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Tomensides A–D, new antiproliferative phenylpropanoid sucrose esters from *Prunus tomentosa* leaves

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ABSTRACT

To search for novel cytotoxic constituents against cancer cells as lead structures for drug development, four new 3-phenylpropanoid-triacetyl sucrose esters, named tomensides A–D (**1**–**4**), and three known analogs (**5**–**7**) were isolated from the leaves of *Prunus tomentosa*. Their structures were elucidated by spectroscopic analyses (1D, 2D NMR, CD and HRESIMS). The cytotoxic activities of all isolates against four human cancer cell lines (MCF-7, A549, HeLa and HT-29) were assayed, and the results showed that these isolates displayed stronger inhibitory activities compared with positive control 5-fluorouracil. Tomenside A (**1**) was the most active compound with IC_{50} values of 0.11–0.62 μM against the four tested cell lines. The structure–activity relationship (SAR) of the isolates was also discussed. The primary screening results indicated that these 3-phenylpropanoid-triacetyl sucrose esters might be valuable source for new potent anticancer drug candidates.

Natural products based drug discovery has become a major strategy in modern pharmaceutical research and development, and roughly half of the currently used drugs are directly or indirectly derived from natural products.[1,2] To search for novel cytotoxic constituents against cancer cells as lead structures for drug development, many medicinal plants have been screened using the in vitro cytotoxic activity assay. Of these plants, the 70% ethanol extract of the leaves of *Prunus tomentosa* was found to exhibit a potent cytotoxic effect. The wild *Prunus tomentosa*, which is widely distributed in China, Japan and Korea, belongs to the Rosaceae family.[3] Bioassay-directed fractionation of this extract led to the isolation of four new 3-phenylpropanoid-triacetyl sucrose esters, tomensides A–D (**1**–**4**), along with three known analogs (**5**–**7**). This study describes the isolation and structural elucidation of these isolates, as well as the evaluation of their inhibitory effects on cancer cells. Moreover, the structure–activity relationships of some compounds are summarized in the Letter.

Phenylpropanoid sucrose esters (PSEs) belong to the phenylpropanoid glycoside (glycoconjugate) class of compounds. As the name indicates, PSEs have a sucrose core connected to one or more Ph–CH=CH–CO– moieties through an ester linkage.[4] Over the past three decades, nearly 150 PSEs have been isolated from the families *Rosaceae*, *Sparganiaceae*, *Polygonaceae*, *Rutaceae*, *Liliaceae*, *Arecaceae* and *Smilacaceae*.[4–14] The PSEs have broad range of biological activities such as antitumor,[9,12–16] antioxidant[10,11] and anti-inflammatory.[10]

Repeated column chromatography of the 70% ethanol extract of the leaves of *Prunus tomentosa* resulted in the isolation of four new 3-phenylpropanoid-triacetyl sucrose esters (**1**–**4**) and three known analogs (**5**–**7**)[17] (Fig. 1). The chemical structures of the known compounds were identified as mumeose C (**5**),[18] 3-*p*-*O*-coumaroyl-1,3′,6′-*O*-triacetyl sucrose (**6**)[19] and 3-*p*-*O*-coumaroyl-1,2′,6′-*O*-triacetyl sucrose (**7**)[20] by comparing the spectroscopic data with those reported literatures.

Tomenside A (**1**)[21] was obtained as yellow amorphous powder. The molecular formula of **1** was established as $C_{27}H_{34}O_{16}$ on the basis of a quasi-molecular ion at *m*/*z* 637.1745 $[M+Na]^+$ (calcd for $C_{27}H_{34}O_{16}Na$, 637.1739) in HRESIMS. The ^1H NMR spectrum (Table 1) showed characteristic signals at δ_H 6.81 (d, J = 8.6 Hz, 2H), 7.52 (d, J = 8.6 Hz, 2H) of *p*-hydroxyphenyl group and a set of *trans*-double-bond signals [δ_H 6.42, (d, J = 15.8 Hz), 7.72 (d, J = 15.8 Hz)], together with the carbon signals (Table 2) at δ_C 168.4, 114.3, 147.9 suggesting the presence of *p*-coumaroyl moiety. The characteristic anomeric proton signal was appeared at δ_H 5.39 with a small coupling constant (J = 3.6 Hz), as well as twelve-oxygenated carbon signals including those for two anomeric carbons (δ_C 103.3, 93.0) in ^{13}C NMR spectrum, together with the correlations between δ_H 5.39 (H-1′) and δ_C 103.3 (C-2) in HMBC

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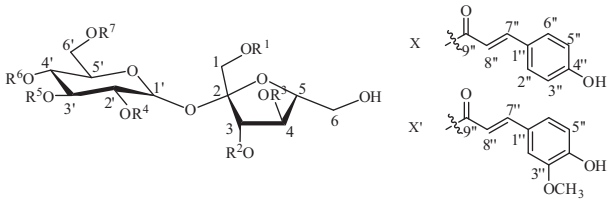

	R^1	R^2	R^3	R^4	R^5	R^6	R^7
1	$COCH_3$	X	$COCH_3$	H	H	H	$COCH_3$
2	$COCH_3$	X	H	H	H	$COCH_3$	$COCH_3$
3	H	X	H	H	$COCH_3$	$COCH_3$	$COCH_3$
4	$COCH_3$	X'	H	$COCH_3$	H	H	$COCH_3$
5	H	X	H	$COCH_3$	$COCH_3$	H	$COCH_3$
6	$COCH_3$	X	H	H	$COCH_3$	H	$COCH_3$
7	$COCH_3$	X	H	$COCH_3$	H	H	$COCH_3$

Figure 1. Structures of compounds **1–7**.

spectrum confirmed that **1** possessed a disaccharide moiety. Moreover, the result of alkaline hydrolysis[22,23] and detailed analysis of ^{1}H NMR, ^{13}C NMR, HMBC and HSQC showed that the two sugars were comprised of β-D-fructose and α-D-glucose moieties connected through a 2 → 1 linkage (a sucrose moiety).

On further inspection of the HMBC spectrum, the structure of **1** could be assigned unambiguously due to the correlations observed between H-1 (δ_H 4.14, 4.12) and δ_C 172.9, H-4 (δ_H 4.43) and δ_C 172.6, H-6′ (δ_H 4.55, 4.10) and δ_C 172.1, together with the correlation between H-3 (δ_H 5.47) and the carbonyl carbon of *p*-coumaroyl (δ_C 168.4) group (Fig. 2). Thus, the structure of **1** was elucidated as 3-*p*-*O*-coumaroyl-1,4,6′-*O*-triacetyl sucrose.

Tomenside B (**2**)[24] was obtained as yellow amorphous powder with the molecular formula $C_{27}H_{34}O_{16}$, based on quasi-molecular ion *m*/*z* 637.1747 [M+Na]$^+$ (calcd for $C_{27}H_{34}O_{16}Na$, 637.1739). The IR, UV and NMR spectra indicated that **2** possessed a *p*-coumaroyl moiety, two sugars moieties and three acetyl groups. The result of alkaline hydrolysis[22,23] revealed the existence of a sucrose moiety. In HMBC spectrum, there are the correlations between H and carbonyl carbons of acetyl, H-1 (δ_H 4.22, 4.17) and δ_C 172.7, H-4′ (δ_H 4.76) and δ_C 172.2, H-6′ (δ_H 4.09, 4.20) and δ_C 172.0, together with correlation between H-3 (δ_H 5.43) and C-9″ (δ_C 168.1), indicated that **2** had the structure of 3-*p*-*O*-coumaroyl-1,4′,6′-*O*-triacetyl sucrose.

The molecular formula of tomenside C (**3**)[25] was assigned $C_{27}H_{34}O_{16}$ according to the quasi-molecular ion at *m*/*z* 637.1744 [M+Na]$^+$ (calcd for $C_{27}H_{34}O_{16}Na$, 637.1739) in HRESIMS. The IR, UV and NMR spectra showed **3** possessed a *p*-coumaroyl moiety, two sugars moieties and three acetyl groups, in a manner similar to **1**, except for the positions of the three acetyl groups. The two sugars moieties were identified as sucrose moiety by alkaline hydrolysis.[22,23] The correlation between H-3 (δ_H 5.40) and C-9″ (δ_C 168.1) in HMBC spectrum indicated the *p*-coumaroyl was located at the same position with **1**. The structure of **3** was identified due to the correlations observed between H-3′ (δ_H 4.88) and δ_C 172.5, H-4′ (δ_H 5.23) and δ_C 172.1, H-6′ (δ_H 4.16) and δ_C 171.5. Thus, the structure of **3** was characterized as 3-*p*-*O*-coumaroyl-3′,4′, 6′-*O*-triacetyl sucrose.

The molecular formula of tomenside D (**4**)[26] was determined to be $C_{28}H_{36}O_{17}$ by HRESIMS at *m*/*z* 667.1846 [M+Na]$^+$ (calcd for $C_{28}H_{36}O_{17}Na$, 667.1845). The IR and UV spectra together with ^{1}H and ^{13}C NMR suggested a similar structure to **1**, except for the presence of a methoxyl group at C-3″ and the positions of three acetyl groups. This evidence suggested that **4** possessed one feruloyl

Table 1
^{1}H NMR data of compounds **1–4** (δ_H values, measured in CD_3OD, at 600 MHz)[a]

H	1	2	3	4
1	4.14 d (11.6) 4.12 d (11.6)	4.22 d (11.8) 4.17 d (11.8)	3.68 d (12.3) 3.60 d (12.3)	4.15 d (11.5) 4.06 d (11.5)
3	5.47 d (8.6)	5.43 d (8.1)	5.40 d (7.0)	5.42 d (8.4)
4	4.43 m	4.34 t (8.2)	3.81 m[b]	4.33 dd (8.4)
5	4.08 m	3.96 m	3.99 m	3.93 m
6	4.38 dd (12.0, 2.8) 4.44 m[b]	3.82 m[b] 2H	4.33 m[b] 2H	3.86 m[b] 2H
1-OAc	2.11 s	2.11 s		2.11 s
4-OAc	2.08 s			
1′	5.39 d (3.6)	5.49 d (3.6)	5.54 d (3.6)	5.57 d (3.6)
2′	3.39 dd (9.8, 3.6)	3.49 dd (9.9, 3.6)	3.72 dd (9.9, 3.6)	4.62 dd (10.1, 3.6)
3′	3.57 dd (9.8, 9.4)	3.70 t (9.9)	4.88 dd (9.9, 9.7)	3.84 m[b]
4′	3.25 t (9.4)	4.76 dd (9.9, 9.5)	5.23 t (9.7)	3.37 t (9.4)
5′	4.12 m	4.26 m	3.81 m	4.10 m
6′	4.55 br d (10.5) 4.10 m[b]	4.09 dd (12.0, 2.1) 4.20 dd (12.0, 5.8)	4.16 m[b] 2H	4.51 dd (11.8, 1.7) 4.16 dd (11.8, 6.2)
2′-OAc				2.10 s
3′-OAc			1.79 s	
4′-OAc		1.95 s	1.99 s	
6′-OAc	2.11 s	2.08 s	2.07 s	2.09 s
2″	7.52 d (8.6)	7.52 d (8.2)	7.54 d (8.6)	7.28 d (1.8)
3″	6.81 d (8.6)	6.82 d (8.2)	6.81 d (8.6)	
5″	6.81 d (8.6)	6.82 d (8.2)	6.81 d (8.6)	6.82 d (8.2)
6″	7.52 d (8.6)	7.52 d (8.2)	7.54 d (8.6)	7.14 dd (8.2, 1.8)
7″	7.72 d (15.8)	7.72 d (15.9)	7.74 d (15.9)	7.71 d (15.8)
8″	6.42 d (15.8)	6.40 d (15.9)	6.42 d (15.9)	6.47 d (15.8)
3″-OCH_3				3.90 s

[a] Assignments are based on ^{1}H,^{13}C NMR, HMBC, and HSQC experiments.
[b] The signals were overlapped.

Table 2
^{13}C NMR data of compounds **1–4** (δ_C values, measured in CD_3OD, at 125 MHz)[a]

C	1	2	3	4
1	65.9	64.3	65.4	66.2
2	103.3	103.5	105.6	103.4
3	78.6	79.6	79.9	79.3
4	73.8	73.6	74.7	73.6
5	81.0	84.4	85.4	84.2
6	66.0	63.5	63.5	63.7
1-OAc	172.1	172.2		172.1
	20.7	20.9		21.0
4-OAc	172.6			
	20.9			
1′	93.0	93.1	92.8	90.8
2′	72.7	72.4	69.7	72.1
3′	74.7	72.6	74.4	74.2
4′	72.0	72.6	70.3	72.1
5′	72.2	70.1	70.9	71.7
6′	65.7	66.6	63.9	65.0
2′-OAc				172.6
				20.6
3′-OAc			171.5	
			20.5	
4′-OAc		172.0	172.1	
		20.7	20.7	
6′-OAc	172.9	172.7	172.5	172.8
	20.7	20.8	20.8	20.8
1″	127.1	127.1	127.3	127.6
2″	131.5	131.4	131.5	112.0
3″	116.8	116.9	116.8	149.4
4″	161.5	161.7	161.4	150.9
5″	116.8	116.9	116.8	116.5
6″	131.5	131.4	131.5	124.5
7″	147.9	147.7	147.7	148.2
8″	114.3	114.3	114.6	114.5
9″	168.4	168.1	168.1	168.3
3″-OCH_3				56.5

[a] Assignments are based on ^1H,^{13}C NMR, HMBC, and HSQC experiments.

moiety, two sugars moieties and three acetyl groups. The result of alkaline hydrolysis[22,23] also revealed the existence of a sucrose moiety. Correlations between H-1 (δ_H 4.15, 4.06) and δ_C 172.8, H-2′ (δ_H 4.62) and δ_C 172.6, H-6′ (δ_H 4.51, 4.16) and δ_C 172.1, together with correlation between H-3 (δ_H 5.42) and C-9″ (δ_C 168.3) in HMBC spectrum, suggested that **4** had the structure of 3-*O*-feruloyl-1,2′,6′-*O*-triacetyl sucrose.

Some PSEs were found to possess anti-tumour activity, and their structure–activity relationships were also reported in previous researches.[9,12–16] In our study, the isolated PSEs (**1–7**) were evaluated in vitro for their inhibitory abilities against the growth of MCF-7, A549, HeLa and HT-29 cancer cells using the MTT method.[27–30] The IC_{50} values of the PSEs along with the positive control 5-fluorouracil are summarized in Table 3. The results showed that all PSEs displayed stronger inhibitory activities compared with positive control. Among all tested compounds, Tomenside A (**1**) showed the most potent inhibitory activities with IC_{50} values of 0.11–0.62 μM against the four tested cell lines.

Table 3
Cytotoxic activities of compounds **1–7** in vitro (IC_{50}, μM)

Compounds	IC_{50} (μM)			
	MCF-7[a]	A549[a]	HeLa[a]	HT-29[a]
1	0.62	0.29	0.11	0.18
2	1.22	0.49	0.97	1.48
3	2.93	1.47	3.80	2.78
4	0.77	0.45	1.09	0.46
5	0.98	1.12	4.32	2.45
6	1.44	2.56	1.33	2.10
7	3.44	3.20	2.49	3.96
5-Fu[b]	11.50	44.05	7.34	17.20

[a] Key to cell lines used: A549 (human lung cancer cells), MCF-7 (human breast cancer cells), HeLa (Henrietta Lacks strain of cancer cells) and HT-29 (colon cancer cell line).
[b] 5-Fu (5-fluorouracil) was used as positive control.

The structural requirements of the active constituents were examined. Panda et al. reported that the substitution on phenyl ring did not influence the cytotoxic activity against Hela cancer cell.[15,16] Whereas, the stronger cytotoxic activities of **4** compared to **7** imply the importance of the substitution on phenyl ring in mediating the cytotoxic activities of the PSEs. Kuo et al.[23] and Panda et al.[15,16] independently suggested that acetyl moieties on the sucrose core in PSEs might be responsible for mediating the observed activities. In our research, we also found that the activity data seems to vary with the position of acetyl moieties on the sucrose core. Compound **1** was the most active compound against the four cancer cell lines in the isolated PSEs. In consideration of the structures of **1–3**, **5–7**, it was suggested that the acetyl moiety at the position of C-4 maight enhance the cytotoxicities of the PSEs. Saeed et al.[31] also concluded that increased lipophilicity of molecules seemed to be responsible for enhanced cytotoxicity. In addition, the lipophilicity can be influenced by the number of the acetyl units on the sucrose core. It is notable that the 3-phenylpropanoid-triacetyl sucrose esters display higher cytotoxic activities compared to the PSEs with one or two acetyl

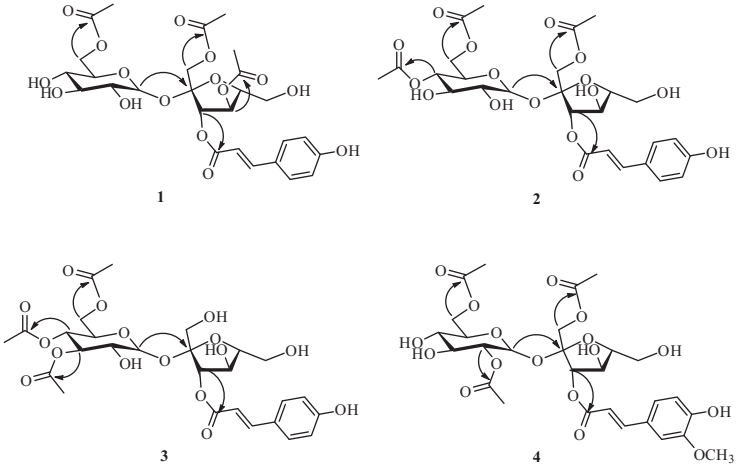

Figure 2. Observed key HMBC correlations of compounds **1–4**.

units from *Fagopyrum tataricum* (L.) Gaertn.[13] The results suggest that the number of acetyl moieties also plays a useful role in the cytotoxic activities of the PSEs.

In summary, four new and three known PSEs were isolated from the leaves of *Prunus tomentosa*. The cytotoxic activities of all isolates against four were assayed and the SAR of the PSEs was also discussed. This study not only helps to provide sufficient materials for the SAR studies, but also indicates that PSEs may be a potentially valuable source for new potent anticancer drug candidates.

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References and notes

1. Newman, D. J.; Cragg, G. M. *J. Nat. Prod.* **2007**, *70*, 461.
2. Popsavin, V.; Francuz, J.; Zelenović, B. S.; Benedeković, G.; Popsavin, M.; Kojić, V.; Bogdanović, G. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 5507.
3. Gao, H. S.; Shi, P. B.; Zhang, J. C. *Food Sci.* **2006**, *27*, 378.
4. Panda, P.; Appalashetti, M.; Judeh, Z. M. A. *Curr. Med. Chem.* **2011**, *18*, 3234.
5. Shirota, O.; Sekita, S.; Satake, M.; Yan, N.; Hua, W. Y. *J. Nat. Prod.* **1996**, *59*, 242.
6. Sun, X. Z.; Zimmermann, M. L.; Campagne, J. M.; Sneden, A. T. *J. Nat. Prod.* **2000**, *63*, 1094.
7. Brown, L. L.; Larson, S. R.; Sneden, A. T. *J. Nat. Prod.* **1998**, *61*, 762.
8. Wang, J. S.; Di, Y. T.; Yang, X. W.; Li, S. L.; Wang, Y. H.; Hao, X. J. *Phytochemistry* **2006**, *67*, 486.
9. Yan, L. L.; Gao, W. Y.; Zhang, Y. J.; Wang, Y. *Fitoterapia* **2008**, *79*, 306.
10. Chang, C. L.; Zhang, L. J.; Chen, R. Y.; Kuo, L. M. Y.; Huang, J. P.; Huang, H. C.; Lee, K. H.; Wu, Y. C.; Kuo, Y. H. *J. Nat. Prod.* **2010**, *73*, 1482.
11. Zhang, L. J.; Liao, C. C.; Huang, H. C.; Shen, Y. C.; Yang, L. M.; Kuo, Y. H. *Phytochemistry* **2008**, *69*, 1398.
12. Takasaki, M.; Kuroki, S.; Kozuka, M.; Konoshima, T. *J. Nat. Prod.* **2001**, *64*, 1305.
13. Zheng, C. J.; Hu, C. L.; Ma, X. Q.; Peng, C.; Zhang, H.; Qin, L. P. *Food Chem.* **2012**, *132*, 433.
14. Takasaki, M.; Konoshima, T.; Kuroki, S.; Tokuda, H.; Nishino, H. *Cancer Lett.* **2001**, *173*, 133.
15. Panda, P.; Appalashetti, M.; Natarajan, M.; Mary, C. P.; Venkatraman, S. S.; Judeh, Z. M. A. *Eur. J. Med. Chem.* **2012**, *58*, 418.
16. Panda, P.; Appalashetti, M.; Natarajan, M.; Chan-Park, M. B.; Venkatraman, S. S.; Judeh, Z. M. A. *Eur. J. Med. Chem.* **2012**, *53*, 1.
17. *Isolation:* The leaves of *Prunus tomentosa* obtained from Liaoning Province, China. Dried leaves (9.3 kg) of *Prunus tomentosa* were extracted with 70% (v/v) EtOH. The combined extract (1200 g) was suspended in 3 L water and partitioned with PE, EtOAc, and water-saturated *n*-butanol, successively, and the EtOAc extract showed stronger cytotoxic activities than other extracts. The EtOAc extract (120 g) was subjected to silica gel column chromatography with a CH_2Cl_2/MeOH gradient solvent system to obtain four fractions (A1–A4), which were combined according to TLC analysis. Fraction A2 (75 g) was subjected to a CHP20 CC to remove chlorophyll by collecting fractions (B1, 69 g) of MeOH/H_2O gradient solvent system. The fraction B1 was chromatographed on Polyamide CC (100 mesh) eluted with a gradient of EtOH/H_2O to obtain 3 fractions, C1 (18 g), C2 (32 g), C3 (19 g). C1 was subjected to silica gel column chromatography (CC) with a CH_2Cl_2/MeOH gradient solvent system to obtain 19 fractions (D1–D19). Fractions D9 (0.5 g), D10 (0.7 g) and D12(0.5 g) were cut into 8 fractions (E1–E8), 9 fractions (F1–F8) and 11 fractions (G1–G11) respectively by preparative HPLC eluted with MeOH/H_2O (40:60). **1** (12 mg, t_R 11.1 min) was obtained from F8 (39 mg) by semi-preparative HPLC eluted with CH_3CN/H_2O (23:77). **2** (22 mg, t_R 9.8 min) and **3** (12 mg, t_R 10.7 min) were obtained from F6 (91 mg) with CH_3CN/H_2O (21:79). **4** (4.5 mg, t_R 14.4 min) was obtained from E8 (48 mg) with CH_3CN/H_2O (20:80). **5** (9 mg, t_R 8.8 min) was obtained from F5 (31 mg) with CH_3CN/H_2O (20:80). **6** (14 mg, t_R 10.3 min) was obtained from E6 (37 mg) with CH_3CN/H_2O (20:80). **7** (32 mg, t_R 14.5 min) was obtained from F7 (56 mg) with CH_3CN/H_2O (23:77).
18. Nakamura, Seikou; Fujimoto, Katsuyoshi; Yoshikawa, Masayuki *Phytochemistry* **2013**, *92*, 128.
19. Yoshinari, K.; Sashida, Y.; Mimaki, Y.; Shimomura, H. *Chem. Pharm. Bull.* **1990**, *38*, 415.
20. Shimazaki, N.; Mimaki, Y.; Sashida, Y. *Phytochemistry* **1991**, *30*, 1475.
21. *Tomenside A* (**1**) yellow amorphous powder; $[\alpha]_D^{20}$ +41.6 (*c* 0.35, CH_3OH); UV (CH_3OH) λ_{max} (logε): 313 (4.11) nm, 228 (3.89) nm, 219 (3.85) nm; IR (KBr) ν_{max}: 3447, 2922, 2851, 1733, 1634, 1517, 1454, 1384, 1268, 1169, 1108, 1052, 838, 619 cm^{-1}; ^1H and ^{13}C NMR data: see Tables 1 and 2; HRESIMS *m*/*z*: 637.1745 $[M+Na]^+$ (calcd for $C_{27}H_{34}O_{16}Na$, 637.1739).
22. *Alkaline hydrolysis:* compounds **1** (20 mg), **2** (1.2 mg), **3** (1.5 mg) and **4** (1.0 mg) were dissolved in 3% KOH in methanol (4 mL) separately, and the solution was stirred at room temperature for 6 h. The reaction mixture was neutralized with 1 N HCl and then extracted with chloroform. From the water layer, sucrose was identified by its TLC behavior [Si gel, developed with chloroform/methanol/water (6:4:1, v/v/v)].
23. Kuo, Y. H.; Hsu, Y. W.; Liaw, C. C.; Lee, J. K.; Huang, H. C.; Kuo, L. M. Y. *J. Nat. Prod.* **2005**, *68*, 1475.
24. *Tomenside B* (**2**) yellow amorphous powder; $[\alpha]_D^{20}$ +27.4 (*c* 0.20, CH_3OH); UV (CH_3OH) λ_{max} (logε): 314 (4.18) nm, 228 (3.90) nm, 219 (3.86) nm; IR (KBr) ν_{max}: 3423, 2920, 2850, 1727, 1605, 1515, 1444, 1384, 1251, 1167, 1048, 836, 591 cm^{-1}; ^1H and ^{13}C NMR data: see Tables 1 and 2; HRESIMS *m*/*z*: 637.1747 $[M+Na]^+$ (calcd for $C_{27}H_{34}O_{16}$ Na, 637.1739).
25. *Tomenside C* (**3**) yellow amorphous powder; $[\alpha]_D^{20}$ +23.9 (*c* 0.25, CH_3OH); UV (CH_3OH) λ_{max} (logε): 313 (4.09) nm, 228 (3.88) nm, 218 (3.84) nm; IR (KBr) ν_{max}: 3447, 2922, 2851, 1742, 1606, 1516, 1450, 1384, 1243, 1170, 1053.6, 836,619, 591 cm^{-1}; ^1H and ^{13}C NMR data: see Tables 1 and 2; HRESIMS *m*/*z*: 637.1744 $[M+Na]^+$ (calcd. for $C_{27}H_{34}O_{16}$ Na, 637.1739).
26. *Tomenside D* (**4**) yellow, amorphous powder; $[\alpha]_D^{20}$ +34.2 (*c* 0.22, CH_3OH); UV (CH_3OH) λ_{max} (logε): 331 (4.17) nm, 236(3.93) nm, 228(3.90) nm; IR (KBr) ν_{max}: 3442, 2920, 2851, 1729, 1632, 1517, 1453, 1384, 1250, 1129, 1050, 619 cm^{-1}; ^1H and ^{13}C NMR data: see Tables 1 and 2; HRESIMS *m*/*z*: 667.1846 $[M+Na]^+$ (calcd for $C_{28}H_{36}O_{17}$ Na, 667.1845).
27. *Cytotoxicity assay:* MTT agent, against MCF-7, A-549, Hela and HT-29, was based on the lit.[28–30] procedure. In brief, all cells were cultured in RPMI-1640 medium. Test samples were prepared at four concentrations. After the cell lines were seeded in a 96-well microplate for 4 h, 20 μL of sample was placed in each well and incubated at 37 °C for 2 days, then 20 μL MTT solution (5 mg/mL) was added. After further 4 h of incubation, 100 μL of 10% sodium dodecyl sulfate in 0.01 M HCl solution was added to each well and the formazan crystals in each well were dissolved by stirring with a pipette, and their absorbances were measured on a microtiter plate reader, at a wavelength of 550 nm. 5-Fluorouracil was used as a positive control.
28. Wang, Y.; Wang, W. J.; Su, C.; Zhang, D. M.; Xu, L. P.; He, R. R.; Wang, L.; Zhang, J.; Zhang, X. Q.; Ye, W. C. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 654.
29. Li, X.; Tian, Y.; Yang, S. X.; Zhang, Y. M.; Qin, J. C. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 2945.
30. Liu, F. Z.; Ren, J. W.; Tang, J. S.; Liu, X. Z.; Che, Y. S.; Yao, X. S. *Fitoterapia* **2013**, *87*, 78.
31. Saeed, S.; Rashid, N.; Jones, P. G.; Ali, M.; Hussain, R. *Eur. J. Med. Chem.* **2010**, *45*, 1323.