Triterpene Glycosides from the Seeds of Dolichos lablab

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Two new triterpene glycosides (1 and 2), together with nine known triterpene glycosides (3–11), were isolated from the seeds of Dolichos lablab (Leguminosae). The structures of the new compounds were determined by spectroscopic analysis, including two-dimensional NMR spectroscopy, and chromatographic analysis of the hydrolyzed products. The isolated compounds did not show cytotoxicity against HL–60 human leukemia cells and HepG2 human hepatoma cells at sample concentrations of 20 µM.

Key words triterpene glycoside; Dolichos lablab; Leguminosae; cytotoxic activity

Introduction
Dolichi Semen is prepared from the seeds of Dolichos lablab L., which belongs to the family Leguminosae (Fabaceae) and is widely distributed in the tropical and subtropical countries of the world with the majority found in South China.1) This crude drug is listed in the 17th edition of the Japanese Pharmacopoeia and has been used for treatment of indigestion, nausea, and diarrhea in Asian countries.2) Several oleanane-type triterpene saponins, named lablabosides A–E,3,4) flavonol glycosides,5) and a brassinosteroid6) have been isolated from D. lablab seeds. As part of our ongoing investigations of traditional medicines, a phytochemical investigation was carried out on the seeds of D. lablab. Two new triterpene glycosides (1 and 2) and nine known triterpene glycosides (3–11) were isolated. In this paper, we describe the structural elucidation of the two new triterpene glycosides (1 and 2) based on spectroscopic analysis, including two-dimensional NMR spectroscopy, and chromatographic analysis of the hydrolyzed products. The isolated compounds (1–11) were evaluated for cytotoxicity against HL–60 human leukemia cells and HepG2 human hepatoma cells.

Results and Discussion
The MeOH extract of the seeds of D. lablab (5.0 kg) was repeatedly subjected to column chromatography, yielding compounds 1–11 (Fig. 1).

Compound 1 was obtained as an amorphous solid and had a molecular formula of C_{42}H_{66}O_{15}, as determined from the high resolution (HR)-electrospray ionization (ESI)-time of flight (TOF)-MS (m/z: 833.4263 [M + Na]^{+}) and ^{13}C-NMR data. The IR spectrum showed the absorptions for the hydroxy (3417 cm\(^{-1}\)) and carbonyl (1725 cm\(^{-1}\)) functionalities. The H-NMR spectrum of 1 contained signals for six methyl groups derived from the triterpene skeleton at δ\(_{H}\) 1.22, 1.18, 0.99, 0.95, 0.86, and 0.72 (each s), an olefinic proton at δ\(_{H}\) 5.30 (brs), an aldehyde proton at δ\(_{H}\) 9.55 (brs), and two anomic protons at δ\(_{H}\) 4.70 (d, J = 7.7 Hz) and 4.47 (d, J = 7.3 Hz). The ^{13}C-NMR spectrum contained two anomic carbon signals of the sugar moiety at δ\(_{C}\) 104.9 and 104.8, and 30 carbon signals of the aglycone moiety including signals for six methyl carbons at δ\(_{C}\) 33.5, 26.2, 24.9, 23.0, 17.6, and 16.1, six quaternary carbons at δ\(_{C}\) 55.9, 44.6, 43.1, 40.9, 37.5, and 32.3, two oxygen-bearing methine carbons at δ\(_{C}\) 91.8 and 69.5, an oxygen-bearing methylene carbon at δ\(_{C}\) 64.4, a set of olefinic carbons at δ\(_{C}\) 143.4 and 124.7, and an aldehyde carbon at δ\(_{C}\) 210.0. Acid hydrolysis of 1 with 1 M HCl in dioxane–H\(_2\)O (1:1) yielded D-galactose and D-glucuronic acid as the carbohydrate moieties, while the aglycone moiety decomposed under acidic conditions. Identification of these sugars, including their absolute configurations, was carried out by direct HPLC analysis of the hydrolysate, which was performed on an amino-propyl bonded silica gel column for D-galactose and on a sulfonated polystyrene ion-exclusion column for D-glucuronic acid. These data suggest that 1 is a triterpene diglycoside. On comparison of the H- and ^{13}C-NMR spectral data for the aglycone moiety of 1, which were assigned by analysis of the H–H shift correlation spectroscopy (COSY), the H-detected heteronuclear single-quantum coherence (HSQC), and the H-detected heteronuclear multiple-bond connectivity (HMBC), with those of lablaboside B (8), the signals due to the ring A–D portions (C-1–C-16 and C-23–C-27) were observed at almost the same positions between the two compounds, indicating the presence of the C-3β oxygen atom bearing the sugar residue, C-12/C-13 double bond, and C-24 hydroxy group. The 4β (45) configuration was ascertained by nuclear Overhauser effect (NOE) correlations between H-3 and Me-23/H-5, and between H-24a and Me-25 in the phase-sensitive NOE spectroscopy spectrum of 1. The H- and ^{13}C-NMR data for the ring E portion of 1 were different from those of 8, and suggested the presence of a hydroxy group (δ\(_{H}\) 3.79/δ\(_{C}\) 69.5) and an aldehyde group (δ\(_{H}\) 9.55/δ\(_{C}\) 210.0). In the HMBC spectrum, the aldehyde proton (δ\(_{H}\) 9.55) showed long-range correlations with the methylene carbon at δ\(_{C}\) 16.3 (C-16), quaternary carbon at δ\(_{C}\) 55.9 (C-17), and methine carbon at δ\(_{C}\) 42.3 (C-18). The hydroxy methine proton (δ\(_{H}\) 3.79) exhibited spin-coupling correlations with methylene protons at δ\(_{H}\) 1.41 and 1.39 (H-21), and HMBC correlations with the quaternary carbon at δ\(_{C}\) 55.9 (C-17) and aldehyde carbon (δ\(_{C}\) 210.0). These correlations allowed an aldehyde group and a hydroxy group to be placed at C-17 and C-22, respectively (Fig. 2). The spin-coupling constants of H-22 (\(^3J_{H-22,H-21axial} = 11.5\) Hz, \(^3J_{H-22,H-21equatorial} = 5.9\) Hz), and NOE correlations between H-18 and H-22/Me-30, H-21α and Me-29, and between H-21β and Me-30 were consistent with the 22α (22S) configuration (Fig. 3). Combined analysis of

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The 1H–1H COSY and HSQC spectra indicated that the diglycoside moiety of 1 was composed of a C-2-substituted β-D-glucuronopyranosyl (4C1) unit (GlcUA) \([\delta_H 4.47 \text{ (d, } J = 7.3 \text{ Hz); } \delta_C 104.9, 80.9, 78.5, 73.5, 76.8, \text{ and } 176.0]\) and a terminal β-D-galactopyranosyl (4C1) unit (Gal) \([\delta_H 4.70 \text{ (d, } J = 7.7 \text{ Hz); } \delta_C 104.8, 73.4, 75.2, 71.2, 76.9, \text{ and } 62.7]\). The anomeric configurations of the GlcUA and Gal residues were determined as β by the large 3J_{H-1, H-2} values of their anomeric protons (7.3 and 7.7 Hz, respectively). In the HMBC spectrum of 1, long-range correlations were observed between the anomeric proton (H-1) of Gal at \(\delta_H 4.70\) and C-2 of GlcUA at \(\delta_C 80.9\), and between H-1 of GlcUA at \(\delta_H 4.47\) and C-3 of the aglycone at \(\delta_C 91.8\). Accordingly, the structure of 1 was established as 3β-[(2- O-β-D-galactopyranosyl-β-D-glucuronopyranosyl)oxy]-22α,24-dihydroxyolean-12-en-28-αl.

Compound 2 was found to have a molecular formula of C_{48}H_{76}O_{19} by HR-ESI-TOF-MS analysis (m/z 979.4849 [M + Na]^+). The 1H- and 13C-NMR spectra of 2 revealed that the aglycone of 2 was the same as that of 1. The deduced molecular formula of 2 was higher than that of 1 by C6H10O5, corresponding to one hexose unit, and the 1H-NMR spectrum of 2 contained signals for three anomeric protons at \(\delta_H 6.32\) (d, \(J = 1.6 \text{ Hz}\)), 4.87 (d, \(J = 7.6 \text{ Hz}\)), and 4.44 (d, \(J = 7.8 \text{ Hz}\)), and the methyl group of a 6-deoxyhexose at \(\delta_H 1.58\) (d, \(J = 6.4 \text{ Hz}\)). Acid hydrolysis of 2 yielded D-galactose, D-glucuronic acid, and L-rhamnose. On comparison of the 1H- and 13C-NMR spectra of 2 with those of 1, a set of six additional signals corresponding to a terminal α-L-rhamnopyranosyl unit (Rha) \([\delta_H 6.32 \text{ (1H, d, } J = 1.6 \text{ Hz); } \delta_C 102.3, 72.3, 72.2, 74.3, 69.6, \text{ and } 18.6]\) appeared, and the signal due to C-2 of the galactosyl moiety shifted downfield by 4.7ppm to be observed at \(\delta_C 78.1\). The anomeric proton of Rha was equatorial, and thus possessed an α-pyranoid anomeric form (C4) due to the large 4J_{C-1, H-1} value (174.2Hz). In the HMBC spectrum...
of 2, long-range correlations were observed between the anomeric proton (H-1) of Rha at δH 6.32 and C-2 of Gal at δC 78.1; H-1 of Gal at δH 4.87 and C-2 of GlcUA at δC 77.3, and between H-1 of GlcUA at δH 4.44 and C-3 of the aglycone at δC 92.3. Thus, the structure of 2 was assigned as 3β-[2-O-α-L-rhamnopyranosyl-(1→2)-O-β-D-galactopyranosyl-(1→β-D-glucopyranosyl)oxoyl]-22α,24-dihydroxyolean-12-en-28-al.

Compounds 3–11 were identified by their physical and spectroscopic data as chikusetsusaponin IVA (3),

gypsogenin 3-O-β-D-glucuronosyl-28-β- D-glucopyranoside (4),

quinoasaponin 9 (5),

udosaponin B (6),

lablaboside A (7),

lablaboside B (8),

lablaboside C (9),

lablaboside F (10),

and lablaboside E (11), respectively. This is the first report concerning the isolation of 4–6 from D. lablab.

The isolated compounds (1–11) were evaluated for their cytotoxic activity against HL-60 and HepG2 cells. They did not exhibit cytotoxicity even at sample concentrations of 20 µM (<50% growth inhibition), whereas cisplatin gave IC50 values with reference to tetramethylsilane (TMS) δH 0.00 and δC 49.4.

In summary, 11 triterpene glycosides (1–11), including two new compounds (1 and 2), were isolated from the seeds of D. lablab, and their cytotoxicity against HL-60 cells and HepG2 cells was evaluated. The isolated compounds did not show cytotoxicity against HL-60 cells and HepG2 cells. Further biological investigation of the isolated compounds is currently under way.

Experimental

Optical rotations were measured by using a JASCO P-1030 (Tokyo, Japan) automatic digital polarimeter. IR spectra were recorded on a JASCO FT-IR 620 spectrophotometer. NMR spectral data were obtained on a DRX-600 (600 MHz for 1H-NMR, Bruker, Karlsruhe, Germany) and DRX-500 spectrometer (500 MHz for 1H-NMR, Bruker) using standard Bruker pulse programs at 300 K. Chemical shifts were presented as δ values with reference to tetramethylsilane (TMS) as an internal standard. ESI-TOF-MS data were recorded on an LCT mass spectrometer (Waters-Micromass, Manchester, U.K.). Diaion HP-20 (Mitsubishi-Chemical, Tokyo, Japan), silica gel (Fuji-Silysia Chemical, Aichi, Japan), and octade- cysilanized (ODS) silica gel (Nacalai Tesque, Kyoto, Japan) were used for column chromatography (CC). TLC was performed on precoated silica gel 60 F254 (0.25 mm thick, Merck, Darmstadt, Germany) and RP18 F254S plates (0.25 mm thick, Merck), and spots were visualized by spraying the plates with 10% H2SO4 aqueous solution and heating. HPLC was performed with a system consisting of a CCPM pump (Tosoh, Darmstadt, Germany) and RP 18 F 254S plates (0.25 mm thick, Merck), and spots were visualized by spraying the plates with 10% H2SO4 aqueous solution and heating. HPLC was performed with a system consisting of a CCPM pump (Tosoh, Tokyo, Japan), a Shodex OR-2 (Showa-Denko, Tokyo, Japan) detector, and a Rheodyne injection port (Rohnernt Park, CA, U.S.A.). HL-60 cells (JCRB0085) and HepG2 cells (JCRB 1054) were obtained from the Japanese Collection of Research Bioresources (JCRB) cell bank (Osaka, Japan). The following materials and reagents were used for the cell culture assays: a Spectra Classic microplate reader (Tecan, Salzburg, Austria), 96-well flat-bottom plates (Iwaki Glass, Chiba, Japan), fetal bovine serum (FBS) (Nichirei Biosciences, Tokyo, Japan), 0.25% trypsin–ethylene-diaminetetraacetic acid (EDTA) solution, RPMI 1640 medium, Dulbecco’s modified Eagle’s medium (DMEM), cisplatin, and 3,4,5,6-tetrahydroxy-2H-pyrimidinone (MTT) (Sigma-Aldrich, St. Louis, MO, U.S.A.), penicillin G sodium salt and streptomycin sulfate (Gibco, Grand Island, NY, U.S.A.), and phosphate buffered saline (PBS) (Wako Pure Chemical Industries, Ltd., Osaka, Japan). All other chemicals used were of biochemical reagent grade.

Plant Material Dolichi Semen (dried seeds of Dolichos lablab) were purchased from Tochimoto Tenkaido Co., Ltd., (Osaka, Japan) in April 2012. A voucher specimen has been deposited in our laboratory (voucher no. DL-2012-001, Department of Medicinal Pharmacognosy).

Extraction and Isolation Dolichi Semen (5.0 kg) was extracted with hot MeOH (20L). The MeOH extract was concentrated under reduced pressure, the viscous concentrate (195 g) was subjected to a Diaion HP-20 column, and successively eluted with 30% MeOH, 50% MeOH, MeOH, EtOH, and EtOAc (each 6L). The 50% MeOH eluted fraction (6.0 g) was separated by silica gel CC eluted with a stepwise gradient mixture of CHCl3–MeOH–H2O (90:10:1) to 10:10:1, and finally with MeOH alone, to produce 10 fractions (A–J). Fraction F was purified by ODS silica gel CC eluted with MeOH–H2O (2:3 and 3:2) and MeCN–H2O (1:3 and 1:2), and silica gel CC eluted with EtOAc–MeOH–H2O (40:10:1 and 20:10:1) to give 1 (5.2 mg), 3 (15.3 mg), and 4 (5.9 mg). Fraction G was purified by ODS silica gel CC eluted with MeCN–H2O (2:7, 2:5, and 2:3) and MeOH–H2O (2:3 and 2:2), and silica gel CC eluted with EtOAc–MeOH–H2O (70:40:1 to 4 (7.0 mg) and 5 (7.7 mg). Fraction H was purified by ODS silica gel CC eluted with MeCN–H2O (2:7, 2:5, and 1:2) to give 2 (9.7 mg) and 6 (3.8 mg). Fraction I was purified by ODS silica gel CC eluted with MeCN–H2O (2:7 and 1:3) and MeOH–H2O (1:1 to 1) to give 7 (9.0 mg), 8 (4.7 mg), 9 (5.0 mg), and 11 (9.0 mg). Fraction J was separated by an ODS silica gel column eluted with MeOH–H2O (1:1) to yield subfractions 1–9. Fraction J-5 was purified by ODS silica gel CC eluted with MeCN–H2O (2:7) and silica gel CC eluted with EtOAc–MeOH–H2O (10:10:1) to give 11 (9.8 mg). Fraction J-6 was purified by silica gel CC eluted with EtOAc–MeOH–H2O (20:10:1, 30:20:1, 10:20:1, and 10:20:1), and ODS silica gel CC eluted with MeOH–H2O (1:1) to give 10 (4.8 mg).

Compound 1

Amorphous solid; [α]D25 +38.2 (c = 0.10, MeOH); IR ν(νmax) (film) cm⁻¹: 3417 (OH), 2924 (CH), 1725 (C=O); 1H (600 MHz, CD3OD) and 13C-NMR (150 MHz, CD3OD) spectra, see Table 1; HR-ESI-TOF-MS m/z: 833.4263 [M + Na]⁺ (Calc. for C42H69NaO19: 833.4299).

Compound 2

Amorphous solid; [α]D25 -5.3 (c = 0.10, MeOH); IR ν(νmax) (film) cm⁻¹: 3390 (OH), 2928 (CH), 1716 (C=O); 1H (600 MHz, CD3OD) and 13C-NMR (150 MHz, CD3OD) spectra, see Table 1; HR-ESI-TOF-MS m/z: 797.4849 [M + Na]⁺ (Calc. for C38H55NaO17: 797.4879).

Acid Hydrolysis of 1 and 2 Compounds 1 (2.5 mg) and 2 (7.9 mg) were independently dissolved in 1 M HCl (dioxane–H2O, 1:1, 2.0 mL) and were heated at 95°C for 1 h under an Ar atmosphere. After dilution of the reaction mixture with H2O (5 mL), it was extracted with EtOAc (10 mL × 3). The H2O residue was neutralized by the addition of Ag2CO3. The mixture was filtered and then passed through a Sep-Pak C18 cartridge (Waters, Milford, MA, U.S.A.) eluted with H2O (5 mL × 3) to give sugar fractions (1.0 mg from 1, and 3.3 mg from 2). The sugar fractions were each analyzed by HPLC.
Table 1. $^1$H- and $^{13}$C-NMR Chemical Shift Assignments for 1 and 2 ($\delta$, ppm: $J$, Hz)$^a$

| Position | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ |
|----------|-----------|-----------|-----------|-----------|
| 1 eq     | 1.60 m    | 39.6      | 1.61 m    | 39.6      |
| ax       | 1.02 m    |           | 1.03 m    |           |
| 2 eq     | 2.12 m    | 27.0      | 2.11 m    | 27.0      |
| ax       | 1.80 m    |           | 1.80 m    |           |
| 3        | 3.41 br d (9.6) | 91.8 | 3.40 br d (9.5) | 92.3 |
| 4        |          | 44.6      |           | 44.7      |
| 5        | 0.93 m    | 57.3      | 0.92 m    | 57.2      |
| 6 eq     | 1.61 m    | 19.4      | 1.61 m    | 19.3      |
| ax       | 1.34 m    |           | 1.33 m    |           |
| 7 eq     | 1.34 m    | 34.1      | 1.33 m    | 34.1      |
| ax       | 1.52 m    |           | 1.53 m    |           |
| 8        |           | 40.9      |           | 40.9      |
| 9        | 1.56 dd (10.3, 6.9) | 48.9 | 1.56 dd (10.2, 7.1) | 48.8 |
| 10       | 37.5      |           | 37.5      |           |
| 11 (2H)  | 1.89 m    | 24.7      | 1.88 m    | 24.7      |
| 12       | 5.30 brs  | 124.7     | 5.30 brs  | 124.7     |
| 13       | 143.4     |           | 143.4     |           |
| 14       | 43.1      |           | 43.1      |           |
| 15 eq    | 1.78 m    | 27.0      | 1.78 m    | 27.0      |
| ax       | 1.14 m    |           | 1.14 m    |           |
| 16 eq    | 2.04 br d (9.1) | 16.3 | 2.04 br d (9.1) | 16.1 |
| ax       | 1.77 m    |           | 1.77 m    |           |
| 17       | 55.9      |           | 55.9      |           |
| 18       | 2.64 dd (13.6, 4.6) | 42.3 | 2.64 dd (13.6, 3.9) | 42.3 |
| 19 eq    | 1.14 dd (13.6, 4.6) | 46.1 | 1.14 dd (13.6, 3.9) | 46.1 |
| ax       | 1.77 dd (13.6, 13.6) | 1.78 dd (13.6, 13.6) | |
| 20       | 32.3      |           | 32.3      |           |
| 21 eq    | 1.41 m    | 43.1      | 1.40 dd (12.4, 5.9) | 43.1 |
| ax       | 1.39 m    |           | 1.39 dd (12.4, 11.2) | |
| 22       | 3.79 dd (11.5, 5.9) | 69.5 | 3.79 dd (11.2, 5.9) | 69.5 |
| 23       | 1.22 s    | 23.0      | 1.25 s    | 23.4      |
| 24 a     | 4.11 d (11.5) | 64.4 | 4.13 d (11.8) | 64.3 |
| b        | 3.21 d (11.5) |           | 3.18 d (11.8) | |
| 25       | 0.86 s    | 16.1      | 0.85 s    | 16.1      |
| 26       | 0.72 s    | 17.6      | 0.71 s    | 17.6      |
| 27       | 1.18 s    | 26.2      | 1.18 s    | 26.2      |
| 28       | 9.55 s    | 210.0     | 9.56 s    | 210.1     |
| 29       | 0.95 s    | 33.5      | 0.95 s    | 33.5      |
| 30       | 0.99 s    | 24.9      | 0.99 s    | 24.9      |
| GlcUA 1' | 4.47 d (7.3) | 104.9 | 4.44 d (7.8) | 105.5 |
| 2'       | 3.61 dd (9.5, 7.3) | 80.9 | 3.75 dd (9.3, 7.8) | 77.3 |
| 3'       | 3.62 dd (9.5, 9.5) | 78.5 | 3.60 dd (9.3, 9.3) | 78.4 |
| 4'       | 3.47 dd (9.5, 9.5) | 73.5 | 3.42 dd (9.3, 9.3) | 74.1 |
| 5'       | 3.55 d (9.5) | 76.8 | 3.53 d (9.3) | 76.9 |
| 6'       | 176.0     |           | 176.0     |           |
| Gal 1''  | 4.70 d (7.7) | 104.8 | 4.87 d (7.6) | 102.1 |
| 2''      | 3.54 dd (9.7, 7.7) | 73.4 | 3.64 dd (9.6, 7.6) | 78.1 |
| 3''      | 3.43 dd (9.7, 3.4) | 75.2 | 3.53 dd (9.6, 3.3) | 76.3 |
| 4''      | 3.76 br d (3.4) | 71.2 | 3.71 br d (3.3) | 71.6 |
| 5''      | 3.50 m    | 76.9      | 3.48 m    | 76.3      |
| 6'' a    | 3.79 dd (11.8, 1.7) | 62.7 | 3.75 dd (11.7, 1.5) | 62.2 |
| b        | 3.68 dd (11.8, 4.7) |           | 3.69 dd (11.7, 5.4) | |
| Rha 1''  | 6.32 d (1.6) | 102.3 | 6.46 d (9.5, 9.5) | 74.3 |
| 2''      | 4.93 dd (3.3, 1.6) | 72.3 | 4.66 m    | 69.6      |
| 3''      | 4.75 dd (9.5, 3.3) | 72.2 | 4.66 m    | 69.6      |
| 4''      | 4.46 dd (9.5, 9.5) | 74.3 | 4.66 m    | 69.6      |
| 5''      | 4.66 m    | 69.6      | 4.66 m    | 69.6      |
| 6''      | 1.58 d (6.4) | 18.6 | 1.58 d (6.4) | 18.6      |

$^a$ Spectra were measured in CD$_3$OD.
under the following conditions: 1) column, Aminex HPX-87H (7.8 mm i.d. × 300 mm, 5 µm, Bio-Rad Laboratories, CA, U.S.A.); solvent, 5 mM H₂SO₄ in H₂O; flow rate, 0.6 mL/min; detection, t₀ (min) and optical rotation (OR). α-Glucuronic acid was detected in the sugar fractions obtained from 1 and 2 (t₀: 8.23, positive optical rotation). 2) column, Capcell Pak NH₂ UG80 (4.6 mm i.d. × 250 mm, 5 µm, Shiseido, Tokyo, Japan); solvent, MeCN–H₂O (17 : 3); flow rate, 1.0 mL/min; detection, t₀ (min) and OR. α-Galactose was detected in the sugar fractions obtained from 1 and 2 (t₀: 10.58, positive optical rotation), and L-rhamnose in that from 2 (t₀: 6.58, negative optical rotation).

Cell Culture and Assay for Cytotoxic Activity against HL-60 and HepG2 Cells HL-60 cells (JCRB 0085) and HepG2 cells (JCRB 1054) were obtained from the Japanese Collection of Research Bioresources (Osaka, Japan). The cells were cultured in RPMI 1640 medium (HL-60 cells) or DMEM (HepG2 cells) containing heat-inactivated 10% (v/v) FBS supplemented with L-glutamine, 100 unit/mL penicillin G sodium salt, and 100 µg/mL streptomycin sulfate in a humidified incubator at 37°C with an atmosphere of 5% CO₂. The cells (HL-60: 4 × 10⁴ cells/mL, HepG2: 4 × 10⁵ cells/mL) were treated with each compound for 72 h continuously, followed by the measurement of cell viability by using an MTT assay. ¹⁴

Conflict of Interest The authors declare no conflict of interest.

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