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Two new phenolic compounds and antitumor activities of asparinin A from *Asparagus officinalis*

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ABSTRACT

Two new phenolic acid compounds, asparoffin C (**1**) and asparoffin D (**2**), together with four known compounds, asparenyol (**3**), gobicusin B (**4**), 1-methoxy-2-hydroxy-4-[5-(4-hydroxyphenoxy)-3-penten-1-ynyl] phenol (**5**), and asparinin A (**6**), have been isolated from the stems of *Asparagus officinalis*. The structures were established by extensive spectroscopic methods (MS and 1D and 2D NMR). Compound **6** has obvious antitumor activities both *in vitro* and *in vivo*.

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Asparagus officinalis; phenolic compounds; asparinin A; antitumor

1. Introduction

Asparagus officinalis is a dioecious plant which is well known as a popular vegetable at home and abroad [1]. It is also used as a traditional Chinese medicine because of its chemical composition of various activities [2], including saponins [3], saccharides [4], flavonoids [5], acetylenic compounds [6], and sulfur-containing [7] and nitrogen-containing [8] compounds. Acetylenic compounds are commonly distributed across the plant kingdom which always exhibited varieties of biological activities, such as insecticidal effects and anti-feeding activities [9]. Previously, we reported two new acetylenic compounds asparoffins A and B from stems of *A. officinalis* [10]. A further investigation of chemical components of *A. officinalis* led to the isolation of two new phenolic compounds, namely asparoffin C (**1**) and asparoffin D (**2**), as well as four known compounds, asparenyol (**3**), gobicusin B (**4**), 1-methoxy-2-hydroxy-4-[5-(4-hydroxyphenoxy)-3-penten-1-ynyl] phenol (**5**), and asparinin A (**6**) (Figure 1). Compound **6** was evaluated for its antitumor activity both *in vitro* and *in vivo*.

2. Results and discussion

2.1. Structural elucidation

Compound **1** has a molecular formula of $C_{20}H_{22}O_4$ deduced from its HR-ESI-MS ($[M + K]^+$ at *m/z* 365.1138; calcd. 365.1150). The IR spectrum revealed absorption bands at 3450 cm^{-1} (hydroxyl groups) and 1605, 1508, 1456 cm^{-1} (benzene rings). Its ^1H- and ^{13}C-NMR

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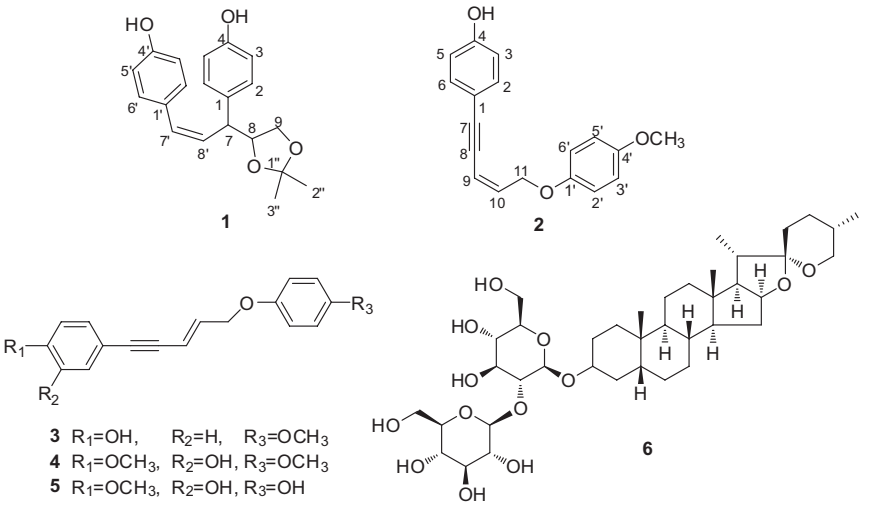

Figure 1. Structures of compounds **1–6**.

Table 1. 1D and 2D NMR data of **1** (methanol-d_4).

Position	δ_H (J, Hz)	δ_C	HMBC(H-C)
1		133.8 C	
2	7.08 (d, 8.5)	130.1 CH	
3	6.73 (d, 8.5)	116.5 CH	C-1
4		157.2 C	
5	6.73 (d, 8.5)	116.5 CH	
6	7.08 (d, 8.5)	130.1 CH	C-4, C-7
7	3.81 (dd, 10.5, 7.8)	47.9 CH	C-1, C-8, C-9, C-7′, C-8′
8	4.28 (ddd, 8.3, 7.1, 6.3)	81.4 CH	C-1, C-8′, C-1″
9	3.55 (dd, 8.3, 7.1)	68.8 CH_2	C-7, C-8, C-1″
	3.73 (dd, 8.3, 6.3)		
1′		130.0 C	
2′	7.10 (d, 8.5)	131.1 CH	C-7′, C-4′
3′	6.71 (d, 8.5)	115.8 CH	
4′		157.5 C	
5′	6.71 (d, 8.5)	115.8 CH	
6′	7.10 (d, 8.5)	131.1 CH	C-7′, C-4′
7′	6.49 (d, 11.5)	130.9 CH	C-8′, C-7
8′	5.88 (dd, 11.5, 10.5)	131.2 CH	C-1, C-1′, C-7, C-8
1″		110.6 C	
2″	1.29 (s)	25.8 CH_3	C-1″
3″	1.29 (s)	26.9 CH_3	C-1″

spectra (Table 1) indicated the presence of two *p*-substituted aromatic rings [δ_H 7.08 (2H, d, J = 8.5 Hz), 6.73 (2H, d, J = 8.5 Hz), δ_C 133.8 (s), 130.1 (d) × 2, 116.5 (d) × 2, 157.2 (s); δ_H 7.10 (2H, d, J = 8.5 Hz), 6.71 (2H, d, J = 8.5 Hz), δ_C 130.0 (s), 131.1 (d) × 2, 115.8 (d) × 2, 157.5 (s)], one double bond [δ_H 5.88 (1H, dd, J = 11.5, 10.5 Hz), 6.49 (1H, d, J = 11.5 Hz); δ_C 131.2 (d), 130.9 (d)], two methines [δ_H 3.81 (1H, dd, J = 10.5, 7.8 Hz), δ_C 47.9; δ_H 4.28 (1H, ddd, J = 8.3, 7.1, 6.3 Hz), δ_C 81.4], and one oxygenated methylene [δ_H 3.55 (1H, dd, J = 8.3, 7.1 Hz, H-9a), 3.73 (1H, dd, J = 8.3, 6.3 Hz, H-9b); δ_C 68.8]. The ^{1}H- and ^{13}C-NMR spectra data revealed structural features very similar to those of isoagatharesinol [11]. The establishment of isoagatharesinol unit was powerfully supported by the HMBC correlations

Table 2. 1D and 2D NMR data of **2** (methanol-d_4).

Position	δ_H (*J*, Hz)	δ_C	HMBC(H-C)
1		114.9, C	
2	7.33, (d, 8.5)	133.2, CH	C-1, C-7
3	6.78, (d, 8.5)	115.5, CH	C-1, C-4
4		156.3, C	
5	6.78, (d, 8.5)	115.5, CH	C-1, C-4
6	7.33, (d, 8.5)	133.2, CH	C-1, C-7
7		96.1, C	
8		83.5, C	
9	5.89 (d*t*, 10.8, 1.2)	112.1, CH	C-7, C-10, C-11
10	6.13 (d*t*, 10.8, 6.1)	137.3, CH	C-8
11	4.85 (dd, 6.1, 1.2)	66.6, CH_2	C-8, C-9, C-10, C-1′
1′		152.5, C	
2′	6.90 (d, 8.9)	115.7, CH	
3′	6.84 (d, 8.9)	114.6, CH	
4′		153.9, C	
5′	6.84 (d, 8.9)	114.6, CH	C-1′
6′	6.90 (d, 8.9)	115.7, CH	C-1′, C-4′
OCH_3	3.77 (s)	55.7, CH_3	C-4′

from H-7 to C-1, C-8, C-9, and C-7′, and from H-2′ and H-6′ to C-7′. Furthermore, the observed HMBC correlations from two methyl signals at δ_H 1.29 (6H, s) to a ketal carbon at δ_C 110.6 established the presence of one acetonide group in **1**. The two positions of the two ether linkages were located at C-8 and C-9 on the basis of the two relatively downfield oxygenated carbons (C-8, 81.4; C-9, 68.8), together with the HMBC correlation from H-8 and H-9 to C-1″. In addition, the *cis* geometries at C-7′ = C-8′ double bond were assigned by the coupling constants (J = 11.5 Hz). On the basis of these results, the complete planar assignment for compound **1** was confirmed. Due to free rotation of bond C-7/C-8, the stereoconfiguration of C-7 and C-8 is not established yet. Thus, the structure of **1** was elucidated as shown and named asparoffin C.

Compound **2** was obtained as a white powder. The molecular formula $C_{18}H_{16}O_3$ of **2** was deduced from HR-EI-MS ($[M - H]^-$ at *m/z* 279.1017; calcd. 279.1027). Its IR (KBr) spectrum showed the presence of hydroxyl group (3423 cm^{-1}), acetylene bond (2202 cm^{-1}), and aromatic ring (1610, 1511, 1450 cm^{-1}). The ^{1}H-NMR data (Table 2) gave signals of two 1,4-disubstituted benzene rings [δ_H 6.78 (2H, d, J = 8.5 Hz), 7.33 (2H, d, J = 8.5 Hz); δ_H 6.90 (2H, d, J = 8.9 Hz), 6.84 (2H, d, J = 8.9 Hz)], as well as the signal of one methoxy groups at δ_H 3.77 (3H, s) and an AMX_2 system [δ_H 6.13 (1H, d*t*, J = 10.8, 6.1 Hz), 5.89 (1H, d*t*, J = 10.8, 1.2 Hz), and 4.85 (2H, dd, J = 6.1, 1.2 Hz)]. Apart from the disubstituted benzene carbons, the ^{13}C-NMR and DEPT spectra of **2** (Table 2) also showed two acetylenic carbons (δ_C 83.5 and 96.1), two olefinic carbons (δ_C 112.1 and 137.3), one oxygenating methylene carbon (δ_C 66.6), and one methoxy group (δ_C 55.7). The ^{1}H-^{1}H COSY correlations of the proton signal at δ_H 6.13 with those at δ_H 5.89 and 4.58 suggested the existence of –CH = $CHCH_2O$–. Furthermore, two substituted benzene rings were linked by –C≡CCH = $CHCH_2O$–, which were deduced from the HMBC correlations of H-9 (δ_H 5.89) with C-7 (δ_C 96.1) and C-11 (δ_C 66.6), and of H-11 (δ_H 4.85) with C-1′ (δ_C 152.5). The methoxy at C-4′ was established by HMBC correlation of δ_H 3.77 (3H, s, OCH_3) with δ_C 153.9 (s, C-4′). The characteristic coupling constant (10.8 Hz) between H-9 and H-10 suggested the *Z* configuration of the double bond. Therefore, the structure of compound **2** was elucidated as shown and named asparoffin D.

Four known compounds were identified as asparenyol (**3**) [6], gobicusin B (**4**) [12], 1-methoxy-2-hydroxy-4-[5-(4-hydroxyphenoxy)-3-penten-1-ynyl] phenol (**5**), and asparinin A (**6**) [13]. Asparinin A was isolated as the main component (80 g).

2.2. In vitro *antitumor activity of asparinin A*

As shown in Table 3, asparinin A (**6**) showed a potential antitumor effect on tumor cells *in vitro* (IC_{50} < 10 μg/ml).

2.3. In vivo *antitumor activity of asparinin A*

Asparinin A (**6**) was evaluated for its antitumor activity *in vivo* using methods of intragastric administration and intraperitoneal injection. H22 and S180 tumor-bearing mice were used to test the antitumor activities of asparinin A *in vivo*. The experimental results were summarized in Tables 4–6. According to these experiments, we demonstrated that asparinin A exhibited multiple antitumor activities against human (K562, HepG-2, A549, HeLa, MCF-7, HCT-116) and mouse (H22, S180) tumor cells. Considering the content of

Table 3. *In vitro* cytotoxic activity of asparinin A (IC_{50}, μg/ml).

Compound	Leukemia K562	Hepatoma HepG-2	Lung A549	Cervical HeLa	Breast MCF-7	Colon HCT-116
Asparinin A	2.7	3.2	4.2	3.9	6.2	4.4
Cisplatin[a]	4.2	1.1	3.1	1.2	3.1	1.5

[a]positive control.

Table 4. *In vivo* effects of asparinin A (**6**) on the growth of H22 inoculated onto the right flank of SPF mice (mean ± SD).

Groups	Doses (mg/kg)	Animal number		Body weight (g)		Tumor weight (g)	Inhibition (%)
		Beginning	End	Beginning	End		
Control	Vehicle	12	12	24.5 ± 0.72	30.8 ± 2.46	1.55 ± 1.02	–
Cisplatin	2.5	12	12	24.7 ± 0.69	17.3 ± 2.04**	0.16 ± 0.07**	89.4
Asparinin A	25	12	12	24.8 ± 1.07	30.9 ± 2.23*	1.52 ± 1.36	1.8
	100	12	12	24.5 ± 1.09	31.5 ± 2.30	1.83 ± 1.37	0
	400	12	12	24.8 ± 1.15	30.8 ± 2.61	1.62 ± 1.03	0

Note: One day after tumor inoculation, mice were given intragastrically asparinin A every day for 10 days (n = 10); compare with control.
*p < 0.05; **p < 0.01.

Table 5. *In vivo* effects of asparinin A (**6**) on the growth of H22 inoculated onto the right flank of SPF mice (mean ± SD).

Groups	Doses (mg/kg)	Animal number		Body weight (g)		Tumor weight (g)	Inhibition (%)
		Beginning	End	Beginning	End		
Control	Vehicle	12	12	25.7 ± 0.84	32.4 ± 1.65	1.70 ± 1.03	–
Cisplatin	2.5	12	12	25.3 ± 0.92	18.9 ± 1.58**	0.34 ± 0.22**	79.9
Asparinin A	25	12	12	25.3 ± 0.83	30.2 ± 2.32*	1.46 ± 0.95	14.3
	100	12	12	25.5 ± 0.98	29.1 ± 2.07**	0.74 ± 0.49**	56.3
	400	12	6				

Note: One day after tumor inoculation, mice were given asparinin A by intraperitoneal injection every day for 10 days (n = 10); compare with control.
*p < 0.05; **p < 0.01.

Table 6. *In vivo* effects of asparinin A (**6**) on the growth of S180 inoculated onto the right flank of SPF mice (mean ± SD).

Groups	Doses (mg/kg)	Animal number		Body weight (g)		Tumor weight (g)	Inhibition (%)
		Beginning	End	Beginning	End		
Control	Vehicle	12	12	23.4 ± 1.84	32.1 ± 2.52	2.0 ± 1.36	–
Cisplatin	2.5	12	12	23.2 ± 1.96	20.0 ± 2.74**	0.4 ± 0.25**	81.5
Asparinin A	25	12	12	22.8 ± 1.67	28.9 ± 2.94**	1.8 ± 1.27	8.6
	50	12	12	23.4 ± 1.92	26.3 ± 3.78**	1.5 ± 0.94	24.9
	100	12	12	23.3 ± 1.77	25.9 ± 1.99**	0.9 ± 0.45*	51.1

Note: One day after tumor inoculation, mice were given asparinin A by intraperitoneal injection every day for 10 days ($n = 10$); compare with control.
*$p < 0.05$; **$p < 0.01$.

asparinin A in *A. officinalis*, these *in vitro* and *in vivo* findings strongly demonstrate that *A. officinalis* can be used potentially for tumor cancer treatment as a chemopreventive agent. However, further investigations, such as screening of antitumor effects of the compounds isolated from *A. officinalis*, and research on mechanisms of actions will be necessary for better understanding the antitumor effects of *A. officinalis*.

3. Experimental

3.1. General experimental procedures

Optical rotations were obtained on a JASCO P-1020 digital polarimeter (Horiba, Kyoto, Japan). IR spectra were taken on a Bruker Tensor 27 FT–IR spectrometer (Bruker, Karlsruher, Germany) with KBr pellets. UV spectra were obtained using a Shimadzu UV-2401A spectrometer (Shimadzu, Kyoto, Japan). Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance III 600 MHz spectrometer (Bruker, Karlsruher, Germany). HRESIMS was measured on a Bruker HCT/Esquire (Bruker, Karlsruher, Germany) instrument. Silica gel 200–300 mesh (Qingdao Marine Chemical Inc., Qingdao, China) and Sephadex LH-20 (Amersham Biosciences, Upssala, Sweden) were used for column chromatography. Medium pressure liquid chromatography (MPLC) was performed on a Buchi Sepacore System equipped with pump manager C-615, pump modules C-605 and fraction collector C-660 (Buchi Labortechnik AG, Flawil, Switzerland), and columns packed with Chromatorex C-18 (40–75 μm, Fuji Silysia Chemical Ltd., Kasugai, Japan). Preparative HPLC was performed on an Agilent 1260 liquid chromatography system equipped with a Zorbax SB-C18 column (9.4 mm × 150 mm) (Agilent Technologies, Santa Clara, CA, USA). MTT (No. X1204P040) was purchased from Sigma Chemical Company (Sigma, St. Louis, MO, USA). Cisplatin was purchased from Jiangsu Hansoh Pharmaceutical Company (No. 141203, Jiangsu, China). FBS (No. 14243922) was purchased from Biological Industries Company in China Marketing Department (Shanghai Xiaopeng Biological Technology Co., Ltd, Shanghai, China).

3.2. Plant material

The stems of *Asparagus officinalis* were collected from Wenshan City of Yunnan province in China, in November 2014, and were identified by Professor Zi-Chao Mao of Yunnan

Agricultural University. A specimen has been deposited in the college of Agriculture and Biotechnology (No. AO201411-2).

3.3. Cell lines

Six human tumor cell lines, K562, HepG-2, A549, HeLa, MCF-7, and HCT-116, and two mouse cell lines, H22 and S180, were used, which were obtained from Kunming Institute of Zoology, Chinese Academy of Sciences.

3.4. Animal material

Kunming mice, female, 18–22 g in weight, was provided by the Guangdong Animal Center, China.

3.5. Extraction and isolation

The fresh, milled stems of *A. officinalis* (35 kg) were extracted three times with EtOH (95%) at room temperature. The extract was suspended in distilled water and partitioned successfully with ethyl acetate. The EtOAc layer was concentrated under reduced pressure to afford a crude extract (650 g), and the residue was subjected to silica gel column chromatography (16 × 80 cm) eluted with step-gradient of $CHCl_3$-MeOH (from 1:0 to 0:1), to yield eight fractions (fr. 1–8). Fr. 2 (100 g) was separated on a silica gel column (5 × 40 cm) using a step-gradient of petroleum ether-acetone (*v*/*v*: 15:1, 10:1, 5:1, 1:1, 0:1) to yield six sub-fractions (2a–2f). Fraction 2e (1.8 g) was separated by gel column Sephadex LH-20 (MeOH), to obtain compounds **1** (7.3 mg), **2** (14 mg), **3** (56.2 mg), **4** (1 mg), and **5** (77.6 mg). Fr. 6 (120 g) was washed with acetone to obtain compound **6** (80 g).

3.5.1. Asparoffin C (1)

Yellowish oil, $[\alpha]_D^{24} + 6.1$ (0.18, MeOH); UV (MeOH) λ_{max} (log ε) 207 (3.20), 238 (4.27), 278 (4.33), 282 (4.12) nm; IR (KBr) ν_{max} 3450, 1605, 1508, 1456 cm^{-1}; ^{1}H-NMR spectral data see Table 1; ^{13}C-NMR spectral data see Table 1; HR-ESI-MS (neg.): *m/z* 365.1138 $[M + K]^+$ (calcd for $C_{20}H_{22}O_4$ K, 365.1150).

3.5.2. Asparoffin D (2)

White powder, UV (MeOH) λ_{max} (log ε) 202 (4.38), 220 (4.24), 286 (4.32), 298 (4.28) nm; IR (KBr) ν_{max} 3423, 2930, 2202, 1610, 1511, 1450, 1349, 1226, 1174, 1035, 832 cm^{-1}; ^{1}H-NMR spectral data see Table 2; ^{13}C-NMR spectral data see Table 2; HR-EI-MS (neg.): *m/z* 279.1017 $[M - H]^-$ (calcd for $C_{18}H_{15}O_3$, 279.1027).

3.6. In vitro *antitumor activity of asparinin A*

The inhibitory effects of asparinin A (**6**) at five concentrations 5, 2.5, 1.25, 0.625, and 0.3125 μg·ml^{-1} on cells viability were measured by MTT colorimetric method [14]. Tumor cells in exponential growth were seeded at a density of 5×10^4 cells/well in a 96-well plate. On the second day, cells were treated with **6** or DMSO (vehicle control, 0.5% DMSO) for 48 or 72 h, respectively. After drug treatments, attached cells were incubated with 20 ul of MTT

(5 mg/ml, 4 h) and the incubation at 37 °C was continued for 12 h. The OD value at 570 nm was measured using Multiskan GO. The cell viability ratio was calculated based on the formula: percent viability = (OD of drug-treated sample)/(OD of non-treated sample) × 100%.

3.7. In vivo *antitumor activity of asparinin A (6)*

The mice are grouped randomly into high-, intermediate-, and low-dose groups, CMC-Na negative control group and positive control (cisplatin) group; 12 mice were used for each sample and dose. Ascites of the H22 and H180 mouse with tumor were drawn out under aseptic conditions and then diluted with aseptic physiological saline. The diluted solution was used to conduct hypoderm inoculation on the axillary region of the right limb with 0.2 ml per mouse. One day after tumor inoculation, mice were given intragastrically (or intraperitoneal injection) **6**, and the dose of **6** was 400, 100, and 25 mg/kg (or 100, 50, and 25 mg/kg) for high-, intermediate-, and low-dose groups and 2.5 mg/kg for cisplatin. Negative group was injected the same volume of 0.5% CMC-Na, and every dose was given to every group mice once a day for 10 days consecutively. The treated mouse was killed the second day after the end of the treatment. The body weight of the mouse and the tumor weight were measured to calculate the tumor inhibition rate and analyze the relationship between the medicine doses and their efficacy. The tumor growth inhibitory ratio was calculated based on the formula: Inhibition = (tumor weight of control group - tumor weight of treated group)/tumor weight of control group × 100%.

3.8. *Statistical analysis*

The inhibition rates of tumor cells were calculated by LOGIT method, which were expressed as mean ± standard deviation (SD). The test was used to compare the mean differences between samples using the statistical software SPSS version 17.0. In all cases, $p < 0.05$ was considered statistically significant.

Disclosure statement

No potential conflict of interest was reported by the authors.

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References

[1] E. Caporali, A. Carboni, M.G. Galli, G. Rossi, A. Spada, and G.P.M. Longo, *Sex. Plant Reprod.* **7**, 239 (1994).
[2] D.S. Jang, M. Cuendet, and H.H.S. Fong, *J. Agric. Food Chem*. **52**, 2218 (2004).
[3] X.F. Huang, Y.Y. Lin, and L.Y. Kong, *J. Integr. Plant Biol.* **50**, 717 (2008).
[4] E. Fukushi, S. Onodera, A. Yamamori, N. Shiomi, and J. Kawabata, *Magn. Reson. Chem*. **38**, 1005 (2000).
[5] D. Jiang, F.Y. Tao, Y.Q. Li, and W. Zhao, *Sci. Technol. Food Ind*. **35**, 357 (2014).

[6] K. Terada, C. Honda, K. Suwa, S. Takeyama, H. Oku, and W. Kamisako, *Chem. Pharm. Bull.* **43**, 564 (1995).
[7] H. Yanagawa, T. Kato, and Y. Kitahara, *Tetrahedron Lett.* **14**, 1073 (1973).
[8] R. Nakabayashi, Z. Yang, T. Nishizawa, T. Mori, and K. Saito, *J. Nat. Prod.* *78*, 1179 (2015).
[9] S.Q. Wan, H.H. Xu, S.H. Zhao, Z.S. Jiang, Z.Z. Shang, and Z. Liu, *Acta Entomol. Sin.* **48**, 526 (2005).
[10] X.M. Li, J.L. Cai, W.X. Wang, H.L. Ai, and Z.C. Mao, *J. Asian Nat. Prod. Res.* **18**, 344 (2016). doi:http://dx.doi.org/10.1080/10286020.2015.1082549.
[11] C.X. Yang, S.S. Huang, X.P. Yang, and Z.J. Jia, *Planta Med.* **70**, 446 (2004).
[12] C.X. Yang, C.S. Yuan, and Z.J. Jia, *Chin. Chem. Lett.* **16**, 365 (2005).
[13] H.J. Zhang, K.M. Sydara, G.T. Tan, C.Y. Ma, B.H. Southavong, D.D. Soejarto, J.M. Pezzuto, and H.H.S. Fong, *J. Nat. Prod.* **67**, 194 (2004).
[14] M. Selvakumaran, D.A. Pisarcik, R. Bao, A.T. Yeung, and T.C. Hamilton, *Cancer Res.* **63**, 1311 (2003).