Absolute Structures of Wedelolide Derivatives and Structure–Activity Relationships of Protein Tyrosine Phosphatase 1B Inhibitory ent-Kaurene Diterpenes from Aerial Parts of Wedelia spp. Collected in Indonesia and Japan

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Two sesquiterpene lactones with the (9R)-eudesman-9,12-olide framework, wedelolides I and J, have been isolated together with five eudesmanolide sesquiterpenes and twelve ent-kaurene diterpenes from the aerial parts of Indonesian Wedelia prostrata. The absolute configurations of wedelolides I and J, proposed in the previous communication, were proven by comparing their experimental Electronic Circular Dichroism (ECD) spectra with the calculated ECD spectrum of wedelolide I. The phytochemical study on the aerial parts of Okinawan Wedelia chinensis led to the isolation of three other eudesmanolide sesquiterpenes in addition to the three sesquiterpenes and eleven diterpenes isolated from the Indonesian W. prostrata as above. However, the wedelolide derivatives found in the Indonesian plant were not detected. Among these compounds, most of the diterpenes inhibited protein tyrosine phosphatase (PTP) 1B activity, and a structure–activity relationship study revealed that the cinnamoyl group enhanced inhibitory activity. Therefore, two ent-kaurene derivatives with and without a cinnamoyl group were examined for the ability to accumulate phosphorylated-Akt (p-Akt) because PTP1B dephosphorylates signal transduction from the insulin receptor such as phosphorylated Akt, a key downstream effector. However, neither compound enhanced insulin-stimulated p-Akt levels in two human hepatoma cell lines (Huh-7 and HepG2) at non-cytotoxic doses.

Key words Wedelia prostrata; Wedelia chinensis; family Asteraceae; eudesmanolide sesquiterpene; ent-kaurene diterpene; protein tyrosine phosphatase 1B

The family Asteraceae (Compositae) is a large taxonomic group, and the genus Wedelia is composed of approximately 60 species that are widely distributed in Japan (mainly in Okinawa), China, and Southeast Asia including Indonesia, India, Burma, and Vietnam. Some species in this genus are used as traditional herbal medicines: Wedelia prostrata has been applied to the treatment of inflammatory diseases, while Wedelia trilobata is used in the prevention and/or treatment of fever and malaria in Vietnam. Chemical studies on this genus have identified more than 120 chemical components, such as sesquiterpenes, diterpenes, triterpenes, flavonoids, and caffeic acid derivatives.

Protein tyrosine phosphatase (PTP) 1B is a key negative regulator in the insulin and leptin signal pathways, and is attracting interest as a drug target for type 2 diabetes and obesity. Although a number of PTP1B inhibitors have been reported from various natural and synthetic origins, a clinical application has not yet been accomplished. Therefore, we have been investigating new classes of PTP1B inhibitors from terrestrial and marine organisms collected in tropical and subtropical regions. 10,11 We reported in the previous communication the isolation of seven sesquiterpene lactones (1–7) including two eudesmanolides, wedelolides I and J (1, 2), and PTP1B inhibitory ent-kaurene diterpenes (8, 9) from the aerial parts of Indonesian W. prostrata 12,13 (Fig. 1). Further bioassay-guided separation afforded ten more ent-kaurene diterpene derivatives (10–19) (Fig. 1) from the remaining fractions of the Indonesian W. prostrata. Therefore, a structure–activity relationship study of these diterpenes on PTP1B inhibitory activity was conducted. Additionally, wedelolide H (20) and two more eudesmanolide sesquiterpenes (21, 22) were identified along with sesquiterpenes 5–7 and diterpenes 8–18 from the aerial parts of Wedelia chinensis collected at Iriomote Island, Okinawa, Japan (Fig. 1). The wedelolides possess a rare (9R)-eudesman-9,12-olide skeleton, and only nine congeners, wedelolides A–H 14–16 and prostrolide A, 17 have been reported from W. trilobata and W. prostrata collected in Vietnam and China. Therefore, wedelolides I and J (1, 2) were the tenth and eleventh examples in this natural product family. The absolute configurations of 1 and 2 tentatively proposed in the previous communication 12,13 were defined by comparing their experimental Electronic Circular Dichroism (ECD) spectra with the calculated ECD spectrum of 1.
We herein report the elucidation of the absolute configurations of 1 and 2 by ECD calculations, a phytochemical study on Okinawan _W. chinensis_, and the biological properties of ent-kaurene diterpenes.

**Results and Discussion**

**Isolation of Compounds 1–19 from Indonesian _Wedelia prostrata_** The EtOH extract from the aerial parts of _W._ _prostrata_ collected at Manado, Indonesia, have been found to inhibit PTP1B activity (approximately 50% inhibition at 50 µg/mL), and bioassay-guided separation of the extract led to the isolation of 8 (4.5 mg) and 9 (25 mg) as active components along with inactive sesquiterpenes 1 (0.9 mg), 2 (1.2 mg), 3 (1.1 mg), 4 (3.3 mg), 5 (2.6 mg), 6 (3.3 mg), and 7 (2.3 mg).12,13) In this study, the remaining fractions were further separated with an octadecyl silane (ODS) column followed by preparative HPLC (ODS) to give compounds 10 (3.5 mg), 11 (4.7 mg), 12 (10 mg), 13 (3.2 mg), 14 (4.6 mg), 15 (12 mg), 16 (8.8 mg), 17 (3.3 mg), 18 (14 mg), and 19 (1.3 mg).

The structures of compounds 10–19 were identified as _ent-3β_-angeloyloxykaur-16-en-19-oic acid,18) _ent-3β_-tigloyloxykaur-16-en-19-oic acid,18) _3α_-cinnamoyloxypterokaurene L1,18) pterokaurene L5,19) _3α_-angeloyloxypterokaurene L1,18) _ent-kaur-9(11),16_-dien-19-oic acid,20) _ent-17_-hydroxykaur-15-en-19-oic acid,21) tetrachryrin,22) and 15-hydroxykaur-9(11),16-dien-19-oic acid,22) respectively, by comparing their spectroscopic data with reported values (Fig. 1).

**Absolute Structures of Wedelolides I and J (1 and 2)** The planar structures of wedelolides I and J (1, 2) have been elucidated from their one dimensional (1D) and 2D-NMR, high resolution-electron ionization (HR-EI)-MS, UV, and IR data as described previously.12,13) The absolute configurations of known wedelolides were elucidated using an X-ray crystallographic analysis and modified Mosher’s method.14,15)

In order to confirm the absolute configurations of 1 and 2, the ECD spectrum of the (1S,4S,5S,6R,7S,8S,9R,10S)-isomer of 1 was calculated with the energy-minimized structures based on nuclear Overhauser effect (NOE) data. The conformational search of (1S,4S,5S,6R,7S,8S,9R,10S)-1 resulted in 8 low-energy conformers in 0.60 kcal/mol as shown in Fig. 2, and the Boltzmann-averaged ECD spectrum of these conformers (dashed line) matched well with the experimental ECD spectrum of 1 (solid line) (Fig. 3). Thus, the absolute configuration of 1 was concluded as shown in Fig. 1.

The absolute configuration of 2 was also decided as 1S, 4S, 5S, 6R, 7S, 8S, 9R, and 10S because compound 2 showed a similar cotton curve to that of 1, and both compounds may be biosynthesized through the same pathway.

**Phytochemistry on Okinawan _Wedelia chinensis_** As a part of the phytochemical study, the aerial parts of _W. chinensis_ were collected at Iriomote Island (Okinawa, Japan) in 2016. The EtOH extract of this plant also inhibited PTP1B activity (approximately 48% at 50 µg/mL) and was purified using an ODS column followed by preparative HPLC to give three eudesmanolide sesquiterpenes 20 (2.8 mg), 21 (5.4 mg), and 22 (5.9 mg) together with the common sesquiterpenes 5–7 and diterpenes 8–18. Compounds 20–22 were identified by
comparing their spectroscopic data with the reported values for wedelolide H,\(^{(16)}\) trilobolide-6-O-isobutyrate,\(^{(18)}\) and trilobolide-6-O-methacrylate,\(^{(18)}\) respectively (Fig. 1). \(^{(9)}\)R\)-Eudesman-9,12-olide-type sesquiterpenes \(^{1–4}\) possessing an acetoxy group at the C-1 position were not obtained from this plant.

**Biological Activity** The PTP1B inhibitory activities of compounds \(^1–22\) and oleanolic acid\(^{24)}\) as a positive control were evaluated, and their IC\(_{50}\) values are listed in Table 1. Among the eudesmanolide sesquiterpenes tested (\(^1–4, 5–7,\) and \(^20–22\)), only wedelolide D (\(^4\)) modestly inhibited PTP1B activity by 32\% at 20 \(\mu\)M (Table 1). Among diterpenes \(^8–11\), diterpene \(^8\) exhibited the most potent PTP1B inhibitory activity with an IC\(_{50}\) value of 8.3 \(\mu\)M. Therefore, the cinnamoyl group at the C-3 position in \(^8\) is more favorable for inhibitory activity than the other functional groups. Although 9-hydroxy derivatives \(^{13–15}\) exhibited markedly weaker activity than the 9-H derivatives \(^{9–11}\), compounds \(^{12}\) (9-OH) and \(^8\) (9-H) possessing a 3-cinnamoyl moiety exhibited similar inhibitory activity (Table 1). Accordingly, the cinnamoyl groups in \(^8\) and \(^12\) enhance the PTP1B inhibitory activity of \(\text{ent-kaurene diterpenes}\). Based on the structure–activity relationships among the tested compounds, compound \(^8\) had the optimal structure for inhibiting PTP1B activity.

Prior to cell-based investigations on \(^8\), cell viability was assessed against four human cancer cell lines: Huh-7 (hepatoma), EJ-1 (bladder), A549 (lung adenocarcinoma), and MCF-7 (breast adenocarcinoma). Compound \(^9\), the decinnamoyl

Table 1. PTP1B Inhibitory Activities of Compounds \(^1–22\)

| Compound | IC\(_{50}\) (\(\mu\)M) |
|----------|-----------------|
| 1        | No inhibition at 21 \(\mu\)M\(^{(2,13)}\) |
| 2        | No inhibition at 21 \(\mu\)M\(^{(2,13)}\) |
| 3        | No inhibition at 21 \(\mu\)M\(^{(2,13)}\) |
| 4        | 32\% inhibition at 20 \(\mu\)M\(^{(2,13)}\) |
| 5        | No inhibition at 24 \(\mu\)M\(^{(2,13)}\) |
| 6        | No inhibition at 25 \(\mu\)M\(^{(2,13)}\) |
| 7        | No inhibition at 20 \(\mu\)M\(^{(2,13)}\) |
| 8        | 8.3\(^{(12,13)}\) |
| 9        | 2\(^{g^{(12,13)}}\) |
| 10       | 12 |
| 11       | 12 |
| 12       | 7.7 |
| 13       | No inhibition at 31 \(\mu\)M |
| 14       | 18\% inhibition at 24 \(\mu\)M |
| 15       | No inhibition at 24 \(\mu\)M |
| 16       | 29\% inhibition at 33 \(\mu\)M |
| 17       | 40\% inhibition at 33 \(\mu\)M |
| 18       | 22 |
| 19       | 13 |
| 20       | No inhibition at 23 \(\mu\)M |
| 21       | No inhibition at 26 \(\mu\)M |
| 22       | No inhibition at 26 \(\mu\)M |
| Oleanolic acid\(^{20}\) | 1.1 |

\(^a\) Positive control.\(^{26}\)
derivative of 8, was simultaneously tested as a control. Each
cancer cell line was treated with 8 or 9 at 50 μM for 48 h, and
cell proliferation was measured by the WST-1 assay.25,26 Compounds 8 and 9 did not affect the cell proliferation of these
cell lines.

PTP1B mainly dephosphorylates signal transduction from
the insulin receptor in the liver,27 and, thus, the phosphoryla-
tion levels of Akt, a key downstream effector in the insulin
pathway, were detected by Western blotting using Huh-7
cells.28 Compounds 8 and 9 did not enhance insulin-stimu-
lated phosphorylated-Akt (p-Akt) levels in Huh-7 cells up
to 50 μM, whereas sodium orthovanadate (SOV), a pan-PTP
inhibitor, increased p-Akt levels at 5 μM.

Experimental

General Experimental Procedures EI-MS and FAB-MS were
measured on a JMS-MS 700 mass spectrometer (JEOL, Tokyo,
Japan). 1H- and 13C-NMR spectra were recorded on a
JNM-AL-400 NMR spectrometer (JEOL) at 400 MHz for
1H and 100 MHz for 13C in CDCl3 (δH 7.24, δC 77.0). Specific
rotations were obtained with the digital polarimeter P-2300
(JASCO, Tokyo, Japan). UV spectra were measured on the
UV-visible (Vis) spectrophotometer U-3310 (Hitachi High
Technologies Co., Ltd., Tokyo, Japan). ECD spectra were
measured with a JASCO J-720 spectropolarimeter. IR spectra
were recorded on the Fourier transform infrared spectrom-
eter FT-710 (Horiba Ltd., Kyoto, Japan). Preparative HPLC
was performed using an L-6200 HPLC system (Hitachi High
Technologies Co., Ltd.).

Materials PTP1B was purchased from Enzo Life Sciences
(Farmingdale, NY, U.S.A.), p-Nitrophenyl phosphate (pNPP)
was purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.).
Oleandric acid was purchased from Tokyo Chemical Industry
(Tokyo, Japan). Plastic plates (96-well) were purchased from
Corning Inc. (Corning, NY, U.S.A.). All other chemicals
including organic solvents were purchased from Wako Pure
Chemical Industries, Ltd. (Osaka, Japan).

Isolation of Compounds 1–22 The aerial parts of Wede-
liella prostrata (family Asteraceae) were collected at Manado,
North Sulawesi (Indonesia) at GPS coordinates (N1°15'.8.82",
E124°51'.23.43") in February 20162,13 and of W. chinensis
(family Asteraceae) at Irımote Islands, Okinawa (Japan) at
GPS coordinates (N24°26'.99.17", E123°84'.51.64") in Septem-
ber 2016. Voucher specimens have been deposited at the Fac-
ulty of Pharmaceutical Sciences, Tohoku Medical and Phar-
maceutical University as TMH-1 (Indonesia) and 16-9-8=P-4
(Japan).

The Indonesian plant (1.0 kg, wet weight) was cut into small
pieces, extracted with EtOH (1.0 L×3) at room temperature,
and filtered. The filtrate was evaporated to make the EtOH ex-
tract (47.0 g), which was chromatographed on an ODS column
(100 g) with a stepwise gradient of CH3OH in H2O (0, 30, 50,
70, 85, 100%) and then with 100% CH3OH containing 0.05%
trifluoroacetic acid (TFA) to give seven fractions (frs. 1–7).

Fraction 4 (275 mg, 70% CH3OH eluate) was further separated into four subfractions (frs. 4-1–4-4) by prepara-
tive HPLC (column, PEGASIL ODS (Senshu Sci. Co., Ltd.,
Tokyo, Japan), i.d. 10×250 mm; solvent, 62% CH3OH in H2O;
flow rate, 2.0 mL/min; detection, UV 210 nm). Compounds 1
(0.9 mg), 2 (1.2 mg), 3 (1.1 mg), and 4 (3.3 mg) were obtained by
HPLC separation (column, PEGASIL ODS, i.d. 10×250 mm;
solvent, 58% CH3OH in H2O; flow rate, 2.0 mL/min; detection,
UV 210 nm) from fr. 4-1 (115 mg). Fraction 4-1 (115 mg) was
subjected to HPLC (column, PEGASIL ODS, i.d. 10×250 mm;
solvent, 50% CH3OH in H2O; flow rate, 2.0 mL/min; detection,
UV 210 nm) to yield compounds 5 (2.6 mg) and 6 (3.3 mg). The
HPLC purification (column, PEGASIL ODS, i.d. 10×250 mm;
solvent, 57% CH3OH in H2O; flow rate, 2.0 mL/min; detection,
UV 210 nm) of fr. 4-2 (108 mg) resulted in 2.3 mg of com-
 pound 7 (2.3 mg). Fraction 6 (2800 mg, 100% CH3OH eluate)
was subjected to repeated HPLC (column, PEGASIL ODS, i.d.
10×250 mm; solvent, 86% CH3OH in H2O; flow rate, 2.0 mL/min; detection, UV 210 nm) to isolate compounds 16
(8.8 mg), 11 (4.7 mg), 10 (3.5 mg), 9 (25 mg), and 8 (4.5 mg). Fraction 5 (641 mg, 85% CH3OH eluate) was divided into four
subfractions (frs. 5-1–5-4) by preparative HPLC (column,
PEGASIL ODS, i.d. 10×250 mm; solvent, 80% CH3OH in
H2O; flow rate, 2.0 mL/min; detection, UV 210 nm). Compounds 13 (3.2 mg), 17 (3.3 mg), 15 (12 mg), 14 (4.6 mg), and
12 (10 mg) were isolated from fr. 5-1 (205 mg) by HPLC sep-
aration (column, PEGASIL ODS, i.d. 10×250 mm; solvent, 71%
CH3OH in H2O, 2.0 mL/min, UV 210 nm). Fraction 5-2 (80 mg)
was separated by preparative HPLC (column, PEGASIL ODS,
i.d. 10×250 mm; solvent, 75% CH3OH in H2O, 2.0 mL/min,
UV 210 nm) to give 19 (1.3 mg) and 18 (1.4 mg).

The Okinawan plant (187 g, wet weight) was extracted with
EtOH (1.0 L×3) using a similar procedure to that described
above. The extract (4.2 g) was separated into seven fractions
(frs. 1–7) using an ODS column (100 g) by stepwise elution
with CH3OH in H2O (0, 30, 50, 70, 85, 100%) and then with
100% CH3OH containing 0.05% TFA.

The isolation of compounds 18 (1.6 mg), 16 (42 mg), 11
(9.9 mg), 10 (4.3 mg), 9 (35 mg), and 8 (4.6 mg) was achieved by
preparative HPLC (column, PEGASIL ODS SP100 (Senshu
Sci. Co., Ltd.), i.d. 10×250 mm; solvent, 89% CH3OH in
H2O; flow rate, 2.0 mL/min; detection, UV 210 nm) from fr.
6 (732 mg, 100% CH3OH eluate). Fraction 5 (255 mg, 85%
CH3OH eluate) was separated by repeated HPLC (column,
PEGASIL ODS SP100, i.d. 10×250 mm; solvent, 75% CH3OH
in H2O; flow rate, 2.0 mL/min; detection, UV 210 nm) to
isolate compounds 13 (6.1 mg), 17 (3.0 mg), 15 (9.1 mg),
14 (4.7 mg), and 12 (6.2 mg). Compounds 22 (5.9 mg), 6 (3.2 mg),
5 (3.4 mg), 21 (5.4 mg), 20 (2.8 mg), and 7 (2.1 mg) were purified
by HPLC separation (column, PEGASIL ODS SP-100, i.d.
10×250 mm; solvent, 58% CH3OH in H2O; flow rate, 2.0 mL/min;
detection, UV 210 nm) from fr. 4 (140 mg, 70% CH3OH eluate).

Wedelolide 1 (1) Colorless solids; [{α}](c = 0.05, CH3OH);
UV (CH3OH) λmax nm (log ε) 203 (4.3), 211 (4.3) nm; ECD
(2.1×10−4 M, CH3CN) λmax (Δε) 216 (−7.2) nm; IR (KBr) νmax
3432, 2947, 1723, 1636, 1455, 1385, 1295, 1244, 1157, 1038, 812 cm−1,
1H- and 13C-NMR in CDCl3, see ref. 12; EI-MS m/z 476 [M]+;
HR-ESI-MS m/z 476.2055 [M]+. (Calcd for C30H32O8, 476.2046).
Wedelolide J (2)

Colorless solids; $[\alpha]_{D}^{25}$ −12.1 (c=0.05, CH$_2$OH); UV (CH$_2$OH) $\lambda_{	ext{max}}$ nm (log $\varepsilon$) 201 (4.0), 211 (3.9) nm; ECD (2.1×10$^{-3}$m, CH$_3$CN) $\lambda_{	ext{max}}$ (Δε) 209 (−2.0) nm; IR (KBr) 3402, 2946, 1737, 1471, 1387, 1245, 1203, 1155, 1033, 805 cm$^{-1}$; 1H and 13C-NMR in CDCl$_3$, see ref. 12; EI-MS m/z 480 [M]$^+$; HR-MS m/z 480.2363 [M]$^+$ (Caled for C$_{25}$H$_{29}$O$_8$ 480.2359).

Calculation of ECD Spectrum A computational analysis of (1S,4S,5S,6R,7S,8S,9R,10S)-1 in the gas phase was performed using the MMFF94 force field. The conformers were further optimized with the density functional theory (DFT) method with the B3LYP functional and 6-31G(d) basis set. Single-point calculations of solvation Gibbs energies in CH$_3$CN were then performed for gas-phase optimized geometries by the SM8 continuum model using the same DFT method as above. These calculations were performed using Spartan’14 (Wavefunction, Inc., Irvine, CA, U.S.A.).

The ECD spectrum of (1S,4S,5S,6R,7S,8S,9R,10S)-1 was calculated using Gaussian 09 (Gaussian, Inc., Wallingford, CT, U.S.A.) by the time-dependent DFT (TDDFT) method with the CAM-B3LYP functional and 6-311G(d) basis set. The calculation was performed using the eight lowest-energy conformers within 0.60 kcal/mol predicted in CH$_3$CN; the energies of the other conformers were higher than the most stable one by more than 1.26 kcal/mol. The eight conformers differed in their relative orientation and/or conformations about the central C–C bonds of the two methacryloyloxy groups. The solvent effect was introduced by the polarizable continuum model (PCM). Twenty-five low-lying excited states were calculated corresponding to the wavelength region to approximately 167 nm. The simulated spectrum for each state was calculated using Gaussian 09 (Gaussian, Inc., Wallingford, CT, U.S.A.) by the SM8 continuum model using the same DFT method as above. These calculations were performed using Spartan’14 (Wavefunction, Inc., Irvine, CA, U.S.A.).

**WST-1 Assay** Cytotoxicity was assessed using the water-soluble tetrazolium (WST-1; sodium 5-(2,4-disulphophenyl)-3-(4-nitrophenyl)-2H-tetrazolium inner salt) assay, which detects metabolically competent cells with an intact mitochondrial electron transport chain. Briefly, 1×10$^4$ cells were seeded on each well of 96-well plastic plates and cultured overnight. Cells were treated with each test compound and incubated for 48 h, and medium containing WST-1 solution (0.5 mM WST-1 and 0.02 mM 1-methoxy-5-methyl-phenazinium methysulfate; 1-PM) was then added to each well. Cells were incubated at 37°C for 60 min, and absorption at 438 nm (reference 620 nm) was measured using an SH-1200 Microplate Reader (Corona Electric). Control cells were treated with 0.1% EtOH. Cell viability was calculated using the formula: absorbance in the treated sample/absorbance in the control×100 (%).

**Western Blotting** Huh-7 or HepG2 cells were grown in a 35-mm cell culture dish and preincubated with a sample dissolved in dimethyl sulfoxide (DMSO) for 1 h. In cell-based assays, the final concentration of DMSO was adjusted to less than 0.1%. Cells were stimulated for 5 min with 3 mIU insulin, washed with phosphate-buffered saline (PBS), and lysed in Celllytic M$^+$ (Sigma-Aldrich) in order to collect whole cell lysates, according to the manufacturer’s instructions. Protein concentrations were measured using a BCA$^+$ protein assay kit (Thermo Fisher Scientific Inc., Waltham, MA, U.S.A.) in accordance with the manufacturer’s instructions. Samples of each protein (10 µg of whole cell lysates) were loaded onto a 10% sodium dodecyl sulfate (SDS)-polyacrylamide gel. After electrophoresis, proteins were transferred to a polyvinylidene difluoride (PVDF) membrane. Proteins were blocked with Blocking One$^+$ (Nacalai Tesque Inc., Kyoto, Japan) for 1 h and reacted with an antibody at 4°C overnight. The membrane was then washed with a solution (PBS containing 0.05% Tween-20) and incubated with a horseradish peroxidase-linked secondary antibody for 1 h. All antibodies used for Western blotting were purchased from Cell Signaling Technology. After washing, protein levels were analyzed by enhanced chemiluminescence with Pierce$^+$ Western blotting substrate (Thermo Fisher Scientific Inc.). The immunoreactivities of p-Akt, total-Akt (t-Akt), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were visualized and measured by densitometry using the “LAS-4000” digital imaging system and “ImageQuant TL” software from GE Healthcare Life Sciences (Little Chalfont, U.K.). The amounts of the p-Akt and t-Akt proteins were expressed as a ratio of those in the control group.

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**Conflict of Interest** The authors declare no conflict of interest.
References

1) Li X., Dong M., Liu Y., Shi Q. W., Kiyota H., Chem. Biodivers., 4, 823–836 (2007).
2) Zhang X. G., Yan W. Q., Lin Y. T., Liu X. J., Liu X. Y., Zhang Y. H., J. Chin. Med. Mat., 34, 383–386 (2011).
3) Li C. L., Liu F. X., Wu H. Z., Zheng Y. K. Fujian, J. Tradit. Chin. Med., 10, 448–449 (1959).
4) Jossang A., El Bitar H., Pham V. C., Sévenet T., J. Org. Chem., 68, 300–304 (2003).
5) Zhang S., Zhang Z. Y., Drug Discov. Today, 12, 373–381 (2007).
6) Barr A. J., Future Med. Chem., 2, 1563–1576 (2010).
7) Zhang Z. Y., Dodd G. T., Tiganis T., Trends Pharmacol. Sci., 36, 661–674 (2015).
8) Popov D., Biochem. Biophys. Res. Commun., 410, 377–381 (2011).
9) Jiang C. S., Liang L. F., Guo Y. W., Acta Pharmacol. Sin., 10, 448–449 (1959).
10) Abdjul D. B., Yamazaki H., Takahashi O., Kirikoshi R., Mangindaan R. E. P., Namikoshi M., Bioorg. Med. Chem. Lett., 25, 904–907 (2015).
11) Yamazaki H., Ukai K., Namikoshi M., Tetrahedron Lett., 57, 732–735 (2016).
12) Abdjul D. B., Yamazaki H., Maarisit W., Losung F., Rotinsulu H., Wewengkang D. S., Sumilat D. A., Namikoshi M., Phytochem. Lett., 20, 191–195 (2017).
13) Abdjul D. B., Yamazaki H., Maarisit W., Losung F., Rotinsulu H., Wewengkang D. S., Sumilat D. A., Namikoshi M., Phytochem. Lett., 24, 131 (2018).
14) That Q. T., Jossang J., Jossang A., Kim P. P. N., Jaureguiberry G., J. Org. Chem., 72, 7102–7105 (2007).
15) Li Y., Hao X., Li S., He H., Yan X., Chen Y., Dong J., Zhang Z., Li S., J. Agric. Food Chem., 61, 3884–3890 (2013).
16) Duc T. P., Thien T. V. N., Jossang A., Kim P. P. N., Grellier P., Jaureguiberry G., That Q. T., Phytochem. Lett., 17, 304–309 (2016).
17) Wu Z. N., Zhang Y. B., Chen N. H., Yang L., Jiang L., Jiang S. Q., Li G. Q., Li Y. L., Wang G. C., Chem. Lett., 45, 1150–1152 (2016).
18) Bohlmann F., Ziesche J., King R. M., Robinson H., Phytochemistry, 20, 751–756 (1981).
19) Murakami T., Iida H., Tanaka N., Saiki Y., Chen C. M., Iitaka Y., Chem. Pharm. Bull., 29, 657–662 (1981).
20) Ma B. J., Wen C. N., Gao Y., Ren F.-C., Wang F., Liu J. K., Nat. Prod. Bioprospect., 3, 107–111 (2013).
21) Bohlmann F., Le Van N., Phytochemistry, 16, 579–581 (1977).
22) Bohlmann F., Suding H., Cuatrecasas J., King R. M., Robinson H., Phytochemistry, 19, 267–271 (1980).
23) Ohno N., Mabry T. J., Zabelt V., Watson W. H., Phytochemistry, 18, 1687–1689 (1979).
24) Zhang Y. N., Zhang W., Hong D., Shi L., Shen Q., Li J. Y., Li J., Hu L. H., Bioorg. Med. Chem., 16, 8697–8705 (2008).
25) Berridge M. V., Herst P. M., Tan A. S., Biotechnol. Annu. Rev., 11, 127–152 (2005).
26) Yamazaki H., Kanno S., Abdjul D. B., Namikoshi M., Bioorg. Med. Chem. Lett., 21, 2307–2309 (2011).
27) Cui L., Na M. K., Oh H., Bae E. Y., Jeong D. G., Ryu S. E., Kim S., Kim B. Y., Oh W. K., Ann J. S., Bioorg. Med. Chem. Lett., 16, 1426–1429 (2006).
28) Yamazaki H., Sumilat D. A., Kanno S., Ukai K., Rotinsulu H., Wewengkang D. S., Ishikawa M., Mangindaan R. E. P., Namikoshi M., J. Nat. Med., 67, 730–735 (2013).