A triterpenoid friedelan-3β-ol isolated from Euphorbia lactea exhibited cytotoxic activity against HN22 cells by inducing an S-phase cell cycle arrest

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ABSTRACT
The anticancer activity of Euphorbia lactea Haw. (E. lactea) has been observed by our lab and other research groups; however, the identity of the bioactive compounds harboring the anticancer effect remains unknown. Here, we report the first isolation of four triterpenoidal compounds, i.e., friedelin [1], friedelan-3β-ol [2], taraxerol [3], and friedelan-3α-ol [4], from the n-hexane fraction of the E. lactea extract. The cytotoxic activities of these compounds were investigated in several cancer cell lines, including HN22, HepG2, HCT116, and HeLa. These compounds exhibited a dose-dependent cytotoxic activity against HN22, HepG2, and HCT116, while the marginal cytotoxic effect was observed in HeLa cells. Among the four bioactive compounds, compound 2 exhibited the most prominent anticancer effect against HN22 cells. Flow cytometry analysis of HN22 cells treated with the compound revealed that compound 2 induced a cell cycle arrest at the S-phase, while apoptosis was not induced at the same concentration and exposure time. In summary, our results highlighted E. lactea as an attractive candidate for anticancer research and identified compound 2 as a chemical constituent of E. lactea harboring anticancer activity.

INTRODUCTION
Cancer is a disease caused by the accumulation of genetic defects within human cells, which culminates in an uncontrolled proliferative state. Current therapy for cancers includes radiation, surgical removal, and pharmacological treatments with various anticancer agents. Rigorous research in the discovery and development of anticancer agents has led to the establishment of many anticancer agents ranging from conventional chemotherapeutic agents to targeted therapy using small-molecule drugs and monoclonal antibodies. However, despite the availability of a vast collection of effective anticancer agents, the acquisition of a drug-resistant phenotype (Holohan et al., 2013; Housman et al., 2014) and serious adverse reactions associated with certain chemical entities still limit the outcome of anticancer pharmacotherapy. Thus, the development of novel anticancer compounds which will add to the variety of treatment options is likely to confer benefits to cancer patients.

Although the pathogenesis of cancers is widely accepted to be heterogenic in nature, driven by an astounding repertoire...
of genetic and epigenetic defects, the deregulation of cell cycle progression and programmed cell death pathways holds a central place in cancer pathogenesis (Evan and Vousden, 2001). Many anticancer agents used clinically and in clinical trials have been shown to directly or indirectly affect cell cycle progression or apoptosis in cancer cells (Sun et al., 2021; Wong, 2011). Therefore, the assessment of the activity of putative anticancer compounds in the interference with cell cycle progression or apoptosis in cancer cells should serve as an additional test to verify cytotoxicity and reveal the anticancer potential of novel therapeutic entities.

Euphorbiaceae is a large botanical family encompassing over 2,000 species of unique flora with distinctive milky latex and flower shape. The medicinal property of Euphorbia has been documented in both traditional medicinal records and contemporary pharmaceutical research. There are over 100 publications documenting the cytotoxic activity of extracts and pure compounds isolated from over 60 species of Euphorbia (Wongrakpanich and Charoensuksai, 2018). Phytochemical research of Euphorbia has led to the isolation of over 500 compounds, especially terpenoids (Shi et al., 2008). Many of these compounds have been shown to exert anticancer activity through the induction of apoptosis (Wongrakpanich and Charoensuksai, 2018).

Endemic to tropical regions of the world and abundantly found in Thailand, Euphorbia lactea Haw. (E. lactea) is a succulent plant with a distinctive triangular stem. Despite the documented anticancer properties of other Euphorbia species, only a few publications have shed light on the anticancer activities of E. lactea. First, El-Manawaty et al. (2013) reported that the methanolic extracts of E. lactea exhibited cytotoxic activity against hepatocellular carcinoma cell line HepG2 and colorectal cancer cell line HCT116. This observation was further echoed by a report from another group describing the cytotoxic effects of the E. lactea crude methanolic extract in HepG2 and breast cancer cell line MCF7 (El-Hlawy et al., 2020). Our lab previously reported the inhibitory effect of the crude ethanolic extract of E. lactea against the viability and migration of HN22 cancer cells (Wongprayoon and Charoensuksai, 2018). Taken together, the anticancer activity of the E. lactea extract has been documented through several independent reports; nevertheless, the chemical entities responsible for the anticancer activity of E. lactea are yet to be identified.

MATERIALS AND METHODS

Plant materials

The E. lactea specimen used in this experiment was propagated from cuttings collected from a mother plant and cultivated in our greenhouse in Bangkok, Thailand. Plant collection was carried out in May 2019. The species identification was made by a comparison of morphologies between the specimen and pictures of E. lactea in plant databases such as http://www.eol.org and https://www.cabi.org. A voucher specimen (SUPY. PC1) was deposited at the Herbarium of the Faculty of Pharmacy, Silpakorn University, Thailand.

Chemicals and reagents

The common solvents, including ethanol, n-hexane, ethyl acetate (EtOAc), and n-butanol (n-BuOH), for extraction and chromatography were purchased from Merck (Germany). Deuterated chloroform (CDCl₃, 99.98% D) for nuclear magnetic resonance (NMR) analysis and cerium (IV) sulfate were purchased from Sigma-Aldrich (St. Louis, MO). Column chromatography was carried out on silica gel (230–400 mesh, Merck). Aluminum sheets of silica gel (60 F254, Merck) were used for thin-layer chromatography (TLC).

Head-and-neck cancer cell line HN22, hepatocellular carcinoma cell line HepG2, colorectal cancer cell line HCT116, and cervical cancer cell line HeLa were kindly provided by Prof. Praneet Opanasopit, Faculty of Pharmacy, Silpakorn University, Thailand. Immortalized human keratinocyte cell line HaCaT was given by Assist. Prof. Dr. Veerawat Teeranaichaidekul, Department of Pharmacy, Faculty of Pharmacy, Mahidol University, Thailand. Dulbecco’s modified Eagle’s medium (DMEM), minimum essential media (MEM), fetal bovine serum (FBS), nonessential amino acids, GlutaMAX, and penicillin/streptomycin solution were purchased from Gibco (Waltham, MA).

Dimethyl sulfoxide (DMSO), Triton-X 100, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) were purchased from Sigma-Aldrich. Hydroxypropyl-β-cyclodextrin (CAVOSIL W7 HP Pharma) from Wacker Chemie AG (Munich, Germany) was given by Prof. Praneet Opanasopit. Irinotecan (Irintotel) was purchased from Fresenius Kabi Oncology, India. DNase-free RNase A was purchased from Bio Basic (Appertst, NY). Propidium iodide was purchased from Life Technologies (Carlsbad, CA). FITC-conjugated annexin-V and 7-AAD was purchased from Thermo Fischer (Waltham, MA).

Extraction and isolation

The aerial parts of E. lactea were cut into thin slices and left to air-dry in a parabola dome solar drying chamber for 5 days. The dried specimens (2.68 kg) were crushed and extracted with 95% ethanol at room temperature (7 l × 3 times). The extract was concentrated under reduced pressure to give a dark brown residue (613.40 g). The residue was resuspended in water (2 l) and then extracted with n-hexane, EtOAc, and n-BuOH (each solvent 1,400 ml × 3), successively. The respective solvents were evaporated to dryness at 40°C under reduced pressure to yield the n-hexane (94.02 g), EtOAc (33.11 g), and n-BuOH (36.82 g) fractions.

The remaining aqueous phase was concentrated to give the H₂O fraction (428.46 g). A portion of the n-hexane fraction (46.15 g) was subjected to flash column chromatography over silica gel and eluted with n-hexane–EtOAc (gradient from 100% n-hexane to 100% EtOAc) to afford 16 fractions (H1–H16). Fraction H4 (0.6030 g) was recrystallized from ethanol to give 1 (0.1069 g). Fraction H15 (0.3297 g) was further subjected to a silica gel column, eluted with n-hexane–EtOAc (99:1–97:3) to yield 2 (0.2006 g). Fraction H6 (0.5303 g) was recrystallized from ethanol to give 3 (0.1879 g). Further purification of fraction H6 (0.5584 g) by column chromatography over a silica gel, using n-hexane–EtOAc (99:1–95:5), afforded 4 (0.1534 g).

Structural identification

The chemical structures of pure compounds 1–4 were determined by mass spectrometry and NMR spectroscopy. High-resolution electrospray ionization mass spectrometry (HRESIMS) experiments were performed on a Micro TOF Bruker Daltonics mass spectrometer. The ¹H and ¹³C-NMR data were recorded at 300 MHz and 75 MHz on a Bruker AVANCE 300 NMR spectrometer using tetramethylsilane as an internal reference.
Column chromatography was carried out using silica gel (230–400 mesh, Merck). TLC was performed, and spots were detected under ultraviolet light at 254 nm or by spraying with a solution of 1% cerium(IV) sulfate in 10% H₂SO₄ followed by heating.

By comparison of ¹H-NMR, ¹³C-NMR, and mass spectral data of each compound (Supplementary Figs. S1–S12) with those reported in the literature, the isolated compounds were identified as pentacyclic triterpenoids including friedelin [1], friedelan-3β-ol [2], taraxerol [3], and friedelan-3α-ol [4]. Physical and spectroscopic data of these compounds are shown as follows.

Friedelin [1] was obtained as white crystals with melting point 254°C–258°C; [H-NMR (Supplementary Fig. S1)] (300 MHz, CDCl₃) δ ppm: 2.40 (1H, m, H-6b), 2.25 (1H, q, J = 6.3 Hz, H-4), 1.97 (1H, m, H-1b), 1.75 (1H, m, H-6a), 1.69 (1H, m, H-1b), 1.18 (3H, s, H-28), 1.05 (3H, s, H-27), 1.01 (3H, s, H-26), 1.00 (3H, s, H-30), 0.95 (3H, s, H-29), 0.89 (3H, d, J = 6.3 Hz, H-23), 0.87 (3H, s, H-25), and 0.73 (3H, s, H-24); [¹³C-NMR (Supplementary Fig. S2)] (75 MHz, CDCl₃) δ ppm: 158.1 (C-14), 116.9 (C-15), 79.1 (C-3), 55.5 (C-5), 45.3 (C-9), 48.7 (C-18), 41.3 (C-7), 39.0 (C-8), 38.8 (C-4), 38.0 (C-10), 37.7 (C-1, C-16), 37.6 (C-13), 36.7 (C-16), 35.8 (C-17), 35.1 (C-22), 33.7 (C-21), 33.4 (C-29), 33.1 (C-12), 29.9 (C-30), 29.8 (C-28), 28.8 (C-20), 28.0 (C-23), 27.1 (C-2), 25.9 (C-26), 21.3 (C-27), 18.8 (C-6), 17.5 (C-11), 15.5 (C-24), and 15.4 (C-25); HRESIMS (Supplementary Fig. S12) m/z 446.4342 [M+Na]+ (calculated mass for C₃₀H₅₅NaO₃, 446.3436).

Friedelan-3β-ol [2] was obtained as white amorphous powder with melting point 274°C–280°C; [H-NMR (Supplementary Fig. S3)] (300 MHz, CDCl₃) δ ppm: 3.73, (1H, m, H-3), 1.90 (1H, m, H-2b), 1.73 (1H, d, J = 9.6, 3.3 Hz, H-6b), 1.22 (1H, m, H-1a), 1.17 (3H, s, H-28), 1.01 (3H, s, H-27), 0.99 (3H, s, H-30), 0.99 (3H, s, H-29), 0.94 (3H, s, H-24), 0.89 (3H, d, J = 6.6 Hz, H-23), 0.81 (3H, s, H-25), and 0.77 (3H, s, H-24); [¹¹H-NMR (Supplementary Fig. S8)] (75 MHz, CDCl₃) δ ppm: 72.2 (C-3), 60.1 (C-10), 53.2 (C-4), 53.0 (C-8), 42.8 (C-18), 41.4 (C-6), 39.7 (C-14), 39.3 (C-22), 38.3 (C-13), 38.1 (C-5), 37.0 (C-9), 36.7 (C-27), 36.1 (C-16), 35.5 (C-11), 35.3 (C-19), 35.0 (C-29), 32.8 (C-21), 32.4 (C-15), 32.1 (C-28), 31.8 (C-30), 30.5 (C-12), 30.0 (C-17), 28.2 (C-20), 20.2 (C-26), 19.6 (C-1), 18.7 (C-27), 18.1 (C-25), 17.8 (C-17), 14.6 (C-24), and 9.9 (C-23); HRESIMS (Supplementary Fig. S12) m/z 451.3855 [M+Na]+ (calculated mass for C₃₀H₅₅NaO₃, 451.3916).

Cell culture

HN22, HepG2, and HCT116 were maintained in DMEM supplemented with 10% FBS and 1% Glutamax. HeLa was maintained in MEM supplemented with 10% FBS, 1% nonessential amino acids, and 1% Glutamax. HaCatT was maintained in DMEM supplemented with 10% FBS. All culture media contained 100 units/mL penicillin and 100 µg/mL streptomycin. Cells were cultured in a humidified atmosphere containing 5% CO₂ and temperature-controlled at 37°C.

Determination of cell viability by MTT assay

The crude extract (n-hexane, EtOAc, and n-BuOH) and H₂O fractions were dissolved in DMSO. The pure compounds 1–4 were dissolved in a mixture solution of DMSO containing 30% w/v hydroxypropyl-β-cyclodextrin (DMSO-HPBCD).

Cell viability determination by the MTT assay was performed following an established protocol (Kumar et al., 2018) with some modifications. Briefly, 8,000 cells were seeded into each well of a 96-well plate and allowed to attach overnight. Due to the limited solubility of the extract, fractions, and pure compounds in the dissolving vehicle, the maximal concentrations investigated were 500 µg/mL for extracts and fractions and 110 µM for pure compounds. Cells were then incubated with varying concentrations of the extracts (500–1,953 µg/mL final concentration) or pure compounds (100–0.176 µM) for 72 hours. 100 µM irinotecan was used as a positive control. The vehicles used to dissolve the test agents, i.e., DMSO for plant extract and fractions or DMSO-HPBCD for 1–4, were used as negative controls. The final concentration of DMSO and DMSO-HPBCD was maintained at 0.5% w/v for all test conditions. Afterward, 25 µl of 5 mg/ml MTT dissolved in phosphate buffer saline (PBS) was added to each well and incubated for 4 hours. Culture media were then discarded, and 100 µl of DMSO was added to each well to dissolve formazan crystals. Absorbance at 550 nm was measured using a microplate reader (Model No. AOPUS01 and A153601; Packard BioScience Company, CT). All experiments were performed in triplicate.

Cell cycle analysis by flow cytometry

The analysis of cell cycle distribution by propidium iodide staining and flow cytometry was performed according to an established protocol (Crowley et al., 2016) with some modifications. Briefly, HN22 cells were cultured in the presence of 110 µM of 2 or vehicle control for 72 hours. Cells were then washed with PBS, harvested, and fixed with 70% ice-cold ethanol. Afterward, cells were washed twice with ice-cold PBS and treated
Figure 1. Cytotoxic activity of *E. lactea* extracts toward HN22 cells. HN22 cells were treated with DMSO (vehicle control), 100 µM irinotecan, or extracts at concentrations ranging from 500 to 1.953 µg/ml for 72 hours as described Materials and Methods in the section. ** *p* < 0.01, * *p* < 0.05, NS *p* ≥ 0.05 compared to vehicle control.
with 100 µg/mL of DNase-free RNase A in PBS containing 0.1% v/v Triton-X 100 for 5 minutes at room temperature. Cells were then stained with 20 µg/ml propidium iodide in PBS containing 0.1% v/v Triton-X 100 for 15 minutes at room temperature in the dark. Cell cycle distribution profiles were obtained with a flow cytometer (Attune NxT, Thermo Fischer). Data were analyzed with Attune NxT Software (Thermo Fischer). All experiments were carried out in triplicate.

Analysis of apoptotic cells by flow cytometry

The flow cytometry analysis of cells undergoing apoptosis by annexin-V and propidium iodide double staining was carried out following an established protocol (Crowley et al., 2016b) with some modifications. Briefly, HN22 cells were cultured in the presence of 110 µM of 2, 20 µM Irinotecan for positive control, or vehicle for negative control for 72 hours. Cells were then collected, resuspended in PBS, and counted. For each condition, 1 × 10^5 cells were collected to stain with 5 µl of FITC-conjugated annexin-V in binding buffer (0.1 M HEPES, 1.5 M NaCl, 50 mM MgCl₂, 50 mM KCl, and 18 mM CaCl₂, pH 7.4) for 15 minutes at room temperature while protected from light. Afterward, propidium iodide was added to a final concentration of 5 µg/ml and incubated for 15 minutes at room temperature while protected from light. Then, the cells were analyzed by a flow cytometer (Attune NxT, Thermo Fischer). Data were analyzed with Attune NxT Software (Thermo Fischer).

Statistical analysis

One-way analysis of variance with Tukey’s HSD post-hoc test was used to analyze the statistical significance of cell viability experiments of the extract, fractions, and pure compounds. Student’s t-test was used for cell cycle experiments. The software GraphPad Prism version 7 (GraphPad Software Inc., La Jolla, CA) was used for statistical analysis. p < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Our group previously reported the cytotoxic activity of the crude hydroalcoholic extract of fresh E. lactea against HN22 cells (Wongprayoon and Charoensuksai, 2018). This prompted us to ask whether E. lactea contains lead compound(s) with potent anticancer activity. First, we sought to study the cytotoxic activity of the crude extract collected from the extraction of dried and ground E. lactea with 95% ethanol. Indeed, the crude extract of E. lactea exhibited a dose-dependent cytotoxic activity against HN22 cells with statistical significance in all concentrations from 15.625 µg/ml and above (Fig. 1A). Next, we sought to further investigate the cytotoxic effect by sequentially partitioning the crude extract with n-hexane, EtOAc, and n-BuOH to collect refined extracts with different polarities. The cytotoxic effect of n-hexane, EtOAc, n-BuOH, and the H₂O fractions was then studied using the same concentration interval of the crude extract. The result revealed that the cytotoxic activity was markedly increased in the n-hexane and EtOAc fractions, while such effect was diminished in the n-BuOH and negligible in the H₂O fraction (Fig. 1).

As the n-hexane fraction exhibited the most potent cytotoxic effect, this fraction was subjected to further purification to obtain pure chemical entities. Purification of the n-hexane fraction by chromatography techniques resulted in the isolation...
of four triterpenoids as the major constituents. The triterpenoids were identified as friedelin [1], friedelan-3β-ol [2], taraxerol [3], and friedelan-3α-ol [4] (Fig. 2). The chemical structures of all compounds were elucidated based on their 1D and 2D NMR spectroscopic data and by comparison of their physical and spectroscopic data with literature reports (Govindachari et al., 1967; Jamal et al., 2009; Koay et al., 2013; Ndwigah et al., 2013). In addition, the molecular mass of all isolated compounds agreed with the measured m/z analyzed by HRESIMS. The isolation of these compounds has been reported in other Euphorbia species. For example, 1 was previously isolated from Euphorbia tortilis (Anju et al., 2018) and Euphorbia geniculata (Farozi et al., 2015). 2 was previously detected in E. neriifolia (Anjeneyulu et al., 1973) and Euphorbia antiquorum (Min et al., 1989). 3 was previously purified from E. antiquorum (Min et al., 1989), Euphorbia myrsinites (Aynehchi et al., 1972), and E. neriifolia (Anjeneyulu et al., 1973). 4 was previously isolated from E. neriifolia (Anjeneyulu et al., 1973). However, to our knowledge, our report describes the first detection and isolation of these compounds from the species E. lactea. These compounds share a core pentacyclic triterpenoid structure, which has been shown to have various pharmacological effects, including anticancer activity (Ghante and Jamkhande, 2019).
Cytotoxic activities of the isolated compounds 1–4 were then investigated in four cancer cell lines, namely HepG2, HN22, HCT116, and HeLa, while HaCaT was used as a representative noncancerous cell line (Fig. 3). Compounds 1–4 exhibited a dose-dependent cytotoxic effect on HepG2, HN22, and HCT116 with more effect on cancer cells than untransformed cells. Among the four compounds, 2 appeared to be the most potent, with the strongest inhibition effect on HN22 and HepG2 cells. Interestingly, all compounds exhibited marginal or no effect on HeLa cells, suggesting that the cytotoxic effect of these triterpenoids is likely dependent on cell type.

Given that 2 exhibited the most prominent cytotoxic effect, we then sought to further explore the underlying molecular mechanism likely associated with such activity, i.e., the induction of cell cycle arrest and apoptosis. First, the effect of 2 on the cell cycle distribution of HN22 and HepG2 cells was studied. No change in cell cycle distribution was detected in HepG2 cells treated with 2 (Fig. 4A and B). However, treatment of HN22 cells with 2 is accompanied by a statistically significant increase in cells at the S-phase, indicative of the induction of an S-phase cell cycle arrest (Fig. 4C and D). Next, an apoptosis assay by flow cytometry analysis of cells stained with propidium iodide and FITC-conjugated annexin-V was performed on HN22 cells treated with 2. While apoptotic cells were markedly increased upon treatment with the positive control irinotecan (Fig. 5), the percentage of early and late apoptotic cells remained unchanged upon treatment with 2, suggesting that the cytotoxic activity of 2 toward HN22 cells was not mediated through apoptosis, at least at the concentration and exposure time we investigated.

Indeed, the cytotoxic activity of 2 has previously been tested against several cancer cell lines. While it generally exhibited mild cytotoxic activity against many cancer cell lines (Monkodaew et al., 2009; Oliveira et al., 2012; Su et al., 2009), 2 markedly suppressed the viability of certain types of cancer. First, Martucciello et al. (2010) reported that 2 inhibited the cell viability of the Kaposi sarcoma cell line by ~30% at 20 µM. Later, Yessoufou et al. (2015) reported that, among the five cancer types tested, including HeLa, MCF7, Jurkat, HT-29, and T24, 2 exhibited the strongest cytotoxic activity against the T24 bladder cancer cell line with an IC₅₀ of 15.61 µg/mL (~35 µM). In line with this observation, we detected >40% reduction in cell viability in HN22 and HepG2 cells treated with 2 at 22 µM, suggesting that

![Figure 4. Cell cycle distribution analysis of cancer cell lines treated with 2. (A) Histogram showing cell cycle distribution of HepG2 cells treated with 110 µM of 2 or vehicle control. (B) Quantitation of HepG2 cells treated with 110 µM of 2 or vehicle control in the G0/G1, S, and G2/M phases of the cell cycle. (C) Histogram showing cell cycle distribution of HN22 cells treated with 110 µM of 2 or vehicle control. (D) Quantitation of HN22 cells treated with 110 µM of 2 or vehicle control in the G0/G1, S, and G2/M phases of the cell cycle. *p < 0.05, NS p ≥ 0.05 compared to vehicle control.](image-url)
the cytotoxic activity of 2 may be more robust toward these types of cancer. Indeed, these observations need to be confirmed in a controlled test. Moreover, the molecular mechanism underlying the anticancer activity of 2 was largely unexplored. Previous reports utilizing molecular docking analysis suggested that 2 may be able to interact with DNA methyltransferase 1 (Wilaputraka et al., 2017) and HER2 and EGFR (Perumal et al., 2016). Our result thus shed some light on the mechanism of action of 2 in that its anticancer effect was mediated through interference with the cell cycle, particularly a cell cycle arrest at the S-phase, and not through the induction of apoptosis like many anticancer compounds (Pistritto et al., 2016). Therefore, it is of particular interest to study the effect of 2 when used in combination with other anticancer agents in susceptible cancer types.

CONCLUSION
This work reported the first isolation of four triterpenoidal compounds, namely friedelin [1], friedelan-3β-ol [2], taraxerol [3], and friedelan-3α-ol [4], from the n-hexane fraction of *E. lactea*. Cytotoxic activity of these compounds was observed in the HN22, HepG2, and HCT116 cell lines. Among the four compounds, 2 exhibited a prominent anticancer effect against HN22 cells. Subsequent analysis of cell cycle distribution and cells undergoing apoptosis using flow cytometry revealed that treatment with 2 was associated with an increase in cells at the S-phase in HN22 while apoptosis was unperturbed. In summary, our results highlighted *E. lactea* as a plant with anticancer activity and identified 2 as a chemical constituent harboring anticancer activity in *E. lactea*.

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AUTHOR CONTRIBUTIONS
All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

CONFLICTS OF INTEREST
The authors report no financial or any other conflicts of interest in this work.

ETHICAL APPROVALS
This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY
All data generated and analyzed are included within this research article.

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ABBREVIATIONS
n-BuOH: n-Butanol
DMEM: Dulbecco’s modified Eagle’s medium
DMSO : Dimethyl sulfoxide
DMSO-HPBCD: 30% w/v hydroxypropyl-β-cyclodextrin in DMSO
EtOAc: Ethyl acetate
L. lactea: Euphorbia lactea Haw.
PBS: Phosphate buffer saline.

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Crowley LC, Chojnowski G, Waterhouse NJ. Measuring the DNA content of cells in apoptosis and at different cell-cycle stages by propidium iodide staining and flow cytometry. Cold Spring Harb Protoc, 2016a; 2016(10).
}


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SUPPLEMENTARY MATERIAL

The online version of this article contains Supplementary Materials: $^1$H-NMR, $^{13}$C-NMR, and HRESIMS spectra of 1–4.

S1. $^1$H NMR spectrum of 1 (300 MHz, CDCl$_3$).

S2. $^{13}$C NMR spectrum of 1 (75 MHz, CDCl$_3$).
S3. $^1$H NMR spectrum of 2 (300 MHz, CDCl$_3$)

S4. $^{13}$C NMR spectrum of 2 (75 MHz, CDCl$_3$)
S5. $^1$H NMR spectrum of 3 (300 MHz, CDCl$_3$)

S6. $^{13}$C NMR spectrum of 3 (75 MHz, CDCl$_3$)
S7. $^1$H NMR spectrum of 4 (300 MHz, CDCl$_3$)

S8. $^{13}$C NMR spectrum of 4 (75 MHz, CDCl$_3$)
## Mass Spectrum List Report

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### Spectra

![S9. HRESIMS spectrum of 1](image)

### Chemical Information

**Chemical Formula:** $C_{30}H_{50}O$

**Exact Mass:** 426.3862

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**calcd. for $C_{30}H_{50}ONa$**

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S10. HRESIMS spectrum of 2
**Mass Spectrum List Report**

### Analysis Info
- **Analysis Name**: OSSSUJJ12112019002.d
- **Method**: Tune_low_1_POS_2019.m
- **Sample Name**: EL-2
- **Acquisition Date**: 11/12/2019 9:16:21 AM
- **Operator**: Administrator
- **Instrument**: microTOF
- **Set Corrector Fill**: 50 V
- **Set Pulsar Pull**: 337 V
- **Set Pulsar Push**: 337 V
- **Set Reflector**: 1300 V
- **Set Flight Tube**: 9000 V
- **Set Detector TOF**: 2285 V

### Acquisition Parameter
- **Source Type**: ESI
- **Scan Range**: n/a
- **Scan Begin**: 50 m/z
- **Scan End**: 3000 m/z
- **Capillary Exit**: 180.0 V
- **Hexapole RF**: 150.0 V
- **Skimmer 1**: 45.0 V
- **Hexapole 1**: 24.3 V

### Mass Spectrum List

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**Chemical Formula**: C<sub>30</sub>H<sub>52</sub>O

**Exact Mass**: 428.4018

**HRMS m/z [M+NH<sub>4</sub>]<sup>+</sup>**: 446.4342

**calcd. for C<sub>30</sub>H<sub>52</sub>O+NH<sub>4</sub>**: 446.4356

S11. HRESIMS spectrum of 3
### Mass Spectrum List Report

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#### Spectrum

**Chemical Formula:** C_{30}H_{52}O  
**Exact Mass:** 428.4018  
**HRMS m/z [M+Na]^+** 451.3855  
**calcd. for C_{30}H_{52}O\text{Na}** 451.3916

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S12. HRESIMS spectrum of 4