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New phloroglucinol derivatives from the whole plant of *Hypericum uralum*

Xiaoqing Li, Yun Li, Jianguang Luo*, Zhongbo Zhou, Guimin Xue, Lingyi Kong*

Jiangsu Key Laboratory of Bioactive Natural Product Research, State Key Laboratory of Natural Medicines, China Pharmaceutical University, 24 Tong Jia Xiang, Nanjing 210009, People's Republic of China

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ABSTRACT

Seven new phloroglucinol derivatives, uraliones L-R (**1**–**7**), were isolated from the whole plant of *Hypericum urialum*. The structures of new compounds were unambiguously elucidated by comprehensive spectroscopic analysis, and the absolute configurations of the *vic*-diol groups were determined by $Mo_2(OAc)_4$-induced electronic circular dichroism experiments. Their capability to reversal the multidrug resistance in doxorubicin-induced resistant MCF-7/DOX sublines showed that compounds **1**, **5**, **6**, **7** exerted a potential effect of doxorubicin susceptibility.

1. Introduction

Investigations of the constituents from the family Guttiferae have revealed a number of biologically and structurally interesting secondary metabolites, including naphtodianthrones, flavonoids, xanthones, benzophenones, and phloroglucinol derivatives [1–5]. Among them, polycyclic polyprenylated acylphloroglucinols (PPAPs) are prominent characteristic metabolites, and feature a highly oxygenated and densely substituted skeleton decorated with prenyl or geranyl side chains [6]. Numerous literatures have been reported that PPAPs exhibit a broad spectrum of pharmacological activities, such as anti-neurodegenerative, antibacterial, anti-inflammatory, antioxidant, and anti-angiogenic activities [7–11]. *Hypericum uralum* Buch.-Ham. ex D. Don is distributed mainly in Tibet and northwest of Yunnan Province, P. R. China. The whole plant of *H. uralum* is used for antiphlogosis, detoxification and antipruritic in the Yunnan province [12]. In our previous study, a series of new PPAPs, uraliones A-K, have been isolated from the petroleum ether extract of the whole plant of *H. uralum,* some of them showed significant protective effects against induced injury in PC12 cells [13]. As part of our ongoing search for bioactive PPAPs from natural sources, further investigations on *H. uralum* led to the isolation of seven new compounds, namely uraliones L-R (**1**–**7**) (Fig. 1). Herein, we report the isolation, structural elucidation, and multidrug resistance (MDR) reversal activity of these isolates.

2. Experimental

2.1. General

Optical rotations were measured with a JASCO P-1020 polarimeter (Jasco, Tokyo, Japan). UV spectra were obtained on a UV-2450 UV/Vis spectrophotometer (Shimadzu, Tokyo, Japan). IR spectra (KBr disks, in cm^{-1}) were acquired using a Bruker Tensor 27 spectrometer (Bruker, Karlsruhe, Germany). A JASCO *J*-810 spectropolarimeter (Jasco, Tokyo, Japan) was used to record ECD spectra. NMR spectra were acquired in $CDCl_3$ at 303 K on Bruker Avance III NMR spectrometer (^{1}H NMR: 500 MHz or 600 MHz; ^{13}C NMR: 125 MHz or 150 MHz) with TMS as the internal standard. Chemical shifts (δ) and coupling constants (*J*) are expressed in parts per million (ppm) and hertz (Hz), respectively. ESIMS data were obtained using an Agilent 1100 Series LC/MSD Trap mass spectrometer, and HRESIMS data were collected using an Agilent 6520B UPLC-Q-TOF mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). Silica gel (100–200 and 200–300 mesh; Qingdao Haiyang Chemical Co., Ltd., Qingdao, China), Sephadex LH-20 (40–70 μm; Amersham Pharmacia Biotech AB, Uppsala, Sweden), and Fuji ODS RP-C_{18} (40–63 μm, Tokyo, Japan) were applied to column chromatography. TLC (GF_{254}, Qingdao Haiyang Chemical Co. Ltd., Qingdao, China) were used for monitoring fractions. Spots were visualized under UV light and by spraying the plates with a 1% vanillin-sulfuric acid solution, followed by heating. All solvents used were of analytical grade (Jiangsu Hanbang Sci. & Tech. Co., Ltd.). Preparative HPLC was performed on a Shimadzu LC-6AD Series instrument equipped with a Shim-pack RP-C_{18} column (20 mm × 200 mm, i.d., 10 μm, Shimadzu, Tokyo, Japan) and 1100 Series Multiple Wavelength

* Corresponding authors.
E-mail addresses: luojg99@163.com (J. Luo), cpu_lykong@126.com (L. Kong).

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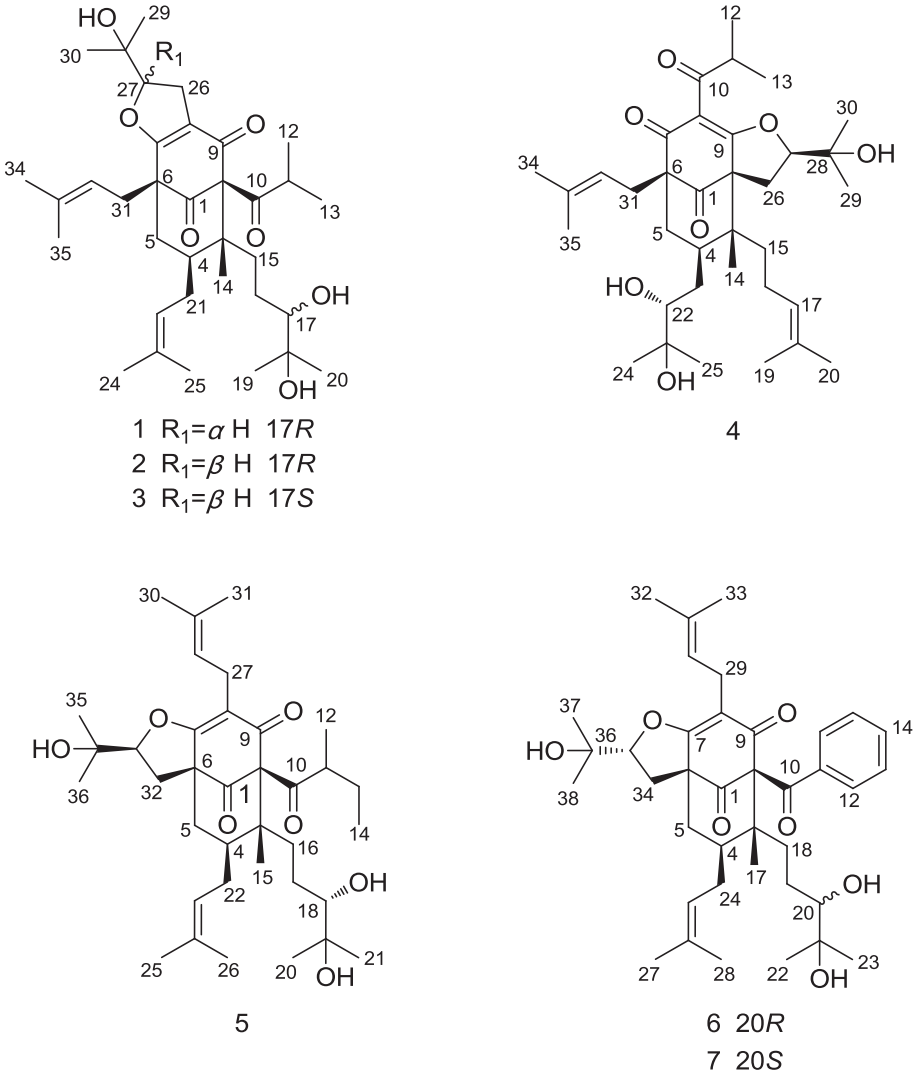

Fig. 1. Structures of compounds **1**–**7**.

Detector. Recycling preparative HPLC was carried out on a Shimadzu LC-20A Series instrument equipped with a Shim-pack RP-C_{18} column (20 mm × 200 mm, i.d., 5 μm, Shimadzu, Tokyo, Japan).

2.2. Plant material

The whole plants of *H. uralum* were collected in Yunnan Province, People's Republic of China, in June 2013 and were authenticated by Prof. Mian Zhang, School of Traditional Chinese Pharmacy, China Pharmaceutical University. Voucher specimens (2013-SEJST) were deposited at the Jiangsu Key Laboratory of Bioactive Natural Product Research, China Pharmaceutical University.

2.3. Extraction and isolation

The air-dried and powdered plant of *H. uralum* (10.0 kg) were extracted by refluxing with 95% aqueous EtOH (3 × 30 L), and then subsequently concentrated under reduced pressure. The obtained crude extract (1.3 kg) was suspended in H_2O (3 L), and partitioned successively with petroleum ether (3 × 3 L) and CH_2Cl_2 (3 × 3 L). The petroleum ether fraction (197 g) was loaded onto silica gel column chromatography (CC) and eluted with a gradient solvent system of petroleum ether-EtOAc (1:0 to 0:1) to yield five fractions (Fr. I-V). Fr. V (18 g) was subsequently chromatographed on a silica gel column using petroleum ether-EtOAc (3:1 to 0:1) as the eluent to obtain six fractions (Fr. VA-VF). Fr. VE (2.1 g) was further separated to Sephadex LH-20 CC (CH_2Cl_2-MeOH, 1:1) to produce three fractions (Fr. VE1–VE3). Fr. VE3 (728 mg) was subjected to an ODS column using the isocratic system of 75% MeOH-H_2O to afford seven fractions (Fr. VE3a–VE3g). Fr. VE3d (86 mg) was then purified by preparative HPLC (MeOH-H_2O, 80:20), followed by recycling HPLC (MeCN-H_2O, 70:30) to yield compounds **1** (12.0 mg), **2** (7.0 mg), **3** (13.0 mg), and **4** (3.2 mg).

The CH_2Cl_2 fraction (106 g) was subjected to chromatography over MCI gel, eluted with a stepwise gradient of MeOH-H_2O (75% to 100%) to provide five fractions (Fr. A-E). Fr. A (29 g) was chromatographed onto a silica gel column, eluted with petroleum ether-EtOAc (10:1 to 0:1), to obtained six fractions (Fr. A1-A6). Fr. A3 (2 g) was separated on a Sephadex LH-20 CC (CH_2Cl_2-MeOH, 1:1) to yield six fractions (Fr. A3a-A3f). Fr. A3a (105 mg) was purified by preparative HPLC (MeOH-H_2O, 75:25), and then by recycling HPLC (MeCN-H_2O, 65:35) to yield compounds **5** (3.5 mg), **6** (4.5 mg) and **7** (10.0 mg).

2.4. Spectroscopic data

2.4.1. Uralione L (1)

Colorless oil; $[\alpha]_D^{25}$ − 29.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.86), 282 (3.94) nm; CD (DMSO) λ_{max} ($\Delta\varepsilon$) 279 (+ 28.1), 302 (− 32.7), 331 (+ 4.3) nm; IR (KBr) ν_{max} 3433, 2974, 2929, 2875, 1726, 1614, 1447, 1408, 1382 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Tables 1 and 2; HRESIMS *m/z*

Table 1
^{1}H NMR data of compounds **1–7**.

Position	1[a]	2[a]	3[a]	4[a]	5[b]	6[a]	7[a]
4	1.67, m	1.60, m	1.70, m	1.60, m	1.61, m	1.84, m	1.86, m
5	1.95, dd (13.6, 4.3)	1.90, dd (13.6, 4.1)	1.90, dd (13.0, 4.0)	1.88, dd (13.5, 4.5)	2.04, m	2.14, m	2.12, m
	1.43, t (13.1)	1.45, t (13.3)	1.44, t (12.9)	1.42, t (13.5)	1.51, t (13.0)	1.63, overlapped	1.62, overlapped
11	2.09, m	2.17, m	2.15, m	2.50, m	1.73, m		
12	1.14, d (6.0)	1.11, d (6.5)	1.13, d (6.5)	1.15, d (6.5)	1.10, d (6.4)	7.43, d (7.4)	7.42, d (7.4)
13	1.04, d (6.0)	1.04, d (6.5)	1.04, d (6.5)	1.16, d (6.5)	1.67, m	7.22, t (7.8)	7.22, t (7.8)
					1.29, m		
14	1.04, s	1.04, s	1.04, s	1.11, s	0.77, t (7.4)	7.39, t (7.4)	7.39, t (7.4)
15	2.26, m	1.95, m	2.19, m	2.11, m	1.07, s	7.22, t (7.8)	7.22, t (7.8)
	1.75, m	1.74, m	1.74, m	1.62, m			
16	1.78, m	1.96, m	1.90, dd (13.0, 4.0)	2.17, m	2.04, m	7.43, d (7.4)	7.42, d (7.4)
	1.78, m	1.73, m	1.64, m	1.88, dd (13.5, 4.5)	1.93, m		
17	3.20, br d (9.5)	3.22, dd (10.0, 1.5)	3.20, br d (10.4)	4.99, t (6.4)	2.15, d (12.4)	1.20, s	1.20, s
18					3.25, d (10.0)	2.31, m	2.12, m
						1.84, m	1.30, m
19	1.19, s	1.18, s	1.19, s	1.67, s		2.14, m	2.12, m
						1.54, m	1.30, m
20	1.11, s	1.13, s	1.12, s	1.65, s	1.13, s	3.28, br d (9.8)	3.28, br d (9.8)
21	2.08, m	2.04, dd (14.3, 5.3)	2.05, dd (13.5, 5.2)	1.53, m	1.19, s		
	1.77, m	1.72, m	1.74, m	1.07, m			
22	4.94, t (6.7)	4.92, t (7.1)	4.91, t (6.5)	3.29, d (10.3)	2.15, m	1.15, s	1.14, s
					1.78, m		
23					4.93, t (7.0)	1.19, s	1.20, s
24	1.57, s	1.55, s	1.56, s	1.17, s		2.14, m	2.21, m
						1.54, m	1.86, m
25	1.70, s	1.67, s	1.67, s	1.13, s	1.70, s	4.99, t (7.5)	4.98, t (7.5)
26	2.97, d (9.2)	2.98, dd (15.0, 10.4)	2.99, dd (15.0, 10.5)	2.99, dd (10.5, 4.5)	1.57, s		
		2.89, dd (15.0, 7.8)	2.89, dd (15.0, 7.6)				
27	4.85, t (9.2)	4.74, dd (10.4, 7.8)	4.76, dd (10.4, 7.7)	4.80, t (10.5)	3.15, dd (14.4, 6.8)	1.72, s	1.72, s
					3.03, dd (14.3, 7.6)		
28					5.05, t (7.2)	1.60, s	1.60, s
29	1.18, s	1.23, s	1.24, s	1.25, s		3.14, dd (14.2, 6.5)	3.14, dd (14.2, 6.5)
						3.07, dd (14.2, 8.1)	3.07, dd (14.2, 8.1)
30	1.32, s	1.28, s	1.28, s	1.37, s	1.70, s	5.06, t (7.3)	5.05, t (7.3)
31	2.54, br d (15.0)	2.52, dd (14.6, 6.6)	2.53, dd (14.5, 6.4)	2.50, dd (13.0, 6.5)	1.65, s		
	2.47, dd (15.0, 9.0)	2.43, dd (14.5, 7.8)	2.43, dd (14.6, 7.9)	1.21, m			
32	4.98, br d (7.7)	5.05, t (7.1)	5.05, t (7.1)	4.99, t (6.4)	2.66, dd (12.9, 11.0)	1.67, s	1.67, s
					1.78, m		
33					4.57, dd (10.9, 5.6)	1.66, s	1.66, s
34	1.70, s	1.68, s	1.68, s	1.65, s		2.68, dd (13.2, 10.5)	2.70, dd (13.0, 10.5)
						1.84, m	1.86, m
35	1.69, s	1.67, s	1.67, s	1.58, s	1.22, s	4.64, dd (10.4, 6.0)	4.65, dd (10.4, 6.0)
36					1.40, s		
37						1.23, s	1.23, s
38						1.40, s	1.40, s

[a] Data were recorded in $CDCl_3$ at 500 MHz.
[b] Data were recorded in $CDCl_3$ at 600 MHz (δ in ppm, J in Hz).

609.3763 [M + Na]$^+$ (calcd. for $C_{35}H_{54}O_7Na$, 609.3762).

2.4.2. Uralione M (2)

Colorless oil; $[\alpha]_D^{25}$ + 28.6 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.90), 282 (3.97) nm; CD (DMSO) λ_{max} ($\Delta\varepsilon$) 279 (+ 35.8), 303 (− 32.1), 337 (+ 2.6) nm; IR (KBr) ν_{max} 3434, 2973, 2930, 1726, 1618, 1448, 1408, 1381 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Tables 1 and 2; HRESIMS *m/z* 609.3761 [M + Na]$^+$ (calcd. for $C_{35}H_{54}O_7Na$, 609.3762).

2.4.3. Uralione N (3)

Colorless oil; $[\alpha]_D^{25}$ + 38.8 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.92), 282 (3.99) nm; CD (DMSO) λ_{max} ($\Delta\varepsilon$) 277 (+ 43.9), 303 (− 35.8), 335 (+ 3.5) nm; IR (KBr) ν_{max} 3433, 2973, 2930, 2875, 1727, 1613, 1447, 1408, 1381 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Tables 1 and 2; HRESIMS *m/z* 609.3765 [M + Na]$^+$ (calcd. for $C_{35}H_{54}O_7Na$, 609.3762).

2.4.4. Uralione O (4)

Colorless oil; $[\alpha]_D^{25}$ + 91.4 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.81), 281 (3.83) nm; CD (DMSO) λ_{max} ($\Delta\varepsilon$) 279 (+ 71.5), 305 (− 29.9), 340 (+ 1.7) nm; IR (KBr) ν_{max} 3435, 2973, 2929, 1724, 1615, 1446, 1383 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Tables 1 and 2; HRESIMS *m/z* 609.3759 [M + Na]$^+$ (calcd. for $C_{35}H_{54}O_7Na$, 609.3762).

2.4.5. Uralione P (5)

Colorless oil; $[\alpha]_D^{25}$ + 27.2 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 203 (3.84), 272 (3.78) nm; CD (DMSO) λ_{max} ($\Delta\varepsilon$) 276 (+ 31.7), 302 (− 11.2), 334 (+ 3.6) nm; IR (KBr) ν_{max} 3434, 2974, 2930, 1729, 1624, 1454, 1381 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) and ^{13}C NMR ($CDCl_3$, 150 MHz) data, see Tables 1 and 2; HRESIMS *m/z* 623.3916 [M + Na]$^+$ (calcd. for $C_{36}H_{56}O_7Na$, 623.2918).

2.4.6. Uralione Q (6)

Colorless oil; $[\alpha]_D^{25}$ − 23.4 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.15), 247 (3.94), 273 (3.84) nm; CD (DMSO) λ_{max} ($\Delta\varepsilon$) 271 (− 51.5), 302 (+ 4.6) nm; IR (KBr) ν_{max} 3435, 2974, 2927, 1727, 1695, 1622, 1448, 1382 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Tables 1 and 2; HRESIMS *m/z* 643.3606 [M + Na]$^+$ (calcd. for $C_{38}H_{52}O_7Na$, 643.3605).

Table 2
^{13}C NMR data of compounds **1–7**.

Position	1[a]	2[a]	3[a]	4[a]	5[b]	6[a]	7[a]
1	206.0	205.8	205.8	205.5	204.1	204.7	204.6
2	83.3	83.2	83.2	74.2	82.8	79.3	79.2
3	48.6	49.0	48.6	47.6	48.9	50.1	49.8
4	41.2	42.4	41.5	40.9	41.7	42.1	42.1
5	38.4	37.9	37.7	37.5	37.8	38.6	38.5
6	54.8	54.8	55.1	63.7	59.5	60.0	60.1
7	177.5	177.3	178.2	190.6	173.8	172.8	173.2
8	119.5	118.2	118.2	120.7	116.8	116.1	116.2
9	188.1	187.8	188.3	171.7	193.7	193.6	193.9
10	209.2	210.1	209.5	208.5	209.0	194.3	194.1
11	42.6	42.4	42.4	39.6	48.3	136.8	136.8
12	21.5	21.3	20.4	21.0	16.6	128.2	128.1
13	20.5	20.4	21.4	20.9	27.4	127.9	127.9
14	15.2	14.5	14.9	14.4	11.5	132.2	132.1
15	33.0	34.2	32.9	37.7	14.8	127.9	127.9
16	25.9	25.9	25.7	24.2	33.0	128.2	128.1
17	78.4	79.2	78.3	124.6	27.6	15.1	15.0
18	72.9	72.9	72.9	131.4	78.6	34.0	33.2
19	26.8	27.3	26.2	26.7	72.9	27.9	27.7
20	23.3	24.0	23.5	17.7	23.4	79.2	78.7
21	27.7	27.6	27.4	31.3	26.2	72.9	72.9
22	122.1	122.4	122.3	75.1	27.3	23.6	23.4
23	133.7	133.7	133.7	73.1	122.0	26.2	26.3
24	18.0	18.1	18.0	23.2	133.7	27.3	27.3
25	25.8	25.1	26.0	25.3	25.9	122.0	122.0
26	26.9	27.3	27.2	26.7	18.0	133.8	133.8
27	93.7	92.5	92.1	94.0	22.2	25.9	26.0
28	71.6	71.9	71.8	71.1	121.0	18.1	18.1
29	26.4	26.8	27.1	26.7	132.6	22.1	22.1
30	23.7	23.7	24.2	25.6	25.6	120.5	120.4
31	29.3	29.0	29.0	29.4	17.8	132.6	132.7
32	119.9	118.4	118.6	119.4	30.2	25.7	25.7
33	134.9	135.0	135.0	134.2	90.3	17.9	17.9
34	18.2	18.0	17.9	25.9	70.8	30.3	30.2
35	25.8	25.0	24.9	18.0	24.1	90.3	90.4
36					26.9	71.0	71.0
37						24.0	24.0
38						26.6	26.6

[a] Data were recorded in $CDCl_3$ at 125 MHz.
[b] Data were recorded in $CDCl_3$ at 150 MHz.

2.4.7. Uralione R (7)

Colorless oil; $[\alpha]_D^{25}$ − 32.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.23), 247 (4.06), 274 (3.96) nm; CD (DMSO) λ_{max} ($\Delta\varepsilon$) 271 (− 89.6), 301 (+ 8.8), 337 (+ 1.6) nm; IR (KBr) ν_{max} 3438, 2977, 2931, 1729, 1697, 1622, 1449, 1371 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Tables 1 and 2; HRESIMS *m/z* 643.3604 [M + Na]$^+$ (calcd. for $C_{38}H_{52}O_7Na$, 643.3605).

2.5. Multidrug-resistance reversal assay

The MCF-7/DOX cells were cultured in RPMI 1640 containing 10% fetal bovine serum and antibiotics (100 U/mL of penicillin and 100 μg/mL of streptomycin) at 37 °C in a 5% CO_2 incubator. After digested with trypsin, the cells suspension (5.0×10^3 cells/mL) were distributed evenly into 96-well culture plates and incubated for 24 h. A series of concentrations for the isolates in DMSO were added to each well. The optical density was measured at a test wavelength of 570 nm using a Universal Microplate Reader (Spectramax Plus 384, Molecular Devices, Sunnyvale, CA, USA) [14].

The MDR reversal assay, as previously reported [15], was performed at noncytotoxic concentration against multidrug resistant cells. MCF-7/DOX cells were distributed into 96-well culture plates at 5×10^3 cells/well. A full range of concentrations of DOX with or without samples or 10 μM verapamil (positive control) were incubated with the cells. Then IC_{50} values of DOX were calculated from plotted results using untreated cells as 100%. The reversal fold (RF) value, as a parameter of reversal potency, was calculated from dividing IC_{50} of DOX alone by IC_{50} of DOX in the presence of test compounds with formula as following: reversal fold (RF) = IC_{50} (MCF-7/DOX cells)/IC_{50} (MCF-7/DOX cells combined with sample treatment). Each concentration was tested in three parallel wells.

3. Results and discussion

Uralione L (**1**) was obtained as colorless oil. It showed a pseudo-molecular ion peak at *m/z* [M + Na]$^+$ 609.3763 in the HRESIMS spectrum, corresponding to a molecular formula of $C_{35}H_{54}O_7$ and indicating nine degrees of unsaturation. Its UV spectrum showed two absorption bands at 204 and 282 nm, which was coincided with the characteristic resonances for PPAPs [16]. The IR spectrum clearly implied the presence of a characteristic band for hydroxyl group at 3433 cm^{-1} and a sharp band for unconjugated carbonyl group at 1726 cm^{-1}. The ^{1}H NMR spectrum indicated the presence of two olefinic protons signals at δ_H 4.98 (1H, br d, J = 7.7 Hz) and 4.94 (1H, t, J = 6.7 Hz), nine singlet methyls and two doublet methyls at high-field resonance. The ^{13}C NMR, with the help of HSQC, exhibited the signals for three carbonyl carbons (δ_C 209.2, 206.0, 188.1), six methylenes (δ_C 38.4, 33.0, 29.3, 27.7, 26.9, 25.9), two oxygen-bearing methines (δ_C 93.7, 78.4), and two methines (δ_C 42.6, 41.2). By analyzing the HSQC, HMBC, and ROESY spectra, all the proton and carbon signals (Tables 1 and 2, Fig. 2) were assigned unambiguously. The HMBC correlations from H-27 (δ_H 4.85) to the C-7 (δ_C 177.5), and C-8 (δ_C 119.4), from H-26 (δ_H 2.97) to the C-7 (δ_C 177.5), C-8 (δ_C 119.4), C-9 (δ_C 188.1), C-27 (δ_C 93.7), and C-28 (δ_C 71.6) confirmed that a dihydrofuran ring was formed between the C-7 enolic hydroxy group and the C-8 isoprenyl side chain. Furthermore, cross-peaks of H_2–31 to C-1 (δ_C 205.9), C-5 (δ_C 38.3), and C-6 (δ_C 54.7), of H_2-21 to C-4 (δ_C 41.2), and C-5 (δ_C 38.3) in HMBC spectrum, verified the location of two isoprenyl groups was at C-6, C-4, respectively. The abovementioned spectroscopic data of **1** revealed its structural features were similar to those of the known compound furohyperforin isomer 1 [17], except that the typical isoprenyl group at C-15 was replaced by a 3-methyl-2, 3-pentanediol moiety in compound **1**. This was further evidenced by the HMBC correlations from H_2-16 (δ_H 1.78) to C-3 (δ_C 48.6), C-17 (δ_C 78.4), and from H-17 (δ_H 3.20) to C-15 (δ_C 33.0) in **1**.

The relative configuration of **1** was determined by ROESY experiment, conjugated with the comparative analysis of the NMR data with its analogues. The chemical shift of C-4 (δ_C 41.2) and the chemical shift difference between H-5β and H-5α ($\Delta\delta_H$ ca. 0.52 ppm) suggested that the configuration of H-4 was in α-orientation [18]. The ROESY correlations of Me-14 (δ_H 1.04)/H-11 (δ_H 2.09), Me-14 (δ_H 1.04)/H-5β (δ_H 1.43), and H-5β (δ_H 1.43)/H-31 (δ_H 2.54), suggested that **1** possessed similar relative configuration at C-2, C-3, and C-6 with those of furohyperforin isomer 1. In addition, the diagnostic ROESY cross-peak of Me-30 (δ_H 1.32)/H-32 (δ_H 4.98) indicated that H-27 was α-oriented [19] (Fig. 2). In order to determine the absolute configuration of the

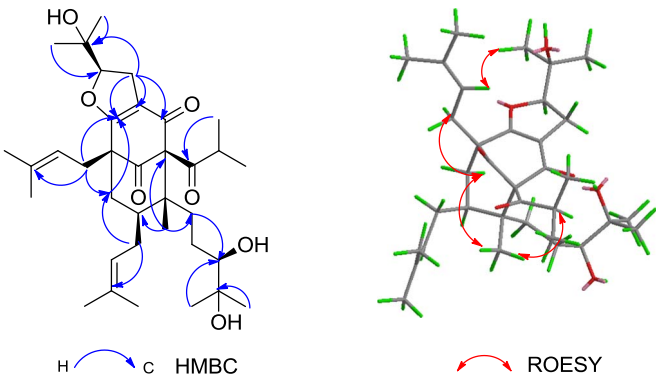

Fig. 2. Selected key HMBC and ROESY correlations of compound **1**.

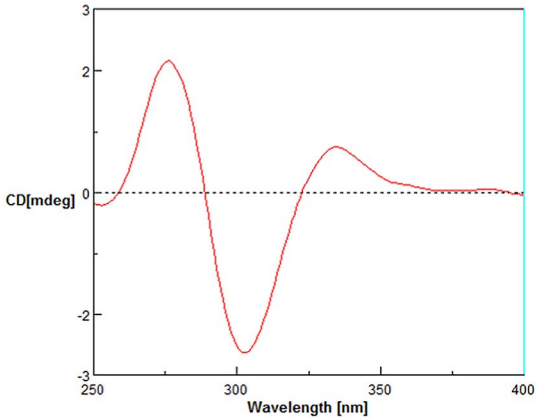

Fig. 3. $Mo_2(OAc)_4$-induced ECD spectrum of **1** in DMSO.

acyclic 17, 18-diol moiety in **1**, the Snatzke's method [20,21], an induced ECD spectrum of in situ complexation with $Mo_2(OAc)_4$ in DMSO solution was applied, in which the sign of the characteristic band at about 310 nm implied the absolute configuration of the chiral centers in the 1, 2-diol moiety [22,23]. Compound **1** showed a negative E band at 303 nm correlating with a 17*R* configuration (Fig. 3). Thus the structure of compound **1** was elucidated as shown in Fig. 1.

Uraliones M (**2**) and N (**3**) were both isolated as colorless oil. Their molecular formula $C_{35}H_{54}O_7$ (nine double bond equivalents), were accommodated collectively by its HRESIMS sodium adduct ion (*m/z* 609.3761 in **2**; *m/z* 609.3765 in **3**) and ^{13}C NMR spectral data. Extensive analysis of their 1D and 2D NMR spectra, the planar structures as well as the stereogenic centers at C-2, C-3, C-4, and C-6 of compounds **2** and **3** were set up as those of compound **1**. Contrary to **1**, no ROESY cross-peak of Me-30/H-32 could be observed in compounds **2** and **3** [19]. However, H-27 showed cross-peaks with Me-29 and Me-30 [24], suggesting that the H-27 should be *β*-oriented. The reported data indicated that the orientation of hydroxypropyl at C-27 resulted in the slight difference of chemical shifts for both proton and carbon at this position. The 27*β*-hydroxypropyl epimer always exhibited chemical shifts at a lower field compared to those of the corresponding 27*α*-hydroxypropyl epimer [16,17,25–28]. It was found that the $\delta_{C/H}$ for C-27 of **2** and **3** ($\delta_{C/H}$ 4.74/92.5 in **2**; 4.76/92.1 in **3**) were at a higher field than those of **1** ($\delta_{C/H}$ 4.85/93.7) and this allowed us to confirm the 27*α*- hydroxypropyl configurations for **2** and **3**. Furthermore, the complex-induced ECD spectrum of compound **2** exhibited a negative E band, indicating the 17*R* configuration, while compound **3** showed a positive E band, coinciding with the 17*S* configuration (Fig. 4). Thus the structures of **2** and **3** were established as depicted in Fig. 1.

Uralione O (**4**), obtained as a colorless oil, was determined to have a molecular formula $C_{35}H_{54}O_7$, by its HRESIMS (*m/z* 609.3759 [M + Na]$^+$) and NMR data, requiring nine double bond equivalents. The ^1H NMR spectrum of **4** revealed the presence of two isoprenyl groups (δ_H 4.99, 2H, t, J = 6.4 Hz), a 2-methylpropionyl moiety [δ_H 2.50 (1H, m), δ_H 1.15 (3H, d, J = 6.5 Hz), δ_H 1.16 (3H, d, J = 6.5 Hz)], a 3-methyl-2, 3-pentanediol moiety [δ_H 3.29 (1H, d, J = 10.3 Hz), δ_H 1.17 (3H, s), δ_H 1.13 (3H, s)], an oxidized 2, 2-dimethyl-2*H*- dihydrofuran ring [δ_H 2.99 (2H, dd, J = 10.5, 4.5 Hz), δ_H 4.80 (1H, t, J = 10.5 Hz), δ_H 1.37 (3H, s), δ_H 1.25 (3H, s)]. These observations indicated that compound **4** was structural similarity to those for attenuatumione E [29]. The significantly difference involving the typical isoprenyl group signals at C-4 was replaced by a set of 3-methyl-2, 3-pentanediol moiety signals in **4**, which was supported by the HMBC correlations from H-21 (δ_H 1.53) to C-4 (δ_C 40.9), C-3 (δ_C 47.6) and C-22 (δ_C 75.1), from H-5 (δ_H 1.88) and H-14 (δ_H 1.11) to C-4 (δ_C 40.9), from H-4 (δ_H 1.60) to C-3 (δ_C 47.6). The ROESY cross-peak of H-27 (δ_H 4.80) with H-15 (δ_H 2.11) indicated that H-27 was *α*-oriented (Fig. S4-9). The in situ $Mo_2(OAc)_4$ complex-induced ECD spectrum of **4** exhibited a negative E band at about 310 nm, indicating a 17*R* configuration (Fig. S0). Therefore, the structure of **4** was assigned as shown in Fig. 1.

Uralione P (**5**) was isolated as a colorless oil. The observed pseudomolecular ion at *m/z* 623.3916 [M + Na]$^+$ in the HRESIMS spectrum and ^{13}C NMR data gave a molecular formula of $C_{36}H_{56}O_7$, indicating nine degrees of unsaturation. Comparison the ^1H NMR and ^{13}C NMR data with those of furoadhyperforin [17], revealed that they differed from the appearance of an oxygenated methine proton (δ_H 3.25, 1H, d, J = 10.0 Hz) and two oxygenated carbons (δ_C 78.6 and δ_C 72.9) in compound **5**. The key HMBC correlations from H-16 to C-2 (δ_C 82.8), C-3 (δ_C 48.9), C-4 (δ_C 41.7), C-15 (δ_C 14.8), C-17 (δ_C 27.6), and C-18 (δ_C 78.6), from H-18 to C-16 (δ_C 33.0), C-17 (δ_C 27.6), and C-19 (δ_C 72.9) indicated that the 3-methyl-2, 3-pentanediol moiety was located at C-3. ROESY cross-peaks of H-5*β*/H-15 and Me-35/H-5*β* indicated that H-33 was in *α*-orientation (Fig. S5-9). The in situ $Mo_2(OAc)_4$ complex-induced ECD spectrum of **5** exhibited a positive E band, consistent with a 18*S* configuration (Fig. S0). Therefore, the structure of **5** was depicted as shown in Fig. 1.

Uraliones Q (**6**) and R (**7**) were both acquired as colorless oil. Based on the sodium adduct ion (*m/z* 643.3606 in **6**; *m/z* 643.3604 in **7**) and ^{13}C NMR data, establishing the molecular formula of **6** and **7** as $C_{38}H_{52}O_7$ with thirteen indices of hydrogen deficiency. The 1D and 2D NMR data of compounds **6** and **7** closely resembled with those of uralione E [13], the only difference lay in that the isoprenyl group at C-18 was oxidized to a 3-methyl-2, 3-pentanediol moiety. Comprehensive analysis of the ROESY spectrum indicated that compounds **6** and **7** possessed the *β*-orientation configuration of H-35 on the basis of the correlations of H-17/H-5*β*, H-35/H-5*β*. The 20*R* configuration in **6** was determined by the induced negative cotton effect, while the positive cotton effect of **7** suggested the 20*S* configuration in **7** (Fig. S0). Thus the structures of **6** and **7** were deduced as shown in Fig. 1.

Compounds **1–7** were other new PPAPs from the whole plant of *H. uralum*. From a biosynthetic perspective, polyprenylated acylphloroglucinols are considered to be produced by prenylation of acylphloroglucinols, which was formed by condensation of one acyl-CoA and three malonyl-CoA units, and subsequently by intramolecular cyclization [6]. The biogenetic starter can be an acyl group or a benzoyl group, both of them are found in the *Hypericum* species [29,31,34]. Compounds **1–5**, with an alkyl starter, maybe derived from leucine or isoleucine [7], while compounds **6** and **7**, with an aromatic starter, maybe derived from phenylalanine [35]. Although many studies on the biosynthesis of PPAPs have been reported, the precursor requires more in-depth research works.

Many phloroglucinol derivatives have been reported to possess moderate to significant cytotoxic activities against different cancer cell lines [30,31], as well as MDR cancer cell line [32,33]. Compounds **1–7** were investigated for their reversal activity towards multidrug resistance in available MCF-7/DOX cancer cells. Among them, compounds **2–4** exhibited inhibition rate less than 50% at the tested

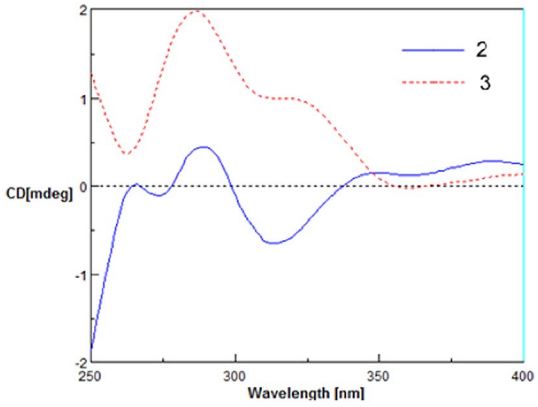

Fig. 4. $Mo_2(OAc)_4$-induced ECD spectra of compounds **2** and **3** in DMSO.

Table 3
MDR reversal effects of the isolates on MCF-7/DOX cells.

Sample	IC_{50} (μM)	RF value
1 (10 μM)	7.72 ± 1.10	4.8
5 (30 μM)	7.48 ± 1.25	4.9
6 (30 μM)	2.32 ± 0.67	16.0
7 (30 μM)	5.61 ± 0.64	6.6
DOX	37.14 ± 3.73	1.0
Ver (10 μM)	1.17 ± 0.12	31.7

Verapamil was used as the positive control at 10 μM.

concentration of 50.0 μM and was considered to be inactive. As shown in Table 3, compounds **5**, **6**, and **7** exerted reversal activity of doxorubicin susceptibility at the tested concentration of 30 μM [Reverse fold (RF): 4.9, 16.0, 6.6-fold, respectively], as compared with the positive control, verpamil (10 μM, RF = 31.7). Compound **1** displayed moderate cytotoxicity against MCF-7/DOX cell at 30 μM, allowing us to assay its reversal effect at the lower concentration of 10.0 μM (RF = 4.8). These results further support the potential of this family of compounds for overcoming MDR in cancer therapy.

Conflict of interest

The authors declare no competing financial interests.

Acknowledgments

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Appendix A. Supplementary data

The original HRESIMS, UV, IR, 1D NMR, 2D NMR, and ECD spectra for compounds **4**–**7** are available as Supporting Information. Supplementary data to this article can be found online at https://doi.org/10.1016/j.fitote.2017.09.020.

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