# Cytotoxic Iridoid Aldehydes from Taiwanese *Viburnum luzonicum*

Yoshiyasu FUKUYAMA,*[,a] Yuka MINOSHIMA,[a] Yoshiko KISHIMOTO,[a] Ih-Sheng CHEN,[b] Hironobu TAKAHASHI,[a] and Tomoyuki ESUMI[a]

[a] *Institute of Pharmacognosy, Faculty of Pharmaceutical Sciences, Tokushima Bunri University; Yamashiro-cho, Tokushima 770–8514, Japan: and* [b] *Department of Pharmacy, Kaohsiung Medical University; Kaohsiung, 807, Taiwan.*
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**Four new iridoid aldehydes bearing (*E*)- or (*Z*)-*p*-coumaroyl group, luzonial A (1), luzonial B (2), luzonidial A (3), and luzonidial B (4), were isolated from a methanol extract of the dried leaves of *Viburnum luzonicum* collected in Kaoshiung, Taiwan and their structures were elucidated by analysis of spectroscopic data. Compounds 1—3 exhibited moderate inhibitory activity against HeLa S3 cancer cells.**

**Key words** *Viburnum luzonicum*; Caprifoliaceae; iridoid; monoterpenoid; cytotoxicity

We have continued to study vibsane-type diterpenes occurring in *Viburnum awabuki*[1] and *V. odoratissimum*[2] from a phytochemical point of view. More than 30 kinds of vibsane-type diterpenes have been found so far, and they can be successfully categorized into three sub-classes.[3,4] As part of our studies on biologically active compounds in *Viburnum* species, we have examined chemical components in the leaves of *V. luzonicum*, an evergreen shrub widely distributed in Taiwan. Since one paper reported that a 70% acetone extract of this plant inhibited KB cell growth,[5] our attention was directed to exploration of the cytotoxic principles, resulting in the isolation of four new iridoid aldehydes **1**—**4** named luzonials A and B, luzonidials A and B. 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 leaves of *V. luzonicum* was fractionated on a silica gel column into fractions 1—17. Fractions 3 and 10 were purified by a combination of silica gel chromatography and preparative HPLC, which yielded the four new iridoids **1**—**4**.

Luzonial A (**1**) had a $[M]^+$ ion peak at *m*/*z* 360.1204 in high-resolution (HR)-EI-MS, corresponding to the molecular formula $C_{19}H_{20}O_7$ and its IR spectrum displayed absorptions due to the presence of hydroxyl (3338 $cm^{-1}$) and carbonyl (1683 $cm^{-1}$) groups, and an aromatic (1587, 1515 $cm^{-1}$) moiety. The UV (313 nm) and NMR data of **1** (Table 1) showed the presence of an (*E*)-*p*-coumaroyl group [$\delta$ 6.80 (2H, d, $J$=8.8 Hz), 7.47 (2H, d, $J$=8.8 Hz), 6.37 (1H, d, $J$=15.9 Hz), 7.64 (1H, d, $J$=15.9 Hz); $\delta$ 168.7], which was supported by the observation of prominent fragment peaks at *m*/*z* 147 and 164 in the EI-MS. The remaining NMR spectral data (Table 1) of **1** indicated the presence of an aldehyde [$\delta$ 9.58 (s); $\delta$ 196.0] and an exo-methylene [$\delta$ 6.24 (s), 6.55 (d, $J$=1.1 Hz); $\delta$ 134.8, 153.4], which formed a conjugated system on the basis of the correlation of the exo-methylene signals with the aldehyde carbonyl in the $^1$H-detetced heteronuclear multi-bond correlation (HMBC), as well as of an acetal moiety [$\delta$ 5.34 (d, $J$=0.8 Hz); $\delta$ 104.6] and an isolated oxymethylene [$\delta$ 3.93 (d, $J$=9.5 Hz), 4.22 (d, $J$=9.5 Hz); $\delta$ 73.5]. The routine analyses of $^1$H–$^1$H shift correlation spectroscopy (COSY) and $^1$H-detected heteronuclear correlation through multiple quantum coherence (HMQC) indicated the presence of a partial structure (bold line), as shown in Fig. 2. This partial structure was found to contain the conjugated *exo*-methylene aldehyde unit at the C-5 position and the *p*-coumaroyl group at the C-7 position according to the HMBC correlations of H-5 and H-7 resonating at $\delta$ 2.99 and 5.22 with the exo-methylene carbons C-4 and C-1 and the ester carbonyl of a *p*-coumaroyl group, respectively. In the HMBC as summarized in Fig. 2, the H-10 oxy-methylene signals showed correlations with the acetal carbon C-1, whereas the H-1 acetal and H-9 signals had cross peaks to the C-8 quaternary oxygen-bearing carbon at $\delta$ 90.7, which further correlated with H-7. These spectral data indicated that C-8 was connected to the *p*-coumaroyl-bearing oxycarbon C-7 and the methine C-9 was involved in the partial unit, and also that C-10 was bonded to C-8 as well as to C-1 through an ether bond, resulting in the formation of an iridoid structure fused with two five-membered rings, as shown in Fig. 1. In addition, stirring a methanol solution of **1** in the presence of Amberlyst 15E gave **1a**, which confirmed the presence of a cyclic acetal ring in **1**. The relative stereochemistry of **1** was elucidated on the basis of the following nuclear Overhauser and exchange spectroscopy (NOESY) correlations as shown in Fig. 2: H-5/H-9, H-5/H-6$\beta$, H-7/H-10$\alpha$, and H-10$\beta$/H-9, which can account for a *p*-coumaroyl group at C-7 and a conjugated aldehyde moiety at C-5 taking a $\beta$- and an $\alpha$-configuration, respectively. However, the configuration on the C-1 acetal carbon was unable to be determined by the NOESY because H-5 observes NOE for H-1$\alpha$ or H-1$\beta$. The most stable conformation for **1** obtained by a MM2 calculation using Marcromodel® indicates that a dihedral angle between H-5 and H-1$\alpha$, and H-5 and H-1$\beta$ is 82° and 39°, respectively. Considering the small $J$ value (0.8 Hz) observed for H-1, the OH located at C-1 should take a $\beta$ configuration. Accordingly, luzonial A (**1**) was represented as **1**.

Luzonial B (**2**) was assigned the same molecular formula $C_{19}H_{20}O_7$ as **1**, obtained from HR-EI-MS at *m*/*z* 360 $[M]^+$, and exhibited physical and NMR data (Table 1) very similar to those of compound **1** except for the NMR data assignable to a *p*-coumaroyl group. Namely, a small $J$ value (12.6 Hz) was observed for two vicinal olefinic protons, indicating the presence of a (*Z*)-*p*-coumaroyl group. It was also evident from the HMBC correlation of the H-7 signal at $\delta$ 5.19 with the ester carbonyl at $\delta_C$ 167.8 that this *p*-coumaroyl group was located at the C-7 position. Other NMR data including HMBC and NOESY were consistent with those of **1**. Thus the structure of luzonial B (**2**) was determined to be 7-*O*-(*Z*)-

* To whom correspondence should be addressed. e-mail: fukuyama@ph.bunri-u.ac.jp

Table 1. $^{13}$C-NMR (150 MHz) and $^{1}$H-NMR (600 MHz) Spectral Data of Compounds **1** and **2** in $CD_3OD$

| Position | **1** | | **2** | |
|---|---|---|---|---|
| | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ |
| 1 | 104.6 | 5.34 d (0.8) | 104.7 | 5.27 d (0.8) |
| 3 | 196.0 | 9.58 s | 196.0 | 9.57 s |
| 4 | 153.4 | | 153.5 | |
| 5 | 39.5 | 2.99 dddd (7.5, 7.1, 6.9, 1.1) | 39.3 | 2.89 dddd (7.1, 7.1, 6.6, 1.1) |
| 6 | 36.6 | 1.99 ddd (13.3, 7.1, 5.9) 2.18 ddd (13.3, 7.5, 5.8) | 35.9 | 1.92 ddd (13.5, 7.1, 6.4) 2.15 ddd (13.5, 7.1, 6.1) |
| 7 | 82.0 | 5.22 dd (5.9, 5.8) | 81.7 | 5.19 dd (6.4, 6.1) |
| 8 | 90.7 | | 90.3 | |
| 9 | 64.4 | 2.31 dd (6.9, 0.8) | 64.0 | 2.27 dd (6.6, 0.8) |
| 10 | 73.5 | 3.93 d (9.5) 4.22 d (9.5) | 73.6 | 3.84 d (9.8) 4.07 d (9.8) |
| 11 | 134.8 | 6.24 s 6.55 d (1.1) | 134.7 | 6.23 s 6.54 d (1.1) |
| Coumaroyl | | | | |
| 1′ | 168.7 | | 167.8 | |
| 2′ | 114.9 | 6.37 d (15.9) | 116.5 | 5.81 d (12.6) |
| 3′ | 147.1 | 7.64 d (15.9) | 145.8 | 6.91 d (12.6) |
| 4′ | 127.1 | | 127.7 | |
| 5′, 9′ | 131.3 | 7.47 d (8.8) | 133.6 | 7.61 d (8.4) |
| 6′, 8′ | 116.9 | 6.80 d (8.8) | 115.9 | 6.75 d (8.4) |
| 7′ | 161.5 | | 160.2 | |

All assignments were made by extensive analyses of 1D and 2D NMR (COSY, DEPT, HMQC, and HMBC).

Table 2. Cytotoxic Activities of Compounds **1**—**3** against HeLa S3[a)]

| Compound | $IC_{50}$ ($\mu$M) |
|---|---|
| **1** | 3.50 |
| **2** | 1.93 |
| **3** | 24.5 |
| **4** | ND[b)] |
| Fluorouracil | 5.40 |
| Cisplatin | 2.46 |

*a*) Human epithelial cancer cell line. *b*) Not done.

*p*-coumaroyl derivative of **1**.

Luzonidial A (**3**) had a molecular formula $C_{19}H_{18}O_6$, determined by HR-EI-MS at *m/z* 342 $[M]^+$ and its IR spectrum displayed absorptions due to a hydroxyl group at 3368 $cm^{-1}$, a conjugated carbonyl group at 1680 $cm^{-1}$, and an aromatic moiety at 1587 and 1515 $cm^{-1}$. The NMR spectral data of **3** showed the presence of two aldehyde groups [$\delta$ 9.85 (s, H-3), 193.5 (C-3); $\delta$ 10.1 (s, H-1), 188.3 (C-1)], an exo-methylene [$\delta$ 6.03 (s, H-11), 6.15 (d, $J$=0.8 Hz, H-11); $\delta$ 134.0 (C-11), 150.3 (C-4)], an isolated oxymethylene [$\delta$ 4.38 (d, $J$=15.1 Hz) and 4.85 (d, $J$=15.1 Hz); $\delta$ 56.3 (C-10)], and a *p*-coumaroyl group with an *E*-geometry ($J$=15.9 Hz). In addition to these structural fragments, one partial structure, $-C_{(5)}H-C_{(6)}H_2-C_{(7)}H-O-$, was obtained from analysis of $^{1}H-^{1}H$ COSY and HMQC. The H-5 signal at $\delta$ 4.26 showed not only a long-range coupling (0.8 Hz) to one of the H-11 exo-methylene protons but also HMBC correlations with C-3, C-4, C-11, and two quaternary $sp^2$ carbons C-8 ($\delta$ 158.2) and C-9 ($\delta$ 141.5), indicating that C-5 connects to both C-9

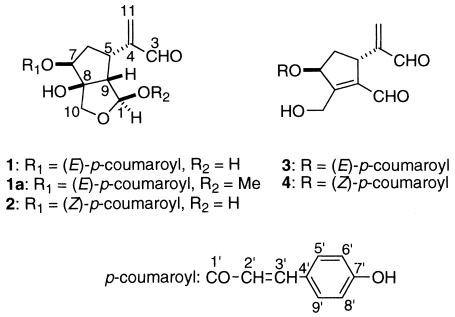


Fig. 1. New Iridoids Isolated from *V. luzonicum*

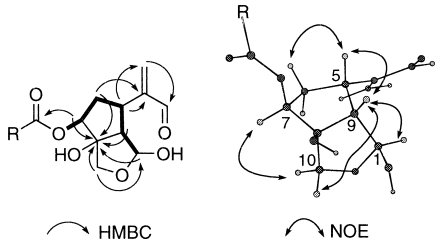


Fig. 2. Representative HMBC and NOESY for **1**

and the exo-methylene C-4 bearing an aldehyde group. According to the additional HMBC, the H-7 signal correlated with the C-8 and C-9 resonances, which further had cross-peaks with the H-10 oxy-methylene and the remaining aldehyde H-1 signal, indicating that both C-7 and C-10 link to C-8 and the other aldehyde function bonds to C-9. The *p*-coumaroyl group was confirmed to be located at the C-7 position on the basis of a HMBC correlation between the H-7 signal resonated at $\delta$ 6.20 and its ester carbonyl at $\delta$ 167.8. Thus, **3** is comprised of a basic iridoid skeleton as shown in Fig. 1. A trans relationship between H-5 and H-7 was elucidated on the basis of the following NOESY: H-5/H-6$\beta$ and H-6$\alpha$/H-7. The structure of luzonidial A was therefore represented as **3**.

Luzonidial B (**4**) was found to have the same molecular formula $C_{19}H_{18}O_6$ as that of **3**, determined by HR-EI-MS at *m/z* 342 $[M]^+$. The spectral data of **4** were similar to those of **3** except for a big difference in the chemical shifts and $J$ values corresponding to the coumaroyl H-1′ and H-2′. A small $J_{1,2}$ value (12.6 Hz) indicated the double bond geometry for this *p*-coumaroyl to be *Z*. The NOESY experiments indicated that the relative stereochemistry of **4** was the same as that of **3**. Thus the structure of **4** was assigned as a *Z* variant of a *p*-coumaroyl group located at the C-7 position in luzonidial A.

All the new iridoids isolated in this study are structurally analogous to those found in many other species of *Viburnum*.[6)] They are characterized by the presence of (*E*)- or (*Z*)-*p*-coumaroyl group at position 7. Although these iridioids bearing (*E*)- or (*Z*)-*p*-coumaroyl group are readily isolated in a pure form, they gradually interconvert into a mixture of *E* and *Z* at room temperature. In a cytotoxicity assay with the HeLa S3 (human epithelial cancer) cell line, iridoid glucosides (**1**) and (**2**) exhibited moderate inhibitory activity at $IC_{50}$ values of 1.9—3.5 $\mu$M as summarized in Table 2.

**Experimental**

**General Procedure** Optical rotations were measured on a JASCO DIP-1000 digital polarimeter. IR spectra were measured on a JASCO FT-IR 5300

infrared spectrophotometer, and 1D- and 2D-NMR spectra were recorded on a Varian Unity 600 instrument. Chemical shifts are given as $\delta$ (ppm) with TMS as an internal standard. MS were recorded on a JEOL AX-500 instrument. Column chromatography was carried out on Kieselgel 60 (70—230 mesh) or Wako gel C-300.

**Plant Material** The leaves of *Viburnum lozonicum* were collected in Kaoshiung, Taiwan in May 2002. Prof. Ih-Sheng Chen identified the plant and a voucher specimen (1714LF) has been deposited at the Institute of Pharmacognosy, TBU.

**Extraction and Isolation** The dried leaves of *V. luzonicum* (7.0 kg) were extracted with MeOH to yield 800 g of the MeOH extract. The extract (150 g) was chromatographed on a silica gel (Kieselgel 60) column eluting with a step gradient of $CH_2Cl_2$ (100%), $CH_2Cl_2$–EtOAc (9 : 1), $CH_2Cl_2$–EtOAc (3 : 2), $CH_2Cl_2$–EtOAc (2 : 3), EtOAc (100%), EtOAc–MeOH (9 : 1), and EtOAc–MeOH (4 : 1) to give seventeen fractions (fr. 1—17).

Fraction 10 (7.7 g) was first subjected to silica gel (Wako C-300) chromatography eluting with $CHCl_3$–EtOAc (1 : 1) to give fractions 18—27. Fraction 25 (195 mg) was separated by silica gel chromatography with $CHCl_3$–MeOH–$H_2O$ (8 : 2 : 0.2), followed by reversed-phase HPLC [Cosmosil 5C18-AR-II, $\phi$ 10×250 mm; $H_2O$–MeOH (11 : 9), 2.0 ml/min] to give compounds **1** (6.7 mg) and **2** (3.1 mg). MeOH soluble portion (1.6 g) of fraction 3 (3.7 g) was separated by column chromatography over silica gel eluting with $CHCl_3$–EtOAc (1 : 1) to nine fractions. The eighth fraction (71 mg) was purified by HPLC (Cosmosil 5C18-AR-II, $\phi$ 10×250 mm) using MeOH–$H_2O$ (11 : 9, 2.5 ml/min) to give compounds **3** (5.4 mg) and **4** (2.2 mg).

Luzonial A (**1**): Yellow oil; $[\alpha]_D^{21}$ −7.1° ($c$=1.04, MeOH); IR $\nu_{max}$ (film) $cm^{-1}$: 3338 (OH), 1683 (conj. C=O), 1587, 1515 (aroma.); UV $\lambda_{max}$ (EtOH) nm: 313 ($\varepsilon$ 11100), 297 ($\varepsilon$ 9000), 210 (9300); EI-MS *m/z* (rel. int.): 360 $[M]^+$ (2), 164 (100), 147 (89); HR-EI-MS *m/z*: 360.1204 ($M^+$ Calcd for $C_{19}H_{20}O_7$: 360.1209); $^1$H- and $^{13}$C-NMR, see Table 1.

Luzonial B (**2**): Yellow oil; $[\alpha]_D^{21}$ −1.9° ($c$=1.17, MeOH). IR $\nu_{max}$ (film) $cm^{-1}$: 3364 (OH), 1688 (C=O), 1586, 1514 (aroma.); UV $\lambda_{max}$ (EtOH) nm: 315 ($\varepsilon$ 10300), 300 ($\varepsilon$ 8600), 225 ($\varepsilon$ 9200), 212 ($\varepsilon$ 10900); EI-MS *m/z* (rel. int.): 360 $[M]^+$ (1), 164 (100), 147 (92); HR-EI-MS *m/z*: 360.1207 ($M^+$ Calcd for $C_{19}H_{20}O_7$: 360.1209); $^1$H- and $^{13}$C-NMR, see Table 1.

Luzonidial A (**3**): Yellow oil; $[\alpha]_D^{21}$ −183.4° ($c$=0.94, $CHCl_3$); IR $\nu_{max}$ (film) $cm^{-1}$: 3368 (OH), 1680 (conj. C=O), 1587, 1515 (aroma.); UV $\lambda_{max}$ (EtOH) nm: 316 ($\varepsilon$ 16000), 228 ($\varepsilon$ 14300); $^1$H-NMR (600 MHz, $CD_3OD$) $\delta$: 2.18 (1H, ddd, $J$=14.1, 7.7, 3.8 Hz, H-6), 2.36 (1H, ddd, $J$=14.1, 9.1, 5.1 Hz, H-6), 4.26 (1H, ddd, $J$=9.1, 3.8, 0.8 Hz, H-5), 4.38 (1H, d, $J$=15.1 Hz, H-10), 4.85 (1H, d, $J$=15.1 Hz, H-10), 6.03 (1H, s, H-11), 6.15 (1H, d, $J$=0.8 Hz, H-11), 6.20 (1H, dd, $J$=7.7, 5.1 Hz, H-7), 6.30 (1H, d, $J$=15.9 Hz, H-2′), 6.86 (2H, d, $J$=8.5 Hz, H-6′, 8′), 7.47 (2H, $J$=8.5 Hz, H-5′, 9′), 7.69 (1H, $J$=15.9 Hz, H-3′), 9.85 (1H, s, H-3), 10.1 (1H, s, H-1); $^{13}$C-NMR (150 MHz, $CD_3OD$) $\delta$: 36.6 (C-6), 40.9 (C-2), 56.3 (C-10), 78.6 (C-7), 114.1 (C-2′), 116.0 (C-6′, 8′), 126.8 (C-4′), 130.3 (C-5′, 9′), 134.0 (C-11), 141.5 (C-9), 146.3 (C-3′), 150.3 ( C-4), 158.1 (C-7′), 158.2 (C-8), 167.8 (C-1′), 188.3 (C-1), 193.5 (C-3); EI-MS *m/z* (rel. int): 342 $[M]^+$, 147 (100); HR-EI-MS *m/z*: 342.1118 ($M^+$ Calcd for $C_{19}H_{18}O_6$: 342.1103).

Luzonidial B (**4**): Yellow oil; $[\alpha]_D^{21}$ −32.4° ($c$=0.54, $CHCl_3$); IR (film) $\nu_{max}$ (film) $cm^{-1}$: 3400 (OH), 1682 (conj. C=O), 1604, 1514; $^1$H-NMR (600 MHz, $CD_3OD$) $\delta$: 2.14 (1H, ddd, $J$=14.1, 7.6, 4.3 Hz, H-6), 2.24 (1H, ddd, $J$=14.1, 9.1, 4.9 Hz, H-6), 4.17 (1H, ddd, $J$=9.1, 4.3, 0.8 Hz, H-5), 4.36 (1H, d, $J$=15.1 Hz, H-10), 4.78 (1H, d, $J$=15.1 Hz, H-10), 5.84 (1H, d, $J$=12.6 Hz, H-2′), 6.03 (1H, s, H-11), 6.09 (1H, dd, $J$=7.6, 4.9 Hz, H-7), 6.13 (1H, d, $J$=0.8 Hz, H-11), 6.83 (2H, d, $J$=8.5 Hz, H-6′, 8′), 6.96 (1H, d, $J$=12.6 Hz, H-3′), 7.62 (2H, d, $J$=8.5 Hz, H-5′, 9′), 9.55 (1H, s, H-3), 10.1 (1H, s, H-1): $^{13}$C-NMR (150 MHz, $CD_3OD$) $\delta$: 36.7 (C-6), 41.0 (C-5), 56.6 (C-10), 78.9 (C-7), 115.1 (C-6′, 8′), 116.0 (C-2′), 127.2 (C-4′), 131.1 (C-11), 132.5 (C-5′, 9′), 141.5 (C-9, C-1′), 145.7 (C-3′), 150.2 (C-4), 157.0 (C-7′), 158.0 (C-8), 188.4 (C-1), 193.5 (C-3); EI-MS *m/z* (rel. int.): 342 $[M]^+$, 147 (100); HR-EI-MS *m/z*: 342.1118 ($M^+$ Calcd for $C_{19}H_{18}O_6$: 342.1103).

**Methylation of 1** A mixture of **1** (2 mg) and Amberlyst 15E (2 pieces) in MeOH (1 ml) was strirred for 12 h at room temperature. After the catalyst was filtered, the filtrate was condensed *in vacuo* to give **1a** (2.1 mg) as yellow oil. $[\alpha]_D^{21}$ −23.3° ($c$=0.62, MeOH); IR $\nu_{max}$ (film) $cm^{-1}$: 3350 (OH), 1689 (C=O), 1586, 1515 (aroma.); UV $\lambda_{max}$ nm ($\varepsilon$): 315 (14800), 298 (11800), 215 (14400); $^1$H-NMR (600 MHz, $CDCl_3$) $\delta$: 2.12 (1H, ddd, $J$=13.5, 7.1, 4.4 Hz, H-6), 2.16 (1H, ddd, $J$=13.5, 9.1, 8.7 Hz, H-6), 2.50 (1H, d, $J$=4.7 Hz, H-9), 3.06 (1H, dddd, $J$=8.7, 4.7, 4.4, 1.1 Hz, H-5), 3.37 (3H, s, OMe), 3.70 (1H, s, 8-OH), 3.84 (1H, d, $J$=9.9 Hz, H-10), 4.22 (1H, d, $J$=9.9 Hz, H-10), 4.92 (1H, s, H-1), 5.20 (1H, dd, $J$=9.1, 7.1 Hz, H-7), 6.17 (1H, s, H-11), 6.33 (1H, d, $J$=15.9 Hz, H-2′), 6.59 (1H, d, $J$=1.1 Hz, H-11), 6.85 (2H, d, $J$=8.5 Hz, H-6′, 8′), 7.45 (2H, $J$=8.5 Hz, H-5′, 9′), 7.66 (1H, $J$=15.9 Hz, H-3′), 9.58 (1H, s, H-3); $^{13}$C-NMR (150 MHz, $CDCl_3$) $\delta$: 34.7 (C-6), 36.7 (C-5), 54.6 ($OCH_3$), 60.9 (C-9), 73.4 (C-10), 82.1 (C-7), 88.7 (C-8), 110.4 (C-1), 114.4 (C-2′), 115.9 (C-6′, 8′), 127.0 (C-4′), 130.3 (C-5′, 9′), 134.2 (C-11), 146.0 (C-3′), 151.8 (C-4), 157.9 (C-7′), 168.2 (C-1′), 194.0 (C-3); EI-MS *m/z* (rel. int.): 374 $[M]^+$ (4), 343 (2), 147 (100); HR-EI-MS *m/z*: 374.1357 ($M^+$ Calcd for $C_{20}H_{22}O_7$: 374.1366).

**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 $\mu$M, 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 further incubated for 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.

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