Diterpenoids from the Seeds of Euphorbia lathyris and their Cytotoxic Acitivity

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Abstract: A total of 23 diterpenoids were isolated from the seeds of Euphorbia lathyris, and one new compound (1) and twenty-two known compounds (2-23) were characterized by using 1D- and 2D-NMR spectra and HRESIMS analysis. Cytotoxicity of the isolated compounds against BT-549 and MDA-MB-231 cancer cell lines were evaluated further. Compounds 3, 10, 14, and 22 were found to exhibit considerable cytotoxic activities against BT-549 cells, with IC50 values ranging from 4.7 to 10.1 μM. Compounds 1, 2, 14 and 22 were able to inhibit the MDA-MB-231 cell line growth with IC50 values of 5.7 to 21.3 μM, while other compounds showed no obvious inhibitory effects.

Keywords: Euphorbia lathyris; lathyrane-type diterpenes; cytotoxic activity. © 2022 ACG Publications. All rights reserved.

1. Introduction

Euphorbia is the largest genus in the Euphorbiaceae family, including more than 2000 known species [1,2]. Previous studies have shown that there are abundant natural diterpenoids in Euphorbia. Up to now, more than 850 diterpenes have been isolated from Euphorbia plants, including cembrane, jatrophone, lathyrane, tigliane, daphnane, and ingol types [1,2]. These diterpenes also show diverse functions, such as cytotoxic, anti-inflammatoriy, and anti-HIV activities [3-6].

Euphorbia lathyris L. a well-known herb plant in the Euphorbia family, is mainly distributed in Europe, North, East Asia and North Africa [7-9]. The seeds of Euphorbia lathyris, is the famous Chinese medicine for treating terminal schistose miasis, hydropsy, ascities, constipation and childhood epilepsy [4,10,11]. Over the past few decades, diterpenoids have always been the research hotspots for the chemical constituents of E. lathyris. Generally, the diterpenes of E. lathyris could be roughly divided into two skeleton types: lathyrane and ingenane [1]. Lathyrane diterpenes are characteristic compounds of E. lathyris and exhibit diverse biological activities, such as anti-HIV [12], anticancer [13,14], melanogenesis inhibitory [15], and multidrug resistance reversal activities [11,16]. Up to now, thirty-four Euphorbia factor series compounds (Euphorbia factors L1-L34) have been isolated from E. lathyris [17-27]. Except for the factors L4, L5 and L6, which belong to the ingenane-type, all the other

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compounds are lathyrane diterpenes. Previous studies have shown that lathyrane-type diterpenoids have cytotoxic activity on human breast cancer cells, such as Euphorbia factors L1 and L3, which have significant inhibitory effects on MCF-7 cells [23]. Despite the abundance of lathyrane-type diterpenoids in E. lathyris, few reports have detailed the inhibitory effect of other Euphorbia factors on breast cancer so far. In our continuing search for novel structure and potential antitumor activity diterpenoids from the medicinal plants, 1 new lathyrane-type diterpenoid and 22 known diterpenoids were isolated and identified from the ethanolic extract of E. lathyris (Figure 1). Herein we described in detail the isolation and structure characterization of these compounds with their cytotoxic activity assays.

2. Materials and Methods

2.1. General Experimental Procedures

Optical rotation was measured on a Rudolph VI polarimeter (Rudolph Research Analytical, Hackettstown, NJ, USA). The UV spectra were recorded using the Shimadzu UV-2600 spectrophotometer (Shimadzu, Kyoto, Japan). NMR spectra were recorded on Bruker Avance DRX-600 spectrometer (Bruker BioSpin AG, Fällanden, Switzerland). ESI-MS was collected by an Agilent 1260-6460. RP-C18 silica gel (Merck KGaA, Darmstadt, Germany), MCI gel (CHP20P, Mitsubishi Chemical Corporation, Tokyo, Japan), and Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden). Thin-layer chromatography was performed on pre-coated silica gel GF254 plates (Yantai Jiangyou Silica Gel Development Co, Yantai, China). Semi-preparative HPLC separations were carried out on an Agilent 1260 instrument (Agilent Technologies Inc., Waldbronn, Germany) equipped with ODS column (YMC-pack ODS-A, 10 × 250 mm, 5 μm).

2.2. Plant Material

The plant Euphorbia lathyris L. was obtained from ‘Juhuayuan’ medicinal materials market in Kunming, Yunnan province in October 2018, and identified by Dr. Jinchuan Zhou (College of Pharmaceutical Science, Linyi University). The sample has been stocked in the School of Biological Science and Technology, University of Jinan, and the specimen deposit number is NPMC-20181014.

2.3. Cell Cultures

Breast cancer cell lines MDA-MB-231, BT-549 cells were gained from the Shanghai Cell Bank, Chinese Academy of Sciences. The MDA-MB-231 and BT-549 cells were cultured in DMEM (HyClone, SH30243.01) containing 10% fetal bovine serum (FBS, Gibco, 10270).

2.4. Extraction and Isolation

The dried E. lathyris (8.0 kg) plants were soaked with 95% ethanol three times (about 3*60 L). The 95% EtOH extract was concentrated to provide a residue (2.8 kg), and then extracted with EtOAc and n-BuOH. The resulting extracts were concentrated further to yield 1.98 kg of EtOAc fraction and 78 g of n-BuOH fraction. The EtOAc extract was eluted with a series of 50%, 80%, 95% ethanol on macroporous resin. 80% of the macroporous resin (800 g) was applied to a silica gel column which was eluted with the petroleum ether/acetone (from 50:1 to 1:3) to obtain ten components (Fr.1 - Fr.10). Fr.5 (32.0 g) was prepared on the MCI reverse phase column (MeOH/H2O, 40 : 60 to 90 : 10, v/v) to obtain four components (Fr.5.1 - Fr.5.4). Fr.5.1 (8.1 g) was prepared by semipreparative HPLC (83% MeOH/H2O, 2.0 mL/min) to yield 4 (16.4 mg, ts = 11.9 min), 5 (6.7 mg, ts = 13.3 min), 11 (8.1 mg, ts = 20.1 min), 2 (5.6 mg, ts = 24.5 min) and 18 (11 mg, ts = 30.1 min). Fr.5.4 (2.1 g) was loaded on a RP-C18 reverse phase column (MeOH/H2O, 10 : 90 to 90 : 10) were separated into two components (Fr.5.4.1 - Fr.5.4.2). Fr.5.4.2 (615 mg) was further prepared by semipreparative HPLC (100% MeOH, 2.0 mL/min) to yield 15 (0.6 mg, ts = 12.7 min), 9 (0.8 mg, ts = 14.5 min), 16
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(28 mg, *t*<sub>R</sub> = 16.1 min), 22 (2.1 mg, *t*<sub>R</sub> = 17.2 min) and 7 (9.2 mg, *t*<sub>R</sub> = 21.3 min). Fr.6 (15.0 g) was first separated on a silica gel (200 - 300 mesh) column using petroleum ether/EtOAc (from 30:1 to 1:3) as eluent to obtain four components (Fr.6.1 - Fr.6.4). Fr.6.1 (3.0 g) was prepared by HPLC (65% MeOH/H<sub>2</sub>O, 2.0 mL/min) to yield 1 (0.9 mg, *t*<sub>R</sub> = 15.3 min), 13 (4.1 mg, *t*<sub>R</sub> = 17.9 min), 17 (21 mg, *t*<sub>R</sub> = 18.9 min), 8 (14 mg, *t*<sub>R</sub> = 22.6 min), 14 (15 mg, *t*<sub>R</sub> = 26.7 min), 3 (12 mg, *t*<sub>R</sub> = 32.6 min), 19 (7 mg, *t*<sub>R</sub> = 34.2 min), 20 (5.6 mg, *t*<sub>R</sub> = 36.9 min) and 12 (5.3 mg, *t*<sub>R</sub> = 39.8 min). Fr.9 (31.0 g) was prepared in MCI reverse phase (MeOH/H<sub>2</sub>O, 30:70 to 90:10, v/v) to obtain six components (Fr.9.1 - Fr.9.6). Fr.9.1 (1.8 g) was further purified by CC (CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 30:1 and 20:1), Three subfractions (Fr.9.1.1 - Fr.9.1.3) were obtained. Fr.9.1.2 (908 mg) was prepared by HPLC (68% MeOH/H<sub>2</sub>O, 2.0 mL/min) to yield 6 (9.6 mg, *t*<sub>R</sub> = 16.2 min), 23 (1.1 mg, *t*<sub>R</sub> = 24.3 min), 21 (1.7 mg, *t*<sub>R</sub> = 40.7 min) and 10 (3.7 mg, *t*<sub>R</sub> = 45.6 min).

2.5. Spectroscopic Data

*Compound (1)*: Colorless solid; [α]<sub>D</sub><sup>20</sup> +38.0 (c 0.1, MeOH); UV (MeOH) λ<sub>max</sub> (log ε) 274 (4.58) nm; ECD (MeOH) λ (Δε) 287 (+26.90), 261 (−15.23), 217 (−14.95) nm. <sup>1</sup>H and <sup>13</sup>C NMR data, see Table 2. (+)-HR-ESIMS *m/z* 587.26013 [M + Na]<sup>+</sup>.

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**Figure 1.** Structures of isolated compounds from *Euphorbia lathyris* (1–23)
2.6. Biological Activities

Cell viability was tested by the sulforhodamine B (SRB) method. MDA-MB-231 and BT-549 cells at logarithmic growth phase with about 6000 cells/well were inoculated in 96-well plates (1 × 10⁴ cells per well) separately [28-30]. After 24-hour incubation, according to the results of the pre-experiment, compounds were set at different concentration gradients in MDA-MB-231 and BT-549 cells and then placed in 96-well plates (Figure 3 and 4). Cells were incubated in a 37°C, 5% CO₂ incubator for additional 48 h. After aspirating the culture medium and washing cells with PBS buffer, cell numbers were estimated by SRB analysis. Cells were then stained with 0.4% sulforhodamine B at room temperature for 10 min, washed with 1% acetic acid and combined with dry 150 μL 10 mM Tris-based solution with shaking for 5 min. The absorbance of the mixture was measured with the microplate reader at 540 nm. Three biological replicates were performed for each compound. Cell viability (%) = (OD of treated group/OD of control group) × 100%.

3. Results and Discussion

3.1. Structure Elucidation

Compound 1 was identified as colorless solid. The molecular formula was determined as C₃₀H₄₀O₈ by its HR-ESIMS data (m/z 587.26013 [M + Na]⁺, calcd. 587.26015). The NMR data of compound 1 showed the occurrence of a cinnamoyloxy group [δ_H 7.53 (2H, m), 7.40 (3H, m), 7.74 (1H, d), 6.49 (1H, d); δ_c 166.4, 118.6, 144.8, 134.5, 128.1, 128.1, 129.1, 129.1, 130.3], two acetoxy groups [δ_H 2.20 (3H, s), δ_c 169.7, 22.4; δ_H 2.06 (3H, s), δ_c 172.1, 21.4], two trisubstituted olefinic bonds [δ_H 5.83 (1H, dd, J = 11.5, 5.2 Hz), δ_c 130.2, 135.4 and δ_H 6.49 (1H, m); δ_c 142.7, 134.6], four methyl groups [δ_H 1.77 (3H, s), δ_c 12.1; δ_H 1.30 (3H, s), δ_c 17.2; δ_H 1.19 (3H, s), δ_c 28.7; δ_H 0.96 (3H, d), δ_c 14.1], and a ketocarboxyl carbon (δ_c 197.0) (Table 2). The spin–spin coupling systems of H-1/H-2(H₃-16)/H-3/H-4/H-5, H-5'/H-6'/H-7'/H-8'/H-9' and H-7/H-8/H-9/H-11/H-12 were observed in the H-1/H-2 COSY spectrum, together with the key HMBC correlations from H₃-20 to C-12, C-13, and C-14; from H₂-17 to C-5, C-6, and C-7; and from H₂-18/H₃-19 to C-9, C-10, and C-11, revealing that 1 was a lathyrane-type diterpenoid, especially considering the strong similarity to Euphorbia factor L₇₄ (16) [5] (Figure 2). The difference between them is that the position of the double bond was shifted from Δ5 to Δ6 in 1, as deduced by the HMBC correlations from H₂-17 (δ_H 4.55, 4.41) to C-5 (δ_c 76.4), C-6 (δ_c 130.2) and C-7 (δ_c 135.4), from H-4 (δ_H 2.36) to C-6, and from H-9 (δ_H 1.29) to C-7. Additionally, an OH at C-5 was determined by the molecular formula and the deshielded effect at δ_c 76.4/δ_H 5.32 (1H, d, J = 8.2 Hz).

![Figure 2. Selected HMBC, H-1/H-2 COSY and NOESY correlations of compound 1.](image-url)
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The relative configuration of 1, furnished by a NOESY experiment, also resembled that of 16. The NOE correlations of H-2/H-3, H-2/H-4, H-4/H-17, H-2/H-7, H-7/H-9, H-9/H-11, and H-11/H3-20 suggested that H-2, H-3, H-4, H-9, and H-11 were α-orientated. In addition, the key NOE correlations of H-5/H-12, H-5/H-8, H-4/H-17 and H-17/H-7 suggested the β-orientation for H-5, as well as Z-configuration for the double bond between C-6 and C-7 (Figure 2). Based on comparison with the known compounds, the construction of 1 was determined to be (2S,3S,4S,5R,9S,11R,15R)-15,17-diacetoxy-3-cinnamoyloxy-5-hydroxy-14-oxolathyrin-6Z,12E-diene and called lathyran diterpenes L35.

Table 1. 1H (600 MHz) and 13C (150 MHz) NMR data for compound 1

| position | δH (ppm) | δC (ppm) |
|----------|----------|----------|
| 1        | 3.41 (1H, dd, J = 14.4, 8.7) | 48.1 |
|          | 1.65 (1H, m) |          |
| 2        | 2.26 (1H, m, H-2) | 37.9 |
| 3        | 5.71 (1H, t, J = 3.6) | 78.7 |
| 4        | 2.36 (1H, dd, J = 8.2, 3.5) | 52.3 |
| 5        | 5.32 (1H, d, J = 8.2) | 76.4 |
| 6        |          | 130.2 |
| 7        | 5.83 (1H, dd, J = 11.5, 5.2) | 135.4 |
| 8        | 2.41 - 2.46 (2H, m) | 24.1 |
| 9        | 1.29 (1H, m, H-9) | 30.6 |
| 10       |          | 25.3 |
| 11       | 1.47 (1H, dd, J = 11.6, 8.6) | 27.8 |
| 12       | 6.49 (1H, m) | 142.7 |
| 13       |          | 134.6 |
| 14       |          | 197.0 |
| 15       |          | 93.2 |
| 16       | 0.96 (3H, d, J = 6.7) | 14.1 |
| 17       | 4.55 (1H, d, J = 12.7) | 65.4 |
|          | 4.41 (1H, d, J = 12.7) |          |
| 18       | 1.30 (3H, s, H-18) | 17.2 |
| 19       | 1.19 (3H, s, H-19) | 28.7 |
| 20       | 1.77 (3H, s) | 12.1 |
| 1'       |          | 166.4 |
| 2'       | 6.49 (1H, d') | 118.6 |
| 3'       | 7.74 (1H, d) | 144.8 |
| 4'       |          | 134.5 |
| 5'       |          | 128.1 |
| 6'       | 7.40 (1H, m) | 129.1 |
| 7'       | 7.40 (1H, m) | 130.3 |
| 8'       | 7.40 (1H, m) | 129.1 |
| 9'       | 7.53 (1H, m) | 128.1 |
| 1"       |          | 169.7 |
| 2"       | 2.20 (3H, s) | 22.4 |
| 1""      |          | 172.1 |
| 2""      | 2.06 (3H, s) | 21.5 |

a,b Overlapping signals

The known compounds isolated from *E. lathyris* were identified as 15-O-acetyl-3-O-benzoyl jolkinol-5b,6b-oxide(2) [31], 15-O-acetyl-3-O-phenylacteyljolkinol-5b,6b-oxide(3) [23], (2S*,3S*,4R*,5R*,9S*,11S*,15R*)-5,15-diacetoxy-3-benzoyloxy-14-oxolathyrin-6(17),(12E)-diene (4) [21], Lathyrol-3-phenylacetate-5,15-diacetate (5) [23], euphorbia factor L8 (6) [32],...
(2S,3S,4S,5R,7R,9S,11R,15R)-5,15-diacetoxy-3-cinnamoyloxy-7-benzoyloxy-14-oxolathyra-6(17),12-E-diene(7) [33], (2S,3S,4R,5R,7R,9S,11R,15R)-5,15-diacetoxy-3-benzoyloxy-7-hydroxy-14-oxolathyra-6(17),12E-diene(8) [34], euphorbia factor L_{11} [9] [35], (2S,3S,4S,5R,7R,9S,11R,15R)-7,15-diacetoxy-3-benzoyloxy-5-hydroxy-14-oxolathyra-6(17),12E-diene(10) [31], 15,17-di-O-acetyl-3-O-benzoyl-17-hydroxyjolkinol(11) [23], 15,17-di-O-acetyl-3-O-phenylacetyl-17-hydroxyjolkinol(12) [23], 15-O-acetyl-3-O-phenylacetyl-17-hydroxyjolkinol (13) [23], euphorbia factor L_{22}(14) [23], euphorbia factor L_{23}(15) [36], euphorbia factor L_{7a}(16) [5], euphorbia factor L_{24}(17) [23], 5,15,17-tri-O-acetyl-3-O-benzoyl-17-hydroxyisolathyrol(18) [37], euphorbia factor L_{6}(19) [38], lathyrane diterpenes L_{1}(20) [39]. (2S,3S,4R,5R,6S,11R,15R)-15-acetoxy-5-hydroxy-3 phenylacetoxy-14-oxolathyra-12E-ene-6(17)-epoxide(21) [33], euphorbin B(22) [40], lathyrane A (23) [41].

3.2. Cytotoxic Activity Evaluation

We assayed the cytotoxic activity of 21 isolated compounds against BT-549 (breast) and MDA-MB-231 (breast) cancer cell lines Table 3. The biological activities of compounds 8 and 21 were not measured due to their limited amount. Assay results suggested that compounds 3, 10, 14 and 22 exhibited considerable cytotoxic activities against BT-549 cells with IC_{50} values of 4.7 to 10.1 μM (Figure 3). Compounds 1, 2, 14 and 22 can inhibit the proliferation of MDA-MB-231 cells with IC_{50} values of 5.7 to 21.3 μM (Figure 4). Other compounds showed weak or no activity against cancer cell lines in this study (IC_{50} > 30 μM).

In summary, the seeds of E. lathyrus has become a commonly used traditional Chinese medicine in clinical with its good pharmacological activity for a long time. We obtained one structurally novel diterpenoid and 22 known compounds from the plant. Some of these diterpenoids (1-3, 10, 14, 22) had inhibitory effects on BT-549 cells and MDA-MB-231 cells, which provided reference for future chemical research on diterpenoids in this genus.

![Figure 3. Inhibitory rate of compounds with different concentrations on BT-549 cells.](image1)

![Figure 4. Inhibitory rate of compounds with different concentrations on MDA-MB-231 cells.](image2)
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**Table 2. Cytotoxic activity (IC_{50} in μM)**

| Compound | MDA-MB-231 | BT-549 |
|----------|-------------|--------|
| 1        | 21.3        | >30    |
| 2        | 15.3        | >30    |
| 3        | >30         | 10.1   |
| 10       | >30         | 7.4    |
| 14       | 16.3        | 9.9    |
| 22       | 5.7         | 4.7    |

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**Supporting Information**

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