Heliosterpenoids A and B, two Novel Jatrophone-Derived Diterpenoids with a 5/6/4/6 Ring System from *Euphorbia helioscopia*

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Experimental Section

Biological Assay

**Intracellular Adriamycin Accumulation Assay.** Human breast adenocarcinoma cells (MCF-7) and Adriamycin-resistant MCF-7 (MCF-7/ADR) cells were seeded in a 24-well plate at a density of $1 \times 10^5$ cells/well and incubated for 48 h at 37°C in a 95% relative humidity atmosphere containing 5% CO₂. After preincubation with fresh medium containing either the commonly used P-gp inhibitor Cyclosporin A (CsA) (0.5-20 μM) or compound 1 and 2 (0.5-20 μM) for 10 min, 10 μM adriamycin was added to the medium. The plates were incubated for 1h at 37 °C with gentle shaking. The reaction was terminated by removal of the medium. Cells were then washed three times with 1 mL of ice-cold PBS. The cell monolayers were subsequently lysed with 0.3 mL of 0.1% Triton X-100, and the concentration of adriamycin in the cell lysate was determined by LC-MS/MS. Protein concentrations served as the loading control and were measured using the bicinchoninic acid procedure with bovine serum albumin as the standard (Solarbio, China). MCF-7 cells were used as a positive control for maximum adriamycin accumulation.

**Cytotoxicity Assay.**

MDA-MB-231 (Human breast cancer cell line), A549 (Human lung adenocarcinoma), Hela (Human Cervical adenocarcinoma), U118MFG (human glioblastoma) and RKO (Human colorectal adenocarcinoma) cell lines were maintained in RPMI 1640 medium or DMEM medium containing 10% fetal bovine serum (FBS), 100 units/mL penicillin, and 100 μg/mL streptomycin sulfate. All cell cultures were maintained at 37°C in a humidified atmosphere containing 5% CO₂. The cells ($5 \times 10^3$) were seeded in 96-well plates, allowed to adhere for overnight to obtain 80% confluent monolayer. The cells were changed to fresh medium containing compound 1 and 2. After incubation for 48h, cell viability was determined by measuring the metabolic conversion of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) into purple formazan crystals by viable cells. The MTT assay results were read using an MK3 Wellscan plate reader at 570 nm. Compounds were tested at five concentrations ($10^{-5}$, $10^{-6}$, $10^{-7}$, $10^{-8}$, $10^{-9}$ M) and were dissolved in 100% DMSO with a final concentration of DMSO of 0.1% (v/v) in each well. Adriamycin was used as a positive control. Each concentration of the compounds was tested in three parallels. IC₅₀ values were calculated using Microsoft Excel software.

**Computationla section**

Conformational analyses of 1A and 2A were performed by using the MMFF94
molecular mechanics force field via the MOE software package. Enantiomer 1A showed three conformers (Figure S1), and 2A showed six conformers (Figure S17). The conformers were further optimized at the B3LYP/6-31g(d) level in methanol. The energies, oscillator strengths, and rotational strengths of the first 50 electronic excitations were calculated using the TDDFT methodology at the B3LYP/6-31g (d) level. ECD spectrums of the conformers were simulated using a Gaussian function with a half-bandwidth of 0.35eV. The corresponding theoretical ECD spectrum of the enantiomers 1B and 2B were depicted by inverting that of 1A and 2A, respectively. All quantum computations were performed using Gaussian 09 program package, on an IBM cluster machine located at the High Performance Computing Center of Peking Union Medical College.

**LC-MS analysis of 1 and 2 in the crude extract**

The dried and powdered whole plants of *Euphorbia helioscopia* (30 g) were extracted in 80% EtOH by ultrasound-assisted extraction under dark condition (3 \( \times \) 2 h for each time). The crude extract dissolved in methanol and then filtrated in order to remove undissolved components. After concentrating under vacuum, the residue (3 g) was diluted to 1.0 mL with methanol, which was analyzed using a Agilent 6520 Q-TOF LC-MS (gradient: 0-10 min, 10-60% A; 10-30 min, 60-80% A; 30-40 min, 80-100% A (A: MeCN; B: H\(_2\)O)) with the flow rate of 1.0 mL/min. The YMC column used was a 250 \( \times \) 4.6 mm, i.d., 5 \( \mu \)m, YMC-Pack ODS-A.
**Figure S1.** The optimized conformer of 1.

**Figure S2.** Experimental ECD spectrum of 1 and calculated ECD spectra of 1A and 1B in MeOH.
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Figure S5. IR spectrum of heliosterpenoid A (1)
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| m/z   | Ion   | Formula       | Abundance |
|-------|-------|---------------|-----------|
| 545.2518 | (M+Nai)+ | C31H38NaO7+ | 426790.9 |

**Figure S7.** (+)-HRESIMS data of heliosterpenoid A (1)

![Figure S7](image-url)
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