Cytotoxic Limonoids from Brazilian *Melia azedarach*

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Three new C-*seco* limonoids (1—3) and one new tetracyclic limonoid (4) were isolated from a methanol extract of the ripe fruits of *Melia azedarach* collected in Curitiba, Brazil, and their structures were elucidated by spectroscopic data analysis and comparison of spectral data with those of the previously known compounds. Among the limonoids isolated in the present study, compounds 3 and 4 exhibited significant inhibitory activity against HeLa S3 cancer cells, whereas 1 and 2 showed weak cytotoxicity.

Key words tetranortriterpenoid; limonoid; *Melia azedarach*; cytotoxicity

Melia azedarach is a large tree of worldwide distribution, and its leaves and fruits show a variety of biological effects on insects, such as an anthelmintic, an antifeedant, and other inhibitory activities.[1—3] Hence, the chemical and biological studies on this tree have been undertaken at many regions in the world to isolate a number of tetranortriterpenoids, so called limonoids.[4,5] As part of our ongoing studies on biologically active substances of *Melia azedarach*,[6] we have investigated chemical components of the BST (brine shrimp lethality test)[7] active methanol extract of the ripe fruits of *M. azedarach* collected in Curitiba, Brazil, thereby resulting in the isolation of three new ring C-*seco* limonoids **1**—**3** and one tetracyclic limonoid **4**. In this paper, we report the structure elucidation of these new compounds and their inhibitory activity against HeLa S3 cancer cells.

The methanol extract of the fruits of *M. azedarach*, collected in Curitiba, which showed strong BST lethal activity at 200 μg/ml, was purified by a combination of silica gel, reversed-phase ODS column chromatography, and preparative HPLC to afford four new limonoids, 15-*O*-deacetyl-15-*O*-methylnimbolidin A (**1**), 15-*O*-deacetyl-15-*O*-methylnimbolidin B (**2**), 15-*O*-deacetylnimbolidin B (**3**), and 12-*O*-deacetyltrichilin H (**4**).

Compound **1** had an $[M]^+$ ion peak at *m/z* 692.3187 in the high-resolution EI-MS, corresponding to the molecular formula $C_{39}H_{48}O_{11}$ and its IR spectrum displayed absorptions due to ester groups at 1736 and 1710 cm^{-1}. The NMR spectral data (Table 1) of **1** contained signals corresponding to a β-furan ring [δ 5.78 (d, J=1.2 Hz, H-22), 7.00 (t, J=1.2 Hz, H-21), 7.02 (t, J=1.2 Hz, H-23); δ 109.3 (C-22), 127.4 (C-20), 139.6 (C-21), 142.8 (C-23)], four tertiary methyl groups including an olefinic methyl at δ 1.25 (6H), 1.36, and 1.74, one isolated oxy-methylene [δ 3.39 (d, J=7.3 Hz), 3.47 (d, J=7.3 Hz); δ 78.2 (C-28)], four oxy-methines at δ 4.25 (d, J=6.3 Hz, H-15), 4.39 (t, J=2.9 Hz, H-3), 4.97 (t, J=2.9 Hz, H-1), and 5.54 (d, J=3.0 Hz, H-7), and a methyl ester group [δ 3.58 (3H, s); δ 174.2], which proved to connect to C-11 by HMBC correlation between its carbonyl resonance and the H_2-11 signals at δ 2.43 (dd, J=14.5, 11.5 Hz) and 2.58 (dd, J=14.5, 2.2 Hz), as well as two acetyl groups at δ 2.05 and 2.11, one methoxy group at δ 3.24, and one benzoate moiety, the presence of which was supported by the observation of a base peak at *m/z* 105 in EI-MS. These spectral data implied that **1** is closely related to nimbolidin A (**5**).[8] The HMBC correlations for **1**, as summarized in Fig. 2a, were consistent with the plane structure of nimbolidin A except for the presence of one extra methoxy group in place of an acetoxy group existing at the C-15 position in the case of **5**. In HMBC, this methoxy signal showed a correlation to C-15 resonated at δ 86.0, indicating this one being placed at the C-15 position. The relative stereochemistry for **1** was elucidated on the basis of not only NOESY correlations as shown in Fig. 2b but also small J values for H-1 (t, J=2.9 Hz), H-3 (t, J=2.0 Hz), and H-7 (d, J=3.0 Hz) to be identical to that of nimbolidin A (**5**) except for C-15 and C-17. Taking biosynthesis of ring C-*seco* limonoids into consideration,[9] the C-17 stereogenic center of **1** is dependent upon its corresponding stereochemistry in the tetracyclic biosynthesis precursor bearing the C-15/C-14 epoxy ring. Additionally, a series of NOEs from H-17 to H-15 as shown in Fig. 2b could account for the same relative stereochemistry of C-15 as that of **5**. Thus, compound **1** was assigned as 15-*O*-deacetyl-15-*O*-methylnimbolidin A.

The molecular formula of compound **2** was assigned as $C_{37}H_{50}O_{11}$, obtained from high-resolution EI-MS at *m/z* 670 $[M]^+$, and exhibited physical and NMR data (Table 1) very similar to those of compound **1** except for the presence of a tigloyl group, which was supported by the observation of a base peak at *m/z* 83 in EI-MS, as well as for missing a benzoyl group existing in **1**. Additionally, the analysis of the 2D NMR data of **2** gave the same plane structure having two acetyl and one tigloyl groups as **1**. These spectral feature diclosed that **2** is closely related to nimbolidin B (**6**).[8] In the HMBC data for **2**, the H-1 and H-3 signal at δ 4.49 (t, J=2.8 Hz) and 4.94 (t, J=2.8 Hz) correlated to the acetyl carbonyls at δ 170.6 and 169.7, respectively, whereas the H-7 signal at δ 5.65 (d, J=2.8 Hz) had a cross peak with the ester carbonyl at δ 166.5 due to a tigloyl group. These spectral data indicated that **2** had a tigloyloxy group at the C-7 position and two acetoxy moieties at the same C-1 and C-3 positions as **1**. The relative configurations for all chiral centers of **2** were found to be identical to those of **1** based on the NOESY data and small J values for H-1, H-3, and H-7. Thus, the structure of **2** was assigned as 15-*O*-deacetyl-15-*O*-methylnimbolidin B.

Compound **3** showed a molecular ion peak at *m/z*

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656.3187 in the high-resolution EI-MS, corresponding to the molecular formula $C_{36}H_{48}O_{11}$. The IR spectrum displayed the absorption attributable to a hydroxyl group at 3440 cm^{-1} and carbonyl groups at 1736 and 1715 cm^{-1}. The ^{1}H- and ^{13}C-NMR spectral data (Table 1) of **3** showed signals similar to those of nimbolidin B (**6**) except for the absence of an acetyl group existing in **6**. These spectral similarity disclosed that **3** is 1-*O*-deacetyl or 3-*O*-deacetyl or 15-*O*-deacetyl nimbolidin B. The H-1 and H-3 signals at δ 4.45 (t, J=2.7 Hz) and 4.95 (t, J=2.7 Hz) showed the HMBC correlations to the acetyl carbonyls resonated at δ 169.7 and 170.0, whereas the H-15 signal at δ 4.74 (t, J=8.2 Hz) had no HMBC correlation to any carbonyl signals, suggesting the presence of a free hydroxyl group at the C-15 position. On the other hand, the H-7 signals at δ 5.46 (d, J=3.0 Hz) showed an HMBC correlation to the tigloyl carbonyl at δ 168.4, indicating this one being attached at C-7. From small J values for H-1, H-3, and H-7, all oxy-functional groups at C-1, C-3, and C-7 took axial and α configurations. The NOESY experiments indicated that the relative stereochemistry for **3** was the same as that of **2**. Thus, the structure of **3** was elucidated to be 15-*O*-deacetylnimbolidin B.

Compound **4** gave the molecular formula $C_{34}H_{44}O_{13}$, as determined by the high-resolution EI-MS at m/z 660.2789 $[M]^+$, indicating 13 degrees of unsaturation. Its IR spectrum displayed absorptions due to a hydroxyl group at 3481 cm^{-1}, and carbonyl groups at 1734 and 1722 cm^{-1}. The NMR spectral data (Table 1) of **4** showed the presence of three tertiary methyl groups at δ 0.83, 1.14, and 1.15 (each 3H, s), a β-furan moiety at δ 6.53 (dd, J=1.6, 0.8 Hz), 7.23 (dd, J=1.6, 0.8 Hz), and 7.33 (t, J=1.6 Hz), seven oxy-methines at δ 3.66 (dd, J=2.0, 2.0 Hz, H-7), 3.78 (br s, H-15), 4.11 (s, H-12), 4.58 (d, J=3.6 Hz, H-1), 5.53 (d, J=4.6 Hz, H-3), 5.74 (s, H-29), and 5.91 (dd, J=4.6, 3.6 Hz, H-2), one oxymethylene at δ 4.31 (d, J=13.2 Hz), 4.36 (d, J=13.2 Hz), and 64.1 (C-19), one ketone function at δ 213.1 (C-11) as well as an isopropionyl moiety at δ 1.22 (6H, d, J=6.9 Hz), 2.67 (1H, qq, J=6.9, 6.9 Hz), and 175.8, the presence of which was additionally supported by the detection of a prominent

Table 1. ^{1}H-NMR (150 MHz) Spectral Data of Compounds **1**—**4**[a)]

Position	**1**	**2**	**3**	**4**
1	71.6	72.8	72.9	71.6
2	27.1	27.3	27.2	68.5
3	73.5	71.6	71.5	72.8
4	42.7	42.5	42.8	40.6
5	40.4	40.3	40.2	27.7
6	71.8	72.0	71.3	25.3
7	75.7	75.8	77.6	69.9
8	48.9	47.4	48.4	42.33
9	38.2	38.7	38.5	47.2
10	40.7	40.7	40.7	42.27
11	31.9	32.0	32.1	213.1
12	174.2	173.7	173.7	78.8
13	136.7	135.5	137.6	46.1
14	145.7	144.7	143.4	72.8
15	86.0	88.4	79.2	59.2
16	36.4	33.7	39.8	33.1
17	45.6	45.6	46.2	38.5
18	16.5	17.2	16.5	14.3
19	15.8	16.0	15.6	64.1
20	127.4	128.8	127.7	123.5
21	139.6	139.0	139.5	140.6
22	109.3	110.8	110.6	112.7
23	142.8	142.7	142.6	142.3
28	78.2	77.8	78.0	18.7
29	19.7	19.8	19.6	93.5
30	19.2	19.1	19.0	22.7
C-12 OMe	52.0	51.9	50.0	
C-15 OMe	55.6	55.8		
C-1 OAc	169.7	170.6	169.7	
	21.0	21.3	20.8	
C-2 OAc				169.0
				20.7
C-3 OAc	170.1	169.7	170.0	170.1
	21.5	20.9	21.4	20.9
C-7 OBz				
1	165.3			
2	130.4			
3, 7	129.9			
4, 6	128.1			
5	133.1			
C-7 OTig				
1		166.5	168.4	
2		129.4	127.8	
3		135.7	139.6	
4		12.4	12.0	
5		14.3	14.6	
1′				175.8
2′				34.1
3′				18.7
4′				18.7

a) Measured in $CDCl_3$.

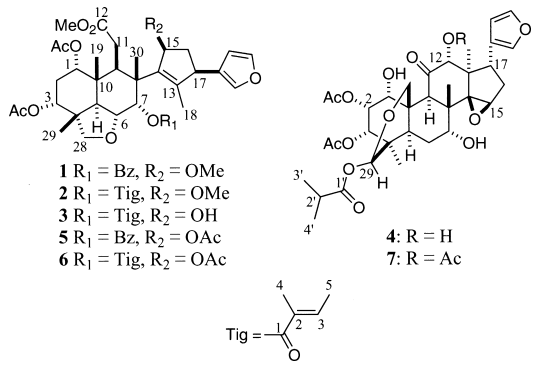
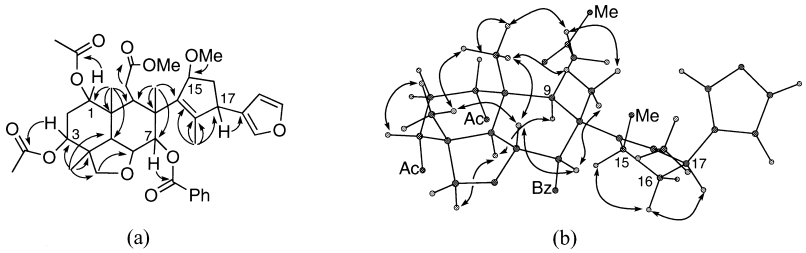


Fig. 1. Limonoids Isolated from *M. azedarach*



(a) (b)

Fig. 2. (a) Representative HMBC Correlations for **1**; (b) NOESY Correlations and Relative Stereochemistry for **1**

Table 2. Cytotoxic Activities of Compounds **1**—**4** against HeLa S3[a)]

Compound	IC_{50} (μM)
1	37.4
2	28.3
3	0.10
4	0.48
Fluorouracil	5.40
Cisplatin	2.46

a) Human epithelial cancer cell line.

peak at *m*/*z* 74 in EI-MS. The routine analyses of H/H COSY and HMQC gave three fragments such as (–O)$C_{(1)}$H–$C_{(2)}$H(O–)–$C_{(3)}$H(O–), $C_{(5)}$H–$C_{(6)}H_2$–$C_{(7)}$H(O–), (–O)$C_{(15)}$H–$C_{(16)}H_2$–$C_{(17)}$H, one isolated methine at δ 4.56 (s) and 47.2 (C-9) as well as five quaternary carbons at δ 40.6 (C-4), 42.33 (C-8), 42.27 (C-10), 46.1 (C-13), and 72.8 (C-14). These spectral data, together with thirteen degrees of unsaturation, suggested that **4** belongs to a tetracyclic limonoid having both the C-19/C-29 acetal bridge and the C-14/C-15 epoxide ring. It turns out to be similar to the structure of trichilin H (**7**). The H-12 signal in **4** was found to be shifted up-field by 1.29 ppm in comparison with that of **7**.[9)] The H-2 and H-3 signals at δ 5.91 and 5.53 showed HMBC correlation to the acetyl carbonyls resonated at δ 169.0 and 170.1, respectively, accounting for the presence of the acetoxy groups at the C-2 and C-3 positions. On the other hand, the H-1, H-7, and H-12 signal had no HMBC correlation to any ester carbonyl resonances, indicating that all the C-1, C-7 and C-12 hydroxy groups must be free. Other HMBC data indicated that the plane structure of **4** was consistent with that of **7** without an acetyl group at C-12. The small *J* values for H-1, H-2, H-3, and H-7 and NOESY correlations for **4** (data not shown) enabled the relative stereochemistry of **4** to assign the same one as **7** without contradictory. Thus, the structure of **4** was determined to be 12-*O*-deacetyltrichilin H.

All limonoids **1**—**4** isolated in the present study were submitted to proliferation assay of the Hela S3 (human epithelial cancer) cell line. As results, compounds **3** and **4** exhibited significant cytotoxic activity (Table 2), whereas compounds **1** and **2** showed weak cytotoxicty in the range of IC_{50} 30—40 μM. Strong cytotoxicty of **4** is anticipated because most tetracyclic sendanin-[10,11)] and trichilin-type[12,13)] limonoids with a 14,15-epoxide ring and a C-19/C-29 acetal bridge exhibit very strong (less than IC_{50} 0.1 μg/ml) cytotoxicity against P388 cells, but it should be noted that compound **3**, a C-*seco* limonoid, showed strong cytotoxicty (less than IC_{50} 0.1 μM).

Experimental

General Procedures Optical rotations were measured on a JASCO DIP-1000 digital polarimeter. IR spectra were measured on a JASCO FT-IR 5300 infrared spectrophotometer. 1D- and 2D-NMR spectra were recorded on a Varian Unity 600 instrument. Chemical shifts are given as δ (ppm) with TMS as internal standard. MS were recorded on a JEOL AX-500 instrument. Column chromatography was carried out on Keiselgel 60 (70—230 mesh and 230—400 mesh).

Plant Material The ripe fruits of *Melia azedarach* were collected in Curitiba, Brazil in August 2002. The plant was identified by Prof. J. D. Fontana and a voucher specimen has been deposited at the Federal University of Paran.

Extraction and Isolation The ripe fruits of *M. azedarach* (500 g) was extracted with MeOH to yield 150 g of the MeOH extract. The extract was chromatographed on a silica gel column eluting with a step gradient of CH_2Cl_2 (100%), CH_2Cl_2–EtOAc (9 : 1), CH_2Cl_2–EtOAc (4 : 1), CH_2Cl_2–EtOAc (1 : 1), CH_2Cl_2–EtOAc (1 : 4), EtOAc (100%), EtOAc–MeOH (9 : 1), and EtOAc–MeOH (4 : 1) to give nine fractions (A—I).

Fraction D (0.91 g) was first subjected to reversed-phase Cosmosil C18-75N chromatography eluting with MeOH–H_2O (3 : 2) to give fractions 1—7. Fraction 3 (99 mg) was separated by reversed-phase HPLC [Cosmosil 5C18-AR-II, ϕ 10×250 mm; MeOH–H_2O (13 : 7), 2 ml/min] to give compounds **1** (2.8 mg, 0.0019%), **2** (2.5 mg, 0.0017%), and **3** (3.0 mg, 0.002%). Fraction 6 (24.1 mg) was separated by reversed-phase HPLC (Cosmosil 5C18-AR-II, ϕ 10×250 mm; MeCN–H_2O (47 : 53), 2 ml/min) to give compound **4** (9.7 mg, 0.0064%).

15-*O*-Deacetyl-15-*O*-methylnimbolidin A (**1**): Colorless amorphous solids, $[\alpha]_D^{21}$ −5.8° (*c*=1.26, $CHCl_3$). IR (film) cm^{-1}: 1736, 1710. EI-MS *m*/*z* (rel. int.): 692 (M^+, 9), 660 (70), 538 (11), 403 (7), 273 (8), 174 (15), 105 (100), 77 (14), 43 (22). ^{1}H-NMR ($CDCl_3$) δ: 1.25 (6H, s, H_3-19, -29), 1.36 (3H, s, H_3-30), 1.60 (1H, m, H-16α), 1.74 (3H, s, H_3-18), 2.05 (3H, s, C1-OAc), 2.11 (3H, s, C3-OAc), 2.18 (1H, m, H-16β), 2.23 (1H, dt, *J*=16.8, 2.9 Hz, H-2α), 2.33 (1H, dt, *J*=16.8, 2.9 Hz, H-2β), 2.43 (1H, dd, *J*=14.5, 11.5 Hz, H-11β), 2.58 (1H, dd, *J*=14.5, 2.2 Hz, H-11α), 3.10 (1H, d, *J*=12.6 Hz, H-5), 3.24 (3H, s, C15-OMe), 3.39 (1H, d, *J*=7.3 Hz, H-28α), 3.47 (1H, d, *J*=7.3 Hz, H-28β), 3.58 (3H, s, C12-OMe), 3.80 (1H, t, *J*=7.8 Hz, H-17), 3.84 (1H, dd, *J*=11.5, 2.2 Hz, H-9), 4.25 (1H, d, *J*=6.3 Hz, H-15), 4.27 (1H, dd, *J*=12.6, 3.0 Hz, H-6), 4.39 (1H, t, *J*=2.9 Hz, H-3), 4.97 (1H, t, *J*=2.9 Hz, H-1), 5.54 (1H, d, *J*=3.0 Hz, H-7), 5.78 (1H, d, *J*=1.2 Hz, H-22), 7.00 (1H, t, *J*=1.2 Hz, H-21), 7.02 (1H, t, *J*=1.2 Hz, H-23), 7.36 (2H, t, *J*=8.1 Hz, Bz-4,6), 7.52 (1H, tt, *J*=7.4, 1.1 Hz, Bz-5), 8.11 (2H, dd, *J*=8.1, 1.1 Hz, Bz-3,7). HR-EI-MS *m*/*z*: 692.3187 (Calcd for $C_{39}H_{48}O_{11}$, 692.3196). ^{13}C-NMR: Table 1.

15-*O*-Deacetyl-15-*O*-methylnimbolidin B (**2**): Colorless amorphous solids, $[\alpha]_D^{21}$ −6.7° (*c*=1.28, $CHCl_3$). IR (film) cm^{-1}: 1736, 1710, 1251. EI-MS *m*/*z* (rel. int.): 670 (M^+, 62), 638 (74), 570 (28), 555 (24), 493 (11), 273 (18), 177 (93), 147 (20), 83 (100), 43 (24). ^{1}H-NMR ($CDCl_3$) δ: 1.15 (3H, s, H_3-19), 1.21 (3H, s, H_3-29), 1.36 (3H, s, H_3-30), 1.67 (3H, dq, *J*=7.1, 1.1 Hz, Tig-5), 1.76 (3H, q, *J*=1.1 Hz, Tig-4), 1.89 (3H, s, H_3-18), 1.91 (1H, m, H-16β), 1.98 (3H, s, C3-OAc), 2.08 (3H, s, C1-OAc), 2.10 (1H, m, H-16α), 2.18 (1H, dt, *J*=16.8, 2.8 Hz, H-2α), 2.21 (1H, dt, *J*=16.8, 2.8 Hz, H-2β), 2.28 (2H, m, H-11), 2.82 (1H, d, *J*=12.5 Hz, H-5), 3.24 (3H, s, C15-OMe), 3.37 (1H, d, *J*=8.5 Hz, H-17), 3.41 (1H, d, *J*=7.5 Hz, H-28α), 3.48 (1H, d, *J*=7.5 Hz, H-28β), 3.57 (3H, s, C12-OMe), 3.63 (1H, dd, *J*=11.0, 2.2 Hz, H-9), 4.17 (1H, dd, *J*=12.5, 2.8 Hz, H-6), 4.22 (1H, d, *J*=6.9 Hz, H-15), 4.49 (1H, t, *J*=2.8 Hz, H-1), 4.94 (1H, t, *J*=2.8 Hz, H-3), 5.65 (1H, d, *J*=2.8 Hz, H-7), 6.29 (1H, br s, H-22), 6.73 (1H, qq, *J*=7.1, 1.1 Hz, Tig-3), 7.20 (1H, br s, H-21), 7.22 (1H, br s, H-23). HR-EI-MS *m*/*z*: 670.3348 (Calcd for $C_{37}H_{50}O_{11}$, 670.3353). ^{13}C-NMR: Table 1.

15-*O*-Deacetylnimbolidin B (**3**): Colorless amorphous solids, $[\alpha]_D^{21}$ −6.7° (*c*=1.28, $CHCl_3$). IR (film) cm^{-1}: 3440, 1736, 1715. EI-MS *m*/*z* (rel. int.): 656 (M^+, 9), 638 (1), 538 (18), 403 (11), 259 (18), 245 (15), 174 (47), 83 (100), 55 (49), 43 (39); ^{1}H-NMR ($CDCl_3$): δ 1.18 (3H, s, H_3-19), 1.24 (3H, s, H_3-29), 1.48 (3H, s, H_3-30), 1.70 (1H, ddd, *J*=14.5, 8.2, 3.4 Hz, H-16β), 1.72 (3H, dq, *J*=7.0, 1.1 Hz, Tig-5), 1.79 (3H, s, H_3-18), 1.88 (3H, q, *J*=1.1 Hz, Tig-4), 2.01 (3H, s, C3-OAc), 2.05 (3H, s, C1-OAc), 2.18 (1H, dd, *J*=13.8, 4.6 Hz, H-11α), 2.20 (1H, dt, *J*=16.8, 2.7 Hz, H-2α), 2.28 (1H, dt, *J*=16.8, 2.7 Hz, H-2β), 2.33 (1H, dd, *J*=13.8, 9.6 Hz, H-11β), 2.51 (1H, ddd, *J*=14.5, 9.3, 8.2 Hz, H-16α), 2.91 (1H, d, *J*=12.6 Hz, H-5), 3.40 (1H, dd, *J*=9.3, 3.4 Hz, H-17), 3.50 (1H, d, *J*=7.4 Hz, H-28α), 3.52 (1H, d, *J*=7.4 Hz, H-28β), 3.57 (3H, s, C12-OMe), 3.64 (1H, dd, *J*=9.6, 4.4 Hz, H-9), 4.18 (1H, dd, *J*=12.6, 3.0 Hz, H-6), 4.45 (1H, t, *J*=2.7 Hz, H-1), 4.74 (1H, t, *J*=8.2 Hz, H-15), 4.95 (1H, t, *J*=2.7 Hz, H-3), 5.46 (1H, d, *J*=3.0 Hz, H-7), 6.28 (1H, d, *J*=1.0 Hz, H-22), 6.81 (1H, qq, *J*=7.0, 1.1 Hz, Tig-3), 7.22 (1H, br s, H-21), 7.30 (1H, t, *J*=1.0 Hz, H-23). HR-EI-MS *m*/*z*: 656.3187 (Calcd for $C_{36}H_{48}O_{11}$, 656.3197). ^{13}C-NMR: Table 1.

12-*O*-Deacetyltrichilin H (**4**): Colorless amorphous solids, $[\alpha]_D^{21}$ −47.5° (*c*=1.06, $CHCl_3$). IR (film) cm^{-1}: 3481, 1734, 1722. EI-MS *m*/*z* (rel. int.): 660 (M^+, 7), 642 (10), 573 (13), 512 (36), 494 (20), 452 (20), 325 (17), 163 (28), 107 (26), 71 (72), 43 (100); ^{1}H-NMR ($CDCl_3$) δ: 0.83 (3H, s, H_3-28), 1.14 (3H, s, H_3-18), 1.15 (3H, s, H_3-30), 1.21 (6H, d, *J*=6.9 Hz, H_3-3′,4′), 1.74 (1H, ddd, *J*=12.0, 12.0, 2.0 Hz, H-6β), 1.98 (1H, ddd, *J*=12.0, 4.1, 2.0 Hz, H-6α), 1.91 (1H, dd, *J*=13.2, 11.0 Hz, H-16α), 2.03 (3H, s, C2-OAc), 2.13 (3H, s, C3-OAc), 2.41 (1H, dd, *J*=13.2, 6.3 Hz, H-16β), 2.67 (1H, qq, *J*=6.9, 6.9 Hz, H-2′), 2.85 (1H, dd, *J*=12.0, 4.1 Hz, H-5), 3.01 (1H, dd, *J*=11.0, 6.3 Hz, H-17), 3.66 (1H, dd, *J*=2.0, 2.0 Hz, H-7), 3.78 (1H, br s, H-15), 4.11 (1H, s, H-12), 4.31 (1H, d, *J*=13.2 Hz, H-19), 4.36 (1H, d,

J=13.2 Hz, H-19), 4.56 (1H, s, H-9), 4.58 (1H, d, J=3.6 Hz, H-1), 5.53 (1H, J=4.6 Hz, H-3), 5.74 (1H, s, H-29), 5.91 (1H, dd, J=4.6, 3.6 Hz, H-2), 6.53 (1H, dd, J=1.6, 0.8 Hz, H-22), 7.23 (1H, dd, J=1.6, 0.8 Hz, H-21), 7.33 (1H, t, J=1.6 Hz, H-23). HR-EI-MS *m/z*: 660.2789 (Calcd for $C_{34}H_{44}O_{13}$, 660.2782). ^{13}C-NMR: Table 1.

Cell Proliferation Assay Cell proliferation assay was carried out using a Cell Counting Kit (Wako Pure Chemical Industries Ltd., Osaka, Japan). In brief, HeLa S3 cells were plated in 384-well plates at a density of 500 cells/well in minimum essential medium. Following overnight culture, drugs were added to final concentrations of 0.1, 1, 10, and 100 μM, and the cells were incubated for 72 h. After 72 h, WST-1 was added according to the manufacturer's protocol and the cells were incubated for a further 2 h. The plates were read at a wavelength of 450 nm using a Microplate Reader Wallac 1420 ARVOsx (Perkin Elmer Life and Analytical Sciences, Inc., Boston, MA, U.S.A.). The assay results are summarized in Table 2.

Supporting Information Available HMBC and NOESY correlations for **2** and **3** can be obtained, on request, from the corresponding author.

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