

Full Paper

New Benzoyl Glucosides and Cytotoxic Pterosin Sesquiterpenes from *Pteris ensiformis* Burm.

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Abstract: Three new compounds: 2*R*,3*R*-pterosin L 3-*O*- β -D-glucopyranoside (**1**), β -D-xylopyranosyl(1 \rightarrow 2)-7-*O*-benzoyl- β -D-glucopyranoside (**2**) and 4-*O*-benzoyl- β -D-xylopyranosyl(1 \rightarrow 2)-7-*O*-benzoyl- β -D-glucopyranoside (**3**), together with nine known compounds, were isolated from the ethyl acetate extract of *Pteris ensiformis*. 5-[2-Hydroxyethylidene]-2(5*H*)-furanone (**4**), which had been synthesized, was isolated from natural sources for the first time. The structures of all isolated compounds were determined on the basis of mass and spectroscopic evidence. Compound **1** and pterosin B (**5**) show cytotoxicity against HL 60 cells (human leukemia) with the IC₅₀ values of 3.7 and 8.7 μ g/mL, respectively.

Keywords: *Pteris ensiformis*; pterosin B; human leukemia

Introduction

For hundreds of years *Pteris ensiformis* Burm. (Pteridaceae) has been one of the most popular constituents of herbal beverages in Taiwan [1]. Some medium polarity extracts of different *Pteris* species show effective antitumor and antibacterial activity [2]. Chemical investigation of more than 30 species of the Pteridaceae has been reported [3]. Phytochemical investigations on the *Pteris* genus led to isolation of various phenolic compounds [4], flavonol glycosides [5-6], kauranes [7], and pterosin-sesquiterpenes [8-9]. In bioactivity reports, for example, pterosin B, one of the main pterosins found in the genus *Pteris*, exhibits potent cytotoxic activity against HL 60 (human leukemia) cells [10-11].

Previously, we identified several antioxidant compounds from the aqueous extract of *P. ensiformis* and found that caffeic acid derivatives and other phenolics attribute to their antioxidant activity [12]. In a large scale screening of crude extracts of Formosan plants, we also found that the whole plant ethyl acetate extract of *P. ensiformis* showed effective inhibition against HL 60 cells with $IC_{50} = 41.6 \mu\text{g/mL}$. Further chemical investigation using bioactivity-guided fractionation led to the isolation of three new compounds: 2*R*,3*R*-pterosin L 3-*O*- β -D-glucopyranoside (**1**), β -D-xylopyranosyl(1 \rightarrow 2)-7-*O*-benzoyl- β -D-glucopyranoside (**2**), 4-*O*-benzoyl- β -D-xylopyranosyl(1 \rightarrow 2)-7-*O*-benzoyl- β -D-glucopyranoside (**3**), together with nine known compounds: 5-[2-hydroxyethylidene]-2(5*H*)-furanone (**4**), pterosin B (**5**), β -D-glucopyranosyl benzoic acid ester (**6**), benzoic acid (**7**), 5-*O*-coumaroylquinic acid (**8**), coumaric acid (**9**), cyclolaudenol (**10**), β -sitosterol-3-*O*- β -D-glucoside (**11**), and β -D-sitosterol (**12**), of which compounds **4**, **6**, **8–10** are identified for the first time from the genus *Pteris* (Figure 1). The structural elucidation of **1–4** and the cytotoxicity inhibition properties of the isolates are described herein.

Results and Discussion

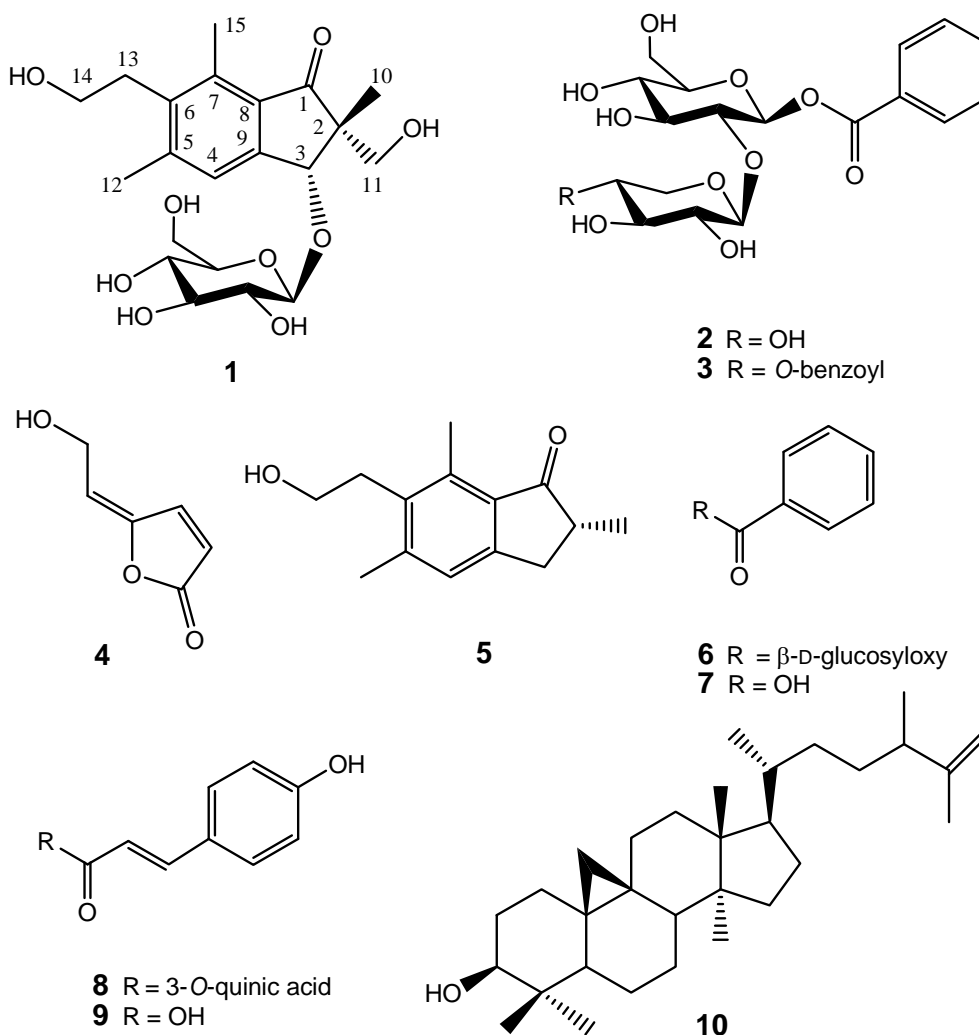
Structure Determinations of Isolated Compounds

The HR ESI-MS of the new compound **1** showed a sodium adduct molecular ion at m/z 449.1787 $[\text{M}+\text{Na}]^+$, corresponding to the molecular formula $\text{C}_{21}\text{H}_{30}\text{O}_9\text{Na}$ (calcd. 449.1782). The IR spectrum indicated the presence of a carbonyl group (1689 cm^{-1}). Characteristic $^1\text{H-NMR}$ signals, including one methyl group at δ 1.21 (3H, s, C-10), two aromatic methyl groups attaching to C-5 and C-7 at δ 2.48 (3H, s) and 2.66 (3H, s), two coupled methylenes of a hydroxyethyl group at δ 3.01 (2H, t, $J = 8.4 \text{ Hz}$) and 3.61 (2H, t, $J = 8.4 \text{ Hz}$), one hydroxymethyl group at δ 3.37 (2H, AB q, $J = 12.1 \text{ Hz}$), one allylic oxygenated methylene at δ 4.97 (1H, s), and one aromatic proton at δ 7.53 (1H, s), indicated the presence of a pterosin-sesquiterpene skeleton [13]. Furthermore, the configuration of the anomeric position was judged to be β from a large $^3J_{\text{H}1',\text{H}2'}$ coupling constant ($J = 7.6 \text{ Hz}$) [14].

The assignments of $^{13}\text{C-NMR}$ were confirmed by 2D NMR techniques. The ^1H -detected heteronuclear multiple bond connectivity (HMBC) spectrum showed the correlations of H-12/C-6, H-12/C-4, H-15/C-6, H-15/C-8, H-14/C-13, H-13/C-6, H-4/C-9, H-3/C-9, H-3/C-2, H-10/C-1, H-10/C-2, and H-10/C-11, which confirmed **1** as a pterosin-type sesquiterpene. In addition, the HMBC correlation of H-3/C-1' corresponds to the linkage between the pterosin moiety and the β -glucopyranose (d, $J = 7.6 \text{ Hz}$) (Fig. 2). The acid hydrolysis of **1** gave the aglycone and D-

glucopyranose, which was confirmed by comparison of the ^{13}C -NMR spectra. The absolute configuration at C-2 and C-3 was deduced from the similarity of the CD spectrum $\{[\theta]_{330}^{20} +19800$ (MeOH) $\}$ of 2*R*,3*R*-pterisin L [13]. Additionally, this configuration (2*R*,3*R*) was confirmed by NOESY correlations of H-10/H-3 and H-11/H-1' (Figure 2). Thus, the structure of compound **1**, 2*R*,3*R*-pterisin L 3-*O*- β -D-glucopyranoside, was determined.

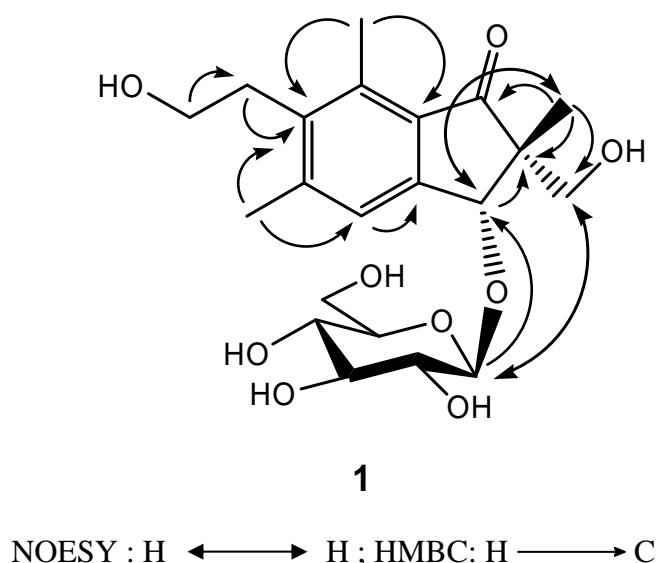
Figure 1. Structures of compounds **1-10**.



The molecular formula of **2**, $\text{C}_{18}\text{H}_{24}\text{O}_{11}$, were established by HR ESI-MS ($[\text{M}+\text{Na}]^+$ m/z 439.1214, calcd. 439,1216). The ^1H - and ^{13}C -NMR spectra of **2** and **3** (Table 1) revealed the presence of glucopyranosyl and xylopyranosyl moieties [15]. The β -anomeric configuration for both glucopyranose and xlyopyranose were determined from a large $^3J_{\text{H}1'\text{H}2'}$ coupling constant (7.6 Hz). Besides the signals of sugars, the ^1H -NMR spectrum of **2** showed a set of proton signals due to a monosubstituted aromatic ring at δ_{H} 8.09 (2H, dd, 8.3, 1.4 Hz), 7.50 (2H, td, 8.3, 1.4 Hz) and 7.63 (1H, tt, 8.3, 1.4 Hz), in combination with the signal for a carbonyl carbon at δ_{C} 166.3 in the ^{13}C -NMR spectrum, suggesting one benzoyl moiety in **2**. The connection of the structural units was determined by HMBC data (Table 1). The β -D-glucopyranose was found to be connected to C-7 of the benzoyl moiety by observation of the HMBC correlation between δ_{H} 5.84 (Glc-H-1) and δ_{C} 166.3 (benzoyl-C-

7). The structure of the sugar chain was assigned by a combination of COSY, HMQC, and HMBC experiments. Starting from the anomeric protons of each sugar unit, all of the proton signals could be identified using a COSY experiment. On the basis of assignments of all proton resonances, the linked carbon atoms were confirmed by HMQC experiment. In $^1\text{H-NMR}$, the anomeric proton of xylose appears at ca. δ 5.0. The high-field anomeric proton signal of the xylose unit at δ 4.50 indicated a sugar-sugar linkage. The down-field shift of C-2' (δ 83.7) compared to the C-2' signal of **6** (δ 74.0) indicated that the xylosyl residue was connected to C-2' of the glucose ring. This linkage was confirmed by the correlation at δ 4.50/83.7 between H-1" and C-2' in the HMBC spectrum. Thus, the structure of **2** was determined to be β -D-xylopyranosyl(1 \rightarrow 2)-7-O-benzoyl- β -D-glucopyranoside.

Figure 2. NOESY and HMBC correlations of compound **1**.



The molecular formula of **3**, $\text{C}_{25}\text{H}_{28}\text{O}_{12}$, was deduced from its HRESIMS (m/z 543.1475 [$\text{M}+\text{Na}$] $^+$, calcd. 543.1473). The $^1\text{H-}$ and $^{13}\text{C-NMR}$ data of **3** (Table 1) were comparable to those of **2**. Extra $^{13}\text{C-NMR}$ signals at 129.6 (2C), 130.6 (2C), 130.9, 134.7, 167.3 suggested an additional benzoyl group. The connection to C-4" of the β -D-xylopyranosyl moiety was deduced from the HMBC correlation between δ_{H} 4.29 (xyl-H-4") and δ_{C} 167.8 (benzoyl-C-7"). Based on the above results, the structure of **3** was established as 4-O-benzoyl- β -D-xylopyranosyl(1 \rightarrow 2)-7-O-benzoyl- β -D-glucopyranoside.

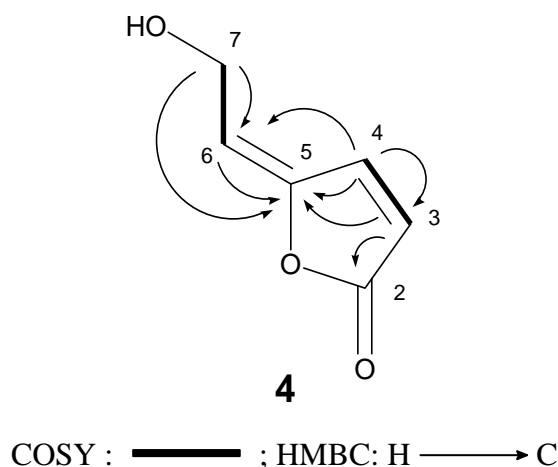
Glycosides with β -D-xylopyranosyl(1 \rightarrow 2)-O- β -D-glucopyranoside moieties were isolated from this family plant, *Pteridium esculentum* [16]. This represents the second time these special disaccharide analogues are found in this family.

In this study 5-[2-hydroxyethylidene]-2(5H)-furanone (**4**) was isolated from a natural source for the first time, although it had been already synthesized by Siegel and Bruckner [17]. The structure of **4** was readily identified by comparison of its spectroscopic data ($^1\text{H-NMR}$, IR, and mass spectrometry data) [15]. We further confirmed its $^{13}\text{C-NMR}$ assignments by $^1\text{H-}^1\text{H}$ COSY, HMQC, and HMBC experiments (Figure 3).

Table 1. ^1H -NMR (400 MHz) and ^{13}C -NMR (100 MHz) spectral data ^a of compounds **2** and **3** in CD_3OD (δ in ppm, J in Hz).

Position	^1H -NMR		^{13}C -NMR	
	2	3	2	3
1			131.0	131.0
2	8.09 (1H, dd, 8.3, 1.4)	8.11 (1H, dd, 8.3, 1.4)	131.0	131.0
3	7.50 (1H, td, 8.3, 1.4)	7.50 (1H, td, 8.3, 1.4)	129.6	129.5
4	7.63 (1H, tt, 8.3, 1.4)	7.62 (1H, tt, 8.3, 1.4)	134.6	134.4
5	7.50 (1H, td, 8.3, 1.4)	7.50 (1H, td, 8.3, 1.4)	129.6	129.5
6	8.09 (1H, dd, 8.3, 1.4)	8.11 (1H, dd, 8.3, 1.4)	131.0	131.0
7			166.3	166.4
7- <i>O</i> - β -glucoside				
1'	5.84 (1H, d, 8.0)	5.86 (1H, d, 8.0)	94.8	94.7
2'	3.69 (1H, m)	3.73 (1H, m)	83.7	83.4
3'	3.68 (1H, m)	3.72 (1H, m)	77.5	77.6
4'	3.46 (1H, m)	3.49 (1H, m)	70.6	70.7
5'	3.45 (1H, m)	3.48 (1H, m)	78.8	78.9
6'a	3.86 (1H, dd, 12.3, 2.1)	3.87 (1H, dd, 12.3, 2.1)		
6'b	3.71 (1H, dd, 12.3, 8.4)	3.73 (1H, dd, 12.3, 8.4)	62.2	62.2
2'- <i>O</i> - β -xylosyl				
1''	4.47 (1H, d, 7.6)	4.63 (1H, d, 7.6)	106.8	106.5
2''	3.15 (1H, dd, 8.8, 7.6)	3.31 (1H, m)	75.7	75.8
3''	3.43 (1H, t, 8.8)	3.70 (1H, m)	77.5	74.8
4''	3.30 (1H, m)	4.77 (1H, ddd, 11.2, 9.6, 5.2)	70.9	73.4
5'' _{ax.}	2.29 (1H, dd, 11.2, 9.6)	3.16 (1H, dd, 11.2, 9.6)		
5'' _{equ.}	3.39 (1H, dd, 11.2, 4.8)	3.68 (1H, dd, 11.2, 4.8)	67.1	63.7
5''- <i>O</i> - β -benzoyl				
1'''				130.9
2'''		8.00 (1H, dd, 8.3, 1.4)		130.6
3'''		7.46 (1H, td, 8.3, 1.4)		129.6
4'''		7.60 (1H, tt, 8.3, 1.4)		134.7
5'''		7.46 (1H, td, 8.3, 1.4)		129.6
6'''		8.00 (1H, dd, 8.3, 1.4)		130.6
7'''				167.3
Key HMBC	H-1'/C-7, H-1''/C-2'	H-1'/C-7, H-1''/C-2', H-4''/C-		

^a Assignments were confirmed by coupling constants, ^1H - ^1H COSY, NOESY, HMQC and HMBC analysis.

Figure 3. COSY and HMBC correlations of compound **4**.

The structures of the known isolates pterosin B (**5**) $\{[\alpha]_{\text{D}}^{25}: -16.0^\circ (c\ 0.20, \text{MeOH})\}$ [18], β -D-glucopyranosyl benzoic acid ester (**6**) [19], benzoic acid (**7**) [19], 5-*O*-coumaroylquinic acid (**8**) $\{[\alpha]_{\text{D}}^{25}: -30.5^\circ (c\ 0.22, \text{MeOH})\}$ [20], coumaric acid (**9**) [21], cyclolaudenol (**10**) $\{[\alpha]_{\text{D}}^{25}: +23.5^\circ (c\ 0.25, \text{MeOH})\}$ [22], β -sitosterol-3-*O*- β -D-glucoside (**11**) $\{[\alpha]_{\text{D}}^{25}: -50.1^\circ (c\ 0.10, \text{MeOH})\}$ [23], and β -D-sitosterol (**12**) $\{[\alpha]_{\text{D}}^{25}: -20.0^\circ (c\ 0.18, \text{MeOH})\}$ [23], were determined by comparison with their spectroscopic data as reported in the corresponding literature.

Cytotoxic activity of Isolated Compounds

In previous studies on plants of this genus, illudin-series compounds were reported as precursors of pterosins. In a recent report [24], a cytokinetic experiment with HL 60 cells indicated that illudin S exerts a primary effect on DNA synthesis. Illudin S could cause a complete block at the G₁-S phase interface of the cell cycle. However, a series of illudin compounds were regarded as anti-cancer and/or carcinogenic substances [11], and the conflict in these biological results suggested that the safety issue needs to be concerned when using extracts from ferns from the family Pteridaceae. The pterosin metabolites were studied for their bioactivity, e.g. antimicrobial and antitumor activity, and displayed less toxicity than illudins [11, 25]. So far, we haven't yet found any illudin-series compounds in this plant by using a cytotoxicity-guided fractionation method. This might explain why Taiwanese people use this plant extract as one ingredient of combination herbal beverages without serious toxic effects. However, it is an important issue to be clarified. Some pterosin glycosides have been identified [13, 25], but 3-*O*- β -D-glucose substituted pterosins are rare and their activity has not yet been reported. All purified compounds were evaluated for the cytotoxicity toward Hep G2 (human liver cancer), A549 (human lung carcinoma), MDA-MB-231 (breast carcinoma), MCF-7 (breast carcinoma), Ca9-22 (human oral squamous carcinoma), and HL 60 (human leukemia) cell lines. Among them, compound **1** and pterosin B (**5**) showed selective activity against HL 60 human leukemia cancer cells with the IC₅₀ values of 3.7 and 8.7 $\mu\text{g/mL}$, respectively. Pterosins are inactive to antioxidant assays, but play an important role to action of cytotoxicity to cancer cell lines.

Experimental

General

Optical rotations were measured on a JASCO DIP-370 digital polarimeter. UV spectra were obtained on a Hitachi 220-20 spectrophotometer. IR spectra were measured on a Hitachi 260-30 spectrophotometer. $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ spectra were recorded on a Varian Inova 500, Varian Unity Plus 400, or Varian Gemini 200 spectrometers using TMS as internal standard. Chemical shifts were reported in parts per million (δ), and coupling constants (J) were expressed in Hertz. LR EI MS were collected on a Bruker APEX II mass or a Quattro GC-MS spectrometer having a direct inlet system. LR ESI-MS and HR ESI-MS were measured on a Bruker APEX II mass spectrometer. Circular dichroism was measured on Jasco J-610. Silica gel 60 (230-400 mesh, Merck) was used for column chromatography. Shimadzu LC-10AT pumps, a SPD-10A UV-vis detector, Hypersil ODS 5 μm (250 x 4.6 mm i.d.), and preparative ODS 5 μm (250 x 21.2 mm i.d.) columns were employed for the HPLC analysis or separation. Pre-coated silica gel plates (Merck, Kieselgel 60 F254, 0.25 mm) were used for preparative thin layer chromatography. TLC was performed on silica gel plates (Merck), spots were detected under UV (254 nm, 366 nm) and by spraying with 50% aqu. H_2SO_4 followed by heating on a hot plate.

Plant Material

Plant material of *Pteris ensiformis* Burm. was collected from Taitung District Agricultural Improvement Station in Taitung, Taiwan, in December, 2006. The material was identified by Dr. Ming-Hong Yen (Associate Professor of the Graduate Institute of Natural Products, Kaohsiung Medical University). A voucher specimen (PE001) was deposited in the Graduate Institute of Natural Products, Kaohsiung Medical University, Kaohsiung, Taiwan.

Extraction and Isolation

Dried whole plants of *P. ensiformis* (2.1 kg) were cut into small pieces, extracted with EtOAc (20 L \times 3) and concentrated to a volume (ca. 2 L) and partitioned with *n*-hexane (2L \times 3). The EtOAc layer was chromatographed on silica gel (2600 g) with different eluents (each 1.5 L, *n*-hexane-EtOAc, 1:0, 1:1, 9:1 and CHCl_3 -MeOH, 20:1, 10:1, 8:1, 6:1, 4:1) to get 8 frs (P1-8). Crystalline fr. P4 (2.9 g) was filtered and washed with MeOH to give **12** (90 mg, TLC: *n*-hexane-EtOAc 1:1, R_f = 0.4). Fr. P5 (2.2 g) was chromatographed over silica gel (20 g) using mixtures of *n*-hexane-EtOAc (each 0.5 L, *n*-hexane-EtOAc, 3:2, 1:1, 1:4) to afford **10** (80 mg, TLC: CHCl_3 100%, R_f = 0.5) and **9** (13 mg, TLC: CHCl_3 -MeOH 100:1, R_f = 0.3). Fr. P7 (9.2 g) was rechromatographed over silica gel (1400 g) using mixtures of CHCl_3 -MeOH (each 1.0 L, CHCl_3 -MeOH, 30:1, 20:1, 10:1) to obtain subfrs (P7.1-10). Subfr. P7-3 (102 mg) was further purified by repeated silica gel chromatography (CHCl_3 -MeOH 20:1) to yield **1** (15 mg, TLC: CHCl_3 -MeOH 20:1, R_f = 0.4), **5** (20 mg, TLC: CHCl_3 -MeOH 20:1, R_f = 0.5), and **6** (10 mg, TLC, CHCl_3 -MeOH 10:1, R_f = 0.6). Subfr. P7-5 (2.0 g) using (each 0.5 L, CHCl_3 -MeOH, 20:1, 10:1) mixtures for elution to afford **11** (120 mg, TLC: CHCl_3 -MeOH 20:1, R_f = 0.4), **7** (20 mg, TLC:

CHCl₃-MeOH 20:1, R_f = 0.5), and **8** (9 mg, TLC: CHCl₃-MeOH 10:2, R_f = 0.6). Crystalline subfr. P7-7 (2.5 g) was filtered and washed with MeOH-Water (1:1) to give **3** (1.2 g, TLC: CHCl₃-MeOH 8:1, R_f = 0.6). Subfr. P7-8 (100 mg) was further separated by preparative reverse-phase HPLC (MeOH-H₂O, 50:50, flow rate = 3.5 mL/min) to give **4** (5 mg, t_R = 23.0 min), **2** (12 mg, t_R = 41.0 min) and **3** (60 mg, t_R = 52.0 min).

2R,3R-Pterodin L 3-O-β-D-glucopyranoside (1): brown amorphous powder; [α]_D²⁵: -38.1° (c 0.31, MeOH); CD [θ]₃₃₀²⁰: +19800 (MeOH); UV: λ_{max} (log ε): 260 (3.90), 219 (4.53) nm; IR (Neat): ν_{max} = 3400, 1689 cm⁻¹; ¹H-NMR (CD₃OD, 400 MHz): ppm δ 7.53 (1H, s, H-4), 4.97 (1H, s, H-3), 3.61 (2H, t, J = 8.4, H-14), 3.37 (2H, AB q, J = 12.1, H-11), 3.01 (2H, t, J = 8.4, H-13), 2.66 (3H, s, H-15), 2.48 (3H, s, H-12), 1.21 (3H, s, H-10); sugar signals: ppm δ 4.66 (1H, d, J = 7.6, H-1'), 3.93 (1H, dd, J = 12.3, 2.1 H-6'a), 3.75 (1H, dd, J = 12.3, 8.1, H-6'b), 3.40 (1H, m, H-3'), 3.38 (1H, m, H-2'), 3.31 (1H, m, H-4'), 3.22 (1H, m, H-5'); ¹³C-NMR (CD₃OD, 100 MHz): ppm δ 208.5 (C-1), 151.3 (C-9), 145.1 (C-4), 137.4 (C-5), 136.7 (C-6), 131.6 (C-7), 125.2 (C-8), 83.8 (C-3), 65.9 (C-11), 60.3 (C-14), 56.1 (C-2), 31.8 (C-13), 20.2 (C-12), 17.5 (C-10), 12.9 (C-15), sugar signals: ppm δ 104.4 (C-1'), 77.0 (C-5'), 76.0 (C-3'), 74.3 (C-2'), 70.4 (C-4'), 61.5 (C-6'); LR ESI-MS m/z 449.0 [M+Na]⁺; HR ESI-MS m/z 449.1787 (calcd. for C₂₁H₃₀O₉Na, 449.1789).

β-D-Xylopyranosyl(1→2)-7-O-benzoyl-β-D-glucopyranoside (2): white amorphous powder; ¹H-NMR (400 MHz, CD₃OD) and ¹³C-NMR (CD₃OD, 100 MHz) see Table 1; LR ESI-MS m/z: 439 [M+Na]⁺, HR ESI-MS m/z: 439.1214 (calcd. for C₁₈H₂₄O₁₁Na⁺, 439.1216).

4-O-Benzoyl-β-D-xylopyranosyl(1→2)-7-O-benzoyl-β-D-glucopyranoside (3): white amorphous powder; ¹H-NMR (400 MHz, CD₃OD) and ¹³C-NMR (CD₃OD, 100 MHz) see Table 1; LR ESI-MS m/z: 439 [M+Na-benzoyl]⁺, 543 [M+Na]⁺; HR ESI-MS m/z: 543.1475 (calcd. for C₂₅H₂₈O₁₂Na⁺, 543.1473).

5-[2-Hydroxyethylidene]-2(5H)-furanone (4): brown amorphous powder; [α]_D²⁵: -20.7° (c 0.31, MeOH); UV (MeOH): λ_{max} (log ε): 264 (3.83) nm; IR (Neat): ν_{max} = 1745 cm⁻¹; ¹H-NMR (CDCl₃, 400 MHz): ppm δ 7.39 (1H, d, J = 5.0 Hz, H-3), 6.24 (1H, d, J = 5.0 Hz, H-4), 5.47 (1H, t, J = 5.0 Hz, H-6), 4.53 (2H, d, J = 5.0 Hz, H-7); ¹³C-NMR (CDCl₃, 100 MHz): ppm δ 169.5 (C-2), 120.6 (C-3), 143.6 (C-4), 149.5 (C-5), 114.1 (C-6), 57.3 (C-7); LR ESI-MS m/z: 149 [M+Na]⁺.

Pterodin B (5): white amorphous powder; [α]_D²⁵: -16.0° (c 0.20, MeOH); ¹H-NMR (200 MHz, CD₃OD): ppm δ 7.09 (1H, s, H-4), 3.76 (2H, t, J = 7.4 Hz, H-13), 3.02 (2H, t, J = 7.4 Hz, H-12), 2.68 (3H, s, H-14), 2.55-2.65 (2H, m, H-3), 2.43 (3H, s, H-11), 1.27 (3H, d, J = 6.3 Hz, H-10); ¹³C-NMR (50 MHz, CD₃OD): ppm δ 210.3 (C-1), 152.6 (C-9), 144.4 (C-5), 138.0 (C-7), 134.8 (C-6), 132.5 (C-8), 125.8 (C-4), 61.6 (C-12), 42.8 (C-2), 34.7 (C-3), 32.8 (C-13), 21.4 (C-11), 16.8 (C-10), 13.7 (C-14); LR ESI-MS m/z: 241 [M+Na]⁺.

β-D-Glucopyranosyl benzoic acid ester (6): white amorphous powder; ¹H-NMR (200 MHz, CD₃OD): ppm δ 8.09 (2H, dd, 8.3, 1.4 Hz, H-2,6), 7.50 (2H, td, 8.3, 1.4 Hz, H-3,4), 7.63 (1H, tt, 8.3, 1.4 Hz, H-

1), sugar signals: ppm δ 5.73 (1H, d, $J = 7.6$, H-1'), 3.86 (1H, dd, $J = 12.1, 2.1$, H-6'a), 3.71 (1H, dd, $J = 12.1, 8.1$, H-6'b), 3.52 (1H, m, H-2'), 3.49 (1H, m, H-3'), 3.44 (1H, m, H-4'), 3.43 (1H, m, H-5'); ^{13}C NMR (CD_3OD , 100 MHz): ppm δ 131.0 (C-1), 130.7 (C-2), 129.6 (C-3), 134.8 (C-4), 129.6 (C-5), 130.7 (C-6), 166.7 (C-7), sugar signals: ppm δ 96.3 (C-1'), 78.0 (C-3'), 78.9 (C-5'), 74.0 (C-2'), 71.0 (C-4'), 62.3 (C-6'); LR ESI-MS: m/z 307 $[\text{M}+\text{Na}]^+$.

Benzoic acid (**7**): white amorphous powder, ^1H -NMR (200 MHz, CD_3OD): ppm δ 8.09 (2H, dd, 8.3, 1.4 Hz, H-2,6), 7.50 (2H, td, 8.3, 1.4 Hz, H-3,4), 7.63 (1H, tt, 8.3, 1.4 Hz, H-1); LR EI-MS: m/z 145 $[\text{M}+\text{Na}]^+$.

5-O-Coumaroylquinic acid (**8**): yellow amorphous powder; $[\alpha]_{\text{D}}^{25}$: -30.5° (c 0.22, MeOH); ^1H -NMR (400 MHz, CD_3OD): ppm δ 7.65 (d, J 16.0 Hz, H-7'), 7.48 (d, J 8.6 Hz, H-2',6'), 6.84 (d, J 8.6 Hz, H-3',5'), 6.34 (d, $J = 16.0$ Hz, H-8'), 5.33 (m, H-5), 3.90 (d, $J = 3.1$ Hz, H-3), 3.77 (dd, $J = 8.6, 3.0$ Hz, H-4), 2.01-2.29 (m, H-2, -6); ^{13}C -NMR (100 MHz, CD_3OD): ppm δ 177.64 (C-7), 169.38 (C-9'), 161.48 (C-4'), 147.34 (C-7'), 131.69 (C-2',6'), 127.70 (C-1'), 117.31 (C-3',5'), 115.68 (C-8'), 76.60 (C-1), 73.82 (C-5), 72.43 (C-3), 72.43 (C-3), 71.66 (C-4), 39.14 (C-6), 38.56 (C-2); LR ESI-MS: m/z 339 $[\text{M}+\text{H}]^+$.

Coumaric acid (**9**): yellow amorphous powder; ^1H -NMR (200 MHz, CD_3OD): ppm δ 7.57 (1H, d, $J = 16.0$ Hz, H-7), 6.28 (1H, d, $J = 16.0$ Hz, H-8), 7.44 (2H, d, $J = 8.0$ Hz, H-2, -6), 6.80 (2H, d, $J = 8.0$ Hz, H-3,-5); LR ESI-MS: m/z 165 $[\text{M}+\text{H}]^+$.

Cyclolaudenol (**10**): amorphous powder; $[\alpha]_{\text{D}}^{25}$: $+23.5^\circ$ (c 0.25, MeOH); ^1H -NMR (400 MHz, CDCl_3): ppm δ 4.67 (2H, m, H-26), 3.28 (1H, dd, $J = 11.5, 4.5$ Hz, H-3), 2.10 (1H, pseudosextet, $J = 7.0$ Hz, H-24), 1.96 (1H, m, H-11 α), 1.87 (1H, m, H-7 β), 1.75 (1H, m, H-2 α), 1.64 (3H, brs, H-27), 1.60 (2H, m, H-12), 1.58 (1H, m, H-6 α), 1.56 (m, H-1 α , H-2 β , H-17), 1.50 (1H, m, H-8), 1.42 (1H, m, H-23a), 1.36 (1H, m, H-20), 1.33 (2H, m, H-16a, H-22a), 1.28 (4H, m, H-5, H-7 α , H-15a,b), 1.24 (1H, m, H-1 β), 1.10 (H, m, H-11 β , H-16b, H-23a), 1.00 (3H, d, $J = 7.0$ Hz, H-31), 0.97 (3H, s, H-28), 0.96 (3H, s, H-18), 0.92 (H, m, H-22b), 0.89 (3H, s, H-30), 0.86 (3H, d, $J = 6.5$ Hz, H-21), 0.81 (3H, s, H-29), 0.80 (1H, m, H-6 β), 0.55, 0.33 (each 1H, d, $J = 4.5$ Hz, H-19); ^{13}C -NMR (CDCl_3 , 100 MHz): δ 150.2 (C-25), 109.4 (C-26), 78.9 (C-3), 52.3 (C-17), 48.8 (C-13), 48.0 (C-8), 47.1 (C-5), 45.3 (C-14), 41.6 (C-24), 40.5 (C-4), 36.0 (C-20), 35.6 (C-15), 33.9 (C-22), 32.9 (C-12), 32.0 (C-1), 31.5 (C-23), 30.4 (C-2), 29.9 (C-19), 28.1 (C-7), 26.5 (C-11), 26.1 (C-10), 26.0 (C-16), 25.4 (C-28), 21.1 (C-6), 20.1 (C-31), 20.0 (C-9), 19.3 (C-30), 18.7 (C-27), 18.3 (C-21), 18.0 (C-18), 14.0 (C-29); LR EI-MS: m/z 440 $[\text{M}]^+$.

β -Sitosterol-3-O- β -D-glucoside (**11**): white amorphous powder; $[\alpha]_{\text{D}}^{25}$: -20.0° (c 0.18, MeOH); ^1H -NMR (200 MHz, CDCl_3): δ 5.25 (1H, d, $J = 4.5$ Hz, H-6), 5.12 (1H, dd, $J = 12.3, 8.3$ Hz, H-23), 5.02 (1H, dd, $J = 12.3, 8.3$ Hz, H-22), 4.35 (1H, d, $J = 7.6$ Hz, aromatic H), 3.45 (1H, m, H-3), 3.10-3.90 (5H, m, sugar moiety H), 1.01 (3H, s, H-19), 0.68 (3H, s, H-18), 0.94 (3H, d, $J = 6.5$ Hz, H-21), 0.86 (3H, t, $J = 7.1$ Hz, H-29), 0.84 (3H, s, H-27); LR EI-MS: m/z 576 $[\text{M}]^+$.

β -D-Sitosterol (**12**): white amorphous powder; $[\alpha]_{\text{D}}^{25}$: -50.1° (c 0.10, MeOH); $^1\text{H-NMR}$ (200 MHz, CDCl_3): δ 5.36 (1H, br s, H-6), 5.12 (1H, dd, $J = 16.1, 8.3$ Hz, H-23), 5.02 (1H, dd, $J = 16.1, 8.3$ Hz, H-22), 1.01 (3H, s, H-19), 0.92 (3H, d, $J = 6.4$ Hz, H-21), 0.86 (3H, t, $J = 7.0$ Hz, H-29), 0.84 (3H, d, $J = 6.8$ Hz, H-27), 0.81 (3H, d, $J = 6.8$ Hz, H-26), 0.68 (3H, s, H-18); LR EI-MS: m/z 412 $[\text{M}]^+$.

Cytotoxicity Assays

Compounds were assayed for cytotoxicity against Hep G2, A549, MCF-7, MDA-MB-231, Ca9-22 and HL 60 cell lines using the MTT method [26]. Freshly trypsinized cell suspensions were seeded in 96-well microtitre plates at densities of 5,000-10,000 cells per well and tested compounds were added from a DMSO stock solution. After 3 days in culture, attached cells were incubated with MTT (0.5 $\mu\text{g/ml}$, 1 h) and subsequently dissolved in DMSO. The absorbance was measured at 550 nm using a microplate reader. The IC_{50} is the concentration of agent reduced cell growth by 50% under the experimental conditions. Results represent the mean two to three separate experiments, each performed in triplicate.

Acid hydrolysis of compound 1

A solution of compound **1** (5.0 mg) in 6% aqueous HCl (3.5 mL) was refluxed for 2 h. The reaction mixture was diluted with water and then extracted with ethyl acetate. The resulting glucose (in water) and 2*R*,3*R* pterisin L (in ethyl acetate) were identified by their corresponding $^{13}\text{C-NMR}$ spectra.

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