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Cucurbitane Glucosides from the Root of *Machilus yaoshansis*

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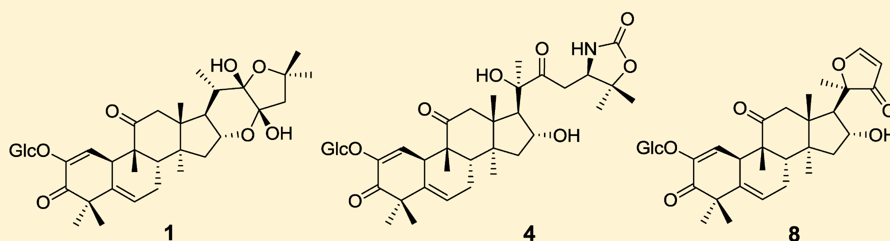
Supporting Information



ABSTRACT: Seven new cucurbitane triterpene glucosides (**1**−**5**, **8**, and **9**) and five known analogues (**6**, **7**, **10**, cucurbitacin I 2-*O*-*β*-D-glucopyranoside, and khekadaengoside K) have been isolated from an ethanol extract of roots of *Machilus yaoshansis*. Compounds **1** and **2** have an unusual 16,23:22,25-diepoxy unit, **4** is an uncommon cucurbitane 25-carbamate with the carbamoyl amino group attached at C-24 to form an oxazolidinone ring in the side chain, and **8** is the first example of a trinorcucurbitane derivative. The configurations in several pairs of C-24 epimeric cucurbitacins with 24,25-dihydroxy-22-one side chains were assigned, and the validity of $J_{23a,24}$ and $J_{23b,24}$ values to differentiate the configuration at C-24 in these cucurbitane derivatives is discussed. Compounds **2**−**4** showed in vitro activity against protein tyrosine phosphatase 1B with IC_{50} values of 8.63, 2.81, and 4.26 μM, respectively. Cucurbitacin E 2-*O*-*β*-D-glucopyranoside (**10**) showed selective cytotoxicity against BGC-823 and A549 cancer cells with IC_{50} values of 4.98 and 3.20 μM, respectively.

Species of the genus *Machilus* have long been used for the treatment of edema, abdominal distension, pain, and inflammation in China.[1,2] As part of a program to assess the chemical and biological diversity of *Machilus* plants,[3] we investigated *Machilus yaoshansis* S. Lee et F. N. Wei (Lauraceae), which is widely distributed in southern China and used as a folk medicine by the ethnic Zhuang in Guangxi Province for the treatment of rheumatism. Four cucurbitane derivatives with cytotoxic and TNF-*α* inhibitory activities were isolated previously from the bark of this plant.[4] We now report the isolation of seven new (**1**−**5**, **8**, and **9**) and five known cucurbitane glycosides (**6**, **7**, **10**, cucurbitacin I 2-*O*-*β*-D-glucopyranoside, and khekadaengoside K) from roots of *M. yaoshansis*. Compounds **1** and **2** possess an unusual 22,23-dihydroxy-16,23:22,25-diepoxy unit, **4** is a cucurbitane 25-carbamate with the carbamoyl amino group attached at C-24 to form an oxazolidinone ring in the side chain, and **8** is the first example of a trinorcucurbitane derivative. In addition, the configuration at C-24 in several pairs of C-24 epimeric cucurbitacins with 24,25-dihydroxy-22-one side chains, including cucurbitacin K 2-*O*-*β*-D-glucopyranoside (**6**), cucurbitacin J 2-*O*-*β*-D-glucopyranoside (**7**), and their aglycones (**6a** and **7a**), is assigned. The assignments are based on the recent X-ray crystallographic analysis of the relative configurations of isocucurbitacins G and H and cucurbitacins G and J[5] and the earlier X-ray crystallographic determination of the absolute configuration of the tetracyclic ring and C-20 of cucurbitacins,[6] together with $Mo_2(AcO)_4$-induced CD data of **6a** and **7a**. The validity of $J_{23a,24}$ and $J_{23b,24}$ values to determine the configuration at C-24 in these cucurbitane derivatives is discussed.

RESULTS AND DISCUSSION

Compound **1** had the molecular formula $C_{36}H_{52}O_{12}$, as indicated by its HRESIMS and NMR data. The IR spectrum of **1** showed the presence of OH (3390 cm^{-1}) and conjugated carbonyl (1686 cm^{-1}) functionalities. The ^{1}H NMR spectrum of **1** in DMSO-d_6 (Table 1) displayed resonances attributable to two olefinic methines at δ_H 5.82 (d, J = 1.5 Hz, H-1) and 5.70 (brs, H-6), an oxymethine at δ_H 4.21 (ddd, J = 10.0, 9.0, and 3.0 Hz, H-16), eight methyls at δ_H 0.69 (s, H_3-18), 0.88 (d, J = 6.0 Hz, H_3-21), 0.89 (s, H_3-19), 1.16 (s, H_3-28/29), 1.22 (s, H_3-26), 1.24 (s, H_3-27), and 1.27 (s, H_3-30), and partially overlapped resonances due to methylenes and methines between δ_H 1.29 and 2.32. Also present were resonances

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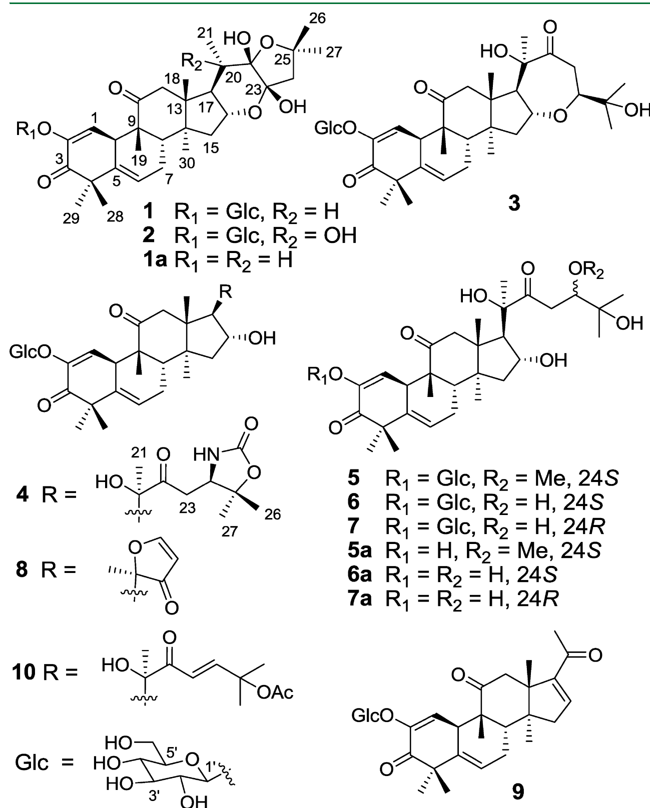

indicating a β-glucopyranosyl moiety (Table 1 and Supporting Information, Table S1) and two exchangeable tertiary OH protons at δ_H 4.98 (s, O*H*-22) and 6.14 (s, O*H*-23). The presence of the β-D-glucopyranosyl moiety was confirmed by enzymatic hydrolysis of **1** using a protocol described previously.[7] The ^{13}C NMR and DEPT spectra of **1** showed 36 carbon resonances (Table 2) corresponding to the above units and 11 quaternary carbons (two carbonyls, two olefinic, and two acetal). These data suggested that **1** was an unusual cucurbitane glycoside with a highly oxygenated side chain,[4,8] which was confirmed by 2D NMR data analysis. The ^{1}H−^{1}H COSY spectrum of **1** demonstrated six isolated spin systems (Supporting Information, Figure S7). HMBC correlations (Supporting Information, Figure S9), together with the shifts of these proton and carbon resonances, indicated a cucurbita-1,5-diene-3,11-dione nucleus for **1**. In addition, a HMBC correlation of H-1′/C-2 confirmed that the β-D-glucopyranosyl moiety was located at C-2. HMBC correlations of O*H*-23/C-22 and H-16/C-23 located one OH at C-23 and an oxygen bridge between C-16 and C-23. HMBC correlations of O*H*-22/C-20, C-22, and C-23, in combination with the molecular formula, placed the remaining OH at C-22 and another oxygen bridge between C-22 and C-25. Thus, the planar structure of **1** was elucidated as 16,23:22,25-diepoxy-2,22,23-trihxydroxycucurbita-1,5-diene-3,11-dione 2-*O*-β-D-glucopyranoside.

The CD spectrum of **1** showed negative Cotton effects at 335 ($\Delta\varepsilon$ −3.10) and 241 ($\Delta\varepsilon$ −2.90) nm and positive Cotton effects at 300 ($\Delta\varepsilon$ +3.20) and 275 ($\Delta\varepsilon$ +4.98) nm, which were consistent with those of reported cucurbitane analogues.[4,9] This suggested that the configuration of the tetracyclic nucleus was identical to that of cucurbitacins having the same chromophores[10] and for which the configuration was determined by X-ray crystallographic analysis.[6] In the NOE difference experiment of **1**, irradiation of H-16 enhanced H$_3$-18, H-20, O*H*-22, and O*H*-23; in turn, irradiation of H$_3$-18 enhanced H-8, H-16, H$_3$-19, and H-20. In addition, H-17 was enhanced by irradiation of H$_3$-21. The enhancements revealed that H-16, H-20, O*H*-22, and O*H*-23 were β-oriented, and H-17 and H$_3$-21 were α-oriented, demonstrating the 20*S*,22*S*,23*S* configuration of the side chain in **1**. Therefore, **1** was determined to be (16α,20*S*,22*S*,23*S*)-16,23:22,25-diepoxy-2,22,23-trihydroxycucurbita-1,5-diene-3,11-dione 2-*O*-β-D-glucopyranoside.

Compound **2** had the molecular formula $C_{36}H_{52}O_{13}$, as indicated by HRESIMS and the NMR data (Tables 1 and 2). Resonances for the CH-20 in **1** were replaced by those of an exchangeable hydroxy proton at δ_H 5.02 (O*H*-20) and a quaternary carbon at δ_C 74.4 (C-20) in **2**. In addition, H-16, H-17, H-18, and H-21 and C-17, C-18, C-20, and C-21 in **2** were deshielded by $\Delta\delta_H$ +0.56, +0.28, +0.16, and +0.23 and $\Delta\delta_C$ +1.3, +1.6, +35.1, and +9.8 ppm, respectively, as compared with those in **1**, and C-16 and C-22 were shielded by $\Delta\delta_C$ −5.4 and −1.1 ppm, respectively. This indicated that **2** was a 20-hydroxy derivative of **1**, which was confirmed by the 2D NMR, NOE, and CD data of **2** (Supporting Information, Figures S13 and S18−S20). HMBC correlations of H$_3$-21/C-17, C-20, and C-22 in combination with their shifts verified the OH group at C-20, and NOESY correlations of H-17 with H$_3$-21 and H$_3$-30 indicated the 20*R* configuration for **2**. Thus, **2** was identified as (16α,20*R*,22*S*,23*S*)-16,23:22,25-diepoxy-2,20,22,23-tetrahydroxycucurbita-1,5-diene-3,11-dione 2-*O*-β-D-glucopyranoside.

The spectroscopic data of **3** indicated that it was an isomer of **1**. Comparison of the NMR data of **3** and **1** demonstrated that they differed in the side chain moiety and that the side chain of **3** contained a carbonyl, two OH-substituted quaternary carbons, and an oxymethine. HMBC correlations of **3** between H$_3$-21/C-17, C-20, and C-22; O*H*-20/C-17; H$_3$-26 and H$_3$-27/C-25; and O*H*-25/C-25 and C-26 indicated a 20,25-dihydroxy-22-one side chain. In addition, HMBC correlations of H-16/C-24; H$_2$-23 and H-24/C-22; and H$_3$-26 and H$_3$-27/C-24 indicated an oxygen bridge between C-16 and C-24. In the ROESY spectrum of **3**, correlations of H-16 with H$_3$-18, H-23a, H$_3$-26, and H$_3$-27 demonstrated that these protons were cofacial and β-oriented on the ring system. These correlations also supported the presence of the 16,24-epoxy bridge in **3**. In addition, ROESY correlations between H-23b and H-24 and of H-17 with H-24, H$_3$-21, and H$_3$-30 revealed that these protons were α-oriented. Therefore, the structure of **3** was determined as (16α,20*R*,24*S*)-16,24-epoxy-2,20,25-trihydroxycucurbita-1,5-diene-3,11,22-trione 2-*O*-β-D-glucopyranoside. The configuration was supported by similarity of the CD data between **3** and **1**.

Cucurbitacins S[11] and T[8a] and colocyntins A−C,[12] with the 16,24-epoxy side chain similar to that of **3**, were reported from cucurbitaceous plants. Although the only difference between colocyntin B and **3** was substitution of the 25-OH in **3** by the 25-OMe in colocyntin B, the NMR data of the oxepanone moieties in the two compounds were different. Comparison of the ^{1}H NMR data of **3** in MeOH-d_4 (Supporting Information, Table S1) with those of colocyntin B in the same solvent[12] demonstrated that H-16 and H-23a in **3** were deshielded more than +0.50 ppm, whereas H-17, H-23b, H-24, H$_3$-26, and H-27 were shielded more than −0.30 ppm. The coupling constants of $J_{23a,24}$ and $J_{23a,23b}$ were 8.0 and 17.0 Hz in colocyntin B and 12.0 and 12.0 Hz in **3**, respectively. This indicated that the configuration at C-24 of colocyntin B differed from that of **3** though the same 24*S* configuration had been assigned for colocyntin B with no detailed evidence.[12] This was supported by the opposite specific rotations of **3** and colocyntin B. In

Table 1. ^{1}H NMR Data for Compounds **1**−**5**, **8**, and **9** in DMSO-d_6 (δ, mult., J in Hz)[a]

no.	1	2	3	4	5[b]	8	9
1	5.82 d (1.5)	5.81 brs	5.77 brs	5.79 brs	5.79 brs	5.77 d (2.0)	5.97 brs
6	5.70 brs	5.70 brs	5.70 brs	5.71 brs	5.71 brs	5.70 brs	5.71 brs
7α	1.96 brd (19.5)	1.97 brd (19.8)	1.96 brd (18.6)	1.94 brd (19.5)	1.94 brd (18.0)	1.94 brd (18.0)	2.05 brd (19.0)
7β	2.30 dd (19.5, 8.0)	2.29 dd (19.8, 8.4)	2.29 dd (18.6, 7.8)	2.28 dd (19.5, 8.0)	2.27 dd (18.0, 7.5)	2.27 dd (18.0, 8.0)	2.36 dd (19.0, 8.0)
8	1.98 d (8.0)	1.99 d (8.4)	1.96 d (7.8)	1.91 d (8.0)	1.91 d (7.5)	1.91 d (8.0)	2.25 d (8.0)
10	3.63 d (1.5)	3.62 brs	3.60 s	3.62 brs	3.63 s	3.65 brs	3.65 brs
12α	3.27 d (15.0)	3.27 d (15.0)	3.34 d (15.0)	3.39 d (15.0)	3.43 d (15.0)	3.41 d (15.0)	3.25 d (16.0)
12β	2.15 d (15.0)	2.24 d (15.0)	2.38 d (15.0)	2.44 d (15.0)	2.47 d (15.0)	2.34 d (15.0)	2.83 d (16.0)
15α	1.29 dd (12.0, 3.0)	1.33 dd (12.6, 3.0)	1.49 d (12.6)	1.29 d (12.0)	1.28 d (12.0)	1.32 d (12.0)	2.17 d (17.5)
15β	1.76 dd (12.0, 9.0)	1.77 dd (12.6, 10.2)	1.91 dd (12.6, 10.2)	1.72 dd (12.0, 9.0)	1.70 dd (12.0, 8.5)	1.72 dd (12.0, 9.0)	2.33 d (17.5)
16	4.21 ddd (10.0, 9.0, 3.0)	4.77 ddd (10.2, 9.6, 3.0)	4.96 dd (10.2, 6.6)	4.44 m	4.36 m	4.24 m	6.92 brs
17	2.10 dd (10.0, 8.5)	2.38 d (9.6)	1.58 d (6.6)	2.30 d (7.0)	2.37 d (7.0)	2.42 d (7.0)	
18	0.69 s	0.85 s	0.91 s	0.81 s	0.81 s	0.72 s	0.84 s
19	0.89 s	0.88 s	0.87 s	0.86 s	0.86 s	0.86 s	0.96 s
20	1.74 m						
21	0.88 d (6.0)	1.11 s	1.25 s	1.25 s	1.26 s	1.31 s	2.24 s
23a			3.76 t (12.0)	3.01 dd (19.0, 5.5)	2.98 brd (17.5)	5.72 d (2.5)	
23b			2.11 dd (12.0, 3.0)	2.88 dd (19.0, 6.0)	2.68 dd (17.5, 8.5)		
24a	2.09 d (12.5)	2.06 d (12.0)	3.40 dd (12.0, 3.0)	3.73 dd (6.0, 5.5)	3.42 brd (8.5)	8.62 d (2.5)	
24b	1.76 d (12.5)	1.81 d (12.0)					
26	1.22 s	1.23 s	1.04 s	1.18 s	1.00 s		
27	1.24 s	1.25 s	1.05 s	1.38 s	1.08 s		
28	1.16 s	1.16 s	1.16 s	1.17 s	1.17 s	1.17 s	1.16 s
29	1.16 s	1.16 s	1.15 s	1.17 s	1.17 s	1.18 s	1.14 s
30	1.27 s	1.28 s	1.20 s	1.28 s	1.31 s	1.31 s	1.11 s
1′	4.54 d (7.5)	4.53 d (7.8)	4.54 d (7.8)	4.55 d (7.5)	4.55 d (7.5)	4.54 d (7.5)	4.58 d (8.0)
2′	3.12 ddd (8.5, 7.5, 5.0)	3.12 ddd (8.4, 7.8, 4.5)	3.11 ddd (8.4, 7.8, 4.2)	3.13 ddd (8.5, 7.5, 5.0)	3.12 ddd (8.5, 7.5, 5.0)	3.12 ddd (9.0, 7.5, 5.0)	3.12 dd (8.5, 8.0)
3′	3.19 ddd (8.5, 8.5, 4.0)	3.19 dd (8.4, 8.4)	3.18 dd (8.4, 8.4)	3.19 ddd (8.5, 8.5, 4.5)	3.17 ddd (8.5, 8.5, 4.5)	3.19 dd (9.0, 9.0)	3.20 dd (8.5, 8.5)
4′	3.26 ddd (8.5, 8.5, 5.0)	3.27 dd (8.4, 8.4)	3.27 dd (8.4, 8.4)	3.27 ddd (8.5, 8.5, 5.0)	3.27 ddd (8.5, 8.5, 5.5)	3.26 dd (9.0, 9.0)	3.26 dd (8.5, 8.5)
5′	3.07 ddd (8.5, 5.0, 3.0)	3.07 ddd (8.4, 5.0, 2.4)	3.06 ddd (8.4, 4.8, 2.4)	3.09 ddd (8.5, 6.0, 2.0)	3.08 ddd (8.5, 5.0, 3.0)	3.06 ddd (9.0, 6.0, 2.0)	3.10 brd (8.5)
6′a	3.71 ddd (12.0, 5.5, 3.0)	3.71 ddd (12.0, 5.0, 2.4)	3.71 dd (12.0, 2.4)	3.72 ddd (12.0, 6.0, 2.0)	3.71 dd (12.0, 3.0)	3.73 ddd (12.0, 5.0, 2.0)	3.69 brd (11.0)
6′b	3.60 ddd (12.0, 5.5, 5.0)	3.62 ddd (12.0, 5.0, 5.0)	3.60 dd (12.0, 4.8)	3.62 ddd (12.0, 6.0, 6.0)	3.61 dd (12.0, 5.0)	3.59 ddd (12.0, 6.0, 5.0)	3.59 brd (11.0)

[a] ^{1}H NMR data were measured at 600 MHz for **2** and **3** and 500 MHz for others. The assignments were based on DEPT, $^{1}H-^{1}H$ COSY, HSQC, and HMBC experiments. Data for exchangeable protons of **1**−**9**, see Supporting Information Tables S1 and S2. [b] Data for O*Me* of **5**: δ 3.26 s.

addition, the similarity of the NMR data between colocyntin B[12] and cucurbitacin T[8a] and between colocyntin C[12] and cucurbitacin S,[8a] in combination with the reported $J_{23a,24}$ values (5.0−8.4 Hz), suggested that the reported configuration at C-24 of these analogues should be revised from *S* to *R*.

The molecular formula $C_{37}H_{53}NO_{13}$ of **4** was indicated by HRESIMS and NMR data. Comparison of the NMR data of **4** and **3** suggested the presence of an additional *N*-substituted carbamate unit [δ_H 7.02 (brs, exchangeable N*H*COO) and δ_C 157.1 (NHCOO)] in **4** and replacement of the tertiary O*H*-25 in **3** by a secondary O*H*-16 [δ_H 4.62 (d, J = 5.0 Hz)] in **4** (Supporting Information, Table S1). In addition, H_2-15, H-16, and H-23a and C-16 and C-24 in **4** were shielded, as compared with those in **3**, whereas H-17, H-23b, and H-24 and C-15, C-17, C-23, and C-25 were deshielded significantly. This suggested that **4** was a 16-hydroxy-25-carbamate analogue of **3** with the carbamoyl amino group substituted at C-24 to form an oxazolidinone ring in the side chain. This was supported by HMBC correlations from N*H*COO, H_2-23, H_3-26, and H_3-27 to C-24 and C-25 and from N*H*COO and H-24 to NHCOO (Supporting Information, Figure S39). The CD spectrum of **4** displayed Cotton effects similar to those of **3**, indicating that they had the same configuration in the tetracyclic ring system. NOE correlations of O*H*-20 with H-16 and H_3-18, of H_3-21 with H-12β and H-17, and the absence of a NOE correlation between H-16 and H_3-21 indicated restricted rotation of the single bond between C-17 and C-20 (Supporting Information, Figures S40 and S41). This suggested the 20*R* configuration for **4**, which was consistent with that of the reported cucurbitane derivatives,[6,10] and supported by comparison of the shifts of C-17, C-18, C-20, and C-21 of **4** with those of 16,20-dihydroxycucurbita-22-one analogues.[8b,d,9,13] Furthermore, the NOESY correlations of N*H* with H-23b and O*H*-16 and between H-23a and H_3-26 and the absence of NOESY correlations of O*H*-16/H-23a, H-24, and H_3-27 indicated that the free rotations of the single bonds between C-21/C-22 and C-22/C-23 were also restricted to give a major conformation in the solution state, suggesting the 24*R* configuration for **4**. This

Table 2. ^{13}C NMR Data for Compounds 1−5, 8, and 9 in DMSO-d_6 (δ)[a]

no.	1	2	3	4	5	8	9
1	120.6	120.5	120.4	120.5	120.5	120.5	120.8
2	145.1	145.1	145.2	145.1	145.2	145.2	145.1
3	196.0	196.0	196.0	196.0	196.0	196.0	196.0
4	48.7	48.7	48.7	48.7	48.7	48.7	48.7
5	136.5	136.5	136.4	136.3	136.3	136.4	135.8
6	119.8	119.9	120.0	120.0	120.0	119.9	119.7
7	23.4	23.2	23.3	23.2	23.3	23.2	22.9
8	41.4	40.9	40.5	41.0	41.0	41.1	39.7
9	48.5	48.5	48.2	48.2	48.2	48.3	48.5
10	34.0	34.0	34.1	34.2	34.3	34.2	34.7
11	213.3	213.5	213.5	213.7	213.8	213.3	213.1
12	48.1	48.5	48.9	49.1	49.1	48.7	45.1
13	48.7	48.7	50.7	49.8	50.0	49.5	51.7
14	46.3	47.4	47.2	47.5	47.5	47.2	49.3
15	40.0	40.0	43.2	45.5	45.7	45.7	42.3
16	74.7	69.3	73.8	69.1	69.1	68.3	145.6
17	49.1	50.4	55.6	58.4	57.7	57.9	149.9
18	18.0	19.6	19.7	19.8	20.0	19.6	22.7
19	19.9	19.8	19.6	19.7	19.7	19.6	20.5
20	39.3	74.4	77.6	79.3	79.2	88.6	196.3
21	11.4	21.2	22.0	25.0	25.1	22.4	26.8
22	102.7	101.6	212.7	214.7	214.1	206.3	
23	105.8	106.4	35.2	39.3	38.7	105.8	
24	48.5	49.2	80.9	56.5	83.7	176.8	
25	77.6	78.3	71.8	81.7	71.4		
26	29.2	29.1	24.4	22.0	24.7		
27	31.7	31.6	27.5	27.5	26.9		
28	20.4	20.4	20.3	20.3	20.3	20.3	20.5
29	27.0	27.1	27.0	27.1	27.1	27.1	26.8
30	20.0	19.9	17.7	17.8	17.7	17.3	18.4
1′	98.7	98.7	98.8	98.7	98.7	98.8	98.7
2′	72.7	72.7	72.7	72.7	72.7	72.7	72.7
3′	76.8	76.8	76.8	76.8	76.8	76.8	76.8
4′	68.8	68.9	68.8	68.8	68.9	68.8	68.9
5′	76.9	76.8	76.9	76.9	76.9	76.9	77.0
6′	59.9	60.0	60.0	60.0	60.0	60.0	60.1
O*Me*					59.5		
HNCOO				157.1			

[a] ^{13}C NMR data (δ) were measured at 150 MHz for **2** and **3** and 125 MHz for others. The assignments were based on DEPT, $^1H-^1H$ COSY, HSQC, and HMBC experiments.

was supported by molecular modeling of the 24*R* and 24*S* epimers of **4** (Supporting Information, Figure S40). The lowest energy 3D conformation of the 24*R* epimer, obtained by Monte Carlo searching with the MMFF molecular mechanics force field using the SPARTAN 04 program,[14] predicted NOE correlations that were consistent with those observed. Thus, compound **4** was elucidated as (16*α*,20*R*,24*R*)-24*N*,25-carbamoyloxy-2,16,20-trihydroxycucurbita-1,5-diene-3,11,22-trione 2-*O*-*β*-D-glucopyranoside.

The spectroscopic data of compound **5** demonstrated that it was a methyl analogue of the co-occurring cucurbitacin K 2-*O*-*β*-D-glucopyranoside (**6**) or cucurbitacin J 2-*O*-*β*-D-glucopyranoside (**7**).[8b,15] The NMR data of **5** showed that C-24 was deshielded about $\Delta\delta_C$ +10 ppm, as compared with that of **6** or **7**, whereas C-23 was shielded about $\Delta\delta_C$ −1 ppm. This revealed that **5** was a 24-methoxy analogue of **6** or **7**, which was confirmed by correlations of OCH_3, *O*H-25, H_3-26, and H_3-27/C-24 and H-24/OCH_3, C-22, C-23, C-25, C-26, and C-27 in the HMBC spectrum of **5**. Comparison of the CD and NMR data of **5** and **5a** with those of **6** and **7** and **6a** and **7a** (**5a**−**7a** were generated by enzymatic hydrolysis of **5**−**7**, respectively) confirmed that the tetracyclic ring and C-20 of these cucurbitane derivatives had the same configuration. The absolute configuration of several pairs of C-24 epimeric cucurbitacins, e.g., compounds **6** and **7**,[8b,9,15] cucurbitacins G and H,[5,16] and cucurbitacins J and K,[8a,b,17] was previously undetermined. From recent X-ray crystallographic analysis of the relative configurations of isocucurbitacins G and H and cucurbitacins G and J,[5] combined with the earlier X-ray crystallographic determination of the absolute configuration of the tetracyclic ring and C-20 for datiscoside and cucurbitacins,[6] the 24*S* configuration can be assigned for isocucurbitacin H, cucurbitacins H and K, and **6**, and the 24*R* for isocucurbitacin G, cucurbitacins G and J, and **7**. The absolute configuration at C-24 in **6a** and **7a** was supported by using the in situ dimolybdenum CD method,[18] which was employed to assign the configurations of acyclic 1,2-diols.[19] According to the empirical rule proposed by Snatzke,[18] the bands around 310 nm (band IV) and 400 nm (band II) in the $Mo_2(AcO)_4$-induced CD spectrum showing the same sign with the O−C−C−O torsion angle in the favored conformation will allow the assignment of the absolute configuration.[19a] In the $Mo_2(AcO)_4$-induced CD spectrum of **6a** (Supporting Information, Figure S60), positive Cotton effects at 318 and 409 nm supported the 24*S* configuration, while a negative Cotton effect at 296 nm in the $Mo_2(AcO)_4$-induced CD spectrum of **7a** (Supporting Information, Figure S68) supported the 24*R* configuration. ^1H NMR data analysis of these cucurbitane derivatives, in C_5D_5N,[8b,20] MeOH-d_4,[15] and DMSO-d_6 (Supporting Information, Table S2) for the glycosides and in CD_3Cl,[5,8a−c] C_5D_5N,[21] and Me_2CO-d_6 (Supporting Information, Table S4) for the aglycones, showed that in the 24*S* analogues the coupling constants of $J_{23a,24}$ (<2.5 Hz) were smaller than those of $J_{23b,24}$ (>8.0 Hz), whereas the values of $J_{23a,24}$ (>8.0 Hz) were larger than those of $J_{23b,24}$ (<2.5 Hz) in the 24*R* analogues (wherein H-23a is defined to be deshielded more than H-23b). This demonstrated that in the solution state the side chains of the 24*S* and 24*R* epimers possessed major conformations with gauche and anti orientations of H-23a and H-24, respectively, as shown in Figure S70 in the Supporting Information, which were consistent with X-ray crystallographic analysis of isocucurbitacins G and H and cucurbitacins G and J,[5] which indicated the major conformation in the solution state was identical to the conformation of the side chain in the crystal state. Therefore, the coupling constants of $J_{23a,24}$ and $J_{23b,24}$ are applicable to determine the configuration at C-24 in these cucurbitane derivatives. On the basis of the above analysis, the $J_{23a,24}$ value of **7**[22] and the $J_{23a,24}$ and $J_{23b,24}$ of cucurbitacin H[23] were not consistent with those assigned by 2D NMR data for the same compounds.[5,8b,15] The ^1H NMR data of cucurbitacin H in the literature[23] were more consistent with those of cucurbitacin G,[5] suggesting that the data in the literature[23] should be reassigned for cucurbitacins G and H. The ^1H NMR spectra of **5** and **5a** showed that the values of $J_{23a,24}$ (≤2.5 Hz) were smaller than those of $J_{23b,24}$ (>8.0 Hz), indicating the 24*S* configuration. Thus, compound **5** was assigned as (16*α*,20*R*,24*S*)-2,16,20,25-tetrahydroxy-24-methoxycucurbita-1,5-diene-3,11,22-trione 2-*O*-*β*-D-glucopyranoside.

Compound **8**, $C_{33}H_{44}O_{11}$, showed spectroscopic data similar to those of **7**. The NMR data of **8** indicated the absence of the

terminal 25-hydroxyisopropyl unit of **7** and the presence of a conjugated double bond [δ_H 5.72 (d, J = 2.5 Hz, H-23) and 8.62 (d, J = 2.5 Hz, H-24) and δ_C 105.8 (C-23) and 176.8 (C-24)] in the side chain. This and the molecular formula suggested that **8** was a derivative of **7** with a 25,26,27-trinor-20,24-epoxy-23-en-22-one side chain and was proved by correlations from both H-24 and H-23 to C-20 and C-22 in the HMBC spectrum. NOE correlations of H-12β/H_3-21, H-16/H_3-18, and H-17/H_3-30 in the NOESY spectrum (Supporting Information, Figure S79), combined with the CD data (Experimental Section), indicated the 16α-hydroxy and 20*R* configuration for **8**. Thus, compound **8** was determined to be (16α,20*R*)-20,24-epoxy-2,16-dihydroxy-25,26,27-trinorcucurbita-1,5,23-triene-3,11,22-trione 2-*O*-β-D-glucopyranoside. Although cucurbitanes containing a similar furanone ring in the side chain and hexanor- and octanor-cucurbitanes were reported,[8b,24] compound **8** is the first example of the trinorcucurbitane skeleton.

The spectroscopic data of compound **9** indicated that it was a glycosidic hexanorcucurbitane derivative with the molecular formula $C_{30}H_{40}O_9$. Comparison of the NMR data of **9** and **8** revealed that the side chain moiety in **8** was replaced by an acetyl group (δ_H 2.24 and δ_C 196.3 and 26.8) in **9**. In addition, the methine (CH-17) and hydroxymethine (CHOH-16) units in **8** were substituted by a conjugated trisubstituted double bond (δ_H 6.92 and δ_C 149.9 and 145.6) in **9**. This demonstrated that **9** was a (22−27)-hexanorcucurbita-1,5,16-triene-3,11,20-trione derivative, which was confirmed by 2D NMR data analysis (Supporting Information, Figures S85−S87). In particular HMBC correlations of H-16/C-13, C-14, C-15, C-17, and C-20; H_3-18/C-12, C-13, C-14, and C-17; and H_3-21/C-17 and C-20, together with the shifts of these proton and carbon resonances, proved the presence of the 16-en-20-one moiety in **9**. Therefore, compound **9** was identified as 2-hydroxy-(22−27)-hexanorcucurbita-1,5,16-triene-3,11,20-trione 2-*O*-β-D-glucopyranoside.

The known compounds were identified by comparison of spectroscopic data with those reported in the literature as cucurbitacin E 2-*O*-β-D-glucopyranoside (**10**),[13] cucurbitacin I 2-*O*-β-D-glucopyranoside,[13] and khekadaengoside K.[8b]

Compounds **2**−**4** showed inhibitory activity in vitro against protein tyrosine phosphatase 1B (PTP1B), with IC_{50} values of 8.63, 2.81, and 4.26 μM, respectively. The positive control oleanolic acid gave an IC_{50} value of 3.84 μM. Although the cytotoxicity of many cucurbitacins is well known,[10,25] compounds obtained in this study were inactive (IC_{50} > 10 μM) to the A2780 ovary, HCT-8 colon, Bel-7402 hepatoma, BGC-823 stomach, and A549 lung cancer cell lines, except that compound **10** showed selective cytotoxic activity against the BGC-823 stomach (IC_{50} 4.98 μM) and A549 lung (IC_{50} 3.20 μM) cells. The positive control camptothecin gave IC_{50} values of 0.26−11.8 μM. This confirmed the role of acetylation of OH-25 in enhancing the cytotoxicity of cucurbitacins postulated earlier.[26] The isolates were also assessed for a TNF-α secretion inhibitory activity of mouse peritoneal macrophages,[27] but were inactive at 10 μM.

■ EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a Rudolph Research Autopol III automatic polarimeter. UV and CD spectra were recorded on a JASCO J-815 spectropolarimeter. IR spectra were recorded on a Nicolet 5700 FT-IR microscope spectrometer (FT-IR microscope transmission). 1D and 2D NMR spectra were obtained at 500 or 600 MHz for ^{1}H and 125 or 150 MHz for ^{13}C, respectively, on INOVA 500 or 600 MHz spectrometers in DMSO-d_6, MeOH-d_4, or acetone-d_6 with solvent peaks used as references. ESIMS data were measured with a Q-Trap LC/MS/MS (Turbo Ionspray source) spectrometer. HRESIMS data were measured using an AccuToFCS JMS-T100CS spectrometer. Column chromatography (CC) was performed with HPD-100 macroporous adsorbent resin (Cangzhou Bonchem Co., Ltd., China), silica gel (200−300 mesh, Qingdao Marine Chemical Inc., China), and Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Sweden). HPLC was performed using a Waters 600 controller, a Waters 600 pump, and a Waters 2487 dual λ absorbance detector with an Alltima (250 × 10 mm i.d.) preparative C_{18} (5 μm) column. TLC was carried out with glass precoated silica gel GF_{254} plates. Spots were visualized under UV light or by spraying with 7% H_2SO_4 in 95% EtOH followed by heating.

Plant Material. Roots of *M. yaoshansis* (10 kg) were collected at Dayao Mountain, Guangxi, China, in December 2007. The plant was identified by Mr. Guang-Ri Long (Guangxi Forest Administration, Guangxi 545005, China). A voucher specimen (no. 07114) was deposited at the Herbarium of Guangxi Forest Administration, China.

Extraction and Isolation. The air-dried roots of *M. yaoshansis* were powdered and extracted with 95% EtOH (3 × 15 L) at room temperature (3 × 48 h). The EtOH extract was evaporated under reduced pressure to yield a dark brown residue (1050 g). The residue was suspended in H_2O (5 L) and then partitioned with EtOAc (5 × 5 L). The aqueous phase was applied to a HPD-100 macroporous adsorbent resin (1500 g, dried weight) column. Successive elution of the column with H_2O, 30% EtOH, 70% EtOH, and 95% EtOH (10 L each) yielded four corresponding portions after removing solvents. The portion (170 g) eluted by 70% EtOH was separated over silica gel eluting with a gradient of increasing MeOH in $CHCl_3$ (2−100%) to give eight fractions (A−H) on the basis of TLC analysis. Separation of fraction B (25 g) by RP-MPLC eluting with a gradient of increasing EtOH in H_2O (0−95%) gave 12 subfractions (B_1−B_{12}). Subfraction B_8 (3.1 g) was further fractionated via Sephadex LH-20 CC, eluting with MeOH−H_2O (1:1), to yield five mixtures ($B_{8\text{-}1}$−$B_{8\text{-}5}$). Fraction $B_{8\text{-}3}$ (2.0 g) was subjected to reversed-phase preparative HPLC, mobile phase MeOH−H_2O (50:50), to afford **5** (25 mg), **6** (11 mg), **7** (22 mg), **8** (8 mg), and **9** (10 mg). Fraction B_9 (1.1 g) was separated on Sephadex LH-20, eluting with MeOH−H_2O (1:1), to give four mixtures ($B_{9\text{-}1}$−$B_{9\text{-}4}$). Fraction $B_{9\text{-}3}$ (440 mg) was purified by reversed-phase preparative HPLC using the mobile phase MeOH−H_2O (57:43) to afford compounds **1** (12 mg), **2** (3 mg), **3** (5 mg), and **4** (7 mg).

*(16α,20S,22S,23S)-16,23:22,25-Diepoxy-2,22,23-trihxydroxycucurbita-1,5-diene-3,11-dione 2-O-β-D-glucopyranoside (**1**):* amorphous solid; $[\alpha]^{20}_D$ +1.2 (c 0.50, MeOH); UV (MeOH) λ_{max} (log ε) 256 (3.78) nm; CD (MeOH) 241 ($\Delta\varepsilon$ −2.90), 275 ($\Delta\varepsilon$ +4.98), 300 ($\Delta\varepsilon$ +3.20), 335 ($\Delta\varepsilon$ −3.10) nm; IR ν_{max} 3390, 1686, 1641, 1380, 1078, 1023 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) data, see Table 1; ^{13}C NMR (DMSO-d_6, 125 MHz) data, see Table 2; ESIMS m/z 699 $[M + Na]^+$ and 675 $[M - H]^-$; HRESIMS m/z 699.3359 $[M + Na]^+$ (calcd for $C_{36}H_{52}O_{12}Na$, 699.3350).

*(16α,20R,22S,23S)-16,23:22,25-Diepoxy-2,20,22,23-tetrahydroxycucurbita-1,5-diene-3,11-dione 2-O-β-D-glucopyranoside (**2**):* amorphous solid; $[\alpha]^{20}_D$ +1.3 (c 0.16, MeOH); UV (MeOH) λ_{max} (log ε) 256 (3.63) nm; CD (MeOH) 242 ($\Delta\varepsilon$ −2.00), 276 ($\Delta\varepsilon$ +3.30), 299 ($\Delta\varepsilon$ +2.14), 334 ($\Delta\varepsilon$ −1.85) nm; IR ν_{max} 3342, 1686, 1666, 1378, 1071, 1047, 1027 cm^{-1}; ^{1}H NMR (DMSO-d_6, 600 MHz) data, see Table 1; ^{13}C NMR (DMSO-d_6, 150 MHz) data, see Table 2; ESIMS m/z 715 $[M + Na]^+$ and 691 $[M - H]^-$; HRESIMS m/z 715.3308 $[M + Na]^+$ (calcd for $C_{36}H_{52}O_{13}Na$, 715.3300).

*(16α,20R,24S)-16,24-Epoxy-2,20,25-trihydroxycucurbita-1,5-diene-3,11,22-trione 2-O-β-D-glucopyranoside (**3**):* amorphous solid; $[\alpha]^{20}_D$ −9.2 (c 0.19, MeOH); UV (MeOH) λ_{max} (log ε) 255 (3.55) nm; CD (MeOH) 241 ($\Delta\varepsilon$ −1.53), 278 ($\Delta\varepsilon$ +2.71), 297 ($\Delta\varepsilon$ +2.45), 336 ($\Delta\varepsilon$ −1.32) nm; IR ν_{max} 3383, 1686, 1667, 1376, 1102, 1076 cm^{-1}; ^{1}H NMR (DMSO-d_6, 600 MHz) data, see Table 1; ^{1}H NMR (MeOH-d_4, 500 MHz) data, see Supporting Information, Table S1;

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^{13}C NMR (DMSO-d_6, 150 MHz) data, see Table 2; ESIMS m/z 699 $[M + Na]^+$ and 711 $[M + Cl]^-$; HRESIMS m/z 699.3362 $[M + Na]^+$ (calcd for $C_{36}H_{52}O_{12}Na$, 699.3350).

*(16α,20R,24R)-24N,25-Carbamoyloxy-2,16,20-trihydroxycucurbita-1,5-diene-3,11,22-trione 2-O-β-D-glucopyranoside (**4**):* amorphous solid; $[\alpha]^{20}_D$ −60.3 (c 0.60, MeOH); UV (MeOH) λ_{max} (log ε) 256 (3.71) nm; CD (MeOH) 242 ($\Delta\varepsilon$ −2.62), 273 ($\Delta\varepsilon$ +2.85), 298 ($\Delta\varepsilon$ +2.20), 335 ($\Delta\varepsilon$ −2.75) nm; IR ν_{max} 3471, 1686, 1643, 1376, 1068 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) data, see Table 1; ^{13}C NMR (DMSO-d_6, 125 MHz) data, see Table 2; ESIMS m/z 742 $[M + Na]^+$ and 754 $[M + Cl]^-$; HRESIMS m/z 742.3437 $[M + Na]^+$ (calcd for $C_{37}H_{53}NO_{13}Na$, 742.3415).

*(16α,20R,24S)-2,16,20,25-Tetrahydroxy-24-methoxycucurbita-1,5-diene-3,11,22-trione 2-O-β-D-glucopyranoside (**5**):* amorphous solid; $[\alpha]^{20}_D$ −52.4 (c 0.84, MeOH); UV (MeOH) λ_{max} (log ε) 255 (3.82) nm; CD (MeOH) 241 ($\Delta\varepsilon$ −3.67), 273 ($\Delta\varepsilon$ +3.36), 298 ($\Delta\varepsilon$ +2.55), 335 ($\Delta\varepsilon$ −3.58) nm; IR ν_{max} 3411, 1689, 1377, 1222, 1098, 1029 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) data, see Table 1; ^{13}C NMR (DMSO-d_6, 125 MHz) data, see Table 2; ESIMS m/z 731 $[M + Na]^+$ and 747 $[M + K]^+$; HRESIMS m/z 731.3617 $[M + Na]^+$ (calcd for $C_{37}H_{56}O_{13}Na$, 731.3613).

*Cucurbitacin K 2-O-β-D-glucopyranoside (**6**):* amorphous solid; $[\alpha]^{20}_D$ −45.0 (c 0.53, MeOH); UV (MeOH) λ_{max} (log ε) 256 (3.79) nm; CD (MeOH) 240 ($\Delta\varepsilon$ −3.29), 272 ($\Delta\varepsilon$ +3.10), 298 ($\Delta\varepsilon$ +2.09), 333 ($\Delta\varepsilon$ −3.62) nm; IR ν_{max} 3410, 1685, 1378, 1221, 1079, 1030 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) data, see Supporting Information, Table S2; ^{13}C NMR (DMSO-d_6, 125 MHz) data, see Supporting Information, Table S3; ESIMS m/z 717 $[M + Na]^+$ and 733 $[M + K]^+$.

*Cucurbitacin J 2-O-β-D-glucopyranoside (**7**):* amorphous solid; $[\alpha]^{20}_D$ −36.0 (c 0.50, MeOH); UV (MeOH) λ_{max} (log ε) 256 (3.80) nm; CD (MeOH) 239 ($\Delta\varepsilon$ −3.36), 273 ($\Delta\varepsilon$ +3.18), 298 ($\Delta\varepsilon$ +2.14), 333 ($\Delta\varepsilon$ −3.72) nm; IR ν_{max} 3411, 1685, 1378, 1220, 1075, 1028 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) data, see Supporting Information, Table S2; ^{13}C NMR (DMSO-d_6, 125 MHz) data, see Supporting Information, Table S3; ESIMS m/z 717 $[M + Na]^+$ and 733 $[M + K]^+$.

*(16α,20R)-20,24-Epoxy-2,16-dihydroxy-25,26,27-trinorcucurbita-1,5,23-triene-3,11,22-trione 2-O-β-D-glucopyranoside (**8**):* amorphous solid; $[\alpha]^{20}_D$ +1.5 (c 0.14, MeOH); UV (MeOH) λ_{max} (log ε) 258 (3.84) nm; CD (MeOH) 228 ($\Delta\varepsilon$ −1.11), 267 ($\Delta\varepsilon$ +5.18), 290 ($\Delta\varepsilon$ +1.91), 332 ($\Delta\varepsilon$ −2.11) nm; IR ν_{max} 3351, 1756, 1686, 1561, 1374, 1074, 1028 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) data, see Table 1; ^{13}C NMR (DMSO-d_6, 125 MHz) data, see Table 2; ESIMS m/z 639 $[M + Na]^+$ and 615 $[M - H]^-$; HRESIMS m/z 639.2768 $[M + Na]^+$ (calcd for $C_{33}H_{44}O_{11}Na$, 639.2781).

*2-Hydroxy-(22−27)-hexanorcucurbita-1,5,16-triene-3,11,20-trione 2-O-β-D-glucopyranoside (**9**):* amorphous solid; $[\alpha]^{20}_D$ −38.3 (c 0.60, MeOH); UV (MeOH) λ_{max} (log ε) 241 (3.69) nm; CD (MeOH) 238 ($\Delta\varepsilon$ −1.19), 280 ($\Delta\varepsilon$ +1.81), 335 ($\Delta\varepsilon$ −0.33) nm; IR ν_{max} 3410, 1687, 1662, 1589, 1378, 1076, 1031 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) data, see Table 1; ^{13}C NMR (DMSO-d_6, 125 MHz) data, see Table 2; ESIMS m/z 567 $[M + Na]^+$ and 583 $[M + K]^+$; HRESIMS m/z 583.2264 $[M + K]^+$ (calcd for $C_{30}H_{40}O_9K$, 583.2309).

Enzymatic Hydrolysis of 1 and 5−7. A solution of each compound (**1** and **5**−**7**, 5−10 mg) in H_2O (3 mL) was hydrolyzed with snailase (30 mg, LJ0427B2011Z, Shanghai Sangon Biotech Co. Ltd.) at 37 °C for 12 h. The reaction mixture was extracted with EtOAc (3 × 3 mL). The H_2O phases of the hydrolysates of **1** and **5**−**7** were separately concentrated to dryness and then eluted with CH_3CN-H_2O (8:1) on a silica gel column to yield glucose with $[\alpha]^{20}_D$ values that ranged from +42.3 to +46.8 (c 0.16−0.22, H_2O). The solvent system $CHCl_3-MeOH-H_2O$ (8:5:1) was used for TLC identification of glucose (R_f, 0.28). EtOAc extracts of the hydrolysates were separately concentrated to dryness and then eluted on a silica gel column with 40−70% EtOAc in petroleum ether to yield **1a** (1.3 mg) from **1**, **5a** (2.2 mg) from **5**, cucurbitacin K (**6a**, 2.8 mg) from **6**, and cucurbitacin J (**7a**, 4.2 mg) from **7**, respectively. **1a**: UV (MeOH) λ_{max} (log ε) 206 (3.76), 224 (3.66), 268 (3.57) nm; CD (MeOH) 207 ($\Delta\varepsilon$ +0.31), 235 ($\Delta\varepsilon$ −0.31), 286 ($\Delta\varepsilon$ +2.11), 331 ($\Delta\varepsilon$ −1.45) nm; ^1H NMR (acetone-d_6, 500 MHz) data, see Supporting Information, Table S4; ESIMS m/z 515 $[M + H]^+$. **5a**: UV (MeOH) λ_{max} (log ε) 201 (3.98), 268 (3.80) nm; CD (MeOH) 202 ($\Delta\varepsilon$ +3.68), 236 ($\Delta\varepsilon$ −1.26), 287 ($\Delta\varepsilon$ +2.81), 330 ($\Delta\varepsilon$ −3.58) nm; ^1H NMR (acetone-d_6, 600 MHz) data, see Supporting Information, Table S4; ESIMS m/z 569 $[M + Na]^+$. **6a**: UV (MeOH) λ_{max} (log ε) 200 (3.65), 268 (3.69) nm; CD (MeOH) 240 ($\Delta\varepsilon$ −0.69), 292 ($\Delta\varepsilon$ +2.11), 330 ($\Delta\varepsilon$ −2.70) nm; $Mo_2(OAc)_4$-induced CD (DMSO) 278 ($\Delta\varepsilon'$ −0.30), 318 ($\Delta\varepsilon'$ +0.25), 350 ($\Delta\varepsilon'$ +0.10), 409 ($\Delta\varepsilon'$ +0.46); ^1H NMR (acetone-d_6, 600 MHz) data, see Supporting Information, Table S4; ESIMS m/z 555 $[M + Na]^+$. **7a**: UV (MeOH) λ_{max} (log ε) 203 (3.46), 270 (3.72) nm; CD (MeOH) 206 ($\Delta\varepsilon$ +1.32), 241 ($\Delta\varepsilon$ −0.73), 290 ($\Delta\varepsilon$ +2.17), 330 ($\Delta\varepsilon$ −2.99) nm; $Mo_2(OAc)_4$-induced CD (DMSO) 259 ($\Delta\varepsilon'$ +1.15), 296 ($\Delta\varepsilon'$ −2.77), 335 ($\Delta\varepsilon'$ +3.31); ^1H NMR (acetone-d_6, 600 MHz) data, see Supporting Information, Table S4; ESIMS m/z 555 $[M + Na]^+$ and 571 $[M + K]^+$.

PTP1B Inhibition Assay. See ref 28.

Cells, Culture Conditions, and Cell Proliferation Assay. See ref 29.

■ ASSOCIATED CONTENT

Supporting Information

MS, CD, IR, and NMR spectra of compounds **1**−**9** and **5a**−**7a**. This can be accessed free of charge via the Internet at http://pubs.acs.org.

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