



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

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ABSTRACT

The insect *Blaps japonensis* 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 japonensis*, 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. japonensis* 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. japonensis*. 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. japonensis*, all the isolates were biologically evaluated for their potential inhibition on human cancer cells, ROCK1, JAK3, and COX-2 with exception of compound **9**.

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*

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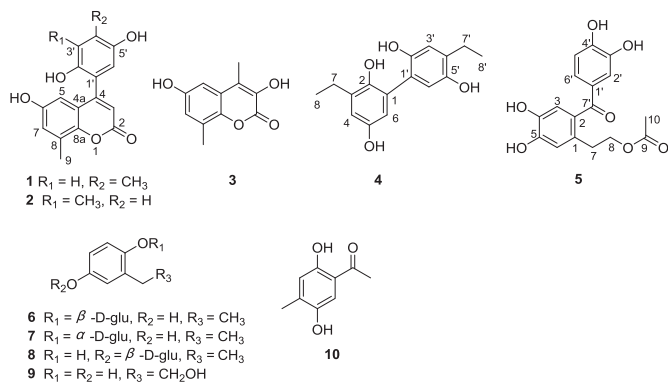


Fig. 1. The chemical structures of compounds 1–10.

Table 1
¹H (600 MHz) and ¹³C NMR (150 MHz) data of **1** and **2** in acetone-*d*₆ (δ, ppm).

No.	1		2	
	δ_{C} , mult	δ_{H} , (mult, <i>J</i> in Hz)	δ_{C} , mult	δ_{H} , (mult, <i>J</i> 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)

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 ¹³C NMR and DEPT spectra contain 17 carbons ascribe to two methyl, five sp² 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 H₃-7'/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 H₃-9/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

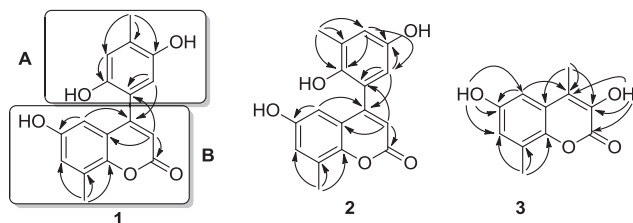


Fig. 2. Key HMBC correlations of 1–3.

derivative. Thus far, the structure of **1** was identified and named as blapsin C.

Compound **2** has the same molecular formula as that of **1** by analysis of its HRESIMS, ¹³C NMR and DEPT spectra. The ¹H and ¹³C 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 H₃-7'/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.

Compound **3** has the molecular formula C₁₁H₁₀O₄ (7 degrees of unsaturation), derived from its HRESIMS, ¹³C 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 ¹³C NMR and DEPT spectra of **3** display 11 signals attributed to two methyl, two sp² methine, and seven quaternary carbons (one carbonyl, six olefinic including three oxygenated). The ¹H and ¹³C NMR data of **3** are similar to those of part B in **1** differing in that a hydroxyl and a methyl is respectively positioned at C-3 and C-4 of structure **3**. This alternation is 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 C₁₆H₁₈O₄ (8 degrees of unsaturation), by analysis of its HREIMS, ¹³C NMR and DEPT spectra. The ¹³C NMR and DEPT spectra of **4** show 16 carbons ascribed to two methyl, two aliphatic methylene, four sp² methine, and eight olefinic quaternary carbons (four oxygenated). Careful inspection of ¹³C 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 ¹H 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 ¹H-¹H 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 are 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.

Compound **5** was determined to be C₁₇H₁₆O₇ (10 degrees of unsaturation) by analysis of its HRESIMS, ¹³C NMR and DEPT

Table 2
¹H (600 MHz) and ¹³C NMR (150 MHz) data of **3** in DMSO-*d*₆ (δ, ppm).

No.	δ _C	δ _H , (m, <i>J</i> in Hz)	δ _C	δ _H , (m, <i>J</i> 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
4a	121.9 s		4-CH ₃	10.6 q
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			

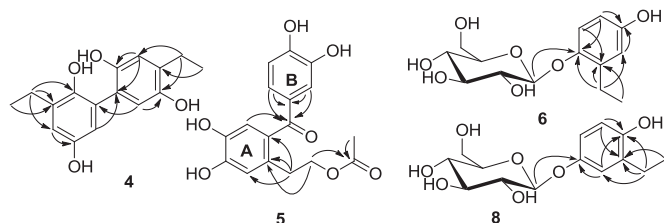


Fig. 3. Key HMBC correlations of 4–6 and 8.

spectra. The ^1H 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 ^1H - ^1H 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 $\text{C}_{14}\text{H}_{20}\text{O}_7$ on the basis of its HRESIMS, ^{13}C NMR and DEPT spectra. The ^1H 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.

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.

Two known compounds were isolated and identified as trans-torine (**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 $10 \mu\text{M}$. 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 IC_{50} value of $6.12 \mu\text{M}$, $10.70 \mu\text{M}$, and $83.20 \mu\text{M}$. 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 $9.77 \mu\text{M}$, $9.31 \mu\text{M}$, and $4.61 \mu\text{M}$. 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 $2.88 \mu\text{M}$, $5.27 \mu\text{M}$, $35.46 \mu\text{M}$, $1.87 \mu\text{M}$, and $6.72 \mu\text{M}$, with **4** is the most active.

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 $250 \text{ mm} \times 9.4 \text{ mm}$, i.d., $5 \mu\text{m}$, Zorbax SB-C₁₈ and a $250 \text{ mm} \times 4.6 \text{ mm}$, i.d., $5 \mu\text{m}$, 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. japonensis 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. japonensis* bodies (50 kg) was soaked with 70% aqueous EtOH ($3 \times 300 \text{ L}$) 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/H₂O, 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/H₂O, 5:1) to produce Fr. 3.2.2.1–Fr. 3.2.2.3. Fr. 3.2.2.2 (80 mg) was purified via semi-preparative HPLC (MeOH/H₂O, 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/H₂O, 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/H₂O, 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/H₂O, 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 via semi-preparative HPLC (MeOH/H₂O, 40:60) to yield **1** (R_t = 20.5 min, 10 mg), **5** (R_t = 28.3 min, 22 mg), 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} ($\log \epsilon$) 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 C₁₇H₁₄O₅, 297.0768). ¹H and ¹³C NMR data, see Table 1.

3.4.2. Blapsin D (**2**)

Yellow solid; UV (MeOH) λ_{\max} ($\log \epsilon$) 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 C₁₇H₁₄O₅, 297.0768). ¹H and ¹³C NMR data, see Table 1.

Table 4

¹H (600 MHz) and ¹³C 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.

3.4.3. Blapsin E (**3**)

Yellowish solid; UV (MeOH) λ_{\max} ($\log \epsilon$) 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 C₁₁H₁₀O₄, 205.0506). ¹H and ¹³C NMR data, see Table 2.

3.4.4. Blapsin F (**4**)

Yellow gum; UV (MeOH) λ_{\max} ($\log \epsilon$) 304 (4.06), 207 (4.64) nm; ESIMS m/z 273 [M–H][–]; HRESIMS m/z 273.1132 [M–H][–] (calcd for C₁₇H₁₄O₅, 273.1132). ¹H and ¹³C NMR data, see Table 3.

3.4.5. Blapsin G (**5**)

Yellowish solid; UV (MeOH) λ_{\max} ($\log \epsilon$) 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 C₁₇H₁₆NaO₇, 355.0788). ¹H and ¹³C NMR data, see Table 4.

3.4.6. Blapsin H (**6**)

Yellowish solid; UV (MeOH) λ_{\max} ($\log \epsilon$) 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 C₁₄H₂₀O₇, 345.1191). ¹H and ¹³C NMR data, see Table 4.

3.4.7. Blapsin I (**7**)

Yellowish solid; UV (MeOH) λ_{\max} ($\log \epsilon$) 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 C₁₄H₂₀O₇, 345.1191). ¹H and ¹³C NMR data, see Table 4.

Table 3

¹H (600 MHz) and ¹³C 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
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
7	24.5 t	2.64 (q, 7.5)	7'	23.7 t
8	14.9 q	1.19 (t, 7.5)	8'	14.5 q

Table 5
Compounds against cancer cells (A549, Huh-7, K562), COX-2, ROCK1, and JAK3.

Compd	IC ₅₀ (μM)					
	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.

3.4.8. Blapsin J (**8**)

Yellowish solid; UV (MeOH) λ_{max} (log ϵ) 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 C₁₄H₂₀O₇, 345.1191). ¹H and ¹³C 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 °C 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: [α]_D²⁵ + 45.0 (c 0.044, H₂O) for **6**, [α]_D²⁵ + 42.3 (c 0.052, H₂O) for **7**, and [α]_D²⁵ + 40.9 (c 0.044, H₂O) for **8**. By comparing optical rotation with D-glucose: [α]_D²⁵ + 42.3 (c 0.106, H₂O), 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 JAK3² as previously described methods.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.tet.2018.12.015>.

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