Photinides A−F, Cytotoxic Benzofuranone-Derived γ-Lactones from the Plant Endophytic Fungus *Pestalotiopsis photiniae*

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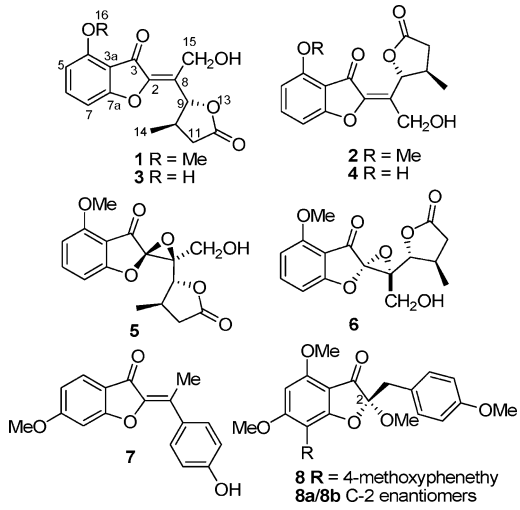
Photinides A−F (**1**−**6**), six new unique benzofuranone-derived γ-lactones, have been isolated from the crude extract of the plant endophytic fungus *Pestalotiopsis photiniae*. The structures of these compounds were elucidated primarily by NMR spectroscopy, and their absolute configurations were assigned by application of the CD excitation chirality method. Compounds **1**−**6** displayed modest cytotoxic effects against the human tumor cell line MDA-MB-231.

Endophytic fungi that inhabit normal tissues of the host plants without causing apparent pathogenic symptoms have been demonstrated to be rich sources of bioactive natural products.[1−4] The widely distributed endophytic fungi *Pestalotiopsis* spp. are capable of producing different classes of compounds, and our prior chemical studies of this genus have led to the isolation of a variety of bioactive metabolites.[5−8] During an ongoing search of new bioactive natural products from species of this genus, a strain of *Pestalotiopsis photiniae* (L461) was isolated from the plant *Roystonea regia* (H.B.K.) Cook (Arecoideae) collected from Jianfeng Mountain, Hainan Province, People's Republic of China. *P. photiniae* was grown in a solid-substrate fermentation culture. An organic solvent extract of the culture inhibited growth of the human tumor cell line MDA-MB-231. Fractionation of the extract led to the isolation of six new cytotoxic benzofuranone-derived γ-lactones, which we named photinides A−F (**1**−**6**). Details of the isolation, structure elucidation, and cytotoxicity of these compounds are reported herein.



Photinide A (**1**) was assigned the molecular formula $C_{16}H_{16}O_6$ (nine degrees of unsaturation) on the basis of its HRESIMS (*m*/*z* 327.0836 $[M + Na]^+$; +0.3 mmu). Analysis of the ^1H and ^{13}C NMR spectroscopic data of **1** (Table 1) revealed two methyl groups (one *O*-methyl), two methylene units (one oxygenated), two methines (one oxygenated), eight olefinic/aromatic carbons (three of which were protonated), one carboxyl carbon, and one ketone carbon. These data, together with one exchangeable proton (δ_H 4.27), accounted for all the ^1H and ^{13}C NMR resonances and suggested that **1** was a tricyclic metabolite. Interpretation of the $^1H-^1H$ COSY NMR data of **1** identified two isolated proton spin systems, which were C-5−C-7 and C-9−C-11 (including C-14). HMBC correlations from H-5 to C-4 and C-3a and H-7 to C-3a and C-7a permitted completion of the trisubstituted aryl ring of **1**. Correlation of H_3-16 with C-4 located the methoxy group at C-4, whereas the long-range *W*-type cross-peak from H-5 to C-3 led to the connection of C-3 to C-3a.[9] Those from H-9, H-10, and H_2-11 to C-12 established the structure of a γ-lactone moiety. In addition, the correlations in the HMBC spectrum from H-9 to C-2, C-8, and C-15 and from H_2-15 to C-2, C-8, and C-9 indicated that C-2, C-9, and C-15 were all attached to C-8, leading to the connection of the C-2/C-8 olefin to the γ-lactone ring at C-9. Considering the ^{13}C NMR chemical shifts of C-2 (δ_C 146.4), C-3 (δ_C 182.3), and C-7a (δ_C 167.0), the ^1H NMR chemical shift of the exchangeable proton (δ_H 4.27), and the unsaturation requirement for **1**, C-2 and C-7a were all connected to the same oxygen atom to form an ether linkage, and C-2 was further linked to the ketone carbon C-3 to complete the benzofuranone moiety. The only exchangeable proton (δ_H 4.27) was assigned to be the OH-15 by default. On the basis of these data, the gross structure of **1** was elucidated as shown.

The relative configuration of **1** was determined by analysis of its NOESY data. The vicinal coupling constant of 7.5 Hz between H-9 and H-10 and the NOESY correlation of H-9 with H_3-14 suggested their *cis* relationship on the γ-lactone ring.[10] The CD spectrum of **1** showed a negative Cotton effect at 210 nm, which reflected the chirality of the γ-lactone chromophore,[11] suggesting the 9*R* absolute configuration. Collectively, these data permitted assignment of the *R* absolute configuration of the other stereogenic center C-10.

Photinide B (**2**) gave a pseudomolecular ion $[M + Na]^+$ peak at *m*/*z* 327.0841 (Δ −0.2 mmu) by HRESIMS, which is consistent with an elemental formula of $C_{16}H_{16}O_6$ (nine degrees of unsaturation). Analysis of the ^1H and ^{13}C NMR data of **2** revealed structural features similar to those present in **1**, except that the resonance for the oxymethine proton (H-9) was shifted significantly upfield from δ_H 6.19 to 5.29, whereas that for H-15a was shifted downfield from δ_H 4.70 to 5.12 in the ^1H NMR spectrum of **2**, implying an orientation change for the γ-lactone moiety attached to the C-2/C-8 olefin in **2**. This postulation was confirmed by analysis of the $^1H-^1H$ COSY and HMBC data of **2**, allowing determination of the same gross structure as **1**.

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Table 1. NMR Spectroscopic Data for Photinides A (**1**) and B (**2**) in Acetone-d_6

	photinide A (**1**)			photinide B (**2**)	
position	δ_H[a] (*J* in Hz)	δ_C[b]	HMBC (H → C#)	δ_H[a] (*J* in Hz)	δ_C[c]
2		146.4, qC			146.7, qC
3		182.3, qC			182.3, qC
3a		112.2, qC			112.1, qC
4		159.6, qC			159.7, qC
5	6.79, d (8.0)	106.4, CH	3, 3a, 4, 7, 7a	6.84, d (8.0)	106.5, CH
6	7.68, dd (8.5, 8.0)	140.2, CH	4, 5, 7, 3a	7.69, t (8.0)	140.3, CH
7	6.84, d (8.5)	104.9, CH	3a, 5, 7a	6.81, d (8.0)	104.9, CH
7a		167.0, qC			167.1, qC
8		128.6, qC			129.5, qC
9	6.19, d (7.5)	80.4, CH	2, 8, 10, 11, 12, 14, 15	5.29, d (7.0)	83.0, CH
10	2.86, m	37.4, CH	8, 9, 11, 12, 14	2.83, m	36.9, CH
11	2.78, dd (17, 8.5)	37.0, CH_2	9, 10, 12, 14	2.83, m	36.8, CH_2
	2.30, dd (17, 8.5)			2.35, dd (17, 9.0)	
12		176.7, qC			176.5, qC
14	1.22, d (7.5)	17.9, CH_3	9, 10, 11	1.24, d (7.0)	17.6, CH_3
15	4.70, br d (13)	56.5, CH_2	2, 8, 9	5.12, br d (13)	54.3, CH_2
	4.41, br d (13)			4.41, br d (13)	
16	3.96, s	56.5, CH_3	4	3.95, s	56.6, CH_3
OH-15	4.27, br s				

[a] Recorded at 500 MHz. [b] Recorded at 100 MHz. [c] Recorded at 150 MHz.

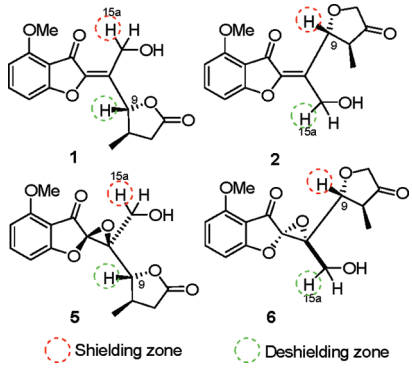

Figure 1. Shielding and deshielding zones for compounds **1**, **2**, **5**, and **6**.

The relative configuration of **2** was assigned on the basis of NOED data. Upon irradiation of H_3-14, enhancement was observed for H-9 in the NOE difference spectrum of **2**, suggesting a *cis* relationship between H-9 and H_3-14 with respect to the γ-lactone ring. The absolute configuration of C-9 in **2** was also assigned as *R* considering the negative Cotton effect observed at 219 nm in its CD spectrum.[11]

To account for the above-mentioned chemical shift differences for H-9 and H-15a between **1** and **2**, the geometry of the C-2/C-8 olefin unit had to be different in these compounds. However, this difference could not be identified by NOESY or NOED experiments due to the lack of cross-peaks for relevant protons. The shielding or deshielding effect on nearby protons resulting from the diamagnetic anisotropy of the ketone group (C-3) was used to assign the *cis* or *trans* double bond of the exocyclic α,β-unsaturated ketone moiety.[12] The ketone functionality usually displays a shielding effect on the substituent *cis* to it (in its shielding zone) with respect to the double bond, resulting in an upfield shift for corresponding protons, whereas its deshielding effect on the substituent *trans* to it (in its deshielding zone) will cause a downfield shift for relevant protons.[12] The relatively downfield chemical shift of H-9 (δ_H 6.19) in **1** indicated that it was located in the deshielding zone of the α,β-unsaturated ketone functionality (Figure 1), whereas the relatively upfield chemical shift of H-15a (δ_H 4.70) placed it in the shielding zone. Therefore, the C-2/C-8 olefin in **1** was assigned the *Z*-geometry. On the basis of similar considerations, the *E*-geometry was proposed for the C-2/C-8 olefin in **2**.

Photinides C (**3**) and D (**4**) were obtained as an inseparable mixture of two epimers in a 2:1 ratio, as determined by integration of some well-resolved ^{1}H NMR resonances for each compound. Exhaustive efforts to separate the mixture using column chromatography and HPLC with various stationary and mobile phases were unsuccessful. Thus, the structure elucidation was performed on the mixture. Compounds **3** and **4** were each assigned a molecular formula of $C_{15}H_{14}O_6$ (nine degrees of unsaturation) by HRESIMS (*m/z* 313.0680 $[M + Na]^+$; Δ +0.3 mmu). The ^{1}H and ^{13}C NMR spectra of **3** and **4** displayed resonances nearly identical to those found in the spectra of **1** and **2**, except that the resonances for OMe-16 were not observed, suggesting that **3** and **4** were the desmethyl analogues of **1** and **2**. Analysis of their ^{1}H−^{1}H COSY and HMBC data confirmed the above observations and permitted assignment of **3** and **4** as the desmethyl analogues of **1** and **2**, respectively. The relative and absolute configurations of **3** and **4** were presumed to be analogous to those of **1** and **2**.

The molecular formula of **5** was established as $C_{16}H_{16}O_7$ (nine degrees of unsaturation) by its HRESIMS (*m/z* 343.0785 $[M + Na]^+$; Δ +0.3 mmu), one more oxygen atom than **1** and **2**. Analysis of the ^{1}H and ^{13}C NMR data of **5** (Table 2) revealed its structural similarity to **1**, except that the oxymethine proton (H-9) was shifted significantly upfield to δ_H 4.88, and the oxymethylene protons (H_2-15) were also shifted upfield to δ_H 4.24 and 3.90, respectively, in the ^{1}H NMR spectrum of **5**. In addition, the ^{13}C NMR resonances for the C-2/C-8 olefin were replaced by those for two oxygenated sp^3 quaternary carbons (δ_C 69.6 and 90.6, respectively). Considering the unsaturation requirement for **5**, C-2 and C-8 had to be attached to the extra oxygen atom to form an epoxide moiety to complete its gross structure as shown.

The relative configuration of **5** was also assigned by analysis of its NOESY data. The distinct cross-peak of H-9 with H_3-14 revealed their *cis* relationship with respect to the γ-lactone ring, whereas the absence of a NOSEY correlation of H-9 with H_2-15 confirmed their *trans* configuration (Figure 2). The absolute configuration of C-9 was established by analysis of its CD spectrum, which exhibited a negative Cotton effect at 228 nm, indicating the 9*R* absolute configuration.[11]

Photinide F (**6**) was assigned the same molecular formula $C_{16}H_{16}O_7$ as **5** by HRESIMS (*m/z* 343.0789 $[M + Na]^+$; Δ −0.1 mmu), and interpretation of its ^{1}H and ^{13}C NMR data established the same gross structure as **5**. However, the resonance for H-9 was shifted upfield from δ_H 4.88 to 4.44, whereas that for H-15a was shifted downfield from δ_H 3.90 to 4.14 in the ^{1}H NMR spectrum of **6**, implying that **6** was a stereoisomer of **5**.

Considering the chemical shift variations for H-9 and H-15a between **5** and **6**, H-9 was placed in the deshielding zone of the

Table 2. NMR Spectroscopic Data for Photinides C−F (**3**−**6**) in Acetone-d_6

	photinide C (**3**)		photinide D (**4**)		photinide E (**5**)		photinide F (**6**)	
position	δ_H[a] (J in Hz)	δ_C[b]	δ_H[a] (J in Hz)	δ_C[b]	δ_H[a] (J in Hz)	δ_C[b]	δ_H[a] (J in Hz)	δ_C[b]
2		146.4, qC		146.4, qC		90.6, qC		90.0, qC
3		184.0, qC		184.0, qC		189.1, qC		188.6, qC
3a		111.1, qC		111.4, qC		110.1, qC		110.4, qC
4		157.8, qC		157.9, qC		159.1, qC		159.0, qC
5	6.74, d (8.0)	110.0, CH	6.74, d (8.0)	110.0, CH	6.82, d (8.5)	106.3, CH	6.84, d (8.5)	106.4, CH
6	7.57, t (8.0)	140.2, CH	7.57, t (8.0)	140.2, CH	7.76, dd (8.5, 8.0)	141.9, CH	7.76, t (8.5)	141.7, CH
7	6.64, d (8.0)	103.5, CH	6.64, d (8.0)	103.5, CH	6.79, d (8.0)	105.8, CH	6.82, d (8.5)	105.5, CH
7a		166.1, qC		166.1, qC		171.0, qC		170.2, qC
8		128.9, qC		129.9, qC		69.6, qC		70.0, qC
9	6.17, d (6.5)	80.4, CH	5.29, d (6.5)	83.0, CH	4.88, d (6.0)	84.5, CH	4.44, d (7.0)	87.3, CH
10	2.87, m	37.0, CH	2.87, m	37.3, CH	3.00, m	36.6, CH	3.00, m	36.5, CH
11	2.77, dd (17, 8.5) 2.30, dd (17, 8.0)	36.7, CH_2	2.82, dd (17, 8.5) 2.34, dd (17, 9.5)	37.0, CH_2	2.85, m 2.18, dd (17, 7.0)	32.8, CH_2	2.77, m 2.22, dd (17, 7.5)	32.8, CH_2
12		176.8, qC		176.8, qC		176.7, qC		176.6, qC
14	1.22, d (6.5)	17.8, CH_3	1.25, d (6.5)	17.7, CH_3	1.07, d (6.0)	19.0, CH_3	1.20, d (7.0)	18.8, CH_3
15	4.71, d (13) 4.42, d (13)	56.5, CH_2	5.15, d (13) 4.42, d (13)	54.2, CH_2	4.24, d (13) 3.90, d (13)	60.5, CH_2	4.20, d (13) 4.14, d (13)	57.0, CH_2
16					3.97, s	56.7, CH_3	3.98, s	56.6, CH_3
OH-15					4.55, br s		4.28, br s	

[a] Recorded at 500 MHz. [b] Recorded at 150 MHz.

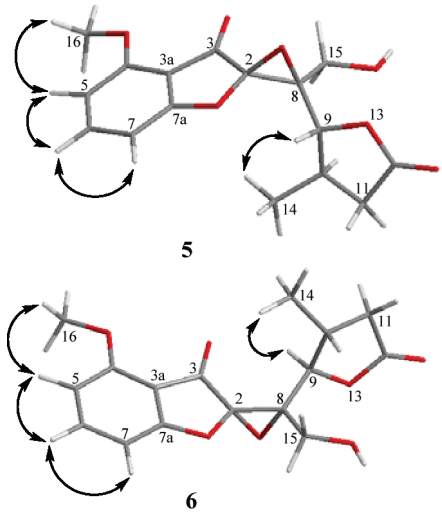

Figure 2. Key NOESY correlations for photinides E (**5**) and F (**6**).

ketone group in **5** versus the shielding zone in **6**, whereas H-15a was placed in the shielding zone in **5** versus the deshielding zone in **6**, as exemplified in **1**−**4**. The NOESY spectrum of **6** showed identical correlations for relevant protons (Figure 2), and the CD spectrum of **6** was also nearly identical to that of **5**, suggesting the 9*R* and 10*R* absolute configuration for both compounds.

The absolute configuration of C-2 in **5** and **6** was also confirmed by analysis of their CD data. The CD spectrum of **5** showed a negative Cotton effect in the 330−380 nm region resulting from the n→ π transition, which was similar to that of a model compound, 7-(4-methoxyphenethyl)tetra-*O*-methylmaesopsin enantiomer **8b**, suggesting the 2*R* absolute configuration;[13] thus C-8 was determined to be *S* in **5**, whereas the opposite Cotton effect appearing in the same region in the CD spectrum of **6** matched that of the other model compound, 7-(4-methoxyphenethyl)tetra-*O*-methylmaesopsin enantiomer **8a**, indicating the 2*S* absolute configuration in **6**.[13]

Photinides A−F (**1**−**6**) were evaluated for cytotoxic activity against two human tumor cell lines, MDA-MB-231 and HeLa. Compounds **1**−**6** showed modest but selective cytotoxicity against MDA-MB-2311, with inhibitory rates of 24.4%, 24.2%, 23.1%, 24.4%, and 24.6%, respectively, when tested at 10 μg/mL, whereas none of these compounds displayed cytotoxicity against HeLa cells at the same concentration.

Photinides A−F (**1**−**6**) are new members of the benzofuranone class of metabolites, and they are structurally related to the aurones with 6-methoxy-9-methyl-4′-hydroxyaurone (**7**) as the closest precedent.[14] However, **1**−**6** differ significantly from the known aurones by having a 4-methyl-γ-lactone moiety and an oxymethylene unit attached to C-8 instead of the *para*-hydroxyphenyl ring and the methyl group. To our knowledge, **1**−**6** are the first examples of the benzofuranone-derived γ-lactones that are structurally related to aurones. From the biosynthetic point of view, aurones were originated from chalcone,[15] whereas most of the known fungal γ-lactones are considered to be biosynthesized through the polyketide pathway. Most likely, the biosynthesis of **1**−**6** proceeds in a manner similar to that of known γ-lactone precedents.[16] In addition, **5** and **6** could originate from **1** and **2** by an epoxidation of the C-2/C-8 olefin.

Photinides A−F (**1**−**6**) are the first secondary metabolites to be reported from *P. photiniae* and indicate that *Pestalotiopsis* spp. warrant further chemical investigations to explore their potential as producers of bioactive natural products.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 polarimeter, and UV data were recorded on a Shimadzu Biospec-1601 spectrophotometer. The CD spectra were recorded on a JASCO J-815 spectropolarimeter, using CH_3OH as solvent. IR data were recorded using a Nicolet Magna-IR 750 spectrophotometer. ^1H and ^{13}C NMR data were acquired with Varian Mercury-400, -500, and -600 spectrometers using solvent signals (acetone-d_6; δ_H 2.05/δ_C 29.8, 206.0) as references. The HMQC and HMBC experiments were optimized for 145.0 and 8.0 Hz, respectively. ESIMS data were recorded on a Bruker Esquire 3000^{plus} spectrometer, and HRESIMS data were obtained using Bruker APEX III 7.0 T and APEXII FT-ICR spectrometers, respectively.

Fungal Material. The culture of *P. photiniae* was isolated from the plant *Roystonea regia* (H.B.K.) Cook (Arecoideae) collected from Jianfeng Mountain, Hainan Province, People's Republic of China, in April 2005. The isolate was identified by one of authors (L.G.) on the basis of morphology and sequence analysis of the ITS region of the rDNA and assigned the accession number L461 in L.G.'s culture collection at the Institute of Microbiology, Chinese Academy of Sciences, Beijing. The fungal strain was cultured on slants of potato dextrose agar (PDA) at 25 °C for 10 days. The agar plugs were used to inoculate 250 mL Erlenmeyer flasks, each containing 50 mL of media (0.4% glucose, 1% malt extract, and 0.4% yeast extract), and the final pH of the media was adjusted to 6.5 before sterilization. Flask cultures were incubated at 25 °C on a rotary shaker at 170 rpm for 5 days. Ten 500 mL Erlenmeyer flasks, each containing 150 mL of liquid media (6% dextrin, 2% maltose, 0.75% cottonseed meal, 0.7% peptone, 0.25% $CaCO_3$, 0.25% $MgSO_4 \cdot 7H_2O$, 0.1% $FeSO_4 \cdot 7H_2O$, 0.001% $ZnSO_4$; final

pH 6.0) and 30 g of vermiculite were individually inoculated with 15 mL of the seed culture and incubated at 25 °C under static conditions for 40 days.

Extraction and Isolation. The fermented material was extracted with methyl ethyl ketone (3 × 500 mL), and the organic solvent was evaporated to dryness under vacuum to afford a crude extract (3.0 g), which was fractionated by silica gel column chromatography (CC) (5 × 25 cm) using CH_2Cl_2−MeOH gradient elution. The fraction (50 mg) eluted with 99:1 CH_2Cl_2−CH_3OH was further separated by semi-preparative RPHPLC (Agilent Zorbax SB-C_{18} column; 5 μm; 9.4 × 250 mm; 30% MeOH in H_2O for 5 min, followed by 30−55% for 60 min; 2 mL/min) to afford photinide A (**1**; 20.0 mg, t_R 18.0 min) and a mixture of photinides B and F (**2** and **6**; 5.5 mg, t_R 20.5 min). Further separation of the mixture of **2** and **6** by HPLC (35% MeCN in H_2O for 5 min, followed by 35−55% for 50 min) afforded photinides B (**2**; 2.5 mg, t_R 20.0 min) and F (**6**; 2.0 mg, t_R 20.5 min). The fraction (20 mg) eluted with 98:2 CH_2Cl_2−CH_3OH was purified by HPLC (40% MeOH in H_2O for 2 min, followed by 40−100% for 40 min) to afford a mixture of photinides C and D (**3** and **4**; 3.0 mg, t_R 16.0 min). Another fraction eluted with 98:2 CH_2Cl_2−CH_3OH was separated by Sephadex LH-20 CC using MeOH as eluent to afford a subfraction of 15 mg, and further purification of this subfraction by HPLC (40% MeOH in H_2O for 2 min, followed by 40−60% for 40 min) afforded photinide E (**5**; 2.5 mg, t_R 17.5 min).

Photinide A (1): amorphous, pale yellow powder; $[\alpha]_D$ −289 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (ε) 374 (12 500), 280 (12 900), 229 (14 100) nm; IR (neat) ν_{max} 3474 (br), 2966, 1775, 1698, 1603, 1494 cm^{-1}; ^1H, ^{13}C, and HMBC data see Table 1; key NOESY correlations (acetone-d_6, 600 MHz) H-5 ↔ H_3-16, H-6; H-6 ↔ H-5, 7; H-9 ↔ H_3-14; HRESIMS *m/z* 327.0836 $[M + Na]^+$ (calcd for $C_{16}H_{16}O_6Na$, 327.0839).

Photinide B (2): pale yellow oil; $[\alpha]_D$ +16 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (ε) 371 (10 300), 273 (36 000), 207 (8450); IR (neat) ν_{max} 3474 (br), 2967, 1772, 1697, 1599, 1493 cm^{-1}; ^1H and ^{13}C data see Table 1; key NOED data (acetone-d_6, 600 MHz) H-9 ↔ H_3-14; HRESIMS *m/z* 327.0841 $[M + Na]^+$ (calcd for $C_{16}H_{16}O_6Na$, 327.0839).

Photinides C and D (3 and 4): pale yellow oil; $[\alpha]_D$ +29 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (ε) 371 (8400), 279 (12 900), 229 (14 000) nm; IR (neat) ν_{max} 3462 (br), 2966, 1775, 1698, 1606, 1495 cm^{-1}; ^1H and ^{13}C data see Table 2; key NOESY correlations (acetone-d_6, 600 MHz) H-5 ↔ H_3-16, H-6; H-6 ↔ H-5, 7; H-9 ↔ H_3-14; HRESIMS *m/z* 313.0680 $[M + Na]^+$ (calcd for $C_{15}H_{14}O_6Na$, 313.0683).

Photinide E (5): pale yellow oil; $[\alpha]_D$ −230 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (ε) 342 (6200), 276 (12 700), 223 (13 500) nm; IR (neat) ν_{max} 3450 (br), 2934, 1778, 1715, 1609 cm^{-1}; ^1H and ^{13}C data see Table 2; key NOESY correlations (acetone-d_6, 600 MHz) H-5 ↔ H_3-16, H-6; H-6 ↔ H-5, 7; H-9 ↔ H_3-14; HRESIMS *m/z* 343.0785 $[M + Na]^+$ (calcd for $C_{16}H_{16}O_7Na$, 343.0788).

Photinide F (6): pale yellow oil; $[\alpha]_D$ +13 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (ε) 343 (5000), 279 (11 500), 219 (12 700) nm; IR (neat) ν_{max} 3450 (br), 2935, 1780, 1716, 1609 cm^{-1}; ^1H and ^{13}C data see Table 2; key NOESY correlations (acetone-d_6, 600 MHz) H-5 ↔ H_3-16, H-6; H-6 ↔ H-5, 7; H-9 ↔ H_3-14; HRESIMS *m/z* 343.0789 $[M + Na]^+$ (calcd for $C_{16}H_{16}O_7Na$, 343.0788).

MTT Assay.[17] In 96-well plates, each well was plated with 10^4 cells. After cell attachment overnight, the medium was removed, and each well was treated with 50 μL of medium containing 0.2% DMSO or appropriate concentration of test compounds (10 mg/mL as stock solutions of a compound in DMSO and serial dilutions). Cells were treated at 37 °C for 4 h in a humidified incubator at 5% CO_2 first, and then the medium was changed to fresh DMEM. MTT (Sigma) was dissolved in serum-free medium or PBS at 0.5 mg/mL and sonicated briefly. In the dark, 50 μL of MTT/medium was added into each well after the medium was removed from wells, and incubated at 37 °C for 3 h. Upon removal of MTT/medium, 100 μL of DMSO was added to each well and agitated at 60 rpm for 5 min to dissolve the precipitate. The assay plate was read at 540 nm using a microplate reader.

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Supporting Information Available: ^1H, ^{13}C NMR, and CD spectra of photinides A−F (**1**−**6**). This material is available free of charge via the Internet at http://pubs.acs.org.

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