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Isolation and cytotoxicity evaluation of taxanes from the barks of *Taxus wallichiana* var. *mairei*

Zhang-Hua Sun [a,†], Yu Chen [a,†], Yan-Qiong Guo [a], Jie Qiu [b], Cui-Ge Zhu [a], Jing Jin [a], Gui-Hua Tang [a], Xian-Zhang Bu [a,*], Sheng Yin [a,*]

[a] School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou, Guangdong 510006, People's Republic of China
[b] School of Chinese Materia Medica, Guangzhou University of Chinese Medicine, Guangzhou, Guangdong 510006, People's Republic of China

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ABSTRACT

Fifteen taxanes (**1**–**15**) including a new taxane glucoside, 7β,9α,10β-triacetoxy-13α-hydroxy-5α-*O*-(β-D-glucopyranosyl)taxa-4(20),11-diene (**1**), were isolated from the barks of *Taxus wallichiana* var. *mairei*. Compounds **1**–**15** representing three sub-types of 6/8/6-taxane were evaluated in vitro for anti-proliferative activity against a panel of parental and drug-resistant cancer cells. Potent compounds were found while several exhibited selective cytotoxicity. Especially, **3**, **8**, and **10** showed selective inhibition to breast carcinoma cell line MCF-7, while **13** selectively inhibited taxol resistant human ovarian carcinoma cell line A2780/TAX (IC_{50} = 0.19 μM), being more potent than the clinical drugs taxol (IC_{50} = 4.4 μM) and docetaxol (IC_{50} = 0.42 μM), and less cytotoxic to mouse embryonic fibroblast cell line NIH-3T3, a cell line close to normal cell line. The possible P-glycoprotein evasion mechanism of **13** against A2780/TAX and the preliminary structure–activity relationships (SARs) of this group of compounds were also discussed.

The discovery of paclitaxel (taxol) as a potent anticancer drug, initially isolated from *Taxus brevifolia*, has spurred several groups all over the world to conduct research work on other *Taxus* species, to isolate potentially more effective paclitaxel derivatives or as starting materials for semisynthesis. So far more than 550 taxanes have been identified, and new taxanes continue to be isolated from the needles, bark, stem, and roots of *Taxus* species.[1] Although taxol and its derivatives are widely used in clinical applications, the chemotherapy often fails due to its acquired-drug resistance, neuro-cytotoxicity, and low oral bioavailability. Thus, there is continuously demanding of new information facilitating the novel taxane-based drug design and discovery. However, previous pharmaceutical study on taxanes mainly focused on the modification of the taxol prototype with a 6/8/6-ring system,[2–7] and systematically evaluation of the anticancer properties of taxanes with different sub-types remains scarce.

Taxus wallichiana var. *mairei* (Lemée & H. Léveillé) L. K. Fu & Nan Li, also known as *Taxus chinensis* (Pilger) Rehder var. *mairei* (Lemee & H. Léveillé) W.C. Cheng & L.K. Fu, an evergreen tree that is only distributed in China.[8] Previous phytochemical studies on this plant have led to the isolation a number of taxanes, some of which showed significant cytotoxicity.[9–14] In our screening program aimed at the discovery of structurally unique and biologically significant taxanes from *Taxus* plants, a new taxane glucoside, 7β,9α,10β-triacetoxy-13α-hydroxy-5α-*O*-(β-D-glucopyranosyl) taxa-4(20),11-diene (**1**), and 14 known analogues (Fig. 1), 2-deacetoxytaxinine J[15] (**2**), 2-deacetoxyaustrospicatine[16] (**3**), cephalomannine[17] (**4**), 10-deacetyl-7-*epi*-taxol[18] (**5**), taxol[19] (**6**), 7-xylosyl-10-deacetyl taxol[20] (**7**), 7-β-xylosyl-cephalomannine[20] (**8**), 7-β-xylosyl-taxol[20] (**9**), 7-β-xylosyl-10-deacetyl-cephalomannine[20] (**10**), 7-xylosyl-10-deacetyl taxol C[20] (**11**), taxinine M[21] (**12**), taxacin[22] (**13**), 10-deacetylbaccatin III[23] (**14**), and baccatin III[17,20] (**15**) were isolated from the barks of *T. wallichiana* var. *mairei*.

Subsequent cytotoxic screening against a panel of cancer cell lines revealed that some of the 6/8/6-taxanes with different sub-types exhibited selective inhibition to some of the cell lines, such as the MCF-7 and taxol-resistant A2780/TAX. Herein, details of the isolation, structural elucidation, cytotoxic activity as well as a preliminary structure–activity relationship (SAR) of these compounds are described.

The air-dried barks of *T. wallichiana* var. *mairei* was extracted with refluxing MeOH to yield an extract, which was partitioned into EtOAc-soluble and EtOAc-insoluble fractions. After a series of column chromatography (CC) steps over silica gel and reversed-phase silica gel, followed by preparative TLC, the EtOAc-soluble

* Corresponding authors. Tel./fax: +86 020 39943054 (X.-Z.B.); tel: +86 020 39943090/31; fax: +86 020 39943090 (S.Y.).
E-mail addresses: phsbxzh@mail.sysu.edu.cn (X.-Z. Bu), yinsh2@mail.sysu.edu.cn (S. Yin).
† These authors contributed equally to this work.

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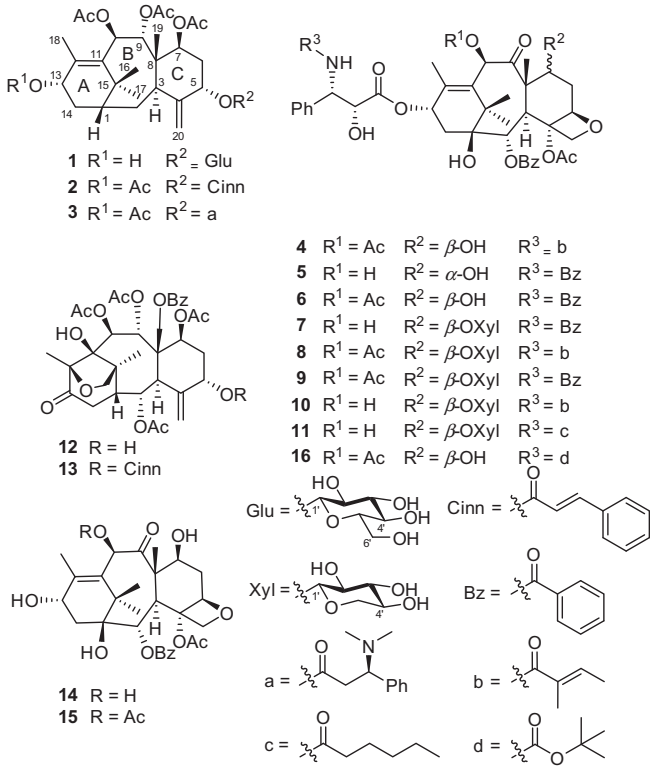

Figure 1. Structures of compounds **1**–**15** and clinical drug docetaxol (**16**).

fraction afforded compounds **1**–**15** (Supplementary data, experimental section).

Compound **1**[24] was isolated as a colorless oil. The molecular formula of $C_{32}H_{48}O_{13}$ was determined by the HRESIMS ion at *m*/*z* 663.2959 $[M+Na]^+$ (calcd for 663.2987) and ^{13}C NMR data. The 1D NMR spectra (Table 1) resolved 32 carbon resonances attributable to four methyls (δ_H 0.81, 1.01, 1.56, and 2.29; δ_C 13.7, 16.1, 27.7, and 31.5), three acetoxys (δ_H 1.93, 2.01, and 2.03; δ_C 21.0, 21.3, 21.4, 169.4, 170.2, and 170.7), a pair of exoethylene protons [δ_H 4.86 (1H, s) and 5.16 (1H, s)], and a glucose moiety [δ_H 4.26 (1H, d, *J* = 7.7 Hz); δ_C 63.0 (CH_2), 71.6 (CH), 74.2 (CH), 77.4 (CH), 77.5 (CH) and 104.8 (CH)]. The aforementioned data were similar to those of the known taxane taxezopidine H[25] except that the cinnamoyl unit in taxezopidine H was replaced by a glucose moiety in **1**. This was supported by the downfield-shifted C-5 signal (δ_C 81.9) in **1** due to the glycosylation shift effect (C-5 at δ_C 75.2 in taxezopidine H). The D-configuration of the glucosyl unit was determined by HPLC analysis.[26] The gross structure of **1** was further confirmed by detailed 2D NMR analysis including HMBC and COSY spectra (Fig. 2).

Table 1
^1H and ^{13}C NMR data of **1** in acetone-d_6[a]

No.	δ_H	δ_C	No.	δ_H	δ_C
1	1.80 (1H, m)	41.4	16	1.01 (3H, s)	31.5
2	1.81 (1H, m)	28.1	17	1.56 (3H, s)	27.7
3	2.96 (1H, d 4.7)	37.9	18	2.29 (3H, s)	16.1
4		150.7	19	0.81 (3H, s)	13.7
5	4.23 (1H, dd 3.7, 2.0)	81.9	20	4.86 (1H, s)	112.9
				5.16 (1H, s)	
6	1.75 (1H, m)	36.9	7-OAc	2.01 (3H, s)	21.4, 170.2
	2.09 (1H, m)				
7	5.60 (1H, dd 11.5, 5.3)	71.2	9-OAc	2.03 (3H, s)	21.3, 170.7
8		47.0	10-OAc	1.93 (3H, s)	21.0, 169.4
9	5.84 (1H, d 11.1)	77.5	1′	4.26 (1H, d 7.7)	104.8
10	6.20 (1H, d 11.1)	73.3	2′	3.21 (1H, m)	74.2
11		133.7	3′	3.36 (1H, m)	77.4
12		142.7	4′	3.33 (1H, m)	71.6
13	4.71 (1H, m)	68.5	5′	3.28 (1H, m)	77.5
14	1.19 (1H, m),	35.0	6′	3.63 (1H, m)	63.0
	2.63 (1H, m)			3.81 (1H, m)	
15		39.9			

[a] Data were recorded at 400 MHz for ^1H NMR and 100 MHz for ^{13}C NMR. (*J* in Hz, δ in ppm).

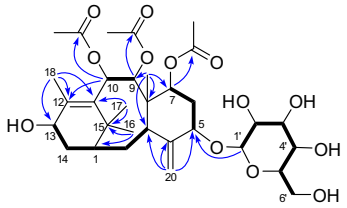

Figure 2. Key HMBC (H→C) and ^1H–^1H COSY (▬) correlations of **1**.

The relative configuration of **1** was determined by analysis of its ^1H–^1H coupling constant and NOESY data. The fusion patterns of A/B/C ring system and the orientations of the substituents (acetyl and hydroxyl groups) on rings A and B were assigned as the same as those of taxezopidine H by comparison of their 1D NMR data. The NOESY correlations of H-7/H-3 and CH_3-19/H-6β indicated that H-7, H-3, CH_3-19, and H-6β adopted the axial bonds of the quasi chair-conformational ring C. The small coupling constant of H-5 [δ_H 4.23 (dd, *J* = 3.7 and 2.0 Hz)] indicated that H-5 adopted equatorial bond and was in β-orientation. Thus, compound **1** was assigned as 7β,9α,10β-triacetoxy-13α-hydroxy-5α-*O*-(β-D-glucopyranosyl)taxa-4(20),11-diene.

The cytotoxicity of compounds **1**–**5** and **7**–**15** were evaluated by sulforhodamine B (SRB) assay with docetaxol (**16**) and taxol (**6**) used as positive controls (Supplementary data, experimental section). Ten human cancer cell lines including human ovarian carcinoma cell A2780 with the taxol resistant cell A2780/TAX, human ileocecum carcinoma cell HCT-8 with the vincristine resistant cell HCT-8/VCT, breast carcinoma cell line MCF-7 with the doxorubicin resistant cell MCF-7/DOX, human lung carcinoma cell line A549 with the *cis*-platinum resistant cell A549/CDDP, colon carcinoma cell SW480, and hepatoma carcinoma cell line HepG2 were chosen for this biological activity assay. The experiments were conducted in three independent replicates.

The bioassay results (Table 2) showed that most of the taxanes exhibited moderate to good inhibitory activity (IC_{50} <10 μM) against some of the cell lines, and compounds **4** and **5** sharing the most structural features with taxol showed broad inhibitory effects against most of the cell lines, with the activities comparable to those of the positive control (most IC_{50s} <0.1 μM). Compared to **4**–**6** and **16**, the introduction of an *O*-β-xylose in R^2 (**7**–**11**) or loss of the C-13 ester side chain (**14**–**15**) greatly decrease their activities, which is consistent with the reported SAR of taxol analogues.[27]

Interestingly, despite of the general low activity, the *O*-β-xylose bearing compounds **7**–**11** reserved their anti-proliferative activity and showed selective cytotoxicity towards certain cancer cell lines. Compounds **8** and **10** selectively inhibited MCF-7 with IC_{50} values at 0.029 and 0.14 μM, respectively. Further analysis showed *N*-butanoylphenylisoserine substitution at R^3 in **8** and **10** led to generally lower cytotoxicity to most of cell line but more selective and cytotoxic to MCF-7, as compared to those with phenyl substitution in **7** and **9**. Meanwhile, **11** selectively inhibited drug resistant cell lines A549/CDDP (IC_{50} = 0.64 μM) and A2780 (IC_{50} = 1.5 μM), respectively.

Compounds **1**–**3**, **12**, and **13**, which possess $\Delta^{4,20}$ terminal double bonds, showed greatly decreased activities to most of cancer cell lines. However, selective cytotoxicity was also observed within

Table 2
Antiproliferative activity of the compounds (**1**–**16**) towards cancer cells and NIH-3T3 cell

Compd	Cytotoxic activities IC_{50} (μM)										
	A549	A549/CDDP	MCF-7	MCF-7/DOX	A2780	A2780/TAX	HCT-8	HCT-8/VCT	Sw480	HepG2	NIH-3T3
1	2.2 ± 1.5	>25	>25	>25	>25	>25	>25	>25	16 ± 3.1	6.1 ± 2.8	>25
2	9.4 ± 1.5	7.1 ± 2.3	>25	>25	9.7 ± 1.6	>25	11 ± 2.1	12 ± 1.8	>25	>25	15 ± 4.7
3	12 ± 0.53	14 ± 3.4	0.040 ± 0.021	>25	5.1 ± 1.4	>25	>25	>25	>25	15 ± 1.2	10 ± 1.0
4	0.0080 ± 0.001	0.0050 ± 0.001	0.0040 ± 0.004	7.0 ± 1.9	0.013 ± 0.0007	4.9 ± 0.8	>25	7.2 ± 1.6	0.033 ± 0.007	0.020 ± 0.004	0.6 ± 0.04
5	0.011 ± 0.003	0.024 ± 0.011	0.012 ± 0.0083	>25	0.030 ± 0.001	7.2 ± 0.9	13 ± 1.2	11 ± 1.2	0.059 ± 0.015	0.095 ± 0.06	2.2 ± 0.60
6	0.0030 ± 0.0006	0.0060 ± 0.003	0.0030 ± 0.002	6.2 ± 1.5	0.0060 ± 0.002	4.4 ± 0.4	0.066 ± 0.02	10 ± 0.59	0.021 ± 0.005	0.0090 ± 0.005	0.58 ± 0.16
7	2.1 ± 1.2	2.5 ± 0.70	0.80 ± 0.35	1.4 ± 0.31	1.6 ± 0.18	>25	0.26 ± 0.06	>25	16 ± 2.0	3.0 ± 1.2	>25
8	2.2 ± 0.54	>25	0.029 ± 0.008	>25	>25	17 ± 1.9	>25	>25	7.2 ± 0.8	5.8 ± 2.7	>25
9	0.39 ± 0.24	0.92 ± 0.07	0.16 ± 0.028	>25	0.85 ± 0.08	>25	5.9 ± 1.5	>25	5.6 ± 1.2	1.2 ± 0.79	>25
10	1.9 ± 0.49	3.1 ± 0.19	0.14 ± 0.11	>25	3.5 ± 0.9	>25	>25	>25	20 ± 1.7	3.9 ± 1.3	>25
11	19 ± 6.4	0.64 ± 0.18	>25	>25	1.5 ± 0.02	>25	8.5 ± 0.6	>25	>25	>25	>25
12	12 ± 2.5	>25	>25	>25	>25	>25	>25	>25	16 ± 1.1	>25	>25
13	15 ± 1.0	>25	>25	>25	>25	0.19 ± 0.03	>25	>25	ND	ND	6.6 ± 1.1
14	21 ± 1.4	25 ± 0.06	17 ± 5.7	>25	>25	16 ± 1.8	>25	>25	ND	ND	>25
15	>25	24 ± 0.29	7.4 ± 2.5	>25	>25	>25	12 ± 1.2	>25	ND	ND	>25
16	<0.002	<0.002	<0.002	<0.002	<0.002	0.42 ± 0.07	<0.002	0.89 ± 0.17	ND	ND	<0.002

ND: not determined.

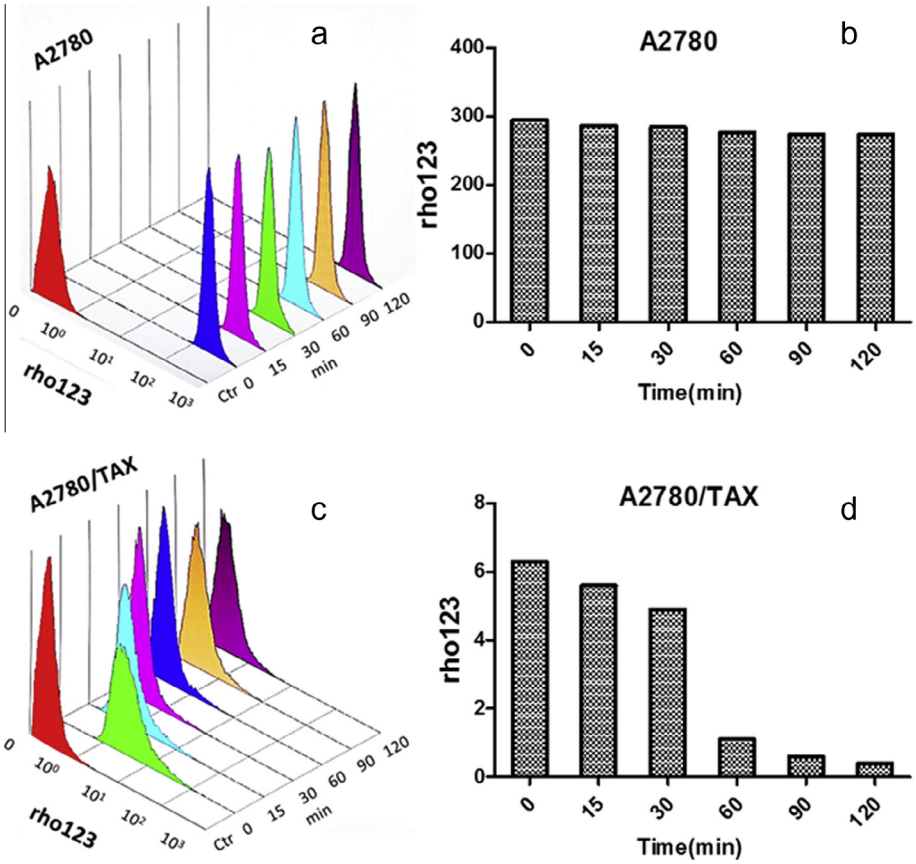

Figure 3. Detection of intracellular P-gp function by Rho123 accumulation and efflux assay. A2780 (a and b) and A2780/TAX (c and d) were incubated with 2 μM Rho123 for 30 min at 37 °C, respectively. Then cells were washed and re-suspended in medium without Rho123 for 0, 15, 30, 60, 90, and 120 min at 37 °C, respectively. After centrifugation the fluorescence of intracellular Rho123 was measured. Fluorescence distribution (a and c) and fluorescence intensity (b and d).

this sub-class. Compounds **3** and **13** showed excellent cytotoxic activities against MCF-7 (IC_{50} = 0.040 μM) and A2780/TAX (IC_{50} = 0.19 μM), respectively, implying that β-aminophenylpropanoic group might contribute the selectivity to MCF-7 in **1**–**3** while cinnamoyloxy was important to the selectivity to A2780/TAX in **12** and **13**.

To evaluate the preliminary safety of the active compounds, we tested their cytotoxicity by using the mouse embryonic fibroblast cell line NIH-3T3, a cell line close to normal cell line, as a model. The results (Table 2) showed that the isolated compounds were much less active than the clinical drugs docetaxol (**16**). In particular, comparing with taxol and docetaxol, **13** with a taxagifine-type structural features (possessing C-12,17-ether ring and $\Delta^{4,20}$) exhibited stronger inhibitory activity on A2780/TAX but weaker activity on NIH-3T3, suggesting it might be served as a drug lead with little side effects to treat ovarian cancer.

A main hurdle of taxol in clinical application is the acquirement of drug resistance, and P-glycoprotein (P-gp) mediated drug efflux was believed as the main cause of the taxol tolerance.[28] To preliminarily understand the underlying action mechanism of **13** in

overcoming the drug tolerance of A2780/TAX, we examined the intracellular P-glycoprotein (P-gp) function by rhodamine 123 (Rho123) accumulation and efflux assay in A2780/TAX and its parental cell line A2780, respectively (Supplementary data, experimental section). The results (Fig. 3) showed that Rho123 accumulated very well in A2780, and no obvious efflux was observed during the examined time. In contrast, much lower Rho123 accumulation was observed in A2780/TAX, and the accumulated Rho123 was gradually eliminated by the time consumption. These results validated A2780/TAX possessed a much elevated intracellular P-gp function. Reasonably, **13** possessing the potent activities against A2780/TAX may evade the P-gp mechanism and exert its effect. However, it should be pointed out that **13** showed no activity against drug sensitive cell line A2780, implying that there may have other complex action mechanism for **13** to specifically exert its effect in A2780/TAX, which is worth further extensively studying in future.

Previous pharmaceutical study on taxanes mainly focused on the modification of the substitutes on the taxol prototype, and revealed that the C-13 esterification chain and oxetane ring between C-5 and C-20 were prerequisite to the anticancer activity.[2] In our current study, fifteen taxanes (**1**–**15**) representing three sub-types of 6/8/6-taxane from *T. wallichiana* var. *mairei*, were systematically evaluated in *vitro* for cytotoxicity against ten human cancer cell lines. Compound **13** with the C-12,17 oxygen bridge but lack of the C-13 esterification chain and oxetane ring showed selectively activity against A2780/T, with IC_{50} value of 0.19 μM, being stronger than the clinical drugs taxol and docetaxol, while **3**, **8**, and **10** showed selective inhibition to MCF-7. The possible P-gp evasion mechanism of **13** against drug-resistant cell line A2780/TAX was explored and the preliminary structure–activity relationships (SARs) of this group of compounds were also discussed. The selectivity of the different sub-types of 6/8/6-taxane implied that these compounds may possess unique action mechanism to certain cancer cell lines, which is worth exploring in future, and **13** with potent activity to drug resistant cell line but weak activity to normal cell line may serve as a drug lead for relative cancer therapy.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.01.056.

References and notes

1. Wang, Y. F.; Shi, Q. W.; Dong, M.; Kiyota, H.; Gu, Y. C.; Cong, B. *Chem. Rev.* **2011**, *111*, 7652.
2. Kingston, D. G. I. *J. Nat. Prod.* **2000**, *63*, 726.
3. Nevarez, D. M.; Mengistu, Y. A.; Nawarathne, I. N.; Walker, K. D. *J. Am. Chem. Soc.* **2009**, *131*, 5994.
4. Kingston, D. G. I. *Pharmacol. Therapeut.* **1991**, *52*, 1.
5. Verma, R. P.; Hansch, C. *Eur. J. Med. Chem.* **2010**, *45*, 1470.
6. Guenard, D.; Gueritte-Voegelein, F.; Potier, P. *Acc. Chem. Res.* **1993**, *26*, 160.
7. Reddy, K. P.; Bid, H. K.; Nayak, V. L.; Chaudhary, P.; Chaturvedi, J. P.; Arya, K. R.; Konwar, R.; Narender, T. *Eur. J. Med. Chem.* **2009**, *44*, 3947.
8. Fu, L. G.; Li, N.; Mill, R. R. In *Flora of China. English and updated version*; Science Press: Beijing, 1999; Vol. 4,; p 91.
9. Shi, Q. W.; Li, L. G.; Li, Z. P.; Cao, C. M.; Kiyota, H. *Tetrahedron Lett.* **2005**, *46*, 6301.
10. Shi, Q. W.; Zhao, Y. M.; Si, X. T.; Li, Z. P.; Yamada, T.; Kiyota, H. *J. Nat. Prod.* **2006**, *69*, 280.
11. Shi, Q. W.; Li, Z. P.; Wang, C. L.; Zhang, M. L.; Choony, N.; Kiyota, H. *J. Asian Nat. Prod. Res.* **2006**, *8*, 431.
12. Shen, Y. C.; Ko, C. L.; Cheng, Y. B.; Chiang, M. Y.; Khalil, A. T. *J. Nat. Prod.* **2004**, *67*, 2136.
13. Shi, Q. W.; Oritani, T.; Sugiyama, T. *Planta Med.* **1999**, *65*, 356.
14. Zhou, Z. H.; Qu, W.; Liu, M. Z.; Sun, J. B.; Wang, M. H.; Liang, J. Y.; Wu, F. H. *Nat. Prod. Res.* **2014**, *28*, 530.
15. Liang, J.; Min, Z.; Niwa, M. *Huaxue Xuebao* **1988**, *46*, 1053.
16. Guo, Y.; Diallo, B.; Jaziri, M.; Vanhaelen-Fastre, R.; Vanhaelen, M.; Ottinger, R. *J. Nat. Prod.* **1995**, *58*, 1906.
17. Miller, R. W.; Powell, R. G.; Smith, C. R., Jr.; Arnold, E.; Clardy, J. *J. Org. Chem.* **1981**, *46*, 1469.
18. McLaughlin, J. L.; Miller, R. W.; Powell, R. G.; Smith, C. R., Jr. *J. Nat. Prod.* **1981**, *44*, 312.
19. Wani, M. C.; Taylor, H. L.; Wall, M. E.; Coggon, P.; McPhail, A. T. *J. Am. Chem. Soc.* **1971**, *93*, 2325.
20. Senilh, V.; Blechert, S.; Colin, M.; Guenard, D.; Picot, F.; Potier, P.; Varenne, P. *J. Nat. Prod.* **1984**, *47*, 131.
21. Beutler, J. A.; Chmurny, G. M.; Look, S. A.; Witherup, K. M. *J. Nat. Prod.* **1991**, *54*, 893.
22. Yoshizaki, F.; Fukuda, M.; Hisamichi, S.; Ishida, T.; In, Y. *Chem. Pharm. Bull.* **1988**, *36*, 2098.
23. Kingston, D. G. I.; Hawkins, D. R.; Ovington, L. *J. Nat. Prod.* **1982**, *45*, 466.
24. *7β,9α,10β-Triacetoxy-13α-hydroxy-5α-O-(β-D-glucopyranosyl)taxa-4(20),11-diene* (**1**): Colorless oil; $[\alpha]_D^{20}$ +26.8 (*c* 0.5, MeOH); IR (KBr) ν_{max} 3365, 2942, 1742, 1437, 1371, 1256 cm^{-1}; HRESIMS *m*/*z* 663.2959 $[M+Na]^+$ (calcd for $C_{32}H_{48}O_{13}Na$, 663.2987).
25. Wang, X. X.; Shigemori, H.; Kobayashi, J. *J. Nat. Prod.* **1998**, *61*, 474.
26. Lin, T. T.; Huang, Y. Y.; Tang, G. H.; Cheng, Z. B.; Liu, X.; Luo, H. B.; Yin, S. *J. Nat. Prod.* **2014**, *77*, 955.
27. Kingston, D. G. I. *Phytochemistry* **2007**, *68*, 1844.
28. Zhu, C. G.; Zuo, Y. L.; Wang, R. M.; Liang, B. X.; Yue, X.; Wen, G. S.; Shang, N. N.; Huang, L.; Chen, Y.; Du, J.; Bu, X. Z. *J. Med. Chem.* **2014**, *57*, 6364.