CrossMark

ORIGINAL ARTICLE

Prenylated Coumarins from *Heracleum stenopterum*, *Peucedanum praeruptorum*, *Clausena lansium*, and *Murraya paniculata*

Xiang-Mei Li · Xian-Jun Jiang · Ku Yang ·
Li-Xia Wang · Shi-Zhen Wen · Fei Wang

Abstract Four hitherto unknown prenylated coumarins, namely $6''$-O-β-D-apiofuranosylapterin (**1**), $4'$-O-isobutyroylpeguangxienin (**2**), 6-(3-methyl-2-oxobutyroyl)-7-methoxycoumarin (**3**), and 6-hydroxycoumurrayin (**4**), were isolated from the ethanol extract of *Heracleum stenopterum*, *Peucedanum praeruptorum*, *Clausena lansium*, and *Murraya paniculata*, respectively. Their chemical structures were established on the basis of extensive spectroscopic analysis. Compound **2** exhibited in vitro cytotoxic activity against five human cancer cell lines (HL-60, A-549, SMMC-7721, MCF-7, and SW-480) with IC_{50} values ranging from 15.9 to 23.2 μM.
Graphical Abstract
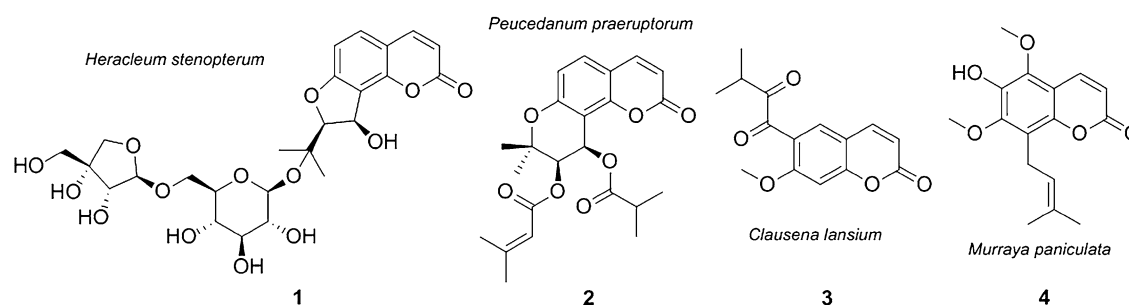
Keywords *Heracleum stenopterum* · *Peucedanum praeruptorum* · *Clausena lansium* · *Murraya paniculata* · Prenylated coumarin · Cytotoxicity

X.-M. Li · X.-J. Jiang · K. Yang · L.-X. Wang · S.-Z. Wen · F. Wang (✉)
BioBioPha Co., Ltd., Kunming 650201, People's Republic of China
e-mail: f.wang@mail.biobiopha.com

1 Introduction

Coumarins are typical secondary metabolites accumulated in the families Apiacee (Umbelliferae) [1] and Rutaceae [2]. The widespread incorporation of prenyl units resulted in the structural diversity of coumarins in both families [2]. Particularly, the genera *Heracleum* and *Peucedanum* from Apiaceae can be characterized by the dominance of furanocoumarins [3] and pyranocoumarins [4], respectively. Coumarins possessed diverse pharmacological properties, in

Springer

which the cytotoxic effects were most extensively examined [5]. As part of a BioBioPha (http://www.chemlib.cn/) objective to assemble a large-scale natural product library valuable in the discovery of new drug leads from nature [6, 7], four new prenylated coumarins Fig. 1, namely 6″-O-β-D-apiofuranosylapterin (**1**), 4′-O-isobutyroylpeguangxienin (**2**), 6-(3-methyl-2-oxobutyroyl)-7-methoxycoumarin (**3**), and 6-hydroxycoumurrayin (**4**), were isolated from the ethanol extract of *Heracleum stenopterum*, *Peucedanum praeruptorum*, *Clausena lansium*, and *Murraya paniculata*, respectively. This paper described the structure elucidation of new coumarins and their cytotoxicity evaluation against five human cancer cell lines.

2 Results and Discussion

Compound **1**, obtained as white amorphous powder, possessed a molecular formula of $C_{25}H_{32}O_{14}$, as evidenced by positive HRESIMS at m/z 579.1681 [M + Na]$^+$ (calcd. for $C_{25}H_{32}O_{14}Na$, 579.1684), requiring 10 degrees of unsaturation. The characteristic UV absorptions at 221 (sh), 248, 258, 325 nm indicated the presence of a 7-oxygenated coumarin chromophore [8]. The ^{1}H NMR spectrum (Table 1) showed two pairs of aromatic doublets at δ_H 6.26 (d, J = 9.6 Hz, H-3), 8.00 (d, J = 9.6 Hz, H-4), 7.59 (d, J = 8.4 Hz, H-5), and 6.91 (d, J = 8.4 Hz, H-6), indicative of the existence of a 7,8-disubstituted coumarin moiety, two vicinal oxymethine protons of dihydrofuran ring at δ_H 4.51 (d, J = 6.4 Hz, H-2′) and 5.45 (dd, J = 8.8, 6.4 Hz, H-3′), and a *gem*-dimethyl group at δ_H 1.47 (s, H-5′, H-6′). The ^{13}C NMR spectrum (Table 1) displayed a total of 25 carbon resonances, including nine coumarin carbons, and a set of carbons at δ_C 91.8 (d, C-2′), 68.2 (d, C-3′), 77.1 (s, C-4′), 24.6 (q, C-5′) and 23.0 (q, C-6′), which suggested that **1** should be an angular dihydrofuranocoumarin [9]. The remaining 11

oxygenated carbons were assignable to two sugar units. By comparison of its NMR spectra with published data [9, 10], an hexose was determined as a C-6 glycosylated glucopyranosyl [δ_H 4.55 (d, J = 7.8 Hz, H-1″); δ_C 97.4 (d, C-1″), 73.3 (d, C-2″), 76.7 (d, C-3″), 69.7 (d, C-4″), 74.9 (d, C-5″) and 66.4 (t, C-6″)], and the pentose as a terminal apiofuranosyl moiety [δ_H 4.68 (d, J = 2.5 Hz, H-1‴); δ_C 109.0 (d, C-1‴), 75.9 (d, C-2‴), 78.8 (s, C-3‴), 73.3 (t, C-4‴), and 63.4 (t, C-5‴)]. In the HMBC spectrum (Fig. 2), the anomeric proton of glucose showed a strong correlation with C-4′, meanwhile, the anomeric one of apiose with the downfield shifted C-6″ ($\Delta\delta \approx$ +5.0 ppm) of glucose, which unambiguously established an api(1 → 6)glc sugar chain at C-4′. The same linkage pattern also occurred in the structure of heraclenol 3′-O-β-D-apiofuranosyl-(1 → 6)-β-D-glucopyranoside, isolated from this plant in our current research [11]. Based on comparison of the coupling constants (*cis*: ~6.0 Hz, *trans*: ~3.5 Hz) [12, 13], the *cis* configuration of H-2′/H-3′ was concluded. Therefore, compound **1** was identified as 6″-O-β-D-apiofuranosylapterin.

Compound **2**, white amorphous powder, had a molecular formula of $C_{23}H_{26}O_7$ by positive HRESIMS at m/z 437.1573 [M + Na]$^+$ (calcd. for $C_{23}H_{26}O_7Na$, 437.1571). The ^{1}H NMR spectrum (Table 2) also indicated the existence of a 7,8-disubstituted coumarin moiety [δ_H 6.21 (d, J = 9.5 Hz, H-3), 7.59 (d, J = 9.5 Hz, H-4), 7.34 (d, J = 8.6 Hz, H-5), and 6.80 (d, J = 8.6 Hz, H-6)], two vicinal oxymethines bearing ester function at δ_H 5.36 (d, J = 5.0 Hz, H-3′) and 6.56 (d, J = 5.0 Hz, H-4′), a *gem*-dimethyl group at 1.44 (s, H-5′) and 1.40 (s, H-6′), a 3-methyl-2-butenoyl unit [δ_H 5.66 (s, H-2″), 2.16 (s, H-4″), 1.89 (s, H-5″)], and an isobutyroyl group [δ_H 2.57 (heptet, J = 7.0 Hz, H-2‴), 1.21 (d, J = 7.0 Hz, H-3‴), and 1.17 (d, J = 7.0 Hz, H-4‴)]. The ^{13}C NMR spectrum (Table 2) exhibited a total of 23 carbon resonances, including a set of signals due to a dihydropyran ring [δ_C 77.7 (s, C-2′), 68.7 (d, C-3′), 60.8 (d, C-4′), 22.9 (q, C-5′) and 24.5 (q, C-6′)], as well as two ester carbonyl carbons [δ_C 165.2 (s, C-1″) and 175.7 (s, C-1‴)]. Comparison of its NMR data with those of hyuganin D [14], also isolated in our current research, revealed that **2** was an analogue of the angular dihydropyranocoumarin. The obvious difference was that a 3-methyl-2-butenoyl moiety replaced the acetyl group. The acylated positions were determined by the HMBC correlations from H-3′ to C-1″ of 3-methyl-2-butenoyl group, and from H-4′ to C-1‴ of isobutyroyl group. The *cis* configuration of H-3′ and H-4′ was concluded based on the diagnostic coupling constant [15]. From the above results, the structure of compound **2** was established as 4′-O-isobutyroylpeguangxienin.

Compound **3**, yellow amorphous powder, possessed a molecular formula of $C_{15}H_{14}O_5$ according to the positive HRESIMS at m/z 297.0737 [M + Na]$^+$ (calcd. for
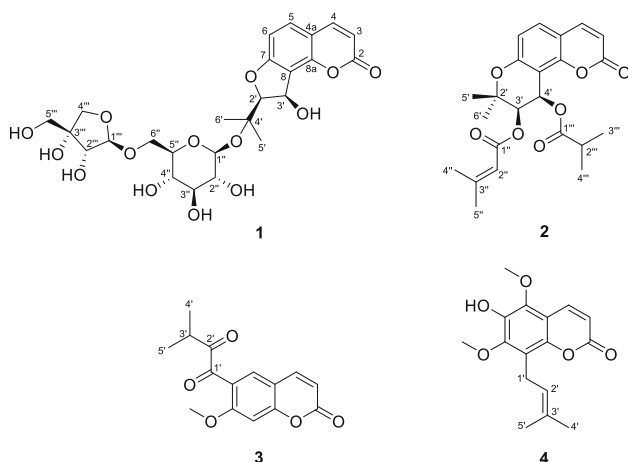

Fig. 1 Structures of new coumarins **1**−**4**

Table 1 NMR spectroscopic data for 6″-*O*-β-D-apiofuranosylapterin (**1**) in DMSO-d_6 (δ_H 2.49, δ_C 39.5 ppm)

No.	δ_H	δ_C	No.	δ_H	δ_C
2		160.0 (s)	3″	3.13 (m)	76.7 (d)
3	6.26 (d, 9.6)	111.8 (d)	4″	3.02 (m)	69.7 (d)
4	8.00 (d, 9.6)	144.9 (d)	5″	3.13 (m)	74.9 (d)
4a		112.9 (s)	6″	3.21−3.27 (m)	66.4 (t)
5	7.59 (d, 8.4)	130.9 (d)	1‴	4.68 (d, 2.5)	109.0 (d)
6	6.91 (d, 8.4)	107.4 (d)	2‴	3.63 (dd, 6.3, 2.5)	75.9 (d)
7		162.8 (s)	3‴		78.8 (s)
8		116.8 (s)	4‴	3.72 (d, 9.4)	73.3 (t)
				3.47 (d, 9.4)	
8a		151.5 (s)	5‴	3.30 (dd, 11.5, 5.6)	63.4 (t)
				3.26 (dd, 11.5, 5.6)	
2′	4.51 (d, 6.4)	91.8 (d)	3′-OH	5.15 (d, 8.8)	
3′	5.45 (dd, 8.8, 6.4)	68.2 (d)	2″-OH	4.98 (d, 4.8)	
4′		77.1 (s)	3″-OH	4.94 (d, 5.0)	
5′	1.47 (s)	24.6 (q)	4″-OH	4.95 (d, 5.6)	
6′	1.47 (s)	23.0 (q)	2‴-OH	4.93 (d, 6.3)	
1″	4.55 (d, 7.8)	97.4 (d)	3‴-OH	4.41 (s)	
2″	2.89 (m)	73.3 (d)	5‴-OH	4.69 (t, 5.6)	

$C_{15}H_{14}O_5Na$, 297.0733). The NMR data (Table 3) revealed the presence of a 6,7-disubstituted coumarin skeleton [δ_H 6.33 (d, *J* = 9.6 Hz, H-3), 7.69 (d, *J* = 9.6 Hz, H-4), 7.97 (s, H-5), and 6.83 (s, H-8)], a methoxy group (δ_H 3.89; δ_C 56.5), and a set of signals originated from a prenyl unit [δ_H 3.15 (heptet, *J* = 7.0, H-3′), 1.24 (d, *J* = 7.0, H-4′ and H-5′); δ_C 193.6 (s, C-1′), 205.6 (s, C-2′), 36.3 (d, C-3′), 17.2 (q, C-4′ and C-5′)], which were further established as a 3-methyl-2-oxobutyroyl unit by the HMBC correlations of H-4′ and H-5′ with C-2′ as well as comparison with literature data [16]. This unit was attached to C-6 from the HMBC correlation of H-5 to C-1′, while the methoxy at C-7 by a weak but clear ^4J correlation from the methoxy protons to C-8. Accordingly, compound **3** was determined as 6-(3-methyl-2-oxobutyroyl)-7-methoxycoumarin.

Compound **4**, white amorphous powder, had a molecular formula of $C_{16}H_{18}O_5$ determined by positive HRESIMS at *m/z* 313.1042 [M + Na]$^+$ (calcd. for $C_{16}H_{18}O_5Na$, 313.1046). The NMR spectra (Table 3) showed the presence of a 5,6,7,8-tetrasubstituted coumarin skeleton [δ_H 6.33 (d, *J* = 9.6 Hz, H-3) and 7.92 (d, *J* = 9.6 Hz, H-4); δ_C 161.2 (s, C-2), 114.5 (d, C-3), 138.3 (d, C-4), 140.9 (s, C-5), 138.4 (s, C-6), 149.4 (s, C-7), 118.9 (s, C-8), 110.0 (s, C-4a), and 146.0 (s, C-8a)], two methoxys [δ_H 3.96, 3.91 (each s); δ_C 62.1, 61.7 (each q)], a prenyl [δ_H 3.50 (d, *J* = 7.0 Hz, H-1′), 5.22 (br t, *J* = 7.0, H-2′), 1.68 (br s, H-4′), and 1.83 (br s, H-5′); δ_C 22.5 (t, C-1′), 121.3 (d, C-2′), 132.8 (s, C-3′), 25.7 (q, C-4′), 18.0 (q, C-5′)], and a phenolic hydroxy group [δ_H 5.56 (s, 6-OH)]. All of the

above spectroscopic data were generally consistent with those of 6-methoxycoumurrayin [16], except that a methoxy group was replaced by a hydroxy group. The two methoxy groups were located at C-5 and C-7 on the basis of the HMBC correlations from H-4 to C-5, 5-OCH$_3$ to C-5, H-1′ to C-7/C-8a, and from 7-OCH$_3$ to C-7. Thus, compound **4** was identified as 6-hydroxycoumurrayin.

The in vitro cytotoxicity of these new coumarins (**1**–**4**) was evaluated against five human cancer cell lines (HL-60, A-549, SMMC-7721, MCF-7, and SW-480) using the MTS method. DDP (cisplatin) and paclitaxel were used as positive controls. Compound **2** exhibited cytotoxic activity with IC$_{50}$ values ranging from 15.9 to 23.2 μM for all tested cell lines, while the other compounds were inactive (IC$_{50}$ values >40 μM).

3 Experimental Section

3.1 General Experimental Procedures

Optical rotations were measured on a Jasco P-1020 automatic digital polarimeter. UV data were obtained from HPLC online analysis. NMR spectra were carried out on a Bruker AV-400, DRX-500, Avance III 600, or AV-800 spectrometer with deuterated solvent signals used as internal standards. ESI and HRESIMS were performed with a Shimadzu LC-IT-TOF mass spectrometer equipped with an ESI interface (Shimadzu, Kyoto, Japan). Silica gel 200–300
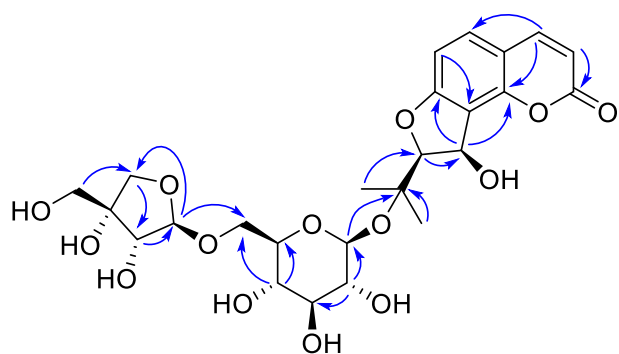
Fig. 2 Key HMBC correlations of 6″-O-β-D-apiofuranosylapterin (**1**)

Table 2 NMR spectroscopic data for 4′-O-isobutyroylpeguangxienin (**2**) in CDCl₃ (δ_H 7.26, δ_C 77.0 ppm)

No.	δ_H	δ_C	No.	δ_H	δ_C
2		159.8 (s)	5′	1.44 (s)	22.9 (q)
3	6.21 (d, 9.5)	113.1 (d)	6′	1.40 (s)	24.5 (q)
4	7.59 (d, 9.5)	143.3 (d)	1″		165.2 (s)
4a		112.5 (s)	2″	5.66 (s)	115.0 (d)
5	7.34 (d, 8.6)	129.0 (d)	3″		158.9 (s)
6	6.80 (d, 8.6)	114.4 (d)	4″	2.16 (s)	20.3 (q)
7		156.8 (s)	5″	1.89 (s)	27.5 (q)
8		107.2 (s)	1‴		175.7 (s)
8a		154.0 (s)	2‴	2.57 (heptet, 7.0)	34.1 (d)
2′		77.7 (s)	3‴	1.21 (d, 7.0)	18.7 (q)
3′	5.36 (d, 5.0)	68.7 (d)	4‴	1.17 (d, 7.0)	18.8 (q)
4′	6.56 (d, 5.0)	60.8 (d)			

mesh (Qingdao Marine Chemical Inc., Qingdao, China), Chromatorex C-18 (40–75 μm, Fuji Silysia Chemical Ltd., Japan) and Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) were used for normal pressure column chromatography. Prep-HPLC separation was performed using an Agilent 1260 series HPLC system equipped with a Zorbax SB-C18 column (5 μm, 21.2 × 150 mm). Fractions were monitored and analyzed by TLC, in combination with an Agilent 1200 series HPLC system equipped by an Extend-C18 column (5 μm, 4.6 × 150 mm).

3.2 Plant Material and Isolation (See Table 4)

The retention times (t_R) of **1**–**4** on an analytical HPLC Extend-C18 column (20 → 100 % MeOH in H₂O over 8.0 min followed by 100 % MeOH to 13.0 min, 1.0 ml/min, 25 °C) were 5.86, 9.69, 7.83, and 8.77 min, respectively.

3.3 6″-O-β-D-Apiofuranosylapterin (**1**)

White amorphous powder; UV (MeOH) λ_{max}: 221 (sh), 248, 258, 325 nm; $[\alpha]_D^{25}$ +114.7 (c 0.20, MeOH); ¹H NMR and ¹³C NMR data: see Table 1; ESIMS (pos.): m/z 579 [M + Na]⁺; HRESIMS (pos.): m/z 579.1681 [M + Na]⁺ (calcd. for C₂₅H₃₂O₁₄Na, 579.1684).

3.4 4′-O-Isobutyroylpeguangxienin (**2**)

White amorphous powder; UV (MeOH) λ_{max}: 218, 256 (sh), 296 (sh), 323 nm; $[\alpha]_D^{25}$ +38.8 (c 0.20, MeOH); ¹H NMR and ¹³C NMR data: see Table 2; ESIMS (pos.): m/z 437 [M + Na]⁺; HRESIMS (pos.): m/z 437.1573 [M + Na]⁺ (calcd. for C₂₃H₂₆O₇Na, 437.1571).

3.5 6-(3-Methyl-2-oxobutyroyl)-7-methoxycoumarin (**3**)

Yellow amorphous powder; UV (MeOH) λ_{max}: 215 (sh), 227 (sh), 261, 308, 340 nm; ¹H NMR and ¹³C NMR data: see Table 3; ESIMS (pos.): m/z 297 [M + Na]⁺;

Table 3 NMR spectroscopic data for 6-(3-methyl-2-oxobutyroyl)-7-methoxycoumarin (**3**) and 6-hydroxycoumurrayin (**4**) in CDCl₃ (δ_H 7.26, δ_C 77.0 ppm)

No.	**3**		**4**	
	δ_H	δ_C	δ_H	δ_C
2		159.7 (s)		161.2 (s)
3	6.33 (d, 9.6)	114.6 (d)	6.33 (d, 9.6)	114.5 (d)
4	7.69 (d, 9.6)	143.0 (d)	7.92 (d, 9.6)	138.3 (d)
4a		113.0 (s)		110.0 (s)
5	7.97 (s)	131.1 (d)		140.9 (s)
6		120.9 (s)		138.4 (s)
7		162.2 (s)		149.4 (s)
8	6.83 (s)	100.1 (d)		118.9 (s)
8a		159.3 (s)		146.0 (s)
1′		193.6 (s)	3.50 (d, 7.0)	22.5 (t)
2′		205.6 (s)	5.22 (br t, 7.0)	121.3 (d)
3′	3.15 (heptet, 7.0)	36.3 (d)		132.8 (s)
4′	1.24 (d, 7.0)	17.2 (q)	1.68 (br s)	25.7 (q)
5′	1.24 (d, 7.0)	17.2 (q)	1.83 (br s)	18.0 (q)
5-OCH₃			3.96 (s)	62.1 (q)
6-OH			5.56 (s)	
7-OCH₃	3.89 (s)	56.5 (q)	3.91 (s)	61.7 (q)

HRESIMS (pos.): m/z 297.0737 [M + Na]⁺ (calcd. for C₁₅H₁₄O₅Na, 297.0733).

3.6 6-Hydroxycoumurrayin (**4**)

White amorphous powder; UV (MeOH) λ_{max}: 232 (sh), 307, 357 (sh) nm; ¹H NMR and ¹³C NMR data: see Table 3; ESIMS (pos.): m/z 313 [M + Na]⁺; HRESIMS (pos.): m/z 313.1042 [M + Na]⁺ (calcd. for C₁₆H₁₈O₅Na, 313.1046).

Table 4 Plant material and isolation

	Family	Species	Plant parts	Place of origin	Plant wt. (kg)	Extract wt. (g)	Compd. wt. (mg)
1	Apiaceae	*H. stenopterum*	Whole plant	Yunnan, CN	5.5	460	20
2	Apiaceae	*P. praeruptorum*	Roots	Sichuan, CN	10.0	1300	234
3	Rutaceae	*C. lansium*	Twigs and leaves	Yunnan, CN	12.0	200	201
4	Rutaceae	*M. paniculata*	Twigs and leaves	Yunnan, CN	8.5	700	23

3.7 Cytotoxicity Assays

Five human tumor cell lines (HL-60, A-549, SMMC-7721, MCF-7, and SW-480) obtained from ATCC (Manassas, VA, USA) were used in the cytotoxicity assay. All cells were cultured in RPMI-1640 or DMEM medium (Hyclone, Logan, UT, USA), supplemented with 10 % fetal bovine serum (Hyclone) at 37 °C in a humidified atmosphere containing 5 % CO_2. Cell viability was assessed by conducting colorimetric measurements of the amount of insoluble formazan formed in living cells based on the reduction of MTS (Sigma, St. Louis, MO, USA). Briefly, 100 μL of adherent cells were seeded into each well of a 96-well cell culture plate and allowed to adhere for 12 h before drug addition, while suspended cells were seeded just before drug addition, both with an initial density of 1×10^5 cells/mL in 100 μL medium. Each cell line was exposed to the test compound at various concentrations in triplicate for 48 h, with cisplatin and paclitaxel as positive controls. After the incubation, 20 μL MTS and 100 μL medium was added to each well after removal of 100 μL medium, and the incubation continued for 2–4 h at 37 °C. The optical density was measured at 492 nm using a Multiskan FC plate reader (Thermo Scientific, USA). The IC_{50} value of each compound was calculated according to the Reed and Muench method.

Compliance with Ethical Standards

References

1. M. Berenbaum, Ecology **62**, 1254–1266 (1981)
2. A.I. Gray, P.G. Waterman, Phytochemistry **17**, 845–864 (1978)
3. Ł. Cieśla, A. Bogucka-Kocka, M. Hajnos, A. Petruczynik, M. Waksmundzka-Hajnos, J. Chromatogr. A **1207**, 160–168 (2008)
4. P. Sarkhail, J. Ethnopharmacol. **156**, 235–270 (2014)
5. I. Kostova, Curr. Med. Chem. -Anti-Cancer Agents **5**, 29–46 (2005)
6. F. Wang, X.L. Cheng, Y.J. Li, S. Shi, J.K. Liu, J. Nat. Prod. **72**, 2005–2008 (2009)
7. F. Wang, M.F. Mao, G.Z. Wei, Y. Gao, F.C. Ren, J.K. Liu, Phytochemistry **95**, 428–435 (2013)
8. K. Panthong, Y. Srisud, V. Rukachaisirikul, N. Hutadilok-Towatana, S.P. Voravuthikunchai, S. Tewtrakul, Phytochemistry **88**, 79–84 (2013)
9. M. Hisamoto, H. Kikuzaki, H. Ohigashi, N. Nakatani, J. Agric. Food Chem. **51**, 5255–5261 (2003)
10. W.L. Xiao, S.H. Li, Y.H. Shen, X.L. Li, H.D. Sun, Heterocycles **65**, 1189–1196 (2005)
11. X.M. Niu, S.H. Li, L.X. Wu, L. Li, L.H. Gao, H.D. Sun, Planta Med. **70**, 578–581 (2004)
12. Y.F. Zou, M. Lobera, B.B. Snider, J. Org. Chem. **70**, 1761–1770 (2005)
13. F. Wang, X.L. Li, G.Z. Wei, F.C. Ren, J.K. Liu, Nat. Prod. Bioprospect. **3**, 238–242 (2013)
14. H. Matsuda, T. Murakami, N. Nishida, T. Kageura, M. Yoshikawa, Chem. Pharm. Bull. **48**, 1429–1435 (2000)
15. I.S. Chen, C.T. Chang, W.S. Sheen, C.M. Teng, I.L. Tsai, C.Y. Duh, F.N. Ko, Phytochemistry **41**, 525–530 (1996)
16. H.N. Lv, S. Wang, K.W. Zeng, J. Li, X.Y. Guo, D. Ferreira, J.K. Zjawiony, P.F. Tu, Y. Jiang, J. Nat. Prod. **78**, 279–285 (2015)