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A Thiopyranchromenone and Other Chromone Derivatives from an Endolichenic Fungus, *Preussia africana*

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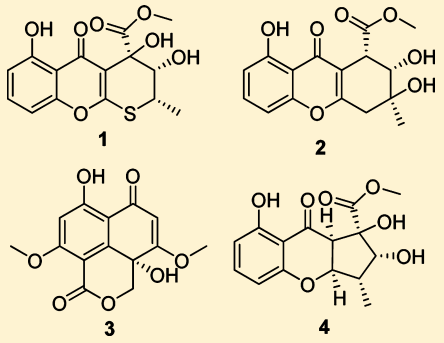
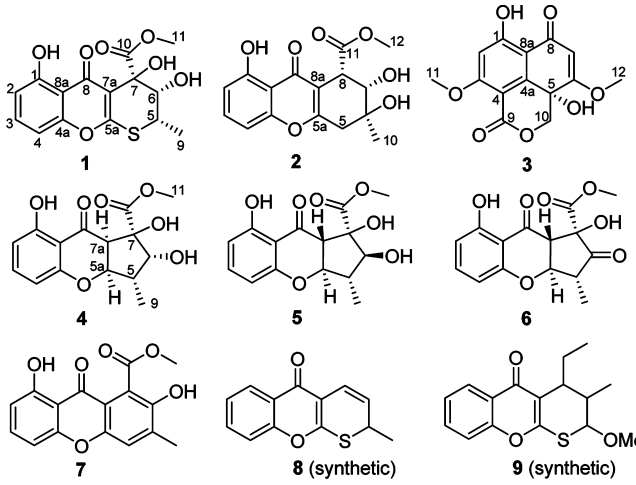
Supporting Information

ABSTRACT: The first example of a naturally occurring thiopyranchromenone, preussochromone A (**1**), and five other new chromone derivatives, preussochromones B–F (**2**–**6**), were isolated from solid cultures of an endolichenic fungus, *Preussia africana*. The structures of **1**–**6** were established primarily by NMR experiments, and **2** and **4** were further confirmed by X-ray crystallography. The absolute configurations of **1** and **2** were determined by the application of electronic circular dichroism (ECD), whereas those of C-5 in **3**, C-6 in **4**, and the 6,7-diol in **5** were deduced via the CD data of the *in situ* formed $[Rh_2(OCOCF_3)_4]$ complex, the modified Mosher method, and Snatzke's method, respectively. Compounds **1** and **3** showed significant cytotoxicity against A549 cells.



Lichens are combinations of a fungus (the mycobiont) and an algal partner (the photobiont or phycobiont). In addition to fungal mycobionts, some nonobligate microfungi, endolichenic fungi, are also found to live asymptomatically in the bodies (thalli) of lichens.[1] Although endolichenic fungi inhabit the lichen thalli similarly to endophytes living in the intercellular spaces of healthy plant tissues, the chemistry of this class of fungi remained largely unexplored. To date, only a limited number of investigations have been documented regarding the isolation and identification of bioactive secondary metabolites from their organisms.[1−6] Our prior work on the endolichenic fungus *Coniochaeta* sp. grown in a solid-substrate fermentation culture afforded four relatively rare ring-expanded (oxepinochromenone) and one ring-contracted (furochromenone) xanthones,[4] as well as the first examples of naturally occurring thienols and thiepinols possessing unique 2,3-dihydrothieno[2,3-*b*]chromen-4-one and 4,5-dihydro-2*H*-thiepino[2,3-*b*]chromen-6(3*H*)-one skeletons, respectively.[5]

During an ongoing search for new cytotoxic natural products from this unique source, the fungus *Preussia africana* (Sporormiaceae) isolated from the lichen *Ramalina calicaris* (L.) Fr. (Ramalinaceae), which was collected from Zixi Mountain, Yunnan Province, People's Republic of China, was subjected to chemical investigation. Fractionation of an EtOAc extract prepared from a solid-substrate fermentation culture led to the isolation of a naturally occurring thiopyranchromenone named preussochromone A (**1**), five other new chromone derivatives, preussochromones B–F (**2**–**6**), and a known xanthone (**7**).[7] Details of the isolation, structure elucidation, and cytotoxicity of these metabolites are reported herein.



RESULTS AND DISCUSSION

Preussochromone A (**1**) gave a pseudomolecular ion $[M + Na]^+$ by HRESIMS, consistent with a molecular formula of $C_{15}H_{14}O_7S$ (nine degrees of unsaturation). Its ^1H and ^{13}C NMR spectra showed resonances for three exchangeable protons (δ_H 5.71, 5.97, and 12.42, respectively), two methyl groups (one methoxy), two methines including an oxymethine, eight aromatic/olefinic carbons with three protonated, one oxygenated sp^3 quaternary carbon, one carboxylic carbon (δ_C 173.5), and one α,β-unsaturated

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Table 1. NMR Data for 1−3

	1			2		3	
pos.	δ_C,[a] mult.	δ_H[b] (J in Hz)	HMBC[a]	δ_C,[c] mult.	δ_H[d] (J in Hz)	δ_C,[c] mult.	δ_H[d] (J in Hz)
1	159.8, qC			160.0, qC		167.4, qC	
2	111.3, CH	6.80, d (8.4)	1, 4, 8a	110.7, CH	6.77, d (8.0)	101.1, CH	6.62, s
3	135.9, CH	7.63, t (8.4)	1, 4a	135.4, CH	7.60, t (8.0)	166.7, qC	
4	106.5, CH	7.14, d (8.4)	2, 4a, 8a	107.0, CH	6.99, d (8.0)	105.8, qC	
4a	155.2, qC			156.5, qC		146.4, qC	
5	39.5, CH	3.95, q (7.2)	9	37.3, CH_2	2.57, d (20); 3.17, d (20)	64.9, qC	
5a	168.8, qC			165.0, qC			
6	71.3, CH	3.64, d (7.2)	5, 7, 7a, 9	71.3, qC		174.7, qC	
7	71.3, qC			71.8, CH	4.04, d (3.5)	101.4, CH	5.73, s
7a	115.2, qC						
8	178.3, qC			43.7, CH	4.00, d (3.5)	190.4, qC	
8a	109.0, qC			113.6, qC		106.3, qC	
9	15.7, CH_3	1.37, d (7.2)	5, 5a, 6	182.2, qC		159.1, qC	
9a				110.7, qC			
10	173.5, qC			25.8, CH_3	1.49, s	70.3, CH_2	4.35, d (12); 4.69, d (12)
11	51.9, CH_3	3.60, s	10	171.3, qC		56.8, CH_3	3.95, s
12				51.3, CH_3	3.69, s	57.6, CH_3	3.95, s
OH-1		12.42, s			12.50, s		13.20, s
OH-5							5.80, s
OH-6		5.71, d (7.2)	5, 7		4.28, s		
OH-7		5.97, s	6, 7a		4.58, s		

[a]Recorded at 150 MHz in DMSO-d_6. [b]Recorded at 600 MHz in DMSO-d_6. [c]Recorded at 125 MHz in acetone-d_6. [d]Recorded at 500 MHz in acetone-d_6.

ketone carbon (δ_C 178.3). Analysis of the NMR data of **1** (Table 1) revealed the presence of the same 5-hydroxy-4*H*-chromen-4-one moiety as that typically found in xanthones (e.g., **7**),[7] but the remaining portion was significantly different, warranting a detailed 2D NMR analysis (Figure S13). In addition to the above-mentioned fragment, the ^{1}H−^{1}H COSY NMR data of **1** showed the isolated spin-system of C-9−C-5−C-6 (including OH-6). HMBC correlations from the *O*-methyl proton signal H_3-11 to the carboxylic carbon at 173.5 ppm located the *O*-methyl group at C-10, while correlations from H-6 to C-7 and C-7a and from the exchangeable proton at 4.54 ppm (OH-7) to C-6 and C-7a indicated that C-7 is attached to both C-6 and C-7a. Considering the chemical shifts of the C-5 methine (δ_H/δ_C 3.95/39.5) and by comparison to a similar unit (δ_H/δ_C 3.96/38.2) in an analogous synthetic compound, 2-methylthiopyrano[2,3-*b*]chromen-5(2*H*)-one,[8] the sulfur atom present in **1** was shown to be attached to C-5 to complete a dihydrothiopyran ring; this was supported by a weak, but distinct four-bond *W*-type HMBC correlation from H_3-9 to C-5a.[9] The C-10 carboxylic carbon is now required to attach to C-7 to satisfy the unsaturation requirement of **1**, even though no additional evidence for this linkage was provided by the HMBC data. Collectively, these data permitted assignment of the planar structure of **1**.

The absolute configuration of **1** was determined by comparison of experimental and simulated electronic circular dichroism (ECD) spectra, which were generated by time-dependent density functional theory (TDDFT).[10] Due to the presence of three stereogenic centers, one of the eight stereoisomers, (5*R*,6*R*,7*S*)-**1**, (5*S*,6*S*,7*R*)-**1**, (5*S*,6*S*,7*S*)-**1**, (5*R*,6*R*,7*R*)-**1**, (5*S*,6*R*,7*S*)-**1**, (5*R*,6*S*,7*R*)-**1**, (5*R*,6*S*,7*S*)-**1**, and (5*S*, 6*R*,7*R*)-**1** (**1a**−**1h**; Figure 1), must represent the actual configuration of **1**. A systematic conformational analysis was performed for **1a**−**1h** via the Molecular Operating Environment (MOE) software package using the MMFF94 molecular mechanics force field calculation. The MMFF94 conformational search was further optimized using TDDFT at the B3LYP/6-31G(d) basis set level, affording five lowest energy conformers for the enantiomer pairs **1a** and **1b**, two for **1c** and **1d**, two for **1e** and **1f**, and three for **1g** and **1h**, respectively (Figures S17−S20). The overall calculated ECD spectra of enantiomers **1a** and **1b** were then generated by Boltzmann weighting of the five lowest energy conformers with 86.00, 12.56, 1.35, 0.01, and 0.07% populations, respectively, by their relative free energies. In a similar fashion, the overall calculated ECD spectra of **1c**−**1h** were also generated. The absolute configuration of **1** was then extrapolated by comparison of experimental and calculated ECD spectra of **1a**−**1h** after a UV correction of 20 nm (Figure S21). The overall pattern of the experimental CD spectrum of **1** was comparable only to that of the calculated ECD spectrum of (5*S*,6*S*,7*R*)-**1** (**1b**), both showing similar negative Cotton effects (CEs) in the regions of 200−225 and 250−350 nm, respectively (Figure 1). The specific optical rotations of the two main conformations of **1b** (Con 1 and Con 2) (Figure S17) have been calculated at the B3LYP/6-31++G(d,p) level using B3LYP/6-31G(d) geometries, with the results given in Table S1. The population-averaged specific optical rotations were compared with the experimental data of **1** in MeOH at 589 nm. The calculated specific rotation of **1b** is consistent with the experimental data of **1**, further supporting the absolute configuration deduced from the ECD spectra. Therefore, the absolute configuration of **1** was deduced to be 5*S*, 6*S*, 7*R*.

The elemental composition of preussochromone B (**2**) was established as $C_{16}H_{16}O_7$ (nine degrees of unsaturation) by HRESIMS. Although the ^{1}H and ^{13}C NMR data of **2** (Table 1) revealed the presence of the same 5-hydroxy-4*H*-chromen-4-one moiety as found in **1**, the remaining portion was significantly different. The C-7−C-8 (including OH-7) fragment was established on the basis of ^{1}H−^{1}H COSY correlations observed

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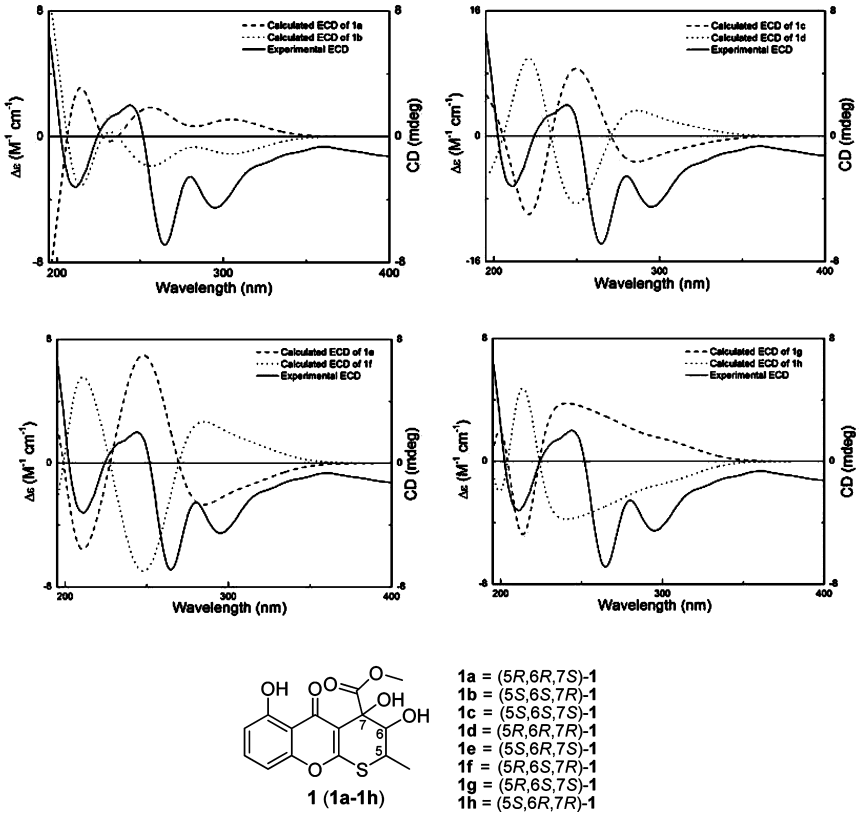

Figure 1. Experimental CD spectrum of **1** in MeOH and the calculated ECD spectra of **1a**−**1h** after a UV correction of 20 nm. Structures **1a**−**1h** represent eight possible stereoisomers of **1**.

for relevant protons. HMBC cross-peaks from H_2-5 to C-5a, C-6, C-7, and C-8a and from H-7 and H-8 to C-8a established the cyclohexene moiety fused to the 5-hydroxy-4*H*-chromen-4-one unit at C-5a/C-8a. HMBC correlations of H_3-10 with C-5, C-5a,[9] and C-7 located the methyl group at C-6, while those from H-8 and H_3-12 to C-11 connected the methyl formate unit to C-8. The remaining exchangeable proton at 4.28 ppm was assigned as OH-6 by default. Collectively, the planar structure of **2** was established, which was further confirmed by X-ray crystallography (Figure 2). The X-ray data also allowed assignment of the relative configuration of **2**.

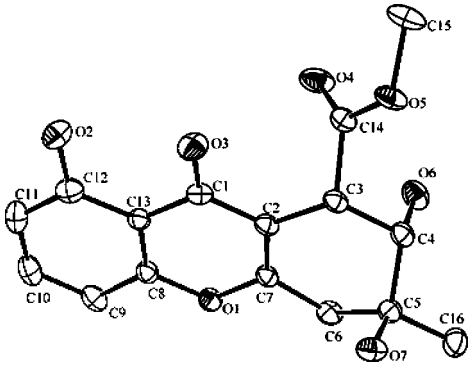

Figure 2. Thermal ellipsoid representation of **2**. (Note: A different numbering system is used for the structural data deposited with the CCDC.)

The absolute configuration of **2** was also determined by comparison of experimental and calculated ECD spectra, which were generated for enantiomers (6*S*,7*S*,8*S*)-**2** (**2a**) and (6*R*,7*R*,8*R*)-**2** (**2b**) after a UV correction of 20 nm (Figure S23). The MMFF94 conformational search followed by B3LYP/6-31G(d) DFT reoptimization afforded four lowest energy conformers (Figure S22). The calculated ECD spectra of enantiomers **2a** and **2b** were then generated by Boltzmann weighting of the four conformers (Figure 3). The CD spectrum

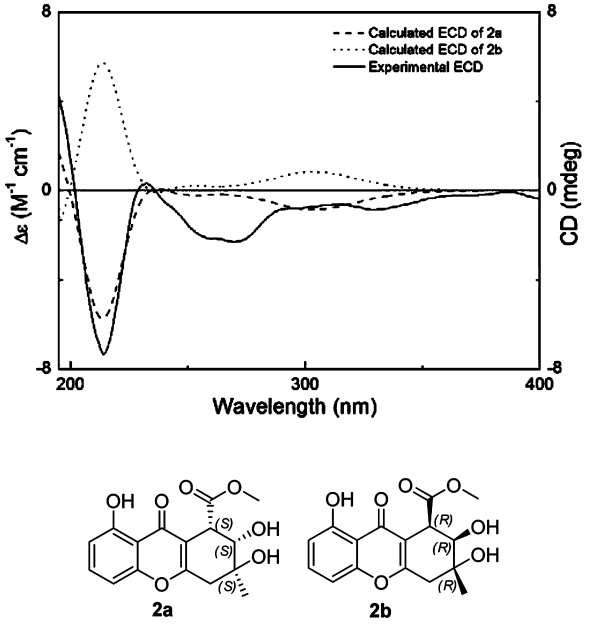

Figure 3. Experimental CD spectrum of **2** in MeOH and calculated ECD spectra of two enantiomers, (6*S*,7*S*,8*S*)-**2** (**2a**) and (6*R*,7*R*,8*R*)-**2** (**2b**), after a UV correction of 20 nm.

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Table 2. NMR Data for 4–6

	4		5		6	
pos.	δ_C,[a] mult.	δ_H[b] (J in Hz)	δ_C,[c] mult.	δ_H[b] (J in Hz)	δ_C,[a] mult.	δ_H[b] (J in Hz)
1	162.8, qC		162.0, qC		162.8, qC	
2	109.0, CH	6.37, d (8.5)	108.8, CH	6.41, d (8.0)	110.4, CH	6.49, d (8.0)
3	139.2, CH	7.38, t (8.5)	138.3, CH	7.39, t (8.0)	139.3, CH	7.43, t (8.0)
4	108.1, CH	6.37, d (8.5)	107.8, CH	6.41, d (8.0)	108.4, CH	6.46, d (8.0)
4a	162.1, qC		161.9, qC		162.0, qC	
5	46.6, CH	2.83, m	43.3, CH	2.53, m	49.9, CH	3.13, m
5a	86.0, CH	4.82, dd (4.0, 8.0)	83.8, CH	4.91, dd (4.0)	79.5, CH	5.31, dd (4.0)
6	82.3, CH	4.23, d (5.5)	77.7, CH	4.29, d (7.5)	210.5, qC	
7	88.0, qC		83.8, qC		79.9, qC	
7a	52.8, CH	3.98, d (8.0)	56.1, CH	3.45, d (4.0)	53.7, CH	3.83, d (4.0)
8	198.0, qC		197.2, qC		196.2, qC	
8a	109.4, qC		109.7, qC		109.4, qC	
9	11.4, CH_3	1.20, d (7.5)	8.2, CH_3	1.27, d (7.0)	7.8, CH_3	1.33, d (7.0)
10	173.2, qC		174.5, qC		171.5, qC	
11	52.8, CH_3	3.79, s	52.5, CH_3	3.78, s	53.8, CH_3	3.82, s
OH-1		11.94, s		11.83, s		11.69, s
OH-6		4.77, s		4.53,[d] s		
OH-7		4.54, br s		4.37,[d] s		5.69, s

[a]Recorded at 100 MHz in acetone-d_6. [b]Recorded at 500 MHz in acetone-d_6. [c]Recorded at 125 MHz in acetone-d_6. [d]These assignments are interchangeable.

recorded for **2** matches the calculated ECD curve of **2a**, but is opposite to that of **2b**. Therefore, **2** was deduced to have the 6*S*, 7*S*, 8*S* absolute configuration.

The molecular formula of preussochromone C (**3**) was determined to be $C_{14}H_{12}O_7$ (nine degrees of unsaturation) on the basis of its HRESIMS data. Analysis of the ^{1}H and ^{13}C NMR data of **3** (Table 2) revealed significantly different structural features from **1** and **2**. On the basis of HMBC cross-peaks from H-2 to C-1, C-4, C-8a, and C-9[4,11] and from the intramolecularly hydrogen-bonded phenolic proton (δ_H 13.2) to C-1, C-2, and C-8a, plus consideration of the chemical shift value of C-4a (δ_C 146.4), an aryl ring was established with a hydroxy group and an upfield carboxylic carbon (δ_C 159.1) attached to C-1 and C-4, respectively. HMBC correlations from the exchangeable proton at 5.80 ppm to C-4a, C-5, and C-10 located OH-5 at C-5 and enabled connection of C-5 to C-4a. Further correlations from H-10a to C-4a and C-5 and from H-10b to C-9 established a δ-lactone moiety fused to the aryl ring at C-4/C-4a. In turn, correlations from H-10b to C-6 and from H-7 to C-5, C-6, C-8, and C-8a indicated that the C-6/C-7 olefin is linked to C-5 and C-8, and C-8 is connected to C-8a, completing the 3,3a-dihydrobenzo[*de*]isochromene-1,6-dione skeleton, as found in corymbiferone.[12] Finally, the remaining two *O*-methyls were attached to C-3 and C-6, respectively, on the basis of relevant HMBC cross-peaks.

The absolute configuration of the C-5 secondary alcohol was deduced via the CD data of the *in situ* formed $[Rh_2(OCOCF_3)_4]$ complex,[13] with the inherent contribution subtracted. The Rh complex of **3** showed a positive E band at ca. 350 nm (Figure S14), correlating to the 5*S* absolute configuration by applying the bulkiness rule.[13,14]

Preussochromone D (**4**) gave a pseudomolecular ion $[M + H]^+$ peak by HRESIMS, indicating the molecular formula $C_{15}H_{16}O_7$ (eight degrees of unsaturation). Its NMR data (Table 2) showed three exchangeable protons (δ_H 4.54, 4.77, and 11.94, respectively), two methyl groups including one *O*-methyl, four methines with two oxygenated, six aromatic carbons (three of which were protonated), one oxygenated sp^3 quaternary carbon, one carboxylic carbon (δ_C 173.2), and one α,β-unsaturated ketone carbon (δ_C 198.0). Analysis of its NMR data revealed the same 1,2,3-trisubstituted aryl ring with a hydroxy group attached to C-1, as appeared in **1** and **2**. The C-8 ketone group (δ_C 198.0) was connected to C-8a on the basis of the downfield ^{1}H NMR chemical shift for OH-1 (δ_H 11.94) and the four-bond *W*-type HMBC correlations from H-2 and H-4 to C-8.[4] The ^{1}H–^{1}H COSY NMR data showed the second isolated spin-system of C-7a–C-5a–C-5/C-9–C-6 (including OH-6). HMBC correlations from H-7a to C-7, C-8, and C-10 and from H-6 to C-7, C-7a, and C-10 completed a cyclopentane ring that was connected to C-8 and C-10 at C-7a and C-7, respectively. An HMBC cross-peak from H_3-11 to C-10 located the *O*-methyl group at C-10. Considering the chemical shift values of C-4a (δ_C 162.1) and C-5a (δ_C 86.0) and the unsaturation requirement for **4**, they were attached to the remaining oxygen atom to complete the planar structure of **4** as shown.

The relative configuration of **4** was assigned by X-ray crystallography (Figure 4), while the absolute configuration was deduced

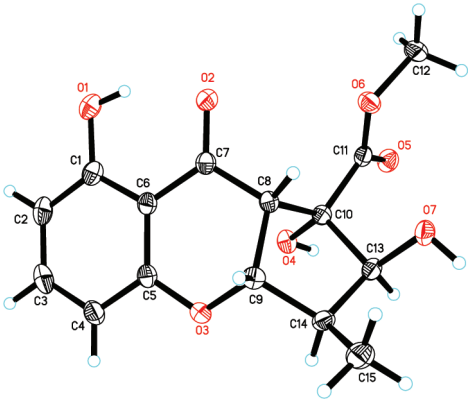

Figure 4. Thermal ellipsoid representation of **4**. (Note: A different numbering system is used for the structural data deposited with the CCDC.)

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using the modified Mosher method.[15] Treatment of **4** with (*S*)- and (*R*)-MTPA Cl afforded the (*R*)- (**4a**) and (*S*)-MTPA (**4b**) monoesters, respectively. The difference in chemical shift values ($\Delta\delta = \delta_S - \delta_R$) for **4b** and **4a** was calculated to assign the 6*R* configuration (Figure S16). Therefore, the 5*S*, 5a*R*, 6*R*, 7*R*, 7a*R* absolute configuration is proposed for **4**.

Preussochromone E (**5**) was assigned the same molecular formula, $C_{15}H_{16}O_7$ (eight degrees of unsaturation), as **4** by HRESIMS. Interpretation of its ^{1}H and ^{13}C NMR spectroscopic data (Table 2) revealed the same planar structure as **4**, which was supported by relevant ^{1}H−^{1}H COSY and HMBC data, suggesting that **5** is a stereoisomer of **4**. The relative configuration of **5** was deduced by analysis of ^{1}H−^{1}H coupling constants and NOESY data. The same coupling constant of 4.0 Hz between H-5 and H-5a in **5** and **4**, together with a NOESY correlation of H-5a with H_3-9, revealed a *cis* relationship for H-5a and H_3-9. A coupling constant of 4.0 Hz observed for H-7a in **5**, compared to 8.0 Hz for the same proton in **4**, suggested a *trans* relationship for H-5a and H-7a, which was also supported by a NOESY correlation of H-5 with H-7a. A coupling constant of 7.5 Hz observed for H-6 (5.5 Hz in **4**) and the NOESY correlations of H-6 with H-5a and H_3-9 established a *trans* relationship for H-6 and H_3-9.[16] A NOESY correlation of H-6 with H_3-11 placed these protons on the same face of the cyclopentane ring, thereby allowing determination of the relative configuration of **5**.

The absolute configuration of the tert/sec 6,7-diol moiety in **5** was assigned using the *in situ* dimolybdenum CD method, developed by Snatzke and Frelek.[17,18] Upon addition of dimolybdenum tetraacteate [$Mo_2(OAc)_4$] to **5** in DMSO solution, a metal complex was generated as an auxiliary chromophore. Since the contribution from the inherent CD resulting from the C-8 and C-10 carbonyls was subtracted to give the induced CD of the complex, the observed sign of the Cotton effect in the induced spectrum originates solely from the chirality of the *vic*-diol moiety expressed by the sign of the O−C−C−O torsion angle. The positive Cotton effect observed at around 310 and 400 nm, respectively, in the induced CD spectrum (Figure S15) permitted deduction of the 6*S* and 7*R* absolute configuration on the basis of the empirical rule proposed by Snatzke. Combining the relative configuration established, **5** was assigned the 5*S*, 5a*R*, 6*S*, 7*R*, 7a*S* absolute configuration.

Preussochromone F (**6**) was isolated as a yellow powder with a molecular formula of $C_{15}H_{14}O_7$ (nine degrees of unsaturation), established by HRESIMS. The ^{1}H and ^{13}C NMR spectra of **6** showed resonances similar to those of **4** and **5**, except that the C-6 oxymethine (δ_H/δ_C 4.23/82.3) was replaced by a ketone carbon (δ_C 210.5), which was confirmed by HMBC correlations from H-5a, H-7, and H_3-9 to C-6. The relative configurations at C-5, C-5a, C-7, and C-7a in **6** were established to be the same as those in **5** by analysis of ^{1}H−^{1}H coupling constants and NOESY data for relevant protons, whereas the absolute configuration of **6** was deduced by a semisynthetic method.[19] Specifically, treatment of **5** with manganese dioxide afforded an oxidation product of OH-6, with its ^{1}H NMR data and specific rotation value identical to those of **6**, suggesting the 5*R*, 5a*R*, 7*R*, 7a*S* absolute configuration for **6**.

The remaining known compound **7** was readily identified as 2,8-dihydroxy-3-methyl-9-oxoxanthene-1-carboxylic acid methyl ester by comparison of its NMR and MS data with those reported.[7]

Compounds **1**−**6** were tested for cytotoxicity against the following four human tumor cell lines, HeLa (cervical epithelial cells), A549 (lung carcinoma epithelial cells), MCF-7 (breast cancer cells), and HCT116 (colon cancer cells) (Table 3). Compounds **1** and **3** showed significant cytotoxic effects against

Table 3. Cytotoxicity of Compounds 1 and 3[a]

	IC_{50} (μM)			
compound	A549	MCF-7	HeLa	HCT116
1	8.34 ± 0.41		25.52 ± 0.73	25.87 ± 2.62
3	5.75 ± 0.69	41.17 ± 1.33	29.96 ± 3.03	15.1 ± 1.11
cisplatin	5.51 ± 0.23	11.62 ± 0.18	9.3 ± 0.46	11.3 ± 0.41

[a]**2** and **4**−**6** were inactive at 20 μg/mL.

A549 cells, with IC_{50} values of 8.34 and 5.75 μM, respectively, while the positive control cisplatin showed an IC_{50} value of 5.51 μM. Other compounds did not show detectable cytotoxicity against the four cell lines at 20 μg/mL.

Preussochromone (**1**) represents the first example of the naturally occurring thiopyranchromenone class of compounds possessing the 3,4-dihydrothiopyrano[2,3-*b*]chromen-5(2*H*)-one skeleton, which was previously found only in synthetic products.[8,20] Initially, the prenylated coumarins from the plant *Ferula communis*[21] showed significant activity against *Mycobacterium tuberculosis*, promoting synthesis of their sulfur isosters (e.g., **8** and **9**)[8,20] for evaluations. Due to assay limitations, **1** was not tested for its antituberculosis activity. Compound **2** is a new member of the xanthone class, which has been encountered frequently as the bioactive principles of plants and fungi.[22] However, **2** differs from the known analogues by having different substituents on the aryl and cyclohexene rings. Compound **3** is closely related to corymbiferone, an antioxidant isolated from *Penicillium hordei*,[12] simonyellin, a phenylbenzoisochromenone isolated from the lichen *Simonyella variegata*,[23] and a few other natural products,[24] but differs from the precedents by having different substituents at C-1, C-3, C-5, C-6, C-7, and C-10. Compounds **4**−**6** belong to a relatively rare type of fungal metabolite incorporating the cyclopentabenzopyran-9-one skeleton. Other examples of this class of natural products include wrightiadione,[25] coniochaetones A and B,[26,27] pseudobruceol I,[28] remisporine A,[29] and diaportheones A and B,[30] but **4**−**6** differ from the above-mentioned compounds by having either different substitution patterns or substituents on the aryl and cyclopentane rings.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 polarimeter, and UV data were obtained on a Shimadzu Biospec-1601 spectrophotometer. CD spectra were recorded on a JASCO J-815 spectropolarimeter. IR data were recorded using a Nicolet Magna-IR 750 spectrophotometer. ^{1}H and ^{13}C NMR data were acquired with Varian Mercury-400, Inova-500, and NMR system-600 spectrometers using solvent signals (acetone-d_6: δ_H 2.05/δ_C 29.8, 206.1; DMSO-d_6: δ_H 2.50/δ_C 39.5) as references. The HMQC and HMBC experiments were optimized for 145.0 and 8.0 Hz, respectively. ESIMS and HRESIMS data were obtained using an Agilent Accurate-Mass-Q-TOF LC/MS 6520 instrument equipped with an electrospray ionization (ESI) source. The fragmentor and capillary voltages were kept at 125 and 3500 V, respectively. Nitrogen was supplied as the nebulizing and drying gas. The temperature of the drying gas was set at 300 °C. The flow rate of the drying gas and the pressure of the nebulizer were 10 L/min and 10 psi, respectively. All MS experiments were performed in positive ion mode. Full-scan spectra were acquired over a scan range of m/z 100−1000 at 1.03 spectra/s.

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Fungal Material. The culture of *Preussia africana* (Sporormiaceae) was isolated from the lichen *Ramalina calicaris* (L.) Fr. (Ramalinaceae) collected from Zixi Mountain, Yunnan Province, People's Republic of China, in November 2006. The isolate was identified by one of the authors (L.G.) based on morphology and sequence (Genbank Accession No. JQ031265) analysis of the ITS region of the rDNA and assigned the accession number 90-1-6-2 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 at 25 °C for 10 days. Agar plugs were cut into small pieces (about 0.5 × 0.5 × 0.5 cm^3) under aseptic conditions, and 15 pieces were used to inoculate three Erlenmeyer flasks (250 mL), each containing 50 mL of media (0.4% glucose, 1% malt extract, and 0.4% yeast extract); the final pH of the media was adjusted to 6.5 and sterilized by autoclave. Three flasks of the inoculated media were incubated at 25 °C on a rotary shaker at 170 rpm for five days to prepare the seed culture. Spore inoculum was prepared by suspension in sterile, distilled H_2O to give a final spore/cell suspension of 1 × 10^6/mL. Fermentation was carried out in eight Fernbach flasks (500 mL), each containing 80 g of rice. Distilled H_2O (120 mL) was added to each flask, and the contents were soaked overnight before autoclaving at 15 psi for 30 min. After cooling to room temperature, each flask was inoculated with 5.0 mL of the spore inoculum and incubated at 25 °C for 40 days.

Extraction and Isolation. The fermented material was extracted repeatedly with EtOAc (4 × 1.0 L), and the organic solvent was evaporated to dryness under vacuum to afford the crude extract (3.0 g), which was fractionated by silica gel vacuum liquid chromatography using petroleum ether−EtOAc gradient elution. The fraction (100 mg) eluted with 10% EtOAc was separated again by Sephadex LH-20 column chromatography (CC) using 1:1 CH_2Cl_2−MeOH as eluents, and the resulting subfractions were further purified by RP HPLC (Agilent Zorbax SB-C_{18} column; 5 μm; 9.4 × 250 mm; 50−75% MeOH in H_2O over 40 min; 2 mL/min) to afford **7** (2.0 mg, t_R 35.9 min). Fractions (350 mg) eluted with 12, 15, and 18% EtOAc were fractionated again by Sephadex LH-20 CC using 1:1 CH_2Cl_2−MeOH as eluent. The resulting subfractions were further purified by semi-preparative RP HPLC (Agilent Zorbax SB-C_{18} column; 5 μm; 9.4 × 250 mm; 43% MeOH in H_2O for 40 min; 2 mL/min) to afford **6** (8.0 mg, t_R 34.0 min). Fractions eluted with 20 (97 mg), 25 (160 mg), 35 (250 mg), and 40 and 45% (300 mg) EtOAc were individually separated by Sephadex LH-20 CC, eluting with 1:1 CH_2Cl_2−MeOH. Further purification of the resulting subfractions by RP HPLC (Agilent Zorbax SB-C_{18} column; 5 μm; 9.4 × 250 mm) afforded **1** (2.0 mg, t_R 36.0 min; 29% CH_3CN in H_2O for 40 min; 2 mL/min) and **5** (1.8 mg, t_R 39.9 min; the same gradient as in purification of **1**), **4** (10.0 mg, t_R 32.4 min; 27% CH_3CN in H_2O for 2 min, followed by 27−30% over 33 min; 2 mL/min), **2** (3.0 mg, t_R 22.9 min; 44% MeOH in H_2O for 30 min; 2 mL/min), and **3** (10.5 mg, t_R 20.7 min; 35% MeOH in H_2O for 2 min, followed by 35−50% over 28 min; 2 mL/min), respectively.

Preussochromone A (1): white powder; $[\alpha]^{25}_D$ −232.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 240 (3.49), 347 (3.36), 368 (3.26) nm; CD (*c* 1.5 × 10^{-3} M, MeOH) λ_{max} (Δε) 207.5 (−2.81), 241 (+1.90), 262 (−6.61), 289.5 (−4.14) nm ; IR (neat) ν_{max} 3466 (br), 2954, 1735, 1646, 1601, 1471, 1413, 1280, 1093 cm^{-1}; ^1H, ^{13}C NMR and HMBC data see Table 1; HRESIMS *m*/*z* 361.0357 (calcd for $C_{15}H_{14}O_7SNa$, 361.0352).

Preussochromone B (2): white needles (MeOH); mp 166−167 °C; $[\alpha]^{25}_D$ −71.9 (*c* 0.16, MeOH); UV (MeOH) λ_{max} (log ε) 239 (3.25), 341 (3.12), 350 (3.15) nm; CD (*c* 1.6 × 10^{-3} M, MeOH) λ_{max} (Δε) 212.5 (−7.02), 252.5 (−1.79), 266.5 (−2.22), 326.5 (−0.83) nm; IR (neat) ν_{max} 3507 (br), 2956, 2916, 1748, 1651, 1622, 1596, 1475, 1349, 1233, 1083 cm^{-1}; ^1H and ^{13}C NMR data see Table 1; HMBC correlations (acetone-d_6, 500 MHz) H-2 → C-1, 4, 9a; H-3 → C-1, 4a; H-4 → C-2, 4a; H_2-5 → C-5a, 6, 7, 8a, 10; H-7 → C-5, 6, 8a, 10; H-8 → C-5a, 7, 8a, 11; H_3-10 → C-5, 5a, 7; H_3-12 → C-11; OH-1 → C-1; HRESIMS *m*/*z* 321.0964 (calcd for $C_{16}H_{17}O_7$, 321.0969).

X-ray Crystallographic Analysis of 2 (ref 31). Upon crystallization from MeOH−H_2O (30:1) using the vapor diffusion method, colorless crystals were obtained for **2**. A crystal (0.40 × 0.30 × 0.20 mm) was separated from the sample and mounted on a glass fiber, and data were collected using a Rigaku RAXIS RAPID IP diffractometer with graphite-monochromated Mo Kα radiation, λ = 0.71073 Å at 293(2) K. Crystal data: $C_{16}H_{16}O_7$, *M* = 320.29, space group orthorhombic, *P*2(1)2(1)2(1); unit cell dimensions *a* = 8.5972(17) Å, *b* = 10.312(2) Å, *c* = 16.512(3) Å, *V* = 1463.9(5) $Å^3$, *Z* = 4, D_{calcd} = 1.453 mg/m^3, μ = 0.115 mm^{-1}, *F*(000) = 672. The structure was solved by direct methods using SHELXL-97[32] and refined by using full-matrix least-squares difference Fourier techniques. All non-hydrogen atoms were refined with anisotropic displacement parameters, and all hydrogen atoms were placed in idealized positions and refined as riding atoms with the relative isotropic parameters. Absorption corrections were performed using the Siemens Area Detector Absorption Program (SADABS).[33] The 1933 measurements yielded 1933 independent reflections after equivalent data were averaged, and Lorentz and polarization corrections were applied. The final refinement gave R_1 = 0.0392 and wR_2 = 0.0605 [$I > 2\sigma(I)$].

Preussochromone C (3): yellow, amorphous solid; $[\alpha]^{25}_D$ +174.5 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 239 (3.32), 341 (3.19), 376 (3.25) nm; CD (*c* 3.4 × 10^{-4} M, CH_2Cl_2) λ_{max} (Δε) 329 (+1.1) nm, 293 (−1.0) nm, 255.5 (+4.6) nm; IR (neat) ν_{max} 3434 (br), 2924, 1709, 1646, 1587, 1460, 1371, 1252, 1210, 1068 cm^{-1}; ^1H and ^{13}C NMR data see Table 1; HMBC data (acetone-d_6, 500 MHz) H-2 → C-1, 4, 8a, 9; H-7 → C-5, 6, 8, 8a; H-10a → C-4a, 5; H-10b → C-4a, 5, 6, 8a, 9; H_3-11 → C-3; H_3-12 → C-6; OH-1 → C-1, 2, 8a; OH-5 → C-4a, 5, 10; HRESIMS *m*/*z* 293.0652 (calcd for $C_{14}H_{13}O_7$, 293.0656).

Absolute Configuration of the Tertiary Alcohol in 3 (refs 13, 14). A sample of **3** (0.5 mg) was dissolved in a dry solution of [Rh_2-$(OCOCF_3)_4$] complex (1.5 mg) in CH_2Cl_2 (200 μL). The first CD spectrum was recorded immediately after mixing, and its time evolution was monitored until stationary (ca. 10 min after mixing). The inherent CD was subtracted. The observed sign of the E band at ca. 350 nm in the induced CD spectrum was correlated to the absolute configuration of the C-5 tertiary moiety.

Preussochromone D (4): white needles (acetone); mp 162−163 °C; $[\alpha]^{25}_D$ −28.2 (*c* 0.17, MeOH); UV (MeOH) λ_{max} (log ε) 300 (3.24), 338 (3.24), 375 (3.32) nm; IR (neat) ν_{max} 3494 (br), 2972, 1707, 1644, 1627, 1460, 1375, 1279, 1211, 1079 cm^{-1}; ^1H and ^{13}C NMR data see Table 2; HMBC data (acetone-d_6, 500 MHz) H-2 → C-1, 4, 8, 8a; H-3 → C-1, 2, 4; H-4 → C-2, 8; H-5 → C-5a, 6, 7, 9; H-5a → C-6, 7, 9; H-6 → C-5a, 7, 7a, 9, 10; H-7a → C-5a, 7, 8, 10; H_3-9 → C-5, 5a, 6; H_3-11 → C-10; OH-1 → C-1, 2, 3; HRESIMS *m*/*z* 309.0968 (calcd for $C_{15}H_{17}O_7$, 309.0969).

X-ray Crystallographic Analysis of 4 (ref 34). Upon crystallization from acetone−H_2O (20:1) using the vapor diffusion method, colorless crystals were obtained for **4**. A crystal (0.56 × 0.14 × 0.14 mm) was separated from the sample and mounted on a glass fiber, and data were collected using a Rigaku RAPID IP diffractometer with graphite-monochromated Mo Kα radiation, λ = 0.71073 Å at 173(2) K. Crystal data: $C_{15}H_{16}O_7$, *M* = 308.28, space group monoclinic, *P*2(1); unit cell dimensions *a* = 9.2581(19) Å, *b* = 5.2295(10) Å, *c* = 14.650(3) Å, *V* = 698.9(2) $Å^3$, *Z* = 2, D_{calcd} = 1.465 mg/m^3, μ = 0.117 mm^{-1}, *F*(000) = 324. The structure was solved by direct methods using SHELXL-97[32] and refined by using full-matrix least-squares difference Fourier techniques. All non-hydrogen atoms were refined with anisotropic displacement parameters, and all hydrogen atoms were placed in idealized positions and refined as riding atoms with the relative isotropic parameters. Absorption corrections were performed using SADABS.[33] The 5776 measurements yielded 1597 independent reflections after equivalent data were averaged, and Lorentz and polarization corrections were applied. The final refinement gave R_1 = 0.0483 and wR_2 = 0.1497 [$I > 2\sigma(I)$].

Preparation of (*R*)- (4a) and (*S*)-MTPA (4b) Esters. A sample of **4** (1.3 mg, 0.004 mmol), (*S*)-MPTA Cl (2.5 μL, 0.013 mmol), and pyridine-d_5 (0.5 mL) were allowed to react in an NMR tube at ambient temperature for 12 h. The mixture was purified by RP HPLC (Agilent Zorbax SB-C_{18} column; 5 μm; 9.4 × 250 mm; 4.6 × 250 mm; 55% MeOH in H_2O for 2 min, followed by 55−100% for 33 min) to afford **4a** (0.8 mg, t_R 27.6 min): white powder; ^1H NMR (acetone-d_6, 500 MHz) δ 11.77 (1H, s, OH-1), 7.45 (1H, t, *J* = 8.5 Hz, H-3), 6.46

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(2H, d, J = 8.5 Hz, H-2/H-4), 5.54 (1H, d, J = 3.0 Hz, H-6), 4.94 (1H, dd, J = 3.0, 7.0 Hz, H-5a), 4.00 (1H, d, J = 7.0 Hz, H-7a), 3.74 (3H, s, H_3-11), 3.19 (1H, m, H-5), 0.97 (3H, d, J = 7.5 Hz, H_3-9).

Similarly, a sample of **4** (0.7 mg, 0.002 mmol), (*R*)-MPTA Cl (2.5 μL, 0.013 mmol), and pyridine-d_5 (0.5 mL) were allowed to react in an NMR tube at ambient temperature for 5 h, and the reaction mixture was processed as described above for **4a** to afford **4b** (0.5 mg): white powder; ^{1}H NMR (acetone-d_6, 500 MHz) δ 11.78 (1H, s, OH-1), 7.45 (1H, t, J = 8.5 Hz, H-3), 6.46 (2H, d, J = 8.5 Hz, H-2/H-4), 5.61 (1H, d, J = 3.0 Hz, H-6), 4.98 (1H, dd, J = 3.0, 7.0 Hz, H-5a), 4.00 (1H, d, J = 7.0 Hz, H-7a), 3.65 (3H, s, H_3-11), 3.24 (1H, m, H-5), 1.23 (3H, d, J = 7.5 Hz, H_3-9).

*Preussochromone E (**5**):* white powder; $[\alpha]^{25}_D$ +30.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 298 (3.28), 334 (3.28), 371 (3.45); CD (*c* 3.2 × 10^{-4} M, DMSO) λ_{max} ($\Delta\varepsilon$) 272 (−3.34), 344 (+0.04) nm; IR (neat) ν_{max} 3380 (br), 2955, 1737, 1639, 1626, 1463, 1463, 1365, 1222, 1061 cm^{-1}; ^{1}H and ^{13}C NMR data see Table 2; HMBC data (acetone-d_6, 500 MHz) H-2 → C-1, 4; H-3 →C-1, 2, 4; H-4 →C-2, 8; H-5 →5a, 6, 7, 9; H-5a → C-6, 7; H-6 → C-7, 10; H-7a → C-7, 8, 10; H_3-9 → C-5, 5a, 6; H_3-11 → C-10; NOESY correlations (acetone-d_6, 500 MHz) H-5 ↔ H-7a; H-5a ↔ H_3-9; H-6 ↔ H-5a, H_3-9, H_3-11; HRESIMS *m*/*z* 309.0966 (calcd for $C_{15}H_{17}O_7$, 309.0969).

Absolute Configuration of the 6,7-Diol Moiety in 5 (refs 17, 18). HPLC grade DMSO was dried with 4 Å molecular sieves. According to the published procedure,[35] a mixture of 1:1.3 diol/$Mo_2(OAc)_4$ for **5** was subjected to CD measurements at a concentration of 0.5 mg/mL. The first CD spectrum was recorded immediately after mixing, and its time evolution was monitored until stationary (about 10 min after mixing). The inherent CD was subtracted. The observed signs of the diagnostic bands at around 310 and 400 nm in the induced CD spectrum were correlated to the absolute configuration of the 6,7-diol moiety.

*Preussochromone F (**6**):* yellow powder; $[\alpha]^{25}_D$ +7.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 298 (3.11), 319 (3.21), 340 (3.19) nm; IR (neat) ν_{max} 3433 (br), 2941, 1755, 1656, 1625, 1583, 1471, 1356, 1215, 1139, 1059 cm^{-1}; ^{1}H and ^{13}C NMR data see Table 2; HMBC data (acetone-d_6, 400 MHz) H-2 → C-1, 4, 8; H-3 → C-1; H-4 → C-2; H-5 → C-5a, 6, 9; H-5a → C-6, 7; H-7a → C-5a, 8; H_3-9 → C-5, 5a, 6; H_3-11 → C-10; OH-1 → C-1, 2; OH-7 → C-6, 7, 7a, 10; NOESY correlation (acetone-d_6, 500 MHz) H-5a ↔ H_3-9, H_3-11; HRESIMS *m*/*z* 307.0808 (calcd for $C_{15}H_{15}O_7$, 307.0812).

*2,8-Dihydroxy-3-methyl-9-oxoxanthene-1-carboxylic acid methyl ester (**7**):* ^{1}H, ^{13}C NMR and the MS data were consistent with literature values.[7]

Computational Details. Systematic conformational analyses for **1** and **2** were performed via the MOE ver. 2009.10. (Chemical Computing Group, Canada) software package using the MMFF94 molecular mechanics force field calculation. The MMFF94 conformational analyses were further optimized using TDDFT at the B3LYP/6-31G(d) basis set level. The stationary points have been checked as the true minima of the potential energy surface by verifying they do not exhibit vibrational imaginary frequencies. The 30 lowest electronic transitions were calculated, and the rotational strengths of each electronic excitation were given using both dipole length and dipole velocity representations. ECD spectra were stimulated using a Gaussian function with a half-bandwidth of 0.3 eV. Equilibrium populations of conformers at 298.15 K were calculated from their relative free energies (ΔG) using Boltzmann statistics. The overall ECD spectra were then generated according to Boltzmann weighting of each conformer. The systematic errors in the prediction of the wavelength and excited-state energies are compensated for by employing UV correlation. All quantum computations were performed using the Gaussian03 package,[36] on an IBM cluster machine located at the High Performance Computing Center of Peking Union Medical College.

MTS Assay (ref 37). The assay was run in triplicate. In a 96-well plate, each well was plated with (2−5) × 10^3 cells (depending on the cell multiplication rate). After cell attachment overnight, the medium was removed, and each well was treated with 100 μL of medium containing 0.1% DMSO or appropriate concentrations of the test compounds and the positive control cisplatin (100 mM as stock solution of a compound in DMSO and serial dilutions; the test compounds showed good solubility in DMSO and did not precipitate when added to the cells). The plate was incubated for 48 h at 37 °C in a humidified, 5% CO_2 atmosphere. Proliferation was assessed by adding 20 μL of MTS (Promega) to each well in the dark, followed by a 90 min incubation at 37 °C. The assay plate was read at 490 nm using a microplate reader.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H and ^{13}C NMR spectra of **1**−**6**, CD spectrum of Rh complex of **3**, CD spectrum of **5** in DMSO containing $Mo_2(OAc)_4$, and UV and CD calculations for **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ DEDICATION

Dedicated to Professor Jiangchun Wei on the occasion of his 80th birthday.

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