New Ring C-*seco* Limonoids from Brazilian *Melia azedarach* and Their Cytotoxic Activity

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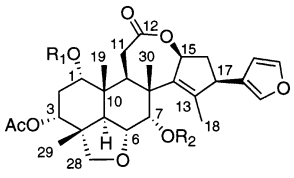
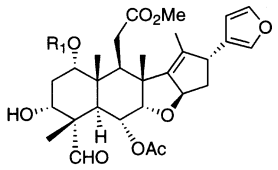
A methanol extract of the ripe fruits of *Melia azedarach* collected in Curitiba, Parana, Brazil, afforded seven new ring C-*seco* limonoids (**1**−**7**) together with three known limonoids (**8**−**10**). The structures of the new compounds were elucidated by NMR and MS analysis and comparison of spectral data with those of previously known compounds. Compounds **4** and **5** exhibited significant inhibitory activity against HeLa S3 cancer cells, whereas **1**, **2**, **3**, and **8** showed weak cytotoxicity.

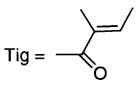
Melia azedarach L. (Meliaceae) is a large tree native of Persia, India, and China, but it has naturalized in Africa, Australia, and the Americas. *M. azedarach* extracts show an array of effects on insects, including anthelmintic, antifeedant, and other inhibitory activities.[1−3] Most of the active principles belong to the group of tetranortriterpenoids called limonoids.[4] Many chemical and biological studies on this tree have been done, and several reviews have been published.[5−7] As part of our ongoing studies on biologically active substances of *M. azedarach*,[8] we have investigated chemical components of the active MeOH extract of the ripe fruits collected in Curitiba, Parana, Brazil, using the BST (brine shrimp lethality test),[9] thereby resulting in the isolation of seven new ring C-*seco* limonoids (**1**−**7**) along with the previously known compounds **8**−**10**. In this paper, we report the structure elucidation of these new compounds and their inhibitory activity against HeLa S3 cancer cells.



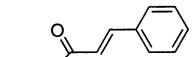
1: $R_1 = R_2 =$ Tig
2: R_1 = Bz, R_2 = Tig
3: R_1= Tig, R_2 = Bz
4: R_1 = H, R_2 = Tig
5: R_1 = H, R_2 = Bz
8: R_1 = Ac, R_2 = Tig
9: R_1 = Ac, R_2 = Bz



6: R_1 = Bz
7: R_1 = Cin
10: R_1 = Tig



Tig =



Cin =

Results and Discussion

As the methanol extract of the fruits of *M. azedarach* showed strong BST lethal activity at 200 μg/mL, it was fractionated on a silica gel column into fractions A−I. The most active fraction (C) was purified by a combination of silica gel chromatography, reversed-phase ODS column chromatography, and preparative HPLC, which yielded ring C-*seco* limonoids **1**−**7**, along with the previously known limonoids ohchnolide B (**8**),[10] ohchnolide A (**9**),[10,11] and ohchinolal (**10**).[12]

Compound **1** had a $[M]^+$ peak at *m*/*z* 664.3223 in HREIMS, corresponding to the molecular formula $C_{38}H_{48}O_{10}$. Its IR spectrum displayed absorptions due to the presence of ester (1735 cm^{-1}) and carbonyl (1716 cm^{-1}) groups. The NMR data implied that **1** was closely related to a ring C-*seco* limonoid, ohchinolide B (**8**).[10,11] The HMBC correlations for **1**, as summarized in Figure S1 (a) (Supporting Information), were consistent with structure **8** except for the presence of one extra tigloyl group and the lack of one acetyl. In the HMBC, the H-1 and H-7 signals at δ 4.88 and 5.78 showed a correlation with the carbonyl signals at δ 166.2 and 166.4 due to the presence of tigloly groups at C-1 and C-7, respectively, and the H-3 signal at δ 5.00 had an additional correlation with an acetyl carbonyl at δ 169.7, indicating tigloyloxy groups were at C-1 and C-7. The relative stereochemistry of **1** was elucidated on the basis of NOESY correlations as shown in Figure S1 (b) (Supporting Information) and *J* values for H-1, H-3, and H-7, which were identical to those of ohchinolide B (**8**).[10] Thus, compound **1** was assigned as 1-*O*-deacetyl-1-*O*-tigloylohchinolide B.

Compound **2** was assigned the molecular formula $C_{40}H_{46}O_{10}$ and exhibited physical and NMR data very similar to those of compound **1** except for the presence of a benzoyl group, which was supported by the observation of a base peak at *m*/*z* 105 in the EIMS, and the absence of one tigloyl group. Analysis of the 2D NMR data of **2** indicated the same planar structure as **1** having a benzoyl, an acetyl, and a tigloyl group. The HMBC data for **2** showed the H-1 signal at δ 5.08 (t, $J = 3.3$ Hz) correlated with the benzoyl carbonyl at δ 164.9, whereas the H-7 signal at δ 5.82 (d, $J = 3.0$ Hz) had a cross-peak with the carbonyl carbon at δ 166.4 due to the presence of a tigloyl group. This meant that **2** had a benzoyloxy group at the C-1 position with remaining ester moieties existing at the same positions as in **1**. The relative configurations for all chiral centers of **2** were identical to those of **1** on the basis of NOESY data and the small *J* values for H-1, H-3, and H-7. Thus, the structure of **2** was assigned as 1-*O*-deacetyl-1-*O*-benzoylohchinolide B.

Compound **3** gave the same molecular formula ($C_{40}H_{46}O_{10}$) as **2**. Its IR spectrum displayed absorptions due to the

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presence of ester carbonyl groups at 1730 and 1721 cm^{-1}. The NMR data of **3** were similar to those of **2** and indicated the presence of the same ester moieties. This suggested that **3** was a positional isomer of **2**. The linked position for these esters was readily differentiated by HMBC experiments; namely, the H-1 and H-7 signals resonating at δ 4.91 and 5.95 showed HMBC correlations with carbonyl signals at δ 166.3 (tigloyl) and 165.0 (benzoyl), thereby confirming tigloyloxy and benzoyloxy groups were at C-1 and C-7, respectively. These spectral data indicated that **3** was ohchinolide A (**9**),[10,11] having a tigloyloxy rather than an acetyl group at C-1. The relative stereochemistry of **3** was identical to that of **2** on the basis of the NOESY interactions. Thus, **3** was determined to be 1-*O*-deacetyl-1-*O*-tigloylohchinolide A.

Compound **4** showed a molecular ion peak corresponding to the molecular formula $C_{33}H_{42}O_9$. The IR spectrum displayed hydroxyl (3449 cm^{-1}) and carbonyl (1730 and 1714 cm^{-1}) absorptions. The ^1H and ^{13}C NMR data of **4** showed signals similar to those of **8** except for the absence of an acetyl group in **8**. These spectral data indicated that **4** was 1-*O*-deacetyl or 3-*O*-deacetyl ohchinolide B. The H-1 signal in **4** appeared at δ 3.61, which was shifted 1.2 ppm upfield in comparison with that of **8**, suggesting the presence of a free hydroxyl group at C-1. The H-3 and H-7 signals at δ 5.10 and 5.74 showed HMBC correlations with the acetyl carbonyl at δ 169.1 and the tigloyl carbonyl at δ 166.2, respectively, indicating actetyloxy and tiglolyloxy groups attached at C-3 and C-7. From small *J* values for H-1, H-3, and H-7, the functional groups at C-1, C-3, and C-7 were in axial and α configurations. NOESY experiments indicated that the relative stereochemistry of **4** was the same as that of **8**. Thus, the structure of **4** was assigned as 1-*O*-deacetylohchinolide B.

Compound **5** gave the molecular formula $C_{35}H_{40}O_9$. Its IR spectrum displayed absorptions due to hydroxyl and carbonyl groups. The NMR data of **5** were similar to those of **4** except for the presence of a benzoyl group, which was supported by the detection of a fragment base peak at *m*/*z* 105 in the EIMS, and the absence of a tigloyl group. These data suggested that **5** was similar to ohchinolide A (**9**) rather than ohchinolide B (**8**). This was confirmed by the HMBC correlation of the H-7 signal at δ 5.92 with the benzoyl carbonyl resonating at δ 164.6. In addition, the OH at C-1 must be free because H-1 resonated at high field (δ 3.66). The other NMR data and NOESY correlations for **5** supported this structure. Thus, **5** was determined to be 1-*O*-deacetylohchinolide A.

Compound **6** was assigned the molecular formula $C_{36}H_{42}O_{10}$. Its IR spectrum displayed hydroxyl (3501 cm^{-1}), carbonyl (1740 and 1719 cm^{-1}), and benzoyl (1650 cm^{-1}) absorptions. The NMR data of **6** implied that **6** was closely related to ohchinalal (**10**).[12] Analyses of COSY and HMQC data of **6** provided four structural fragments: $(-O)C_{(1)}H-C_{(2)}H_2-C_{(3)}H(O-)$, $C_{(5)}H-C_{(6)}H(O-)-C_{(7)}H(O-)$, $C_{(9)}H-C_{(11)}H_2$, $C_{(15)}H(O-)-C_{(16)}H_2-C_{(17)}H$. As shown in Figure S2 (Supporting Information), three tertiary methyl (H_3-19, H_3-29, and H_3-30) signals correlated with the former three partial units that made up a decaline ring, whereas the HMBC correlation of H-15 (δ 5.51) to the C-7 resonance (δ 86.0) indicated an ether bond between C-7 and C-15. Further HMBC correlation between the aldehyde proton signal and the C-4 quaternary carbon (δ 49.0) indicated that the sole aldehyde group was attached to C-4. The benzoyloxy and acetyloxy groups were bonded to C-1 and C-6 according to the HMBC correlations of H-1 and H-6 with the benzoyl and acetyl carbonyls at δ 165.0 and 170.3, respectively. NOESY correlations, in addition to *J* values for H-1, H-3, H-5, and H-6, indicated that the relative stereochemistry of **6** was identical to that of ohchinolal (**10**). Thus, compound **6** was elucidated as 1-*O*-detigloyl-1-*O*-benzoylohchinolal.

Table 1. Cytotoxic Activities of Compounds **1**−**10** against HeLa S3[a]

compound	IC_{50} (μM)
1	33.8
2	33.0
3	29.7
4	0.10
5	2.40
6	inactive
7	inactive
8	40.50
9	inactive
10	inactive
fluorouracil	5.40
cisplatin	2.46

[a] Human epithelial cancer cell line.

Compound **7** showed a molecular ion peak corresponding to the molecular formula $C_{38}H_{44}O_{10}$. The IR spectrum displayed absorptions ascribable to hydroxyl (3445 cm^{-1}), carbonyl (1730 and 1717 cm^{-1}), and cinnamoyl (1636 and 1435 cm^{-1}) moieties. The presence of a cinnamoyl group was supported by the fragment base peak at *m*/*z* 131 and ^1H NMR data. The ^1H and ^{13}C NMR data of **7** were identical to those of **10** except for the presence of a cinnamoyl group and the absence of a benzoyl group. In HMBC experiments, the H-1 signal at δ 5.15 showed a cross-peak with the carbonyl resonance of the cinnamoyl moiety, indicating that **7** had a cinnamoyloxy group at the C-1 position in place of a benzoyloxy group in **6**. NOESY experiments indicated the relative stereochemistry of **7** to be the same as that of **6**. Thus, the structure of **7** was elucidated as 1-*O*-detigloyl-1-*O*-cinnamoylohchinolal.

Limonoids **1**−**10** all showed 100% lethality in the BST assay at 100 μg/mL. They were also evaluated against the Hela S3 (human epithelial cancer) cell line. Compounds **4** and **5** exhibited significant cytotoxic activity (Table 1), whereas compounds **1**−**3** and **8** showed weak cytotoxicty in the range of IC_{50} 30−40 μM. Most tetracyclic sendanin-[13−15] and trichilin-type[16,17] limonoids with a 14,15-epoxide ring and a C-19/C-29 acetal bridge were reported to show strong (less than IC_{50} 0.1 μg/mL) cytotoxicity against P388 cells. The azadirachtin-type limonoids also exhibited significant cytotoxic activity, but to a lesser degree than the sendanin-type limonoids.[17,18] The cytotoxic activity of compounds **4** and **5** was comparable to that of the azadirachtin-type limonoids.

Experimental Section

General Experimental 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 or 400 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 Kieselgel 60 (70−230 mesh and 230−400 mesh).

Plant Material. The ripe fruits of *Melia azedarach* were collected in Curitiba, Parana, Brazil, in August 2002. The plant was identified by Prof. Jose Domingos Fontana, and a voucher specimen (1734FR) has been deposited at the Federal University of Parana.

Extraction and Isolation. The ripe fruits of *M. azedarach* (500 g) were blended with MeOH to yield 150 g of extract. The

extract was chromatographed on a silica gel column eluted 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 C (3.51 g) was first subjected to reversed-phase Cosmosil C18-75N chromatography eluting with MeOH−H_2O (3:2) to give fractions 1−8. Fraction 1 (409 mg) was separated by reversed-phase HPLC (Cosmosil 5C18-AR-II, 10 × 250 mm, partial size 5 μm) to give compounds **6** (2.2 mg), **7** (2.5 mg), and ohchinolal (**10**) (5 mg). Fraction 3 (318 mg) was separated by reversed-phase HPLC (Cosmosil 5C18-AR-II, 10 × 250 mm, partial size 5 μm) using MeOH−H_2O (13:7) to give compounds **1** (5.8 mg), **4** (2.8 mg), and ohchinolide B (**8**) (6.1 mg). Fraction 4 (865.9 mg) was chromatographed on a silica gel column eluting with CH_2Cl_2−EtOAc (2:1) to give fractions 9−12. Fraction 9 (409 mg) was separated by reversed-phase HPLC (Cosmosil 5C18-AR-II, 10 × 250 mm, partial size 5 μm) using MeOH−H_2O (13:7) as eluting solvent to give compounds **2** (4.1 mg), **3** (7.2 mg), **5** (5.1 mg), and ohchinolide A (**9**) (6.8 mg).

1-*O*-Deacetyl-1-*O*-tigloylohchinolide B (1): amorphous solid; $[\alpha]^{21}_D$ −49.5° (*c* 0.94, $CHCl_3$); IR (film) ν_{max} 1735, 1716, 1253, 1154 cm^{-1}; EIMS *m*/*z* (rel int) 664 (M^+, 15), 564 (13), 481 (8), 321 (70), 174 (17), 161 (16), 83 (100), 55 (41), 43 (8); ^{1}H NMR ($CDCl_3$, 400 Mz) δ 7.30 (1H, t, J = 1.6 Hz, H-23), 7.15 (1H, s, H-21), 7.02 (1H, qq, J = 7.3, 1.5 Hz, C-1 tig-3), 6.92 (1H, qq, J = 7.0, 1.1 Hz, C-7 tig-3), 6.31 (1H, d, J = 1.6 Hz, H-22), 5.78 (1H, d, J = 3.3 Hz, H-7), 5.52 (1H, d, J = 7.0 Hz, H-15), 5.00 (1H, t, J = 3.0 Hz, H-3), 4.88 (1H, t, J = 3.0 Hz, H-1), 4.12 (1H, dd, J = 12.6, 3.3 Hz, H-6), 3.55 (1H, d, J = 7.7 Hz, H-28), 3.47 (1H, d, J = 7.7 Hz, H-28), 3.40 (1H, d, J = 9.2 Hz, H-17), 3.21 (1H, dd, J = 12.2, 3.7 Hz, H-9), 2.78 (1H, d, J = 12.6 Hz, H-5), 2.75 (1H, dd, J = 18.7, 12.2 Hz, H-11), 2.63 (1H, dd, J = 18.7, 3.7 Hz, H-11), 2.30 (1H, dt, J = 16.5, 3.0 Hz, H-2), 2.23 (1H, dt, J = 16.5, 3.0 Hz, H-2), 2.11 (1H, ddd, J = 16.5, 9.2, 7.0 Hz, H-16), 1.97 (3H, s, Ac), 1.93 (3H, d, J = 7.3 Hz, C-1 tig-5), 1.91 (3H, d, J = 7.0 Hz, C-7 tig-5), 1.89 (1H, d, J = 16.5 Hz, H-16), 1.85 (3H, s, C-1 tig-4), 1.83 (6H, s, H-18, C-2 tig-4), 1.48 (3H, s, H-30), 1.21 (3H, s, H-29), 1.07 (3H, s, H-19); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.2 (C-12), 169.7 (CH_3CO), 166.4 (C-7 tig-1), 166.2 (C-1 tig-1), 147.6 (C-14), 143.4 (C-23), 139.1 (C-21), 138.2 (C-13), 137.6 (C-1 tig-3), 137.0 (C-7 tig-3), 128.8 (C-1 tig-2), 128.5 (C-7 tig-2), 126.4 (C-20), 109.9 (C-22), 85.7 (C-15), 78.0 (C-28), 74.3 (C-7), 71.8 (C-6), 71.3 (C-1), 71.2 (C-3), 47.1 (C-17), 45.0 (C-8), 42.4 (C-4), 41.3 (C-5), 40.3 (C-10), 37.7 (C-9), 37.5 (C-16), 32.6 (C-11), 27.8 (C-2), 20.8 (Ac), 20.4 (C-30), 18.0 (C-29), 16.1 (C-18), 15.7 (C-19), 14.5 (C-1, C-7 tig-4), 12.3 (C-7 tig-5), 12.2 (C-1 tig-5); HREIMS *m*/*z* 664.3223 (calcd 664.3247 for $C_{38}H_{48}O_{10}$).

1-*O*-Deacetyl-1-*O*-benzoylohchinolide B (2): amorphous solid; $[\alpha]^{21}_D$ −50.8° (*c* 1.06, $CHCl_3$); IR (film) ν_{max} 1730, 1722, 1599, 1530, 1482, 1273 cm^{-1}; EIMS *m*/*z* (rel int) 686 (M^+, 35), 668 (23), 586 (12), 343 (14), 244 (13), 221 (15), 174 (20), 161 (13), 105 (100), 83 (77), 43 (12); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.15 (2H, dd, J = 8.4, 1.5 Hz, Bz-3, 7), 7.63 (2H, tt, J = 7.2, 1.5 Hz, Bz-4, 6), 7.46 (1H, t, J = 7.2 Hz, Bz-5), 7.28 (1H, t, J = 1.8 Hz, H-23), 7.21 (1H, d, J = 0.6 Hz, H-21), 6.96 (1H, qq, J = 7.2, 1.2 Hz, C-7 tig-3), 6.28 (1H, dd, J = 1.8, 0.6 Hz, H-22), 5.82 (1H, d, J = 3.0 Hz, H-7), 5.41 (1H, d, J = 7.1 Hz, H-15), 5.08 (1H, t, J = 3.3 Hz, H-1), 5.03 (1H, t, J = 3.3 Hz, H-3), 4.16 (1H, dd, J = 12.6, 3.0 Hz, H-6), 3.59 (1H, d, J = 7.7 Hz, H-28), 3.52 (1H, d, J = 7.7 Hz, H-28), 3.37 (1H, d, J = 9.2 Hz, H-17), 3.31 (1H, dd, J = 12.4, 3.8 Hz, H-9), 2.89 (1H, d, J = 12.6 Hz, H-5), 2.81 (1H, dd, J = 18.4, 12.4 Hz, H-11), 2.71 (1H, dd, J = 18.4, 3.8 Hz, H-11), 2.39 (1H, dt, J = 16.8, 3.3 Hz, H-2), 2.32 (1H, dt, J = 16.8, 3.3 Hz, H-2), 2.13 (1H, ddd, J = 16.8, 9.2, 7.1 Hz, H-16), 1.97 (3H, t, J = 1.2 Hz, Tig-4), 1.87 (3H, dq, J = 7.2, 1.2 Hz, Tig-5), 1.82 (3H, s, Ac), 1.81 (1H, d, J = 16.8 Hz, H-16), 1.81 (3H, s, H-18), 1.49 (3H, s, H-30), 1.25 (3H, s, H-29), 1.14 (3H, s, H-19); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 171.1 (C-12), 166.4 (Tig-1), 164.9 (Bz-1), 164.8 (CH_3CO), 147.4 (C-14), 143.3 (C-23), 139.1 (C-21), 138.3 (C-13), 137.1 (Tig-3), 133.7 (Bz-4, 6), 129.9 (Bz-2, Tig-2), 129.5 (Bz-3, 7), 128.6 (Bz-5), 126.3 (C-20), 109.9 (C-22), 85.4 (C-15), 78.1 (C-28), 74.3 (C-7), 71.8 (C-6), 71.7 (C-1), 71.3 (C-3), 47.1 (C-17), 45.1 (C-8), 42.4 (C-4), 41.2 (C-5), 40.4 (C-10), 37.9 (C-9), 37.2 (C-16), 32.7 (C-11), 28.0 (C-2), 20.8 (Ac), 20.5 (C-30), 19.2 (C-29), 16.0 (C-18), 15.6 (C-19), 14.6 (Tig-5), 12.4 (Tig-4); HREIMS *m*/*z* found 686.3084 $[M]^+$ (calcd 686.3091 for $C_{40}H_{46}O_{10}$).

1-*O*-Deacetyl-1-*O*-tigloylohchinolide A (3): amorphous solid; $[\alpha]^{21}_D$ −48.4° (*c* 1.59, $CHCl_3$); IR (film) ν_{max} 1730, 1721, 1600, 1549, 1449, 1271 cm^{-1}; EIMS *m*/*z* (rel int) 686 (M^+, 30), 648 (4), 566 (11), 481 (10), 464 (9), 321 (15), 244 (16), 221 (19), 161 (21), 105 (100), 83 (88); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.07 (2H, dd, J = 8.4, 1.2 Hz, Bz-3, 7), 7.62 (1H, tt, J = 7.8, 1.8 Hz, Bz-5), 7.45 (2H, td, J = 7.8, 1.8 Hz, Bz-4, 6), 7.28 (1H, t, J = 1.8 Hz, H-23), 7.23 (1H, t, J = 1.1 Hz, H-21), 7.09 (1H, qq, J = 7.2, 1.4, Tig-3), 6.28 (1H, dd, J = 1.8, 1.1 Hz, H-22), 5.95 (1H, d, J = 3.0 Hz, H-7), 5.51 (1H, d, J = 7.1 Hz, H-15), 5.00 (1H, t, J = 3.3 Hz, H-3), 4.91 (1H, t, J = 3.3 Hz, H-1), 4.20 (1H, dd, J = 12.6, 3.0 Hz, H-6), 3.54 (1H, d, J = 7.7 Hz, H-28), 3.45 (1H, d, J = 7.7 Hz, H-28), 3.35 (1H, dd, J = 12.0, 3.6 Hz, H-9), 3.33 (1H, d, J = 8.2 Hz, H-17), 2.93 (1H, d, J = 12.6 Hz, H-5), 2.79 (1H, dd, J = 18.4, 12.6 Hz, H-11), 2.63 (1H, dd, J = 18.4, 3.3 Hz, H-11), 2.32 (1H, dt, J = 16.8, 3.3 Hz, H-2), 2.26 (1H, dt, J = 16.8, 3.3 Hz, H-2), 1.99 (3H, t, J = 1.4 Hz, Tig-4), 1.98 (3H, s, Ac), 1.92 (1H, m, H-16), 1.88 (3H, s, H-18), 1.81 (3H, dq, J = 7.2, 1.4 Hz, Tig-5), 1.72 (1H, m, H-16), 1.54 (3H, s, H-30), 1.23 (3H, s, H-29), 1.11 (3H, s, H-19); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.2 (C-12), 169.7 (CH_3CO), 166.3 (Tig-1), 165.0 (Bz-1), 147.9 (C-14), 143.3 (C-23), 139.1 (C-21), 138.2 (C-13), 137.7 (Tig-3), 133.3 (Bz-5), 130.3 (Bz-2), 129.1 (Bz-3, 7), 128.9 (Tig-2), 128.6 (Bz-4, 6), 126.3 (C-20), 109.9 (C-22), 85.7 (C-15), 78.1 (C-28), 75.1 (C-7), 71.8 (C-6), 71.3 (C-3), 71.2 (C-1), 47.0 (C-17), 45.1 (C-8), 42.4 (C-4), 41.3 (C-5), 40.4 (C-10), 37.8 (C-9), 37.3 (C-16), 32.6 (C-11), 27.8 (C-2), 20.9 (Ac), 20.4 (c-30), 19.0 (C-29), 16.1 (C-18), 15.7 (C-19), 14.6 (Tig-5), 12.3 (Tig-4); HREIMS *m*/*z* 686.3091 $[M]^+$ (calcd 686.3091 for $C_{40}H_{46}O_{10}$).

1-*O*-Deacetylohchinolide B (4): amorphous solid; $[\alpha]^{21}_D$ −48.5° (*c* 1.59, $CHCl_3$); IR (film) ν_{max} 3449, 1730, 1714, 1254, 1156, 1025 cm^{-1}; EIMS *m*/*z* (rel int) 582 (M^+, 28), 564 (12), 482 (18), 244 (24), 221 (18), 174 (21), 161 (17), 83 (100), 55 (40), 43 (26); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.31 (1H, t, J = 1.8 Hz, H-23), 7.26 (1H, br s, H-21), 6.87 (1H, qq, J = 6.4, 1.2 Hz, Tig-3), 6.33 (1H, ddd, J = 1.8, 0.5 Hz, H-22), 5.74 (1H, d, J = 3.0 Hz, H-7), 5.70 (1H, d, J = 6.9 Hz, H-15), 5.10 (1H, t, J = 3.3 Hz, H-3), 4.11 (1H, dd, J = 12.5, 3.0 Hz, H-6), 3.61 (1H, t, J = 3.3 Hz, H-1), 3.52 (1H, d, J = 7.7 Hz, H-28), 3.49 (1H, dd, J = 12.4, 4.1 Hz, H-9), 3.40 (1H, d, J = 9.3 Hz, H-17), 3.31 (1H, d, J = 7.7 Hz, H-28), 2.88 (1H, dd, J = 18.4, 12.4 Hz, H-11), 2.77 (1H, dd, J = 18.4, 4.1 Hz, H-11), 2.58 (1H, d, J = 12.5 Hz, H-5), 2.35 (1H, dt, J = 16.2, 3.3 Hz, H-2), 2.22 (1H, ddd, J = 16.4, 9.3, 7.1 Hz, H-16), 2.12 (1H, dt, J = 16.2, 3.3 Hz, H-2), 2.06 (3H, s, Ac), 2.03 (1H, m, H-16), 1.87 (3H, t, J = 1.2 Hz, Tig-4), 1.81 (3H, dq, J = 6.4, 1.2 Hz, Tig-5), 1.47 (6H, s, H-18, 30), 1.18 (3H, s, H-29); ^{13}C NMR (150 MHz, $CDCl_3$) δ 172.3 (C-12), 169.1 (CH_3CO), 166.2 (Tig-1), 146.9 (C-14), 143.3 (C-23), 139.1 (C-21), 138.8 (C-13), 137.1 (Tig-3), 128.5 (Tig-2), 126.5 (C-20), 110.1 (C-22), 85.5 (C-15), 77.9 (C-28), 74.4 (C-7), 73.2 (C-3), 71.8 (C-6), 71.0 (C-1), 47.0 (C-17), 44.9 (C-8), 42.5 (C-4), 41.0 (C-10), 39.4 (C-5), 37.3 (C-16), 37.0 (C-9), 32.6 (C-11), 30.1 (C-2), 20.8 (Ac), 20.1 (C-30), 18.8 (C-29), 15.9 (C-18), 15.3 (C-19), 14.5 (Tig-5), 12.3 (Tig-4); HREIMS *m*/*z* 582.2833 $[M]^+$ (calcd 582.2829 for $C_{33}H_{42}O_9$).

1-*O*-Deacetylohchinolide A (5): amorphous solid; $[\alpha]^{21}_D$ −30.9° (*c* 1.12, $CHCl_3$); IR (film) ν_{max} 3443, 1735, 1725, 1376, 1271, 1055 cm^{-1}; EIMS *m*/*z* (rel int) 604 (M^+, 4), 482 (6), 244 (18), 221 (3), 173 (7), 105 (100), 77 (25), 43 (34); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.03 (2H, dd, J = 8.5, 1.4 Hz, Bz-3, 7), 7.59 (2H, tt, J = 7.4, 1.4 Hz, Bz-4, 6), 7.45 (1H, t, J = 7.4 Hz, Bz-5), 7.29 (1H, t, J = 1.8 Hz, H-23), 7.23 (1H, t, J = 1.2 Hz, H-21), 6.31 (1H, dd, J = 1.8, 0.7 Hz, H-22), 5.92 (1H, d, J = 3.0 Hz, H-7), 5.74 (1H, J = 7.0 Hz, H-15), 5.09 (1H, t, J = 3.3 Hz, H-3), 4.18 (1H, dd, J = 12.6, 3.0 Hz, H-6), 3.66 (1H, t, J = 3.3 Hz, H-1), 3.64 (1H, dd, J = 12.5, 4.2 Hz, H-9), 3.49 (1H, d, J = 7.8 Hz, H-28), 3.32 (1H, d, J = 9.1 Hz, H-17), 3.21 (1H, d, J = 7.8 Hz, H-28), 2.92 (1H, dd, J = 18.1, 4.2 Hz, H-11), 2.82 (1H, dd, J = 18.1, 12.5 Hz, H-11), 2.73 (1H, d, J = 12.6 Hz, H-5), 2.38 (1H, dt, J = 16.5, 3.3 Hz, H-2), 2.12 (1H, dt, J =

16.5, 3.3 Hz, H-2), 1.95 (3H, s, Ac), 1.93 (1H, ddd, J = 14.8, 9.1, 7.0 Hz, H-16), 1.87 (3H, s, H-18), 1.78 (1H, d, J = 14.8 Hz, H-16), 1.52 (3H, s, H-30), 1.19 (3H, s, H-29), 1.02 (3H, s, H-19); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 172.2 (C-12), 169.1 (CH_3CO), 164.6 (Bz-1), 146.9 (C-14), 143.3 (C-23), 139.1 (C-21), 138.9 (C-13), 133.3 (Bz-5), 130.5 (Bz-2), 129.1 (Bz-3, 7), 128.7 (Bz-4, 6), 126.5 (C-20), 110.0 (C-22), 85.5 (C-15), 78.0 (C-28), 75.1 (C-7), 73.1 (C-3), 71.9 (C-6), 71.1 (C-1), 47.0 (C-17), 45.0 (C-8), 42.6 (C-4), 41.1 (C-10), 39.4 (C-5), 37.2 (C-9), 37.0 (C-16), 32.7 (C-11), 30.2 (C-2), 20.8 (Ac), 20.2 (C-30), 18.7 (C-29), 15.9 (C-18), 15.3 (C-19); HREIMS m/z 604.2667 $[M]^+$ (calcd 604.6972 for $C_{35}H_{40}O_9$).

1-*O*-Detigloyl-1-*O*-benzoylohchinolal (6): amorphous solid; $[\alpha]^{21}_D$ +99.4° (c 1.41, $CHCl_3$); IR (film) ν_{max} 3501, 2927, 1740, 1719, 1650, 1273, 1232, 1028, 712 cm^{-1}; EIMS m/z (rel int) 634 (M^+, 64), 574 (9), 564 (5), 424 (6), 314 (16), 273 (67), 231 (100), 174 (30), 147 (34), 105 (94); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 9.80 (1H, s, H-28), 8.08 (2H, dd, J = 7.3, 1.2 Hz, Bz-3, 7), 7.63 (2H, tt, J = 7.3, 1.2 Hz, Bz-4, 6), 7.50 (1H, t, J = 8.0 Hz, Bz-5), 7.28 (1H, s, H-23), 7.12 (1H, s, H-21), 6.11 (1H, d, J = 0.7 Hz, H-22), 5.51 (1H, t, J = 5.8 Hz, H-15), 5.32 (1H, dd, J = 12.4, 2.7 Hz, H-6), 5.27 (1H, t, J = 2.6 Hz, H-1), 4.06 (1H, d, J = 2.7 Hz, H-7), 3.82 (1H, d, J = 12.4 Hz, H-5), 3.81 (1H, dt, J = 8.8, 2.9 Hz, H-3), 3.61 (1H, d, J = 8.8 Hz, H-17), 2.92 (1H, dd, J = 7.3, 4.8 Hz, H-9), 2.84[19] (3H, CO_2Me), 2.54 (1H, m, H-11), 2.36 (1H, m, H-11), 2.33 (1H, m, H-16), 2.29 (1H, m, H-2), 2.15 (1H, m, H-16), 2.11 (1H, m, H-2), 2.00 (3H, s, Ac), 1.64 (3H, d, J = 1.5 Hz, H-18), 1.43 (3H, s, H-30), 1.13 (3H, s, H-19), 1.04 (3H, s, H-29); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 206.8 (C-28), 172.2 (C-12), 170.3 (CH_3CO), 165.0 (Bz-1), 145.6 (C-14), 142.9 (C-23), 138.6 (C-21), 136.1 (C-13), 133.5 (Bz-5), 129.9 (Bz-2), 129.6 (Bz-3, 7), 128.7 (Bz- 4, 6), 126.8 (C-20), 110.5 (C-22), 87.7 (C-15), 86.0 (C-7), 74.9 (C-3), 73.1 (C-1), 68.9 (C-6), 51.3 (CO_2Me), 49.5 (C-17), 49.0 (C-4), 46.9 (C-8), 42.3 (C-10), 41.1 (C-16), 39.8 (C-9), 35.0 (C-5), 30.6 (C-11), 28.7 (C-2), 20.9 (Ac), 17.0 (C-30), 16.8 (C-19), 13.9 (C-29), 13.2 (C-18); HREIMS m/z 634.2771 (calcd 634.2778 for $C_{36}H_{42}O_{10}$).

1-*O*-Detigloyl-1-*O*-cinnamoylohchinolal (7): amorphous solid; $[\alpha]^{21}_D$ +62.9° (c 1.02, $CHCl_3$); IR (film) ν_{max} 3445, 1730, 1717, 1636, 1435 cm^{-1}; EIMS m/z (rel int) 660 (M^+, 53), 628 (23), 568 (11), 482 (27), 273 (52), 231 (100), 174 (31), 131 (96), 103 (24), 43 (17); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 9.77 (1H, s, H-28), 7.74 (1H, d, J = 15.9 Hz, Cin-3), 7.58 (2H, m, Cin-5, 9), 7.44 (3H, m, Cin-6, 7, 8), 7.19 (1H, s, H-23), 7.18 (1H, s, H-21), 6.51 (1H, d, J = 15.9 Hz, Cin-2), 6.24 (1H, s, H-22), 5.57 (1H, t, J = 6.6 Hz, H-15), 5.28 (1H, dd, J = 12.2, 2.8 Hz, H-6), 5.15 (1H, t, J = 3.0 Hz, H-1), 4.06 (1H, d, J = 2.8 Hz, H-7), 3.78 (1H, dt, J = 9.3, 2.8 Hz, H-3), 3.70 (1H, d, J = 12.2 Hz, H-5), 3.63 (1H, d, J = 8.5 Hz, H-17), 3.18 (3H, s, CO_2Me), 2.91 (1H, dd, J = 8.8, 2.8 Hz, H-9), 2.33 (1H, m, H-11), 2.30 (1H, m, H-11), 2.28 (2H, m, H-2, 16), 2.11 (1H, m, H-16), 2.07 (1H, m, H-2), 1.99 (3H, s, Ac), 1.65 (3H, d, J = 1.4 Hz, H-18), 1.43 (3H, s, H-30), 1.10 (3H, s, H-19), 1.01 (3H, s, H-29); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 206.9 (C-28), 172.4 (C-12), 170.4 (CH_3CO), 165.4 (Cin-1), 146.1 (Cin-3), 145.9 (C-14), 143.0 (C-23), 138.8 (C-21), 135.7 (C-13), 134.1 (Cin-4), 130.7 (Cin-7), 129.0 (Cin-5, 9), 128.3 (Cin-6, 8), 126.8 (C-20), 117.4 (Cin-2), 110.6 (C-22), 87.6 (C-15), 85.9 (C-7), 75.0 (C-3), 72.8 (C-1), 68.9 (C-6), 51.7 (CO_2Me), 49.5 (C-17), 39.6 (C-9), 35.0 (C-5), 30.2 (C-11), 28.5 (C-2), 20.9 (Ac), 17.1 (C-30), 17.0 (C-19), 13.8 (C-29), 13.0 (C-18); HREIMS m/z 660.2927 $[M]^+$ (calcd 660.2934 for $C_{38}H_{44}O_{10}$).

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 Wallac 1420 ARVOsx microplate reader (Perkin-Elmer Life and Analytical Sciences, Inc., Boston, MA). The assay results are summarized in Table 4.

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Supporting Information Available: HMBC correlations for **1** and **6** and NOESY correlations for **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(19) The chemical shift of the C-12 methyl ester in **6** is shifted upfield due to the anisotropy shielding effect of the benzoyl ring at C-1.

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