b] Institute of Ecology and Biological Resources, Vietnamese Academy of Science and Technology, Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam
[c] Program for Collaborative Research in the Pharmaceutical Sciences, Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, 833 South Wood Street, University of Illinois at Chicago, Chicago, IL 60612, USA

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ABSTRACT

Five new triterpenoids (**1**–**5**), together with two known quassinoids, bruceantin (**6**) and bruceine A (**7**), and a known flavonolignan, (−)-hydnocarpin (**8**), were isolated from the chloroform-soluble subfraction of a methanol extract of the combined twigs, leaves, and inflorescence of *Brucea javanica* collected in Vietnam. The structures of the new compounds **1**–**5** were established on the basis of spectroscopic methods. All isolates were evaluated for cytotoxicity against a small panel of human cancer cell lines. Quassinoids **6** and **7** were found to be highly active against these cell lines. (−)-Hydnocarpin (**8**) showed a potentiating effect when combined with both **6** and **7**, during cytotoxicity testing using the MCF-7 human breast cancer cell line.

1. Introduction

Brucea javanica (L.) Merr. (Simaroubaceae) is an evergreen shrub distributed from southeast Asia to northern Australia. In southeast Asia, all parts of *B. javanica* have been employed as an antimalarial treatment, and the seeds of this plant have also been used for the alleviation of dysentery and skin conditions such as warts and corns.[1–5] Previous investigations on the chemical constituents of *B. javanica* have focused mainly on the fruits and seeds, and have found this plant part to be a rich source of quassinoids,[6–12] which are the bitter-tasting principles of many species in the family Simaroubaceae. Quassinoids have been documented from *B. javanica* with a wide spectrum of biological effects, such as having potential antibabesial, anti-HIV, antimalarial, antitubercular, antitumor, cancer chemopreventive, and cytotoxic activities.[13–20] Research on other plant parts of *B. javanica* is limited, but apotirucallane triterpenoids and β-carboline alkaloids occur as major constituents besides the quassinoids.[3,4]

As part of ongoing investigation of natural anticancer agents from tropical plants, an initial screening procedure was conducted using the MCF-7 human breast cancer cell line to monitor fractionation. The chloroform-soluble partition of a methanol extract from the mixed *B. javanica* leaves, twigs, and inflorescence collected in Vietnam was chosen for phytochemical study and biological evaluation due to its inhibitory effect on this cell line. In the present work, activity-guided fractionation of the chloroform-soluble extract led to the isolation of five new triterpenoids (**1**–**5**), two known quassinoids (**6**, **7**), and a known flavonolignan (**8**). Herein, the isolation and structure elucidation of compounds **1**–**5**, as well as the biological evaluation of all isolates obtained, are described.

2. Results and discussion

2.1. Structure elucidation of new compounds

Bruceajavanone A (**1**) was obtained as a white amorphous resin. The molecular formula was determined as $C_{38}H_{56}O_8$ based on the sodiated molecular ion peak at *m/z* 663.3873 $[M+Na]^+$ (calcd 663.3873) in the HRESIMS. The IR spectrum showed absorptions for hydroxyl (3458 cm^{-1}) and carbonyl (1730, 1671 cm^{-1}) groups. The UV maximum at 228 nm ($\log\varepsilon$ 4.12) suggested the presence of an α,β-unsaturated ketone group. In the ^1H NMR spectrum, seven tertiary methyl groups were observed at the high-field region from

* Corresponding author. Tel.: +1 614 247 8094; fax: +1 614 247 8642.
E-mail address: kinghorn.4@osu.edu (A. D. Kinghorn).

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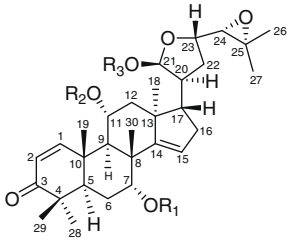
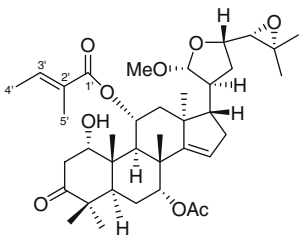
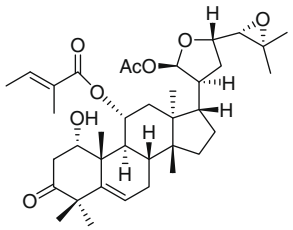
δ_H 0.95–1.35 ppm (see Table 1), and signals for two olefinic protons at δ_H 7.08 (1H, d, J = 10.4 Hz, H-1) and 5.73 (1H, d, J = 10.4 Hz, H-2) were consistent with the presence of an endocyclic double bond conjugated to a ketone group, while another olefinic proton resonating at δ_H 5.34 (s, H-15) was ascribed to a trisubstituted double bond. Moreover, an acetyl group as well as a hexanoate group was recognized from characteristic signals in the ^{1}H and ^{13}C NMR spectra (see Tables 1 and 2). After subtraction of these two ester groups, there were 30 carbon signals remaining in the ^{13}C NMR spectrum, which were sorted by DEPT into seven methyls, four methylenes, four methines, four quaternary carbons, six oxygenated carbons (including five secondary and a tertiary), a carbonyl group, a trisubstituted double bond, and a 1,2-substituted double bond. These characteristic NMR data suggested that compound **1** is an apotirucallane-type triterpenoid, with a structure similar to that of 21-*O*-acetyl-toosendantriol.[21] Compounds based on this triterpenoid skeleton have been obtained previously from the stems and bark of *B. javanica* as well as other species of the plant families Meliaceae and Rutaceae.[4,21–24]

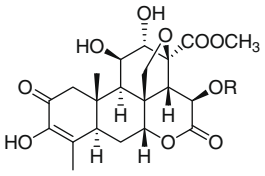


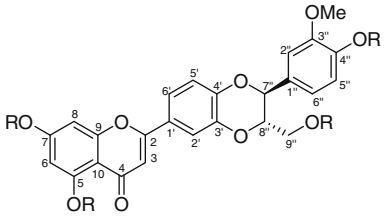




1 R_1 = H, R_2 = Hexanoyl, R_3 = Ac
2 R_1 = Ac, R_2 = Hexanoyl, R_3 = Ac
3 R_1 = Ac, R_2 = Tigloyl, R_3 = Ac

4 **5**





6 R = (*E*)-3,4-Dimethyl-2-pentenoyl
7 R = Isopentanoyl

8 R = H
8a R = Ac

The presence of a five-membered hemiacetyl ring in **1** was confirmed by the HMBC correlations from the H-21 methine proton (δ_H 6.20, d, J = 4.1 Hz) to C-20 (δ_C 44.2), C-22 (δ_C 31.4), as well as C-23 (δ_C 79.8). HMBC correlations from the high-field oxymethine proton (δ_H 2.67, d, J = 7.6 Hz, H-24) as well as two methyl protons (δ_H 1.32, 1.29, s, H-26 and H-27, each 3H) to C-25 (δ_C 57.1) indicated the occurrence of an epoxide moiety with two methyl substituents. The key ^{1}H–^{1}H COSY correlations of H-23 (δ_H 3.90, ddd, J = 9.9, 7.3, 7.3 Hz) with H-24, and H-20 (δ_H 2.35, m) with H-17 (δ_H 1.96, m), established the connectivities of these different moieties. The acetyl and hexanoate groups mentioned above were assigned to C-21 and C-11, respectively, based on the key HMBC correlations between H-21 with the carbonyl of the acetyl group (δ_C 169.7), and between H-11 (δ_H 5.45, dd, J = 9.5, 7.5 Hz) with the carbonyl of the hexanoate group (δ_C 172.9). A free hydroxy group was located at C-7, with HMBC correlations of H-7 (δ_H 4.02, br s) with C-5, C-8, and C-30 observed. The NOE effects between H_3-18 with H-20 and H-17 with H_3-30 indicated that the five-membered hemiacetal ring is located in an α-position at C-17, and the C-24, C-25 epoxide group was found to be β-oriented based on the NOE correlations of H-23 with H_α-22 and H-24 with H_β-22. The α-positions of OH-7 and the C-11 hexanoate group were deduced from the NOE cross peaks of H-7/H-30, H-11/H-30, and H-11/H_β-22. The β orientation of OAc-21 was elucidated from the NOE effects of H-21 with H-20 as well as H-18. The α,β-unsaturated ketone in the A ring obeyed the octant rule, which made it possible to confirm the absolute configuration of **1** using a circular dichroism (CD) spectroscopic measurement.[4,25] The CD curve of this compound showed a negative band at 346 nm ($\Delta\varepsilon = -1.84$) for a n–$\pi^*$ transition, which was in agreement with the predicted negative Cotton effect for the A ring based on the assignments of 5*R* and 10*R*. Thus, the chemical structure of compound **1** was determined as shown, and was accorded the trivial name, bruceajavanone A.

The HRESIMS of compound **2** gave a sodiated molecular ion peak at m/z 705.3978 $[M+Na]^+$, consistent with a molecular formula of $C_{40}H_{58}O_9$. The ^{1}H NMR spectrum of compound **2** was very similar to that of bruceajavanone A (**1**), except for an obvious downfield shift of H-7 from δ_H 4.02 (br s, 1H) to δ_H 5.26 (br s, 1H) (Table 1). Moreover, a second acetate methyl group signal was observed at δ_H 1.97 (s, 3H) besides that resonating at δ_H 2.07 (s, 3H). These observations suggested that the free hydroxy group at C-7 in compound **1** is acetylated in **2**. Corresponding evidence in the ^{13}C NMR spectrum was found from the resonances for a carbonyl group at δ_C 170.0 and the methyl signal at δ_C 20.5, along with a downfield shift of C-7 by 2.8 ppm relative to **1** (Table 2). This analysis was confirmed by a key HMBC correlation between H-7 and the carbonyl of the acetyl group at δ_C 170.0. The absolute configuration of compound **2** was consistent with that of bruceajavanone A (**1**) based on the analysis of NOESY spectrum as well as the CD curve. Thus, the chemical structure of compound **2** (bruceajavanone A 7-acetate) was determined as shown.

The molecular formula of **3** was defined as $C_{39}H_{54}O_9$ from the sodiated molecular ion peak at m/z 689.3651 $[M+Na]^+$ in the HRESIMS. Comparison of the 1D and 2D NMR data of **3** with those of **2** revealed that the only difference between these two compounds was at the C-11 substituent, with the hexanoate group in **2** replaced by a tigloyl group in **3**. The signals of the latter appeared at δ_H 6.85 (1H, qd, J = 7.1, 1.0 Hz, H-3′), 1.84 (3H, br d, J = 7.1 Hz, H-4′) and 1.88 (1H, br s, H-5′) in the ^{1}H NMR spectrum as well as at δ_C 169.8 (s, C-1′), 128.9 (s, C-2′), 138.2 (d, C-3′), 14.6 (q, C-4′) and 12.1 (q, C-5′) in the ^{13}C NMR spectrum. A key HMBC correla-

Table 1
^{1}H NMR chemical shifts of compounds **1**–**5**[a].

Position	1	2	3	4	5
1	7.08, d (10.4)	7.14, d (10.4)	7.07, d (10.4)	3. 66, br s	3.85, br s
2α	5.73, d (10.4)	5.76, d (10.4)	5.72, d (10.4)	2.40, m	2.39, m
2β				3.02, m	2.95, m
5	2.48, m	2.24, m	2.27, m	2.38, m	
6	1.89–1.92, m	1.82–1.90, m	1.83, m, 1.94, m	1.81, m, 2.02, m	5.42, br s
7	4.02, br s	5.26, br s	5.28, br s	5.25, br s	2.23, m[b]
8					2.20, m
9	2.53, d (9.5)	2.53, d (9.5)	2.57, d (9.5)	2.84, d (9.1)	3.14, m
11	5.45, dd (9.5, 7.5)	5.43, dd (9.5, 7.5)	5.49 dd (9.5, 7.5)	5.56, m	5.35, m
12α	1.59, br d (14.8)	1.55, br d (14.8)	1.60, br d (15.0)	1.85, m	1.02, m
12β	1.82, m	1.83, m	1.82, m	1.85, m	2.40, m[b]
15	5.34, br s	5.34, br s	5.34, br s	5.36, br s	1.65, m
16	2.26–2.30, m	2.12–2.18, m	2.17–2.20, m	2.16–2.19, m	1.42, m, 2.00, m
17	1.96, m	1.95, m	1.92, m	1.70, m	1.97, m
18	1.11, s	1.10, s	1.10, s	1.13, s	1.05, s
19	1.24, s	1.28, s	1.28, s	1.23, s	1.09, s
20	2.35, m	2.32, m	2.32, m	2.30, m	2.15, m
21	6.20, d (4.1)	6.20, d (4.1)	6.17, d (4.1)	4.63, d (3.8)	6.10, d (4.1)
22α	2.10, m	2.06, m	2.05, m	1.99, m	2.10, m
22β	1.68, m	1.68, m	1.69, m	1.40, m	1.68, m
23	3.90, ddd (9.9, 7.3, 7.3)	3.90, ddd (9.8, 7.2, 7.2)	3.90, ddd (9.8, 7.2, 7.2)	3.90, ddd (11.6, 7.5, 4.7)	3.91, m
24	2.67, d (7.6)	2.67, d (7.5)	2.66, d (7.5)	2.66, d (7.5)	2.67, d (7.6)
26	1.32, s	1.29, s[b]	1.32, s	1.32, s	1.32, s
27	1.29, s	1.27, s[b]	1.27, s	1.27, s	1.26, s
28	1.17, s	1.08, s	1.08, s	1.08, s	1.08, s
29	0.96, s	1.05, s	1.05, s	1.05, s	0.97, s
30	1.18, s	1.22, s	1.24, s	1.24, s	0.88, s
2′	2.30, m	2.32, m			
3′	1.64, m	1.68, m	6.85, qd (7.1, 1.0)	6.82, qd (7.1, 1.0)	6.82, qd (7.1, 1.0)
4′	1.34, m[b]	1.32, m	1.84, br d (7.1)	1.81, br d (7.1)	1.81, br d (7.1)
5′	1.35, m[b]	1.34, m	1.88, br s	1.87, br s	1.87, br s
6′	0.91, t (6.9)	0.91, t (6.9)			
OAc-7		1.97, s	2.00, s	1.99, s	1.99, s
OAc-21	2.07, s	2.07, s	2.07, s		2.06, s
OMe-21				3.23, s	

[a] Measured at 400 MHz; obtained in $CDCl_3$ with TMS as internal standard; *J* values (Hz) are given in parentheses. Assignments are based on ^{1}H-^{1}H COSY, HSQC, and HMBC spectroscopic data.
[b] Assignments may be interchangeable in the same column.

tion of H-11 (δ_H 5.49 m) with the carbonyl carbon of the tigloyl group at δ_C 169.8 (C-1′) was observed. Analysis of the NOESY spectrum and the CD curve indicated the absolute configuration of compound **3** to be identical with those of compounds **1** and **2**. Therefore, the structure of compound **3** (bruceajavanone B) was assigned as shown.

Bruceajavanone C (**4**) was obtained as white amorphous resin, with the molecular composition determined as $C_{38}H_{56}O_9$ based on the sodiated molecular ion peak at *m/z* 679.3823 $[M+Na]^+$ (calcd for $C_{38}H_{56}O_9Na$, 679.3822) in the HRESIMS. The 1D and 2D NMR spectra of **4** were similar in appearance to those of **1**–**3**. However, in the ^{1}H NMR spectrum of **4**, two olefinic protons belonging to the double bond of the A ring present in **1**–**3** were missing, while proton signals of a methylene group adjacent to a ketone group were recognized at δ_H 2.40 and 3.02 (each 1H, H-2α, and H-2β). In the ^{13}C NMR spectrum, a downfield shift around 10 ppm for C-3 (δ_C 214.2) was observed due to the lack of conjugation with a double bond. Moreover, an oxymethine proton signal appeared at δ_H 3.66 (br s, H-1) and showed COSY correlations with H_2-2. This suggested that the A ring of **4** is saturated, with a hydroxyl group substituted at C-1. HMBC correlations from H-1 to C-3 and C-19, as well as H_2-2 to C-3 confirmed this inference. Another difference between **4** and **3** was that a methoxyl group instead of an acetyl group was located at C-21 in **4**, which was confirmed by the HMBC correlation from the methoxyl protons at δ_H 3.23 (s, 3H) to C-21 (δ_C 110.0). The orientation of the hydroxy group on C-1 in **4** was assigned as α from the small coupling constant value observed between H-1 and H-2, which implied that H-1 adopted an equatorial β orientation. This was confirmed by NOE effects between H-1 with H-19 and $H_β$-2. The α orientation of the methoxy group at C-21 was deduced from the NOE effects between H-21 with H-17 and $H_β$-22. This assignment explained the notable downfield shift of around 4.5 ppm for C-17 (δ 57.0), due to the *cis*-γ substitution effect on H-17 in **1**-**3** compared with a *trans*-γ effect in **4**. In the CD spectrum of **4**, the n–π^* transition of the ketone group on A ring was blue-shifted to 292 nm due to the lack of any conjugation effect, but was still a broad negative band, suggesting that the absolute stereochemistry of A ring in compound **4** was consistent with those of compounds **1**–**3**. Thus, the structure of compound **4** (bruceajavanone C) was elucidated as shown.

The molecular formula of **5** was defined as $C_{37}H_{54}O_8$ from the sodiated molecular ion peak at *m/z* 649.3716 $[M+Na]^+$ in the HRESIMS. Close scrutiny of the NMR data revealed that the skeleton of **5** exhibited subtle differences from those of **1**–**4**. Accordingly, a trisubstituted double bond was located in the B ring at C-5/C-6 instead of in the D ring at C-14/C15 like in compounds **1**–**4**, based on HMBC correlations from the olefinic proton (δ_H 5.42, br s, H-6) to C-8, and from H-9 (δ_H 3.14, m) to C-5 (δ_C 141.6). A tertiary methyl proton signal appeared at δ_H 0.88 (s, H_3-30) and exhibited HMBC correlations with C-13, C-14 as well as C-15. In the ^{1}H-^{1}H COSY spectrum, the H-9 proton signal showed correlations not only with H-11 (δ_H 5.35, m), but also with a methine proton at δ_H 2.20 (H-8). This suggested that the tertiary methyl group of C-30 at C-8 in **1**–**4** was migrated to C-14 in **5**. NOE effects between

Table 2
^{13}C NMR chemical shifts of compounds **1–5**[a,b].

Position	1	2	3	4	5
1	158.4, d	158.7, d	158.4, d	74.5, d	75.5, d
2	124.1, d	124.3, d	123.8, d	41.3, t	41.0, t
3	204.3, s	204.0, s	203.8, s	214.2, s	214.5, s
4	44.4, s	44.4, s	44.2, s	47.5, s	47.8, s
5	43.8, d	45.7, d	45.5, d	40.8, d	141.6, s
6	24.0, t	23.7, t	23.5, t	23.7, t	120.8, d
7	71.3, d	74.1, d	73.8, d	73.9, d	24.6, t
8	44.3, s	42.6, s	42.4, s	42.1, s	45.3, d
9	42.5, d	44.0, d	44.1, d	40.9, d	45.6, d
10	40.7, s	40.7, s	40.5, s	42.7, s	42.7, s
11	69.7, d	69.9, d	69.6, d	71.5, d	70.7, d
12	42.4, t	43.0, t	42.6, t	41.8, t	41.6, t
13	45.6, s	45.8, s	45.6, s	46.0, s	46.0, s
14	160.4, s	158.2, s	158.2, s	158.9, s	51.0, s
15	119.9, d	119.1, d	118.7, d	118.3, d	34.3, t
16	35.2, t	35.5, t	35.1, t	34.7, t	25.1, t
17	52.5, d	52.8, d	52.2, d	57.0, d	45.7, d
18	20.1, q	20.4, q	20.0, q	19.2, q	22.1, q
19	20.0, q	19.5, q	19.9, q	16.7, q	13.9, q
20	44.2, d	44.5, d	44.4, d	46.4, d	46.4, d
21	96.3, d	96.5, d	96.5, d	110.0, d	96.6, d
22	31.4, t	31.6, t	31.3, t	34.8, t	32.2, t
23	79.8, d	80.0, d	79.8, d	77.7, d	80.0, d
24	66.6, d	66.8, d	66.6, d	65.3, d	67.0, d
25	57.1, s	57.4, s	57.1, s	57.3, s	57.3, s
26	24.9, q	25.1, q	24.9, q	25.1, q	25.1, q
27	19.3, q	20.4, q	19.3, q	19.6, q	19.6, q
28	26.5, q	26.7, q	26.2, q	24.8, q	24.4, q
29	21.5, q	21.6, q	21.0, q	21.5, q	27.5, q
30	29.6, q	29.6, q	29.6, q	29.9, q	21.8, q
1′	172.9, s	173.1, s	169.8, s	166.8, s	166.8, s
2′	35.2, t	35.3, t	128.9, s	129.2, s	129.2, s
3′	24.6, t	24.8, t	138.2, d	138.7, d	138.7, d
4′	31.3, t	31.5, t	14.6, q	14.9, q	14.9, q
5′	22.5, t	22.5, t	12.1, q	12.4, q	12.4, q
6′	14.1, q	14.1, q			
OAc-7		170.0, s	170.0, s	170.4, s	
		21.5, q	21.5, q	21.5, q	
OAc-21	169.7, s	170.2, s	169.7, s		170.4, s
	21.4, q	21.6, q	21.4, q		21.5, s
OMe-21				55.7, q	

[a] Measured at 100 MHz; obtained in $CDCl_3$ with TMS as internal standard. Assignments are based on HSQC, and HMBC NMR spectra.
[b] Multiplicity obtained from the DEPT spectra.

H-18 with H-9, as well as between H-30 with H-8 and H-17 indicated a *trans* fusion of the C/D rings. Therefore, compound **5** was found to be a derivative based on a protolimonoid carbon skeleton rather than an apotirucallane skeleton.[26,27] Investigation of the 2D NMR data including the HMBC and NOESY spectra, enabled the assignments of all substituent groups and the relative configuration of **5**, which was comparable with compounds **1–4**. The CD spectrum of **5** exhibited a negative Cotton effect at 289 nm, very similar to that of compound **4**. Thus, the structure of **5** was determined as shown, and has been named bruceajavaninone A.

2.2. Identification of known compounds

The structures of compounds **6** and **7** were identified as bruceantin (**6**) and bruceine A (**7**), respectively, by comparing their physical and spectroscopic data with reported values.[28–30] These two known quassinoids have been isolated previously as active principles from *B. javanica*.[2,20,29] The structure of compound **8** was determined as the known flavonolignan, (−)-hydnocarpin,[31,32] with the ^{1}H and ^{13}C NMR data fully assigned based on HSQC and HMBC spectroscopic data. In order to determine the absolute configuration of **8**, (−)-hydnocarpin 5,7,4″,9″-tetraacetate (**8a**) was produced using standard acetylation conditions with $(Ac)_2O$/pyridine, to yield a methanol-soluble derivative. The CD spectrum of **8a** showed a positive Cotton effect at 223 nm and a negative Cotton effect at 279 nm. These data were very similar to CD data published for *trans*-(2*S*,3*S*)-3-methyl-2-phenyl-1,4-benzodioxane, which was derivatized by a stereoselective procedure to afford a product with established absolute configuration.[33] Thus, the absolute configuration of **8** was assigned as 7″*S* and 8″*S*, respectively. This is first time (−)-hydnocarpin (**8**) has been isolated from *B. javanica*, and the absolute configuration has been clarified in this investigation.

2.3. Biological activity evaluation

All pure compounds obtained in the present investigation were evaluated their cytotoxic activity against three human cancer cell lines (Lu1, LNCaP, and MCF-7) (Table 3). The five new compounds (**1–5**) were considered inactive (ED_{50} >10 μM). The two quassinoids, bruceantin (**6**) and bruceine A (**7**), as shown in Table 3, demonstrated significant inhibitory activities for all the three human cancer cell lines, and for bruceantin (**6**), the cytotoxicities were extremely potent. Bruceantin (**6**) was formerly of interest for development as an anticancer agent by the US National Cancer Institute, but was eventually dropped because of a lack of demonstrated efficacy in human clinical trials.[34] Recently, it has been suggested that this compound may have value in treating human hematological malignancies.[35]

(−)-Hydnocarpin (**8**) did not show any discernible cytotoxicity against the cancer cell lines itself (ED_{50} >10 μM). However, the cytotoxicities against the MCF-7 cell line of bruceantin (**6**) and bruceine A (**7**), were dramatically increased by 7.4-fold and 10-fold, respectively, in the presence of (−)-hydnocarpin (**8**) at a concentration of 20 μg/mL (43 μM) (see Table 3). Hydnocarpin and its analogs have been reported to possess a bacterial multidrug resistance pump-inhibiting effect,[32,36,37] but its effect in potentiating the cytotoxic potency of potential anticancer agents does not seem to have been reported to date.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured on a Perkin-Elmer 241 automatic polarimeter. CD spectra were conducted on a JASCO J-810 spectropolarimeter. IR spectra were obtained on an ATI Mattson Genesis Series FT-IR spectrometer. UV spectra were run on a Perkin-Elmer Lambda 10 UV/vis spectrometer. NMR spectroscopic data were recorded at room temperature on a Bruker Avance DRX-400 spectrometer with tetramethylsilane (TMS) as an internal standard. HRESIMS and ESIMS were obtained on a 3-Tesla Finnigan

Table 3
Cytotoxicity evaluation of compounds **6** and **7**[a].

Compound	Cell line[b]		
	Lu1	LNCaP	MCF-7
6	12	11	2.0
6[c]	NT[d]	NT[d]	0.27
7	79	96	15
7[c]	NT[d]	NT[d]	1.5
Paclitaxel[e]	2.3	4.7	0.70
Camptothecin[e]	29	29	29

[a] Compounds **1–5** and **8** were inactive (ED_{50} >10 μM).
[b] Key to cell lines used: Lu1, human lung cancer; LNCaP, hormone-dependent human prostate cancer; MCF-7, human breast cancer. Results are expressed as ED_{50} values (nM).
[c] (−)-Hydnocarpin (**8**) was added at a concentration of 20 μg/mL (43 μM).
[d] The potentiation effect of (−)-hydnocarpin (**8**) was not tested in these two cell lines, for compounds **6** and **7**.
[e] Used as positive control substances.

FTMS-2000 Fourier transform mass spectrometer. Column chromatography was performed with Sephadex LH-20 (Supelco, Bellefonte, PA), 65–250 or 230–400 mesh Si gel (Sorbent Technologies, Atlanta, GA). Analytical thin-layer chromatography was conducted on precoated 250 μm thickness Partisil Si gel $60F_{254}$ glass plates (Whatman, Clifton, NJ), and preparative thin-layer chromatography was performed on precoated 20 cm × 20 cm, 500 μm or 1000 μm thickness Partisil Si gel $60F_{254}$ glass plates (Whatman, Clifton, NJ). A 150 mm × 19 mm i.d., 5 μm Sunfire PrepC_{18} column (Waters, Milford, MA) and a 10 mm × 19 mm i.d., 5 μm Sunfire guard column were used for preparative HPLC, along with a Waters system composed of a 600 controller, a 717 Plus autosampler, and a 2487 dual wavelength absorbance detector.

3.2. Plant material

The combined leaves, twigs, and inflorescence (4.3 kg) were collected at Hon Ba Mountain (12° 09.428″N, 109° 02.624″E., altitude 150 m), Khanh Hoa Province, Vietnam by Tran Ngoc Ninh and Djaja Djendoel Soejarto in December, 2006, who also identified this plant. A voucher specimen (Collection No. Soejarto and Ninh 13944) has been deposited in the John G. Searle Herbarium of the Field Museum of Natural History, Chicago, Illinois.

3.3. Cytotoxicity evaluation procedures

The cytotoxic activity of extracts, chromatographic subfractions of chloroform-soluble extract and all pure compounds were evaluated against a panel of human cancer cell lines (Table 3), according to established protocols.[38]

3.4. Extraction and isolation

The dried and milled material contained leaves, twigs and inflorescence (4.3 kg) of *B. javanica* was extracted using MeOH (3× 8 L, each for 2 days) at rt. The solvent was removed under reduced pressure to give 900 g of a thick brown syrup, which was suspended in a 3 L mixture of methanol–water (9:1) solution and was then partitioned with hexanes (3× 2 L). To the defatted residue was added 10% water (300 mL) and this was then partitioned with chloroform (3× 2 L). Next, the chloroform partition was washed with water that contained 1% NaCl (3× 2 L) to give 82 g of a partially detannified chloroform-soluble extract. The chloroform-soluble extract was found to be active against the MCF-7 cell line, with an ED_{50} value of <0.16 μg/mL. This was subjected to separation over a Si gel column (9× 45 cm, 65–250 mesh, 1000 g), eluted with a solvent system of CH_2Cl_2–acetone with a gradient polarity (20:1 to 1:1, then pure acetone) to yield 11 fractions, denoted as F1–F11. All these fractions were evaluated against the MCF-7 cell line, and F3 was found to be most active, with an ED_{50} value of <0.16 μg/mL.

Fraction F3 (20 g) was chromatographed on a silica gel column (4× 40 cm, 230–400 mesh, 500 g) and eluted in a gradient manner with hexanes-acetone (10:1 to pure acetone), to give eight subfractions (F301–F308). Subfraction F303 (2 g) was further subjected to passage over a Sephadex LH-20 column (2× 120 cm), eluted with $CHCl_3$ first, to remove chlorophylls, and then with MeOH. Next, subfraction F303 was applied to a silica gel column (1× 40 cm, 230–400 mesh) with a gradient solvent system of $CHCl_3$–acetone (30:1 to pure acetone) to yield 12 subfractions (F30301–F30312). Subfraction F30302 was purified by preparative TLC (20 × 20 cm, 500 μm), developed with a solvent system of CH_2Cl_2–hexanes–acetone (6:1:0.4, R_f = 0.65), to afford **2** (15 mg). Repeated chromatography of subfraction F30303 using Si gel columns with a gradient solvent system of $CHCl_3$–acetone afforded impure **1**, which was finally purified by preparative TLC (20 × 20 cm, 500 μm), using CH_2Cl_2–hexanes–acetone (6:2:1) as developing solvent (R_f = 0.70, 20 mg). F30304 was subjected to preparative TLC (20 × 20 cm, 1000 μm), developed with a solvent system of CH_2Cl_2–hexanes–acetone (6:2:1), to give a mixture of **3** and **4** (R_f = 0.65, 25 mg). This mixture was purified by HPLC with MeCN–H_2O (60:40) as solvent system to afford **3** (7 mg) and **4** (11 mg), respectively. F30305 was purified by preparative TLC (20 × 20 cm, 500 μm), with CH_2Cl_2–hexanes–acetone (6:2:1) as developing solvent, to yield **5** (R_f = 0.50, 8 mg). F308 was subjected to passage over a Si gel column (2× 45 cm, 230–400 mesh) using a solvent system of $CHCl_3$–acetone (10:1 to pure acetone), to yield six fractions (F30801–F30806). F30803 was purified by HPLC, with MeCN–H_2O (35:65) as eluant, to afford **6** (17 mg) and **7** (15 mg), respectively. F30805 was chromatographed on a Sephadex LH-20 column (2× 120 cm) using MeOH as solvent to afford **8** (15 mg).

3.5. Bruceajavanone A (1)

White amorphous resin; $[\alpha]_D^{23}$ −4.6 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 228 (4.12) nm; IR ν_{max} (film) 3458, 2958, 2930, 1730, 1671, 1457, 1379, 1228, 1093, 887 cm^{-1}; CD (*c* 7.8 × 10^{-5} M, MeOH) λ_{max} (Δε) 232 (+11.93), 346 (−1.84) nm; HRESIMS obsd *m/z* 663.3873 $[M+Na]^+$, calcd for $C_{38}H_{56}O_8Na$, 663.3873; ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively.

3.6. Bruceajavanone A 7-acetate (2)

White amorphous resin; $[\alpha]_D^{23}$ +24.1 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 228 (4.16) nm; IR ν_{max} (film) 2958, 2933, 1730, 1671, 1457, 1377, 1244, 1228, 1093, 890 cm^{-1}; CD (*c* 7.3 × 10^{-5} M, MeOH) λ_{max} (Δε) 232 (+12.62), 345 (−1.82) nm; HRESIMS obsd *m/z* 705.3978 $[M+Na]^+$, calcd for $C_{40}H_{58}O_9$ Na, 705.3979; ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively.

3.7. Bruceajavanone B (3)

White amorphous resin; $[\alpha]_D^{23}$ +34.3 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 224 (4.77) nm; IR ν_{max} (film) 2928, 2857, 1740, 1705, 1671, 1458, 1377, 1248, 1244, 1132, 1082, 1031, 953, 817 cm^{-1}; CD (*c* 7.5 × 10^{-5} M, MeOH) λ_{max} (Δε) 222 (+28.24), 346 (−1.93) nm; HRESIMS obsd *m/z* 689.3651 $[M+Na]^+$, calcd for $C_{39}H_{54}O_9Na$, 689.3666; ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively.

3.8. Bruceajavanone C (4)

White amorphous resin; $[\alpha]_D^{23}$ −121.1 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 221 (4.11) nm; IR ν_{max} (film) 3591, 2965, 2933, 1728 (br), 1708, 1647, 1458, 1380, 1248, 1125, 1031, 879 cm^{-1}; CD (*c* 7.6 × 10^{-5} M , MeOH) λ_{max} (Δε) 219 (+27.09), 246 (−3.67), 292 (−2.00) nm; HRESIMS obsd *m/z* 679.3823 $[M+Na]^+$, calcd for $C_{38}H_{56}O_9Na$, 679.3822; ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively.

3.9. Bruceajavaninone A (5)

White amorphous resin; $[\alpha]_D^{23}$ −43.3 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 221 (4.09) nm; IR ν_{max} (film) cm^{-1} 3596, 2990, 2927, 1738, 1711 (br), 1708, 1644, 1456, 1379, 1255, 1227, 1097, 953, 884 cm^{-1}; CD (*c* 8.0 × 10^{-5} M , MeOH) λ_{max} (Δε) 246 (−0.63), 287 (−1.24) nm; HRESIMS obsd *m/z* 649.3716 $[M+Na]^+$, calcd for $C_{37}H_{54}O_8Na$, 649.3716; ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively.

3.10. (−)-Hydnocarpin (8)

Pale yellow amorphous powder; $[\alpha]_D^{23}$ −32.0 (*c* 0.5, DMSO); ^{1}H NMR (400 MHz, DMSO-d_6, TMS) δ 3.37 (1H, m, H-9″b), 3.58 (1H,

m, H-9″a), 3.78 (3H, s, OMe-3″), 4.27 (1H, m, H-8″), 4.99 (1H, t, J = 5.0 Hz, OH-9″), 5.01 (1H, d, J = 8.0 Hz, H-7″), 6.20 (1H, d, J = 1.9 Hz, H-6), 6.52 (1H, d, J = 1.9 Hz, H-8), 6.81 (1H, d, J = 8.0 Hz, H-5″), 6.87 (1H, dd, J = 8.0, 1.5 Hz, H-6″), 6.89 (1H, s, H-3), 7.03 (1H, d, J = 1.5 Hz, H-2″), 7.08 (1H, d, J = 8.5 Hz, H-5′), 7.60 (1H, dd, J = 8.5, 1.9 Hz, H-6′), 7.66 (1H, d, J = 1.9 Hz, H-2′), 9.20 (1H, s, OH-4″), 10.88 (1H, s, OH-7), 12.90 (1H, s, OH-5); ^{13}C NMR (100 MHz, DMSO-d_6, TMS) δ 55.7 (OMe-3″), 60.07 (C- 9″), 76.4 (C-7″), 78.0 (C-8″), 94.1 (C-8), 98.9 (C-6), 103.8 (C-3), 103.9 (C-10), 111.7 (C-2″), 114.8 (C-2′), 115.3 (C-5″), 117.5 (C-5′), 119.9 (C-6′), 120.6 (C-6″), 123.7 (C-1′), 126.9 (C-1″), 143.7 (C-3′), 147.1 (C-4′), 147.2 (C-4″), 147.6 (C-3″), 157.3 (C-9), 161.4 (C-5), 162.9 (C-7), 164.2 (C-2), 181.8 (C-4).

3.11. (−)-Hydnocarpin 5,7,4″,9″-tetraacetate (8a)

$[\alpha]_D^{23}$ −21.2 (c 0.05, MeOH); CD (c 6.5 × 10^{-5} M, MeOH) λ_{max} ($\Delta\varepsilon$) 223 (+8.52), 279 (−0.50) nm; ^{1}H NMR (400 MHz, $CDCl_3$, TMS) δ 2.10 (3H, s, OAc), 2.35 (3H, s, OAc), 2.37 (3H, s, OAc), 2.46 (3H, s, OAc), 3.88 (3H, s, OMe-3″), 4.04 (1H, dd, J = 12.0, 4.5 Hz, H-9″b), 4.31 (1H, m, H-8″), 4.41 (1H, br d, J = 12.0 Hz, H-9″a), 5.02 (1H, d, J = 8.0 Hz, H-7″), 6.60 (1H, d, J = 1.9 Hz, H-6), 6.85 (1H, d, J = 1.9 Hz, H-8), 7.00 (2H, m, H-5″ and H-6″), 7.11 (2H, m, H-3 and H-2″), 7.36 (1H, d, J = 8.5 Hz, H-5′), 7.45 (1H, dd, J = 8.5, 2.0 Hz, H-6′), 7.56 (1H, d, J = 2.0 Hz, H-2′).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2008.10.076.

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