Polycyclic Polyprenylated Acylphloroglucinols and Chromone *O*-Glucosides from *Hypericum henryi* subsp. *uraloides*

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Two new *C(30)*-epimeric polycyclic polyprenylated acylphloroglucinols (PPAPs), named uralodins B and C (**1** and **2**, resp.), were isolated from the aerial parts of *Hypericum henryi* subsp. *uraloides* together with two new chromone glucosides, urachromones A and B (**3** and **4**, resp.), as well as 16 known compounds. Their structures were established by extensive NMR techniques and MS analysis. The epimers **1** and **2** always behaved like a single compound when examined by TLC, and were separated by HPLC. Their configuration was distinguished by comparative analysis of the NMR data with known analogues together with the ROESY experiment. All the isolated PPAPs were evaluated for their cytotoxic activities against HepG2, SGC7901, HL-60, and K562 cell lines. Compound **1** showed modest cytotoxic activities against SGC7901 and HL-60 cell lines, and **2** showed modest cytotoxic activities against HepG2, SGC7901, HL-60, and K562 cell lines.

Introduction. – Polycyclic polyprenylated acylphloroglucinols (PPAPs), with a highly oxygenated and densely substituted bicyclo[3.3.1]nonane-2,4,9-trione or bicyclo[3.2.1]octane-2,4,8-trione core decorated with C_5H_9 or $C_{10}H_{17}$ (prenyl, geranyl, *etc.*) side chains, are a kind of natural products mainly from *Hypericum*, *Clusia*, and *Garcinia* species of the family Guttiferae [1]. This type of compounds showed a wide variety of biological activities such as antimicrobial [2], antidepressant [3], antioxidant [4], cytotoxic [5], and anti-HIV activities [6]. According to the literature, 119 PPAPs have been found in natural products by 2006 [1]. Hyperforin, as the most famous PPAP compound, is one of the active constituents of *H. perforatum* extract clinically used for the treatment of depression [7]. In addition, hyperforin is also well known for its antibacterial [8], anti-inflammatory [8], and antitumoral activity [9]. To search for more bioactive analogues of hyperforin, many investigations about the PPAP constituents of *Clusia*, *Garcinia*, and *Hypericum* species have been conducted and a number of structurally and biologically interesting PPAPs have been found such as garsinol [10], chamones I and II [11], garcinielliptone HF [12], 15,16-dihydro-16-hydroperoxyplukenetione F [13], tomoeones A–H [14], and sinaicinone [15].

To identify new bioactive PPAP constituents, a phytochemical and biological study of *Hypericum* plants was carried out in our laboratory. The genus *Hypericum*, one of the main resources for PPAP compounds, is widely distributed in the world and comprises about 400 species. Among them, 55 species are growing in China [16]. Many

species of *Hypericum* are used in China for the treatment of various kinds of diseases such as bacterial diseases, infectious hepatitis, gastrointestinal disorder, and tumors [17][18]. *H. henryi* subsp. *uraloides* (REHDER) N. ROBSON is mainly distributed in the southwest of China and middle Burma [19]. One PPAP compound, uralodin A, has been previously isolated from this plant collected in Jinping County, Yunnan Province, P. R. China [20]. In the current study, the MeOH extract of this plant collected in Lüchun County was investigated, and two new C(30) epimeric PPAPs (**1** and **2**) were identified together with two new chromone glucosides, **3** and **4**, and 16 other known compounds. Herein, we report on the isolation and structural elucidation of the new compounds and the cytotoxic testing of the PPAP constituents.

Results and Discussion. – 1. *Structure Elucidation*. The MeOH extract of the aerial part of *H. henryi* subsp. *uraloides* was subjected to column chromatography on silica gel, *Sephadex LH-20*, *RP-18*, as well as preparative HPLC to afford four new compounds, **1**–**4** (*Fig. 1*), and 16 known compounds: uralodin A (**5**) [20], furohyperforin (**6**) [21], quercetin [22], quercetin 3-*O*-α-L-rhamnofuranoside [23], quercetin 3-*O*-β-D-glucuronopyranoside [24], 3,4-epoxy-5-hydroxycyclohex-1-enecarboxylic acid

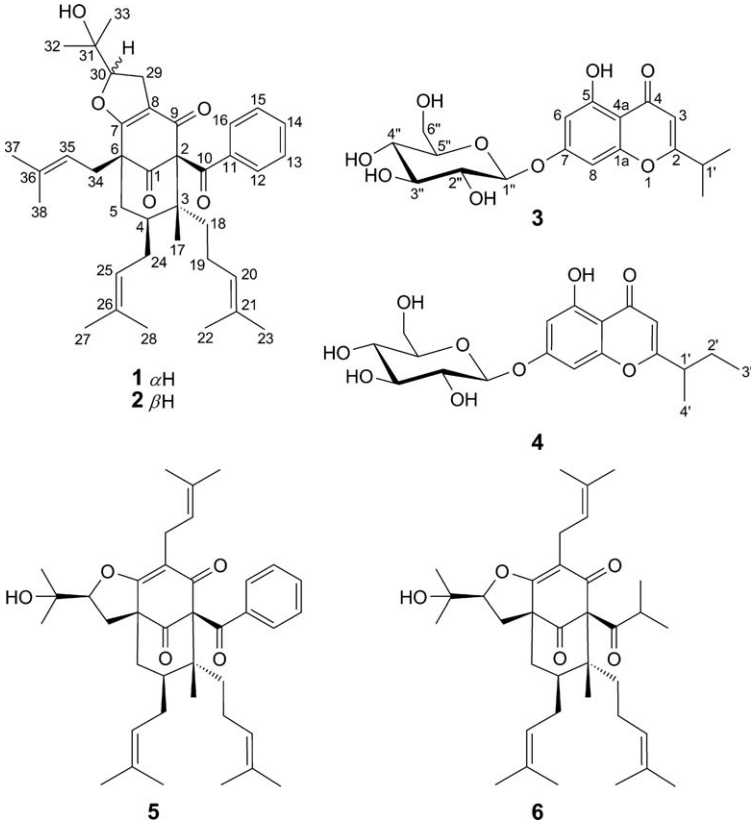

Fig. 1. *Structures of compounds* **1**–**6**

[25], 1,5,6-trihydroxy-3-methoxyxanthone [26], 1,3,7-trihydroxyxanthone [27], 1,3,5-trihydroxyxanthone [28], calycinoxanthone D [29], 1,3,5,8-tetrahydroxyxanthone [29], 1-hydroxyxanthone [30], 2,5-dihydroxyxanthone [31], kielcorin [32], 1,3,5,6-tetrahydroxy-4-prenylxanthone [33], and 'epicatechin-(7,8-*bc*)-4β-(hydroxyphenyl)-dihydroxy-2(3*H*)-pyranone' [34].

Uralodin B (**1**), a colorless oil, had the molecular formula $C_{38}H_{50}O_5$ based on HR-ESI-MS (*m*/*z* 585.3594 ($[M-H]^-$; calc. 585.3580)) and ^{13}C-NMR data analysis. The IR spectrum showed broad bands for OH (3439 cm^{-1}) and conjugated CO (1628 cm^{-1}) groups. The ^{13}C-NMR and DEPT data (*Table 1*) exhibited the signals for two conjugated CO groups (δ(C) 188.2 and 194.7), a nonconjugated CO group (δ(C) 207.5), a benzene ring (δ(C) 129.0, 128.6, 137.9, and 132.8), one angular Me group (δ(C) 14.8), two sp^3 CH_2 groups (δ(C) 39.8 and 37.4), one sp^3 CH group (δ(C) 43.0), three sp^3 quaternary C-atoms (δ(C) 80.3, 49.9, and 56.1), two sp^2 quaternary C-atoms (δ(C) 176.9 and 118.9), and 20 other C-atoms ascribable to three prenyl units and another C_5 unit. The ^{1}H-NMR data showed the presence of a benzene ring, nine *singlet* Me groups, and three vinyl H-atoms. Considering the characteristic ^{13}C- and ^{1}H-NMR data mentioned above, along with the fact that many PPAPs have been isolated from *Hypericum* species, **1** was ascribable to be a PPAP-type compound.

Comparative analysis of the NMR data of **1** with those of furohyperforin isomer 1 indicated they were closely similar to each other [21]. The clearest difference lies in that the signals due to the ^{i}Pr moiety at C(10) in furohyperforin isomer 1 were replaced by the signals for an aromatic ring in **1**, which suggested that **1** was an analogue of furohyperforin isomer 1 with an aromatic ring located at C(10). This conclusion was confirmed by the HMBCs from H–C(12/16) to C(10). In addition, the key HMBCs (*Fig. 2*) of H–C(4) with C(3), C(5), C(17), and C(18), of H–C(5) with C(1), C(4), C(6), and C(34), of Me(17) with C(4) and C(18), of H–C(29) with C(7) and C(8), and of H–C(30) with C(7), C(32), and C(33) verified the core bicyclo[3.3.1]nonane-1,9-dione system and the formation of a furan ring between C(7) and C(30). Thus, the constitutional formula of **1** was established.

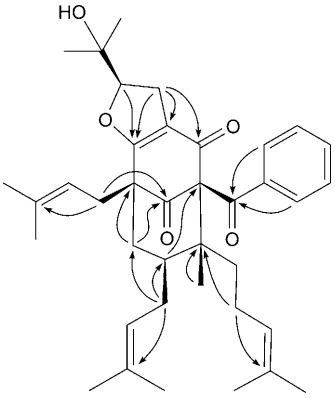

Fig. 2. *Key HMBCs of compound* **1**

The configuration of **1** was determined by a ROESY experiment conjugated with the comparative analysis of the NMR data with its analogues. According to the

Table 1. *^{1}H- and ^{13}C -NMR Data for Compounds **1** and **2** in (D_6)Acetone.* δ in ppm, J in Hz.

	1		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
C(1)	–	207.5 (*s*)	–	207.6 (*s*)
C(2)	–	80.3 (*s*)	–	80.3 (*s*)
C(3)	–	49.9 (*s*)	–	50.3 (*s*)
H−C(4)	1.98–2.03 (*m*)	43.0 (*d*)	1.96–2.01 (*m*)	43.0 (*d*)
H_α−C(5)	1.60–1.62 (*m*)	39.8 (*t*)	1.58–1.61 (*m*)	39.5 (*t*)
H_β−C(5)	2.07–2.11 (*m*)		2.14–2.17 (*m*)	
C(6)	–	56.1 (*s*)	–	56.6 (*s*)
C(7)	–	176.9 (*s*)	–	176.9 (*s*)
C(8)	–	118.9 (*s*)	–	118.9 (*s*)
C(9)	–	188.2 (*s*)	–	188.1 (*s*)
C(10)	–	194.7 (*s*)	–	194.3 (*s*)
C(11)	–	137.9 (*s*)	–	137.2 (s)
H−C(12)	7.58 (*d*, *J*=10.0)	129.0 (*d*)	7.60 (*d*, *J*=6.0)	129.0 (*d*)
H−C(13)	7.25 (*dd*, *J*=9.5, 10.0)	128.6 (*d*)	7.31 (*dd*, *J*=6.0, 7.5)	128.6 (*d*)
H−C(14)	7.44 (*t*, *J*=9.5)	132.8 (*d*)	7.50 (*t*, *J*=6.0)	132.8 (*d*)
H−C(15)	7.28 (*dd*, *J*=9.5, 10.0)	128.6 (*d*)	7.31 (*dd*, *J*=6.0, 7.5)	128.6 (*d*)
H−C(16)	7.58 (*d*, *J*=10.0)	129.0 (*d*)	7.60 (*d*, *J*=7.5)	129.0 (*d*)
Me(17)	1.11 (*s*)	14.8 (*q*)	1.09 (*s*)	14.8 (*q*)
H_α−C(18)	1.70–1.75 (*m*)	37.4 (*t*)	1.73–1.76 (*m*)	37.4 (*t*)
H_β−C(18)	2.01–2.06 (*m*)		1.98–2.03 (*m*)	
H_α−C(19)	2.00–2.03 (*m*)	25.9 (*t*)	1.98–2.05 (*m*)	25.7 (*t*)
H_β−C(19)	2.16–2.23 (*m*)		2.13–2.17 (*m*)	
H−C(20)	5.04–5.10 (*m*)	125.6 (*d*)	5.04–5.07 (*m*)	125.6 (*d*)
C(21)	–	131.4 (*s*)	–	131.4 (*s*)
Me(22)	1.59 (*s*)	18.3 (*q*)	1.59 (*s*)	17.9 (*q*)
Me(23)	1.63 (*s*)	26.1 (*q*)	1.63 (*s*)	25.7 (*q*)
H_α−C(24)	1.95–1.99 (*m*)	28.3 (*t*)	1.87–1.92 (*m*)	28.2 (*t*)
H_β−C(24)	2.13–2.18 (*m*)		2.12–2.16 (*m*)	
H−C(25)	5.11–5.17 (*m*)	123.5 (*d*)	5.09–5.13 (*m*)	123.7 (*d*)
C(26)	–	133.8 (*s*)	–	133.6 (s)
Me(27)	1.55 (*s*)	18.3 (*q*)	1.59 (*s*)	17.9 (*q*)
Me(28)	1.69 (*s*)	26.1 (*q*)	1.67 (*s*)	26.3 (*q*)
CH_2(29)	2.97–3.03 (*m*)	27.8 (*t*)	2.93–2.97 (*m*)	28.0 (*t*)
H−C(30)	4.9 (*dd*, *J*=9.0, 12.5)	94.2 (*d*)	4.91 (*t*, *J*=11.5)	93.4 (*d*)
C(31)	–	71.8 (*s*)	–	71.7 (*s*)
Me(32)	1.30 (*s*)	24.6 (*q*)	1.26 (*s*)	24.5 (*q*)
Me(33)	1.26 (*s*)	26.1 (*q*)	1.29 (*s*)	25.4 (*q*)
CH_2(34)	2.53–2.58 (*m*)	29.3 (*t*)	2.51–2.54 (*m*)	29.2 (*t*)
H−C(35)	5.21–5.30 (*m*)	121.0 (*d*)	5.14–5.20 (*m*)	119.7 (*d*)
C(36)	–	134.5 (*s*)	–	135.3 (*s*)
Me(37)	1.64 (*s*)	18.3 (*q*)	1.65 (*s*)	18.2 (*q*)
Me(38)	1.67 (*s*)	25.6 (*q*)	1.68 (*s*)	25.6 (*q*)

literature, when H−C(4) is in α-orientation, the chemical shift of C(4) is always between 41 and 44 ppm, and the difference of H_β−C(5) and H_α−C(5) is also always between 0.3 and 1.2 ppm, regardless of NMR solvent, PPAP type, or nature of the C(4) substituent [1][35][36]. On the other hand, the difference in chemical shifts of the

H_β–C(5) and H_α–C(5) is 0.0–0.2 ppm and the chemical shift of C(4) is δ(C) 45–49 ppm, if H–C(4) is in β-orientation [35][36]. In compound **1**, the chemical shifts of C(4) (δ(C) 43.0) and the difference of H_β–C(5) and H_α–C(5) ($\Delta\delta$ *ca.* 0.47 ppm) indicated that H–C(4) was in α-orientation. The NOE correlations (*Fig. 3*) of Me(17)/CH_2(24), CH_2(24)/H_β–C(5), H_β–C(5)/CH_2(34), and H–C(12)/H–C(35) showed that **1** had the same relative configuration at C(2), C(3), and C(6) as furohyperforin, which was also isolated in this study. In addition, the ROESY cross-peak of Me(32)/H–C(35) indicated that H–C(30) was in α-orientation. Hence, the structure of **1** was elucidated and named uralodin B.

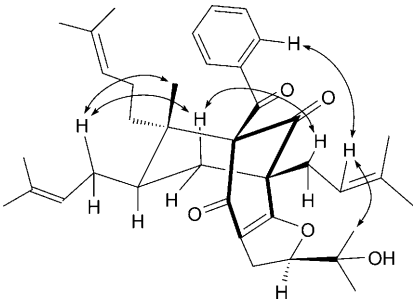

Fig. 3. *Key ROESY correlations of compound* **1**

Uralodin C (**2**) was obtained as a colorless oil. Although **2** and **1** presented a single spot on TLC (silica gel) developed in several solvent systems, they did show two close separated peaks in the HPLC analysis. The molecular formula of **2** was established to be the same as that of **1**, $C_{38}H_{50}O_5$, according to the HR-ESI-MS (*m/z* 585.3597 ($[M-H]^-$); calc. 585.3580). Compound **2** exhibited similar UV, IR, and NMR spectra as **1**, except the different chemical shifts at C(30), C(35), and C(36) in the 1D-NMR spectra, which indicated that **2** should be the epimer of **1** at C(30). The HMBC, HSQC, and ^{1}H,^{1}H-COSY spectra established the connections of all the H- and C-atoms, which confirm that **1** and **2** have the same constitutional formula.

Analogously to compound **1**, the chemical shifts of C(4) (δ(C) 43.0) and the difference of H_β–C(5) and H_α–C(5) ($\Delta\delta$ *ca.* 0.56 ppm) in **2** suggested that H–C(4) was α oriented. In the ROESY experiment, the correlations of Me(17)/CH_2(24), CH_2(24)/H_β–C(5), H_β–C(5)/CH_2(34), and H–C(12)/H–C(35) showed that **2** had the same relative configuration at C(2), C(3), and C(6) as **1**. Contrarily to compound **1**, no ROESY cross-peak of Me(32)/H–C(35) could be observed in **2**, which indicated that H–C(30) was α oriented. Therefore, the structure of **2** was established and named uralodin C. As mentioned in the literature, the different chemical shifts of C(30) (δ(C) 94.2 in **1**, δ(C) 93.4 in **2**), C(35) (δ(C) 121.0 in **1**, δ(C) 119.7 in **2**), and C(36) (δ(C) 134.5 in **1**, δ(C) 135.3 in **2**) can be explained by different orientations of the substituent at C(30) in **1** and **2**, caused by the steric effects between the 1-hydroxy-1-methylethyl group at C(30) and 2-methylbut-2-enyl group at C(6) like in hyperibone A (garciniellipton I) and hyperibone B [37][38]. Our data confirmed that *C(30)*-epimeric furan PPAPs, like uralodin B and uralodin C, can be distinguished by the chemical shifts of C(30), C(35), and C(36).

Urachromone A (**3**), a pale yellow powder, had the molecular formula $C_{18}H_{22}O_9$, deduced from its HR-ESI-MS (*m/z* 417.0940 ($[M+Cl]^-$); calc. 417.0952) and ^{13}C-NMR data. The IR spectrum showed strong absorption bands of OH (3385 cm^{-1}) and conjugated CO groups (1663 cm^{-1}), and of an aromatic ring (1620 cm^{-1}, 1573 cm^{-1}, and 1502 cm^{-1}). The ^{13}C- and DEPT-NMR spectra of **3** indicated the presence of an ^{i}Pr moiety (δ(C) 33.3 and 19.9) and a chromone skeleton (nine downfield C-atom signals) together with one glucopyranose moiety (δ(C) 62.4, 71.2, 74.8, 78.5, 79.3, and 101.7). The ^1H-NMR signals at δ(H) 6.14 (*s*, 1 H), 6.83 (*d*, $J = 1.6$, 1 H), and 6.92 (*d*, $J = 1.6$, 1 H) also indicated the presence of 5,7-dioxygenation in the chromone skeleton. The H-atom signals for an ^{i}Pr moiety (δ(H) 2.58–2.60 (*m*, 1 H); 1.05 (*d*, $J = 6.8$, 6 H)) and for a glucopyranose (δ(H) 4.16–5.79 (5*m*, 2*d*, 7 H)) were also observed in the ^1H-NMR spectrum. The 1D-NMR spectral data of **3** were very similar to those of undulatoside A [39]. The only significant difference was the presence of the signals for an ^{i}Pr group in **3** instead of a Me group in undulatoside A. Replacement of the Me group at C(2) in undulatoside A by an ^{i}Pr group in **3** was confirmed by the correlations of H–C(1′) (δ(H) 2.58–2.60 (*m*)) with C(3) (δ(C) 106.2) and C(2) (δ(C) 175.5), Me(2′) (δ(H) 1.05 (*d*, $J = 6.8$)) with C(2) (δ(C) 175.5) in the HMBC spectrum. In addition, the HMBCs from H–C(3) (δ(H) (*s*, 6.14)) to C(5) (δ(C) 162.2) and from H–C(1″) (δ(H) (5.79, *d*, $J = 7.6$)) to C(7) (δ(C) 164.2) indicated that the glucopyranosyl residue was linked to C(7). Therefore, **3** was established as 5-hydroxy-2-isopropylchromone-7-*O*-glucoside and named urachromone A.

Urachromone B (**4**), a pale yellow powder, had the molecular formula $C_{19}H_{24}O_9$, as determined by HR-ESI-MS (*m/z* 431.1101 ($[M+Cl]^-$); calc. 431.1108). Comparison of the NMR data of **4** with those of **3** suggested that the ^{i}Pr moiety in **3** was replaced by an ^{i}Bu group in **4**. This deduction was proved by the correlations of H–C(1′) (δ(H) 2.36–2.38 (*m*)) with C(2) (δ(C) 174.6) and C(3) (δ(C) 106.6), H_a–C(2′) (δ(H) 1.34–1.36 (*m*)) with C(2), C(3′) (δ(C) 17.7), and C(4′) (δ(C) 11.7), and H_b–C(2′) (δ(H) 1.51–1.55 (*m*)) with C(2), C(3′), and C(4′). Thus, the structure of **4** was established as shown and named urachromone B.

2. *Biological Activity.* Considering that many PPAP compounds are reported to have antitumoral activity, the cytotoxic activity of PPAP constituents, *i.e.*, **1**, **2**, **5**, and **6**, against HepG2, SGC7901, HL-60, and K562 tumor cell lines was evaluated. Compounds **2**, **5**, and **6** showed moderate cytotoxic activity against all the tested cell lines, with IC_{50} values less than 60 μM. On the other hand, **1** showed modest activity against SGC7901 and HL-60 cell lines and showed no activity against HepG2 and K562 cell lines (*Table 2*).

Table 2. *Cytotoxic Activities of PPAPs* (**1**, **2**, **5**, and **6**) *against Tumor Cell Lines*

	IC_{50} [μM]			
	HepG2	SGC7901	HL-60	K562
1	171.0	63.7 ± 4.4	21.8 ± 2.1	171.0
2	28.5 ± 2.6	26.1 ± 1.7	14.3 ± 1.3	32.1 ± 2.1
5	59.7 ± 3.3	27.1 ± 2.2	16.0 ± 0.9	23.7 ± 1.5
6	46.2 ± 3.4	39.5 ± 2.5	18.5 ± 1.9	30.1 ± 3.3
Cisplatin	4.6 ± 0.9	5.8 ± 0.7	1.9 ± 0.3	17.4 ± 1.3

Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 200–300 mesh, *Qingdao Marine Chemical, Inc.*, Qingdao, P. R. China), silica gel *H* (10–40 μm, *Qingdao Marine Chemical, Inc.*, Qingdao, P. R. China), *Sephadex LH-20* (*Amersham Pharmacia Biotech*, Sweden), or reversed-phase C_{18} (*RP-18*) silica gel (*Merck*). Preparative HPLC: *Shimadzu LC-8A* prep. liquid chromatograph with *Shimadzu PRC-ODS (K)* column (30 mm × 25 cm, 5 μm, 25 ml/min) and a UV *SPD-10AVP* (280 nm) detetor. Optical rotations: *Horiba SEPA-300* polarimeter. UV Spectra: *UV-210A* spectrometer. IR Spectra: *Tenor-27* spectrophotometer; KBr pellets; in cm^{-1}. ^{1}H-, ^{13}C-NMR and 2D-NMR spectra: *Bruker AM-400* or *DRX-500* spectrometers; δ in ppm rel. to Me_4Si, *J* in Hz. FAB-MS: *VG Autospec-3000* spectrometer, with glycerine as the matrix. HR-ESI-MS: *Finnigan MAT* instrument; in *m*/*z*.

Plant Material. The aerial parts of *Hypericum henryi* subsp. *uraloides* were collected in Lüchun County, Yunnan Province, P. R. China, in July 2007. The plant was identified by Prof. *X. Cheng* Kunming Institute of Botany, Kunming, P. R. China. A voucher specimen was deposited with Kunming Institute of Botany with identification number 20070715.

Extraction and Isolation. The air-dried plant materials (5 kg) were powdered and extracted with MeOH (3 × 20 l, each 2 d) at r.t. The extract was evaporated to dryness under reduced pressure to obtain a residue (900 g). The residue was suspended in H_2O (1500 ml) and then partitioned with petroleum ether (PE; 1500 ml) and AcOEt (1500 ml), resp. The PE soluble extract (180 g) was subjected to CC (SiO_2; PE/Me_2CO 1:0 to 0:1) to yield ten fractions (*Frs. 1–10*). *Fr. 4* (7.6 g) was subjected to CC (SiO_2; PE/Me_2CO 9:1) to obtain four subfractions (*Frs. 4.1–4.4*). *Fr. 4.4* (1.1 g) was subjected to *Sephadex LH-20* ($CHCl_3$/MeOH 1:1) followed by HPLC (MeOH/H_2O 85:15) to yield compounds **1** (15 mg, t_R 74 min), **2** (32 mg, t_R 69 min), and **5** (12 mg, t_R 62 min). *Fr. 4.2* was further purified by HPLC (MeOH/H_2O 80:20) to yield **6** (25 mg, t_R 65 min). The AcOEt soluble extract (90 g) was subjected to CC (SiO_2; $CHCl_3$/MeOH 95:5 to 0:100) to give six fractions (*Frs. A–F*). *Fr. A* (12.3 g) was purified by *RP-18* (MeOH/H_2O 1:5 to 2:1) and then was subjected to repeated CC (SiO_2; $CHCl_3$/MeOH 50:1) to yield 1,3,5-trihydroxyxanthone (11.2 mg), 2,5-dihydroxyxanthone (7.8 mg), and kielcorin (9.0 mg). *Fr. B* (6.9 g) was further purified using *Sephadex LH-20* ($CHCl_3$/MeOH 1:1) and then subjected to HPLC (MeOH/H_2O 45:55) to yield 1,5,6-trihydroxy-3-methoxyxanthone (21 mg, t_R 46 min), 1,3,7-trihydroxyxanthone (25 mg, t_R 55 min), and calycinoxanthone D (18 mg, t_R 70 min). *Fr. C* (5.2 g) was subjected to CC (SiO_2; $CHCl_3$/MeOH 50:1 to 10:1), followed by *Sephadex LH-20* ($CHCl_3$/MeOH 1:1) to yield 3,4-epoxy-5-hydroxycyclohex-1-enecarboxylic acid (15 mg), 1,3,5,8-tetrahydroxyxanthone (7 mg), 1-hydroxyxanthone (8 mg), and 1,3,5,6-tetrahydroxy-4-prenylxanthone (12 mg). *Fr. D* (6.6 g) was applied to repeated CC (SiO_2; $CHCl_3$/MeOH 60:1 to 10:1) to give **3** (50 mg), **4** (32.6 mg), and epicatechin-(7,8-*bc*)-4*β*-(hydroxyphenyl)dihydroxy-2(3*H*)-pyranone (22 mg). *Fr. E* (11.2 g) was separated by CC (SiO_2; $CHCl_3$/MeOH 15:1) and then recrystallized to yield quercetin (200 mg, $CHCl_3$/MeOH, 9.8:0.2), quercetin 3-*O*-*α*-L-rhamnofuranoside (1.5 g, MeOH/H_2O, 9.5:0.5), and quercetin 3-*O*-*β*-D-glucuronopyranoside (1.63 g, MeOH/H_2O, 9.8:0.2).

Uralodin B (=*(2*R*,5*R*,6*R*,7*S*,9*R*)-3,5,6,7,8,9-Hexahydro-2-(2-hydroxypropan-2-yl)-6-methyl-7,9-bis(3-methylbut-2-en-1-yl)-6-(4-methylpent-3-en-1-yl)-5-(phenylcarbonyl)-5,9-methanocycloocta[*b*]furan-4,10(2*H*)-dione*; **1**). Colorless oil. $[\alpha]_D^{23.3} = -24.6$ ($c = 0.075$, MeOH). UV (MeOH): 204 (6.5), 248 (6.0), 285 (3.0). IR (KBr): 3439, 1765, 1743, 1701, 1688, 1628. ^{1}H- and ^{13}C-NMR: *Table 1*. FAB-MS (neg.): 585 ($[M-H]^-$). HR-ESI-MS (neg.): 585.3594 ($[M-H]^-$, $C_{38}H_{49}O_5^-$; calc. 585.3580).

Uralodin C (=*(2*S*,5*R*,6*R*,7*S*,9*R*)-3,5,6,7,8,9-Hexahydro-2-(2-hydroxypropan-2-yl)-6-methyl-7,9-bis(3-methylbut-2-en-1-yl)-6-(4-methylpent-3-en-1-yl)-5-(phenylcarbonyl)-5,9-methanocycloocta[*b*]furan-4,10(2*H*)-dione*; **2**). Colorless oil. $[\alpha]_D^{23.3} = -55.0$ ($c = 0.010$, MeOH). IR (KBr): 3440, 1723, 1701, 1689, 1628. UV (MeOH): 204 (6.4), 247 (5.9), 284 (2.4). ^{1}H- and ^{13}C-NMR: *Table 1*. FAB-MS (neg.): 585 ($[M-H]^-$). HR-ESI-MS (neg.): 585.3597 ($[M-H]^-$, $C_{38}H_{49}O_5^-$; calc. 585.3580).

Urachromone A (=*5-Hydroxy-4-oxo-2-(propan-2-yl)-4*H*-chromen-7-yl β-*D*-Glucopyranoside*; **3**). Pale yellow powder. $[\alpha]_D^{23.3} = -45.0$ ($c = 0.023$, MeOH). IR (KBr): 3385, 1663, 1620, 1573, 1502, 1075, 827, 610. UV (MeOH): 249 (5.9), 285 (4.7), 314 (6.4). ^{1}H-NMR (C_5D_5N, 500 MHz): 1.05 (*d*, *J* = 6.8, 2 Me(2′)); 2.58–2.60 (*m*, H–C(1′)); 4.16–4.23 (*m*, H–C(5″)); 4.32–4.37 (*m*, H–C(4″)); 4.38–4.42 (*m*, H–C(2″)); 4.38–4.50 (*m*, H_a–C(6″)); 4.40–4.45 (*m*, H–C(3″)); 4.53 (*d*, *J* = 11.8, H_b–C(6″)); 5.79 (*d*,

J=7.6, H–C(1″)); 6.14 (*s*, H–C(3)); 6.83 (*d*, J = 1.6, H–C(6)); 6.92 (*d*, J=1.6, H–C(8)). ^{13}C-NMR (C_5D_5N, 125 MHz): 19.9 (*q*, 2 C(2′)); 33.3 (*d*, C(1′)); 62.4 (*t*, C(6″)); 71.2 (*d*, C(4″)); 74.8 (*d*, C(2″)); 78.5 (*d*, C(3″)); 79.3 (*d*, C(5″)); 95.3 (*d*, C(8)); 100.6 (*d*, C(6)); 101.7 (*d*, C(1″)); 106.2 (*d*, C(3)); 106.5 (*s*, C(4a)); 158.4 (*s*, C(1a)); 162.2 (*s*, C(5)); 164.2(*s*, C(7)); 175.5 (*s*, C(2)); 183.3 (*s*, C(4)). FAB-MS (neg.): 381 ($[M-H]^-$). HR-ESI-MS (neg.): 417.0940 ($[M+Cl]^-$, $C_{18}H_{22}ClO_9^-$; calc. 417.0952).

Urachromone B (=*2-(Butan-2-yl)-5-hydroxy-4-oxo-4*H-*chromen-7-yl β*-D-*Glucopyranoside*; **4**). Pale yellow powder. $[\alpha]_D^{23.3} = -33.3$ (c = 0.060, MeOH). IR (KBr): 3396, 1665, 1622, 1582, 1502, 1422, 1177, 1075. UV (MeOH): 251 (5.6), 256 (4.5), 316 (6.3). ^{1}H-NMR (C_5D_5N, 500 MHz): 0.73 (*dd*, J=6.0, 7.6, Me(3′)); 1.05 (*d*, J=6.0, Me(4′)); 1.34–1.36 (*m*, H_a–C(2′)); 1.51–1.55 (*m*, H_b–C(2′)); 2.36–2.38 (*m*, H–C(1′)); 4.16–4.20 (*m*, H–C(5″)); 4.30–4.37 (*m*, H–C(4″)); 4.31–4.33 (*m*, H–C(2″)); 4.36–4.42 (*m*, H_a–C(6″)); 4.39–4.41 (*m*, H–C(3″)); 4.52 (*d*, J=11.8, H_b–C(6″)); 5.80 (*d*, J=7.8, H–C(1″)); 6.18 (*d*, J = 1.6, H–C(3)); 6.83 (*s*, H–C(6)); 6.97 (*s*, H–C(8)). ^{13}C-NMR (C_5D_5N, 125 MHz): 11.7 (*q*, C(4′)); 17.7 (*q*, C(3′)); 27.6 (*t*, C(2′)); 40.4 (*d*, C(1′)); 62.3 (*t*, C(6″)); 71.1 (*d*, C(4″)); 74.8 (*d*, C(2″)); 78.5 (*d*, C(3″)); 79.2 (*d*, C(5″)); 95.3 (*d*, C(8)); 100.7 (*d*, C(6)); 101.7 (*d*, C(1″)); 106.6 (*d*, C(3)); 107.4 (*s*, C(4a)); 158.4 (*s*, C(1a)); 162.7 (*s*, C(5)); 164.2 (*s*, C(7)); 174.6 (*s*, C(2)); 183.1 (*s*, C(4)). FAB-MS (neg.): 393 ($[M-H]^-$). HR-ESI-MS (neg.): 431.1101 ($[M+Cl]^-$, $C_{19}H_{24}ClO_9^-$; calc. 431.1108).

Cytotoxicity Assay. Cytotoxicity of the PPAPs against HepG2, SGC7901, HL-60, and K562 cell lines was determined using the MTT method with minor modifications [40–42]. Cells were seeded in 96-well plates 24 h before treatment and continuously exposed to different concentrations of compounds (0.064, 0.32, 1.6, 8, and 40 μM). After 48 h, 20 μl of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 5 mg/ml) soln. were added to each well, which were incubated for another 4 h at 37°. Then 100 μl resolving buffer (20% SDS, 50% DMF) were added to each well. After 12 h incubation at 37°, the *OD* value of each well was read at 595 nm with a plate reader. All assays were carried out in triplicate, and cisplatin was used as positive control. Control wells, treated with DMSO alone, were included in all the experiments: Growth inhibition [%] = $(OD_{control} - OD_{treated})/OD_{control} \times 100$. The cytotoxicity of the compounds on tumor cells was expressed as IC_{50} values (the drug concentration reducing by 50% the absorbance in treated cells, with respect to untreated cells) calculated by *Reed* and *Muench*'s method [43].

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