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PII: S0040-4020(18)31480-7

DOI: https://doi.org/10.1016/j.tet.2018.12.015

Reference: TET 30001

To appear in: *Tetrahedron*

Received Date: 28 September 2018

Revised Date: 1 December 2018

Accepted Date: 11 December 2018

Please cite this article as: Yan Y-M, Zhu H-J, Zhou F-J, Tu Z-C, Cheng Y-X, Phenolic compounds from the insect *Blaps japanensis* with inhibitory activities towards cancer cells, COX-2, ROCK1 and JAK3, *Tetrahedron* (2019), doi: https://doi.org/10.1016/j.tet.2018.12.015.

Graphical Abstract

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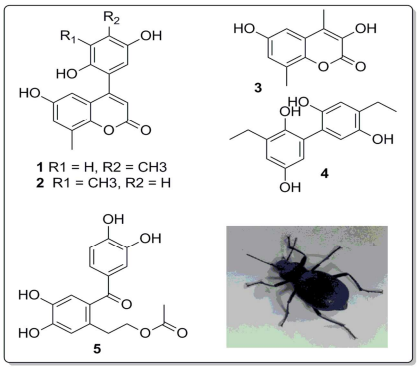

	IC_{50} (μM)	
Compd	JAK3	ROCK1
1	2.88	9.77
2	5.27	9.31
3	35.46	NA
4	1.87	4.61
5	6.72	NA

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Phenolic compounds from the insect *Blaps japanensis* with inhibitory activities towards cancer cells, COX-2, ROCK1 and JAK3

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ARTICLE INFO

Article history:
Received
Received in revised form
Accepted
Available online

Keywords:
Insect
Blaps japanensis
phenolic compounds
Cytotoxicity
COX-2
ROCK1
JAK3

ABSTRACT

The insect *Blaps japanensis* have been used as an ethnomedicine in China for the treatment of several disorders such as cancer and inflammation. Our investigation with this insect led to the isolation of eight new and two known phenolic compounds. The structures of the new compounds, blapsins C–J (**1**–**8**), were identified by spectroscopic data. The inhibitory activities of all the compounds except of **9** against human cancer cells (A549, K562 and Huh-7), COX-2, ROCK1, and JAK3 were evaluated. Several compounds were found to be biologically active in these assays.

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1. Introduction

Insect medicine is well known for its pronounced effects in clinical practice. However, chemical profiling in the insects remains largely unknown. In recent years, we have been interested in searching non-peptide small molecules from medicinal insects and several biologically active and structurally interesting substances have been characterized by us from several insects such as black ants,[1,2] *Aspongopus chinensis*,[3-5] *Periplaneta americana*,[6,7] and *Catharsius molossus*.[8] *Blaps japanensis*, as a commonly used ethnomedicine, has been used for the treatment of some refractory diseases including cancer, infection, rheumatoid arthritis, cardiovascular and inflammatory diseases by Yi-nationality of Yunnan province of China.[9] In contrast to its wide medical use, chemical investigations in this insect is scarce. We have initiated an investigation on *B. japanensis* by using 5 kg of dried material, and identified two compounds as potent 14-3-3 protein-protein interaction inhibitors.[10] In addition, blapsols as derivatives of *N*-acetyldopamine and selective COX-2 inhibitors were also characterized from it.[11] These findings prompted us to conduct an in-depth study in *B. japanensis*. Our follow-up investigation led to the isolation of ten phenolic compounds (Fig. 1), eight of which are new ones. Inspired by the traditional use of *B. japanensis*, all the isolates were biologically evaluated for their potential inhibition on human cancer cells, ROCK1, JAK3, and COX-2 with exception of compound **9**.

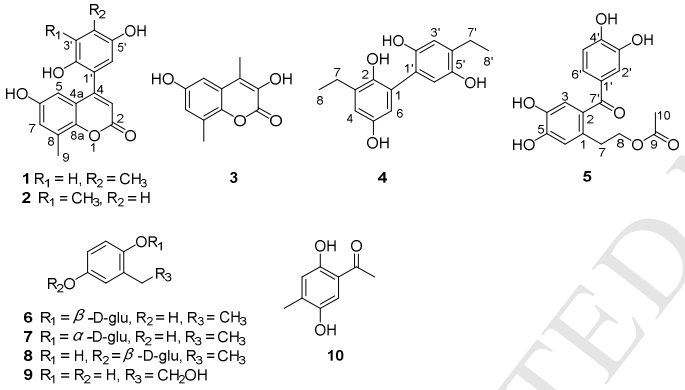

Fig. 1. The chemical structures of compounds **1**–**10**.

2. Results and discussion

2.1. Structure elucidation

Compound **1** was found to have the molecular formula $C_{17}H_{14}O_5$ (11 degrees of unsaturation) derived from its HRESIMS, ^{13}C NMR and DEPT spectra. The ^1H NMR spectrum (Table 1) of **1** contains two *meta* protons (δ_H 6.61, 1H, d, J = 2.7 Hz, H-5; 6.96, 1H, d, J = 2.7 Hz, H-7), suggesting a 1,2,3,5-tetrasubstituted benzene ring, two *para* protons (δ_H 6.80, 1H, s, H-3′; 6.69, 1H, s, H-6′), suggesting a 1,2,4,5-tetrasubstituted benzene rings, along with a resonance for olefinic proton (δ_H 6.24, 1H, s). The ^{13}C NMR and DEPT spectra contain 17 carbons ascribe to two methyl, five sp^2 methine, and ten quaternary carbons (one carbonyl, 9 olefinic including four oxygenated). The structure of **1** was constructed mainly on the basis of an HMBC experiment. HMBC correlations of H_3-7′/C-3′ (δ_C 119.3), C-4′, C-5′ (δ_C 149.3), H-6′/C-1′, C-4, in consideration of the chemical shifts of C-2′ and C-5′ and a *para* relationship between H-3′ and H-6′, indicates the presence of substructure A (Fig. 2). HMBC correlations of H_3-9/C-7 (δ_C 121.6), C-8, C-8a (δ_C 146.8), H-3/C-2, C-4, C-4a, and H-5/C-4, in conjunction with a *meta* relationship of H-5 and H-7, suggest a substructure like that of part B (Fig. 2). Additional HMBC correlations of H-6′/C-4 and H-3/C-1′ allow parts A and B connecting via C-1′–C-4. The observed ROESY correlations of H-3/H-6′, H-9/H-7, and H-7′/H-3′, in consideration of the chemical shift of C-2 and the requirement of the total degrees of unsaturation, further confirmed the presence of part B, which is actually a coumarin derivative. Thus far, the structure of **1** was identified and named as blapsin C.

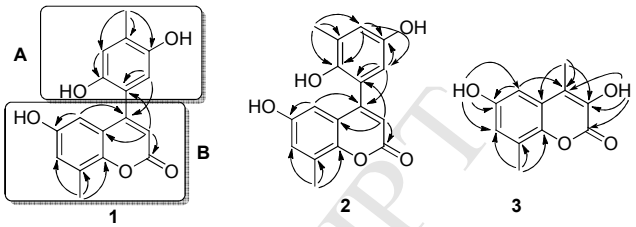

Fig. 2. Key HMBC correlations of **1**–**3**.

Compound **2** has the same molecular formula as that of **1** by analysis of its HRESIMS, ^{13}C NMR and DEPT spectra. The ^1H and ^{13}C NMR spectra of **2** (Table 1) are very similar to those of **1**. The only difference is that a methyl group at C-4′ in **1** was positioned at C-3′ of **2**. This alternation was in accordance with the HMBC correlations of H_3-7′/C-2′, C-3′, C-4′, H-4′/C-6′ (Fig. 2), and an observed *meta* relationship between H-4′ (δ_H 6.80, 1H, d, J = 2.5 Hz) and H-6′. Compound **2** was therefore identified and named as blapsin D.

Table 1
^1H (600 MHz) and ^{13}C NMR (150 MHz) data of **1** and **2** in acetone-d_6 (δ, ppm)

No.	1		2	
	δ_C, mult	δ_H, (mult, J in Hz)	δ_C, mult	δ_H, (mult, J in Hz)
2	161.1 s		160.9 s	
3	116.7 d	6.24 (s)	117.3 d	6.26 (s)
4	154.5 s		154.2 s	
4a	120.8 s		120.8 s	
5	110.6 d	6.61 (d, 2.7)	110.4 d	6.53 (overlap)
6	153.8 s		153.8 s	
7	121.6 d	6.96 (d, 2.7)	121.7 d	6.97 (d, 2.1)
8	127.6 s		127.6 s	
8a	146.8 s		146.9 s	
9	15.6 q	2.37 (s)	15.6 q	2.38 (s)
1′	121.2 s		124.4 s	
2′	147.5 s		145.4 s	
3′	119.3 d	6.80 (s)	128.1 s	
4′	127.9 s		119.5 d	6.80 (d, 2.5)
5′	149.3 s		151.3 s	
6′	116.2 d	6.69 (s)	114.0 d	6.53 (overlap)
7′	16.3 q	2.22 (s)	16.8 q	2.25 (s)

Compound **3** has the molecular formula $C_{11}H_{10}O_4$ (7 degrees of unsaturation), derived from its HRESIMS, ^{13}C NMR and DEPT spectra. The ^1H NMR spectrum of **3** (Table 2) shows two *meta* protons in the olefinic region (δ_H 6.74, 1H, d, J =2.4 Hz, H-5; 6.73, 1H, d, J = 2.4 Hz, H-6), indicating the presence of a 1,2,3,5-tetrasubstituted benzene ring. The ^{13}C NMR and DEPT spectra of **3** displays 11 signals attributed to two methyl, two sp^2 methine, and seven quaternary carbons (one carbonyl, six olefinic including three oxygenated). The ^1H and ^{13}C NMR data of **3** are similar to those of part B in **1** differing in that a hydroxyl and a methyl was respectively positioned at C-3 and C-4 of structure **3**. This alternation was supported by HMBC correlations of Me-4/C-3, C-4, C-5 and OH-3/C-2, C-3, C-4 (Fig. 2). As a result, the structure of **3** was assigned as shown and named as blapsin E.

Compound **4** has a molecular formula $C_{16}H_{18}O_4$ (8 degrees of unsaturation), by analysis of its HREIMS, ^{13}C NMR and DEPT

spectra. The ^{13}C NMR and DEPT spectra of **4** show 16 carbons (= 7.3 Hz). Consequently, the structure of **6** was identified and ascribe to two methyl, two aliphatic methylene, four sp^2 methine, and eight olefinic quaternary carbons (four oxygenated). Careful inspection of ^{13}C NMR spectrum of **3** found that signals appear in pairs, suggesting that **4** might be a dimer. Two *para* protons (H-3′ and H-6′) and two *meta* protons (H-4 and H-6) observed in the ^{1}H NMR spectrum reveal the presence of a 1,2,3,5-tetrasubstituted benzene ring and a 1,2,4,5-tetrasubstituted benzene ring, respectively. The ^{1}H-^{1}H COSY cross peak of H-7/H-8 and HMBC correlations of H-7/C-2, C-3, C-4 (Fig. 3), ROESY correlations of H-6/H-6′, H-3′/H-7′, and H-7/H-4 suggest that the monomer of **4** is a 2-ethylbenzene-1,4-diol. The two monomers were connected via C-1′–C-1 evidenced from H-6/C-1′ and H-6′/C-1 correlations. As a result, the structure of **4** was deduced as shown and named as blapsin F.

Table 2

^{1}H (600 MHz) and ^{13}C NMR (150 MHz) data of **3** in DMSO-d_6 (δ, ppm)

No.	δ_C	δ_H, (m, *J* in Hz)		δ_C	δ_H, (m, *J* in Hz)
2	158.2 s		8	125.7 s	
3	137.9 s		8a	140.2 s	
4	123.7 s		9	15.3 q	2.29 (s)
4a	121.9 s		4-CH_3	10.6 q	2.17 (s)
5	106.3 d	6.74 (d, 2.4)	3-OH		9.71 (s)
6	153.5 s		6-OH		9.52 (s)
7	117.1 d	6.73 (d, 2.4)	8	125.7 s	
4	123.7 s				

Compound **5** was determined to be $C_{17}H_{16}O_7$ (10 degrees of unsaturation) by analysis of its HRESIMS, ^{13}C NMR and DEPT spectra. The ^{1}H NMR spectrum of this substance contains a typical ABX spin system (δ_H 7.32, 1H, d, *J* = 1.8 Hz, H-2′; 6.90, 1H, d, *J* = 8.2 Hz, H-5′; 7.19, 1H, dd, *J* = 8.2, 1.8 Hz, H-6′) and two *para* protons (δ_H 6.83, 1H, s, H-3; 6.87, 1H, s, H-6). The ^{13}C NMR and DEPT spectra of **5** give 17 carbons classified into one methyl, two aliphatic methylene (one oxygenated), five olefinic methine, and nine quaternary carbons (one ketone, one carbonyl, seven olefinic including four oxygenated). The ^{1}H-^{1}H COSY correlation of H-7/H-8 and HMBC correlations of H-8, H-10/C-9 allow the assignment of the side chain (Fig. 3), whose position at the ring A was established by HMBC correlations of H-8/C-1, H-7/C-1, C-2, C-6. The positions of two hydroxyl groups in ring A was deduced as shown from HMBC correlations of H-7/ C-1, C-2, C-6 and H-3/C-7′, C-2 and a *para* relationship between H-3 and H-6. The presence of an ABX system along with HMBC correlations of H-2′, H-6′/C-1′, C-7′ suggests the substituted pattern in ring B which was connected to ring A via C-7′. The structure of **5** was therefore identified and named as blapsin G.

Compound **6** was found to have a molecular formula $C_{14}H_{20}O_7$ on the basis of its HRESIMS, ^{13}C NMR and DEPT spectra. The ^{1}H NMR spectrum (Table 4) of **6** exhibits a typical ABX spin system (δ_H 6.59, 1H, d, *J* = 2.9 Hz, H-3; 7.00, 1H, d, *J* = 8.7 Hz, H-6; 6.54, 1H, dd, *J* = 8.7, 2.9 Hz, H-5). The ^{13}C NMR and DEPT spectra of this substance give 14 resonances attributable to one methyl, two methylene, seven methine (three olefinic and four oxygenated), and three olefinic quaternary carbons (two oxygenated). Inspection of ^{13}C NMR of **6** found that compound **6** is a glycoside. Acid hydrolysis of **6** produced D-glucose, which was identified by TLC comparison with an authentic sample and from its positive sign of optical rotation. In addition to the signals of a glucose residue, the remaining resonances are in accordance with the monomer of **4**. This conclusion could be confirmed by detailed interpretation of its 2D NMR data (Fig. 3). The glucose moiety is connected to C-1 supported by the observed HMBC correlation of H-1′/C-1. The configuration of glycosidic bond was assigned as β-form based on the coupling constant of H-1′ (*J* = 7.3 Hz). Consequently, the structure of **6** was identified and named as blapsin H.

Table 3

^{1}H (600 MHz) and ^{13}C NMR (150 MHz) data of **4** in acetone-d_6 (δ, ppm)

No.	δ_C	δ_H, (m, *J* in Hz)		δ_C	δ_H, (m, *J* in Hz)
1	128.6 s		1′	125.3 s	
2	145.6 s		2′	146.6 s	
3	134.3 s		3′	117.6 d	6.80 (s)
4	116.1 d	6.65 (d, 2.9)	4′	131.9 s	
5	151.7 s		5′	149.7 s	
6	115.4 d	6.57 (d, 2.9)	6′	118.3 d	6.73 (s)
7	24.5 t	2.64 (q, 7.5)	7′	23.7 t	2.61 (q, 7.5)
8	14.9 q	1.19 (t, 7.5)	8′	14.5 q	1.17 (t, 7.5)

Compound **7** has the same molecular formula as that of **6**. Its NMR data are also similar to those **6**, indicating that they might be isomers. Careful comparison of NMR data of **6** and **7** found that they differ only in the configuration of glycosidic bond. The coupling constant of H-1′ is 3.6 Hz in **7** suggests an α-form configuration. In the same manner as that of **6**, acid hydrolysis followed by TLC comparison with an authentic sample and optical rotation measurement confirmed the above conclusion. The structure of **7** was thus identified and named blapsin I.

Compound **8** has the same molecular formula and similar NMR data as those of **6**. Inspection of NMR data of compounds **6** and **8** found that they differ only in the position of the glucose moiety, which could be evidently confirmed by the observed HMBC correlation of H-1′/C-4. Evidence for the presence of a D-glucose moiety in **8** comes from analysis of the acid hydrolysis product in the manner described for **6**. Therefore, the structure of **8** was identified and named blapsin J.

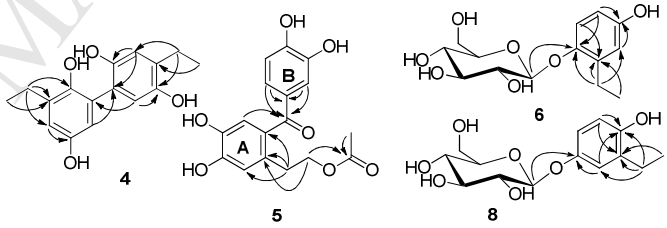

Fig. 3. Key HMBC correlations of **4**–**6** and **8.**

Two known compounds were isolated and identified as transtorine (**9**)[12] and 2,5-dihydroxy-4-methyl acetophenone (**10**)[13] by comparing their spectroscopic properties with those previously reported for these substances. Of note, all the compounds with exception of **5** are hydroquinone derivatives. Compound **4** is a dimer of 2-ethylbenzene-1,4-diol, and **1**–**3** might be hybrids of hydroquinone derivatives with coumarin.

2.2. Biological evaluation

Biological activities of all the isolates except of **9** against cancer cells (A549, Huh-7, K562), COX-2, ROCK1 and JAK3 were evaluated. For cytotoxic activities by using CCK8 assay, we found that compounds **1**, **2**, and **4** are active towards the above three cancer cell lines (Table 5), among them, **1** appears to be the most active with IC_{50} values less than 10 μM. We also noted that compound **3** is not active towards these cell lines, indicating the importance of part A in the structure of **1** for keeping the activity. Activated COX-2 is known to promote inflammation, selective inhibition of COX-2 will be beneficial for anti-inflammatory therapy. In this study, we found that compounds **2**–**4** show COX-2 inhibitory activity with respective

Table 4

^{1}H (600 MHz) and ^{13}C NMR (150 MHz) data of **5**–**8** (δ, ppm)

No.	**5**[a]		**6**[b]		**7**[b]		**8**[b]	
	δ_C	δ_H, (m, *J* in Hz)	δ_C	δ_H, (m, *J* in Hz)	δ_C	δ_H, (m, *J* in Hz)	δ_C	δ_H, (m, *J* in Hz)
1	131.9 s		150.2 s		149.7 s		151.4 s	
2	131.7 s		136.5 s		136.4 s		132.6 s	
3	117.6 d	6.83 (s)	116.5 d	6.59 (d, 2.9)	116.7 d	6.60 (d, 3.0)	119.4 d	6.88 (d, 2.9)
4	143.3 s		153.6 s		153.4 s		152.4 s	
5	147.8 s		113.8 d	6.54 (dd, 8.7, 2.9)	113.8 d	6.53 (dd, 8.7, 3.0)	116.2 d	6.79 (dd, 8.7, 2.9)
6	118.6 d	6.87 (s)	118.4 d	7.00 (d, 8.7)	118.1 d	7.04 (d, 8.7)	116.1 d	6.65 (d, 8.7)
7	32.5 t	2.87 (t, 7.0)	24.1 t	2.65 (q, 7.5)	24.3 t	2.68 (q, 7.5)	24.3 t	2.57 (q, 7.5)
8	65.9 t	4.14 (t, 7.0)	15.0 q	1.17 (t, 7.5)	15.1 q	1.19 (t, 7.5)	14.6 q	1.17 (t, 7.5)
9	170.9 s							
10	20.7 q	1.86 (s)						
1′	130.6 s		104.0 d	4.72 (d, 7.3)	100.3 d	5.31 (d, 3.6)	103.7 d	4.73 (d, 7.2)
2′	117.5 d	7.32 (d, 1.8)	75.1 d	3.42 (overlap)	73.6 d	3.56 (dd, 9.8,3.6)	75.1 d	3.42 (overlap)
3′	145.6 s		78.3 d	3.42 (overlap)	75.0 d	3.86 (t, 9.3)	78.3 d	3.42 (overlap)
4′	150.9 s		71.5 d	3.36 (overlap)	71.7 d	3.43 (t, 9.2)	78.0 d	3.36 (overlap)
5′	115.5 d	6.90 (d, 8.2)	78.0 d	3.36 (overlap)	74.4 d	3.71 (overlap)	71.5 d	3.36 (overlap)
6′a	124.8 d	7.19 (dd, 8.2, 1.8)	62.6 t	3.88 (dd, 12.0, 2.0)	62.5 t	3.77 (dd, 13.5, 4.0)	62.6 t	3.88 (dd, 12.0, 2.0)
6′b				3.70 (dd, 12.0, 5.1)		3.71 (overlap)		3.69 (dd, 12.0, 4.9)
7′	196.1 s							

[a] in acetone-d_6, [b] in methanol-d_4

IC_{50} value of 6.12 μM, 10.70 μM, and 83.20 μM. It was noted that compound **1** is not active towards COX-2 in spite of its slight difference in the structure from **2**. ROCK1 regulates a wide range of fundamental cell functions, inhibition of ROCK1 has been proved to be beneficial for the treatment of oncological, cardiovascular and neurological disorders and cardiorenal syndrome.[14] Our study showed that compounds **1**, **2** and **4** possess ROCK1 inhibitory activity with respective IC_{50} value of 9.77 μM, 9.31 μM, and 4.61 μM. JAK3 is a member of the JAK family of non-receptor protein tyrosine kinases, selective JAK3 inhibitors might be useful as therapeutic agents in the areas of oncology, organ transplantation and autoimmune diseases.[15,16] Therefore JAK3 has become a drug target which attracted great interest in academia and pharmaceutical industry. Since the title insect has been used for treating rheumatoid arthritis that is closely associated with dysimmunity, all the isolates were thus submitted to test their potential against JAK3. It was found that compounds **1**–**5** show potent inhibitory activity with respective IC_{50} value of 2.88 μM, 5.27 μM, 35.46 μM, 1.87 μM, and 6.72 μM, with **4** is the most active.

Table 5

Compounds against cancer cells (A549, Huh-7, K562), COX-2, ROCK1, and JAK3

	IC_{50} (μM)					
Compd	A549	Huh-7	K562	JAK3	ROCK1	COX-2
1	9.96	2.35	4.06	2.88	9.77	NA
2	13.10	3.86	5.45	5.27	9.31	6.12
3	NA[a]	NA[a]	NA[a]	35.46	NA[a]	10.70
4	NA[a]	14.20	8.04	1.87	4.61	83.20
5	NA[a]	NA[a]	NA[a]	6.72	NA[a]	NA[a]
Toxol	0.0009	0.0052	0.0025	NT[b]	NT[b]	NT[b]
Celecoxib	NT[b]	NT[b]	NT[b]	NT[b]	NT[b]	0.0032
Staurosporine	NT[b]	NT[b]	NT[b]	0.0001	0.0074	NT

[a] No activity, [b] No test.

3. Experimental section

3.1. General procedure

Column chromatography was performed on silica gel (200-300 mesh; Qingdao Marine Chemical Inc., P. R. China), on C-18 silica gel (40–60 μm; Daiso Co., Japan), MCI gel CHP 20P (75-150 μm, Mitsubishi Chemical Industries, Tokyo, Japan) and on Sephadex LH-20 (Amersham Pharmacia, Sweden). Optical rotations were recorded on a Horiba SEPA-300 polarimeter. UV spectra were recorded on a Shimadzu UV-2401PC spectrometer. CD spectra were measured on a Chirascan instrument. Semi-preparative HPLC was carried out using an Agilent 1200 liquid chromatograph, the column used was a 250 mm × 9.4 mm, i.d., 5 μm, Zorbax SB-C_{18} and a 250 mm × 4.6 mm, i.d., 5 μm, Daicel Chiralpak IC. NMR spectra were recorded on a Bruker AV-500 or an AV-600 spectrometer, with TMS as an internal standard. EIMS and HREIMS were collected by AutoSpec Premier P776 spectrometer. ESIMS and HRESIMS were collected by API QSTAR Pulsar 1 spectrometer.

3.2. Insect material

B. japanensis was collected from YuanJiang County, Yunnan Province, People's Republic of China in September 2012, and authenticated by Professor Dazhi Dong at Kunming Institute of Zoology, Chinese Academy of Sciences, People's Republic of China. A voucher specimen (CHYX0469) was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China of our institute.

3.3. Extraction and isolation

The air-dried powder of *B. japanensis* bodies (50 kg) was soaked with 70% aqueous EtOH (3 × 300 L) at room temperature. The combined extracts were concentrated to obtain a crude extract (4 Kg), which was suspended in water followed by extracted with EtOAc to afford a EtOAc soluble extract (1.0 kg). The EtOAc extract was divided into 7 parts (Fr.1–Fr.7) by using a MCI gel CHP 20P column eluting with gradient aqueous MeOH (5:95–100:0). Fr. 3 (65 g) was separated by using MCI gel CHP 20P eluted with a gradient of aqueous MeOH (MeOH/H_2O, 10:90–50:50) to yield six fractions (Fr. 3.1–Fr. 3.6). Fr. 3.2 (4.5 g) was subjected to gel filtration on Sephadex LH-20 (MeOH) to give five portions (Fr. 3.2.1–Fr. 3.2.5). Fr. 3.2.2 (400 mg) was separated by preparative TLC (MeOH/H_2O, 5:1) to produce Fr. 3.2.2.1–Fr. 3.2.2.3. Fr. 3.2.2.2 (80 mg) was purified vis semi-preparative HPLC (MeOH/H_2O, 10:90) to obtain **6** (R_t = 24.1 min, 3.0 mg), **7** (R_t = 26.5 min, 8.0 mg) and **8** (R_t = 28.8 min, 5.0 mg). Fr. 5 (55 g) was separated by using a MCI gel CHP 20P column eluting with gradient aqueous MeOH (40:60–100:0) to provide eight portions (Fr. 5.1–Fr. 5.8). Fr. 5.4 (6.2 g) was separated by using Sephadex LH-20 (MeOH) followed by a RP-18 column (MeOH/H_2O, 20:80–70:30) to

produce Fr. 5.4.1–Fr. 5.4.6. Fr. 5.4.2 (2.5 g) was separated by using Sephadex LH-20 (MeOH) to give five portions (Fr. 5.4.2.1–Fr. 5.4.2.5). Fr. 5.4.2.4 (200 mg) was purified by semi-preparative HPLC (MeOH/H_2O, 42:58) to obtain **3** (R_t = 15.3 min, 4.5 mg) and **2** (R_t = 24.5 min, 2.5 mg). Fr. 5.4.2.5 (150 mg) was purified by semi-preparative HPLC (MeOH/H_2O, 38:62) to obtain **9** (R_t = 12.2 min, 8.5 mg) and **10** (R_t = 28.5 min, 25 mg). Fr. 5.4.3 (120 mg) was separated vis semi-preparative HPLC (MeOH/H_2O, 40:60) to yield **1** (R_t = 20.5 min, 10 mg), **5** (R_t = 28.3 min, 22mg), and **4** (R_t = 35.2 min, 15 mg).

3.4. Spectral data of the new compounds

3.4.1. Blapsin C (1). Yellow solid; UV (MeOH) λ_{max} (logε) 350 (3.78), 286 (4.13), 221 (4.44), 205 (4,68) nm; ESIMS *m/z* 297 $[M–H]^-$; HRESIMS *m/z* 297.0777 $[M–H]^-$ (calcd for $C_{17}H_{14}O_5$, 297.0768). ^{1}H and ^{13}C NMR data, see Table **1**.

3.4.2. Blapsin D (2). Yellow solid; UV (MeOH) λ_{max} (logε) 350 (3.68), 286 (4.08), 219 (4.42), 206 (4.62) nm; ESIMS *m/z* 297 $[M–H]^-$; HRESIMS *m/z* 297.0773 $[M–H]^-$ (calcd for $C_{17}H_{14}O_5$, 297.0768). ^{1}H and ^{13}C NMR data, see Table **1**.

3.4.3. Blapsin E (3). Yellowish solid; UV (MeOH) λ_{max} (logε) 341 (4.16), 248 (3.97), 213 (4.53) nm; ESIMS *m/z* 205 $[M–H]^-$; HRESIMS *m/z* 205.0507 $[M–H]^-$ (calcd for $C_{11}H_{10}O_4$, 205.0506). ^{1}H and ^{13}C NMR data, see Table **2**.

3.4.4. Blapsin F (4). Yellow gum; UV (MeOH) λ_{max} (logε) 304 (4.06), 207 (4.64) nm; ESIMS *m/z* 273 $[M–H]^-$; HRESIMS *m/z* 273.1132 $[M–H]^-$ (calcd for $C_{17}H_{14}O_5$, 273.1132). ^{1}H and ^{13}C NMR data, see Table **3**.

3.4.5. Blapsin G (5). Yellowish solid; UV (MeOH) λ_{max} (logε) 320 (3.96), 291 (3.89), 240 (4.03), 207 (4.28) nm; ESIMS *m/z* 355 $[M+Na]^+$; HRESIMS *m/z* 355.0793 $[M+Na]^+$ (calcd for $C_{17}H_{16}NaO_7$, 355.0788). ^{1}H and ^{13}C NMR data, see Table **4**.

3.4.6. Blapsin H (6). Yellowish solid; UV (MeOH) λ_{max} (logε) 286 (3.32), 215 (3.79), 202 (4.08) nm; ESIMS *m/z* 345 $[M+HCOOH–H]^-$; HRESIMS *m/z* 345.1199 $[M+HCOOH–H]^-$ (calcd for $C_{14}H_{20}O_7$, 345.1191). ^{1}H and ^{13}C NMR data, see Table **4**.

3.4.7. Blapsin I (7). Yellowish solid; UV (MeOH) λ_{max} (logε) 286 (3.35), 216 (3.78), 202 (4.09) nm; ESIMS *m/z* 345 $[M+HCOOH–H]^-$; HRESIMS *m/z* 345.1201 $[M+HCOOH–H]^-$ (calcd for $C_{14}H_{20}O_7$, 345.1191). ^{1}H and ^{13}C NMR data, see Table **4**.

3.4.8. Blapsin J (8). Yellowish solid; UV (MeOH) λ_{max} (logε) 286 (3.27), 215 (3.69), 200 (3.93) nm; ESIMS *m/z* 345 $[M+HCOOH–H]^-$; HRESIMS *m/z* 345.1197 $[M+HCOOH–H]^-$ (calcd for $C_{14}H_{20}O_7$, 345.1191). ^{1}H and ^{13}C NMR data, see Table **4**.

3.5. Acid hydrolysis of 6–8

A solution of **6–8** (1.0 mg) in 6 N HCl was stirred at 60□ for 1.5 h. After cooling, the mixtures were extracted with EtOAc. The aqueous layer was concentrated in vacuo followed by TLC examination and optical rotation measurement. The optical rotation of the glucose of **6–8** are as follows: $[\alpha]_D^{25}$ + 45.0 (*c* 0.044, H_2O), $[\alpha]_D^{25}$ + 42.3 (*c* 0.052, H_2O), and $[\alpha]_D^{25}$ + 40.9 (*c* 0.044, H_2O). By comparing optical rotation with D-glucose: $[\alpha]_D^{25}$ + 42.3 (*c* 0.106, H_2O), the glucose in compounds **6–8** were determined to be D-configurations.

3.6. Procedure for biological evaluation

All the isolates except of **9** were evaluated for their inhibitory effects against cancer cells (A549, Huh-7, K562),[17,18] COX-2,[2] ROCK1,[1] and JAK3[2] as previously described methods.

Acknowledgments

This work was financially supported by National Science Fund for Distinguished Young Scholars (81525026), Shenzhen Government's Plan of Science and Technology (JCYJ20170412110504956), and National Natural Science Foundation of China (21172223).

Supplementary material

HRMS, and 1D and 2D NMR spectra of compounds **1**–**8**. This material is available free of charge via the Internet at http://

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