Efficient Synthesis of Jolkinolides A and B from Steviol

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Abstract: Jolkinolides, isolated from *Euphorbia fischeriana Steud*, are naturally occurring tetracyclic *ent*-abietane diterpenes, some of which exhibit promising antitumor and other biological activity. An efficient strategy for the synthesis of jolkinolides A and B is described starting from readily available steviol in 10 and 11 steps with total yields of over 10%, respectively.

Key words: *ent*-abietane diterpenes, jolkinolides, steviol, Wittig–Horner reaction, antitumor activity

The specific structural features and intriguing biological activity of natural products have attracted the attention of researchers. Jolkinolides, isolated from *Euphorbia fischeriana Steud*,[1] are tetracyclic *ent*-abietane diterpenes characterized by the γ-ylidenebutenolide functionality in ring D, some of which exhibit significant antitumor activity with IC_{50} values ranging from 7.1 nM to 0.5 μM.[2] Jolkinolides A (**1**) and B (**2**) (Figure 1) are representative compounds of the tetracyclic abietane lactones and exhibit significant antitumor activity against several tumor cell lines such as Sarcoma 180 and Ehrlich ascites carcinoma in mice.[3] In human leukemic U937 cells, jolkinolide B was found to reduce cell viability and induce apoptosis in a dose- and time-dependent manner through PI3K/Akt and XIAP pathways.[4]

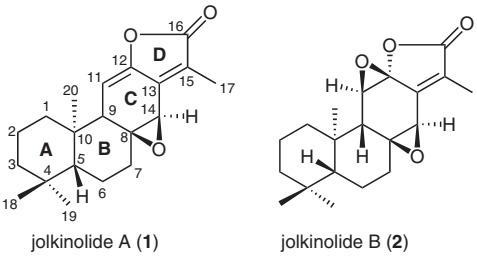
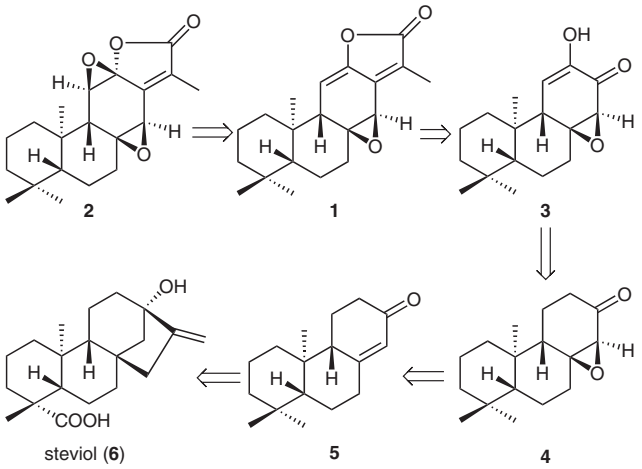


Figure 1 The structure of jolkinolides A and B

Katsumura et al.[5] firstly accomplished the total synthesis of (±)-jolkinolides A, B, and E starting from 10-(methoxycarbonyl)-β-ionone through almost twenty steps, which gave a low yield of the target products. Herein, we describe a concise and scalable approach to the synthesis of jolkinolides A and B from an easily available material, steviol (**6**).

Our retrosynthetic analysis is shown in Scheme 1, the construction of γ-ylidenebutenolide moiety was anticipated from diosphenol **3** through the intramolecular Wittig–Horner reaction developed by Katsumura et al.[5] We envisioned that diosphenol **3** would be available in a single maneuver via the direct oxygenation of **4**, which would be prepared from **5** through stereoselective epoxidation. In the structure of steviol (**6**), the A/B ring is *trans* fused, at C4 there are β-methyl and α-carboxy groups, at C10 there is an α-methyl group, and at C5 and C9 there are β-H, which almost exactly matches those of our target compounds in the structural framework. Therefore, it is reasonable to assume that the key intermediate tricyclic enone **5** could be derived from steviol (**6**), which could be obtained from commercially available stevioside by a literature method.[6]



Scheme 1 Retrosynthetic analysis of jolkinolides A and B

On the basis of the steviol structure skeleton, we anticipated a functional group transformation by converting the C19 carboxy functional group into a methyl group and decomposition of the D ring to get the key intermediate tricyclic enone **5**. Accordingly, the synthetic sequence commenced with reduction of steviol (**6**) with lithium aluminum hydride to afford the diol **7** in a good yield (90%). Barton–McCombie radical deoxygenation[7] was performed on diol **7** to afford **9** in 74% yield (in 2 steps). Allylic oxidation with *tert*-butyl hydroperoxide and selenium dioxide[8] gave **10** in 80% yield. Ozone oxidation

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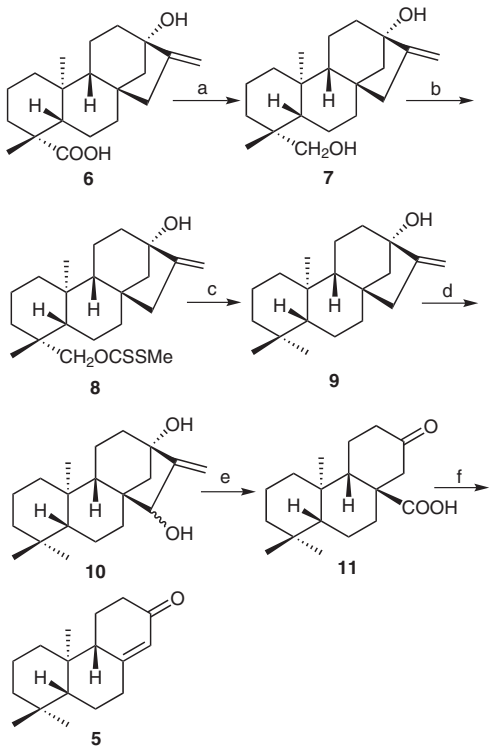
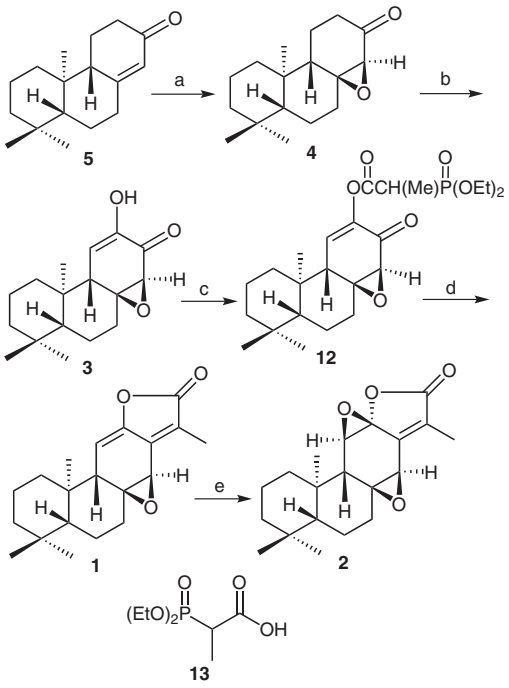
of **10** at –78 °C in a Criegee rearrangement[9] gave compound **11** directly. Decarboxylation of **11** with lead tetraacetate in refluxing benzene provided the key intermediate enone **5** in 68% yield[10] (Scheme 2).



Scheme 2 *Reagents and conditions*: (a) $LiAlH_4$, THF, reflux, 90%; (b) NaH, CS_2, MeI, THF, 89%; (c) Bu_3SnH, AIBN, toluene, reflux, 83%; (d) SeO_2, *t*-BuOOH, THF, r.t., 80%; (e) O_3, MeOH, –78 °C, 69%; (f) $Pb(OAc)_4$, $Cu(OAc)_2$, pyridine, benzene, reflux, 68%.

With a sufficient supply of the key intermediate **5**, we proceeded to prepare the advanced intermediate epoxy ketone **4** with hydrogen peroxide in good yield (81%). Alternatively, treatment of epoxy ketone **4** with a large excess of potassium *tert*-butoxide under air at room temperature directly gave diosphenol **3** in 82% yield. Esterification of diosphenol **3** with 2-(diethoxyphosphoryl)propanoic acid (**13**) in the presence of 4-(dimethylamino)pyridine and *N*,*N*′-dicyclohexylcarbodiimide provided **12**; treatment of **12** with 60% sodium hydride in anhydrous tetrahydrofuran provided jolkinolide A (**1**). The synthesis of jolkinolide B (**2**) was completed by treatment of jolkinolide A (**1**) with 3-chloroperoxybenzoic acid in dichloromethane in 83% yield[5] (Scheme 3).

In the identification of jolkinolide A by its NOESY spectrum (Figure 2), the β-orientation of H9 [δ = 2.64 (d, *J* = 5.2 Hz)] was supported by the NOESY spectrum, in which H9 did not correlate with H20 [δ = 0.73 (s)], but correlated with H5 [δ = 1.19 (m)]. Since H14 did not correlate with H9 [δ = 2.64 (d, *J* = 5.2 Hz)], this confirmed that the epoxy ring of jolkinolide A is β-orientation. The relative configuration of jolkinolide B was also established on the basis of a NOESY experiment. The protons at δ = 4.03 (s, H14) and at δ = 3.67 (s, H11) correlated to



Scheme 3 *Reagents and conditions*: (a) 30% H_2O_2, 4 M NaOH, MeOH, r.t., 81%; (b) *t*-BuOK, O_2, *t*-BuOH, 40 °C, 82%; (c) acid **13**, DCC, DMAP, CH_2Cl_2, r.t., 88%; (d) 60% NaH, THF, r.t., 84%; (e) MCPBA, CH_2Cl_2, r.t., 83%.

H20 [δ = 0.82 (s)], while the proton at δ = 2.28 (s, H9) did not correlate to H14 (δ = 4.03, s), which revealed that both the C8–C14 and C11–C12 epoxides are β-orientation, which is in accordance with literature reports.[11]

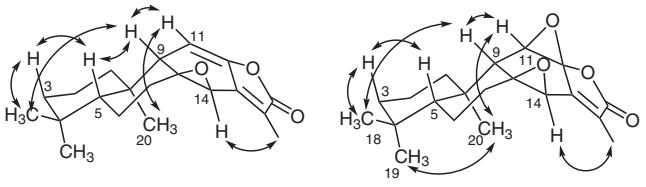


Figure 2 Key NOESY correlations of jolkinolides A and B

The cytotoxic activities[12] of the synthetic jolkinolides A and B were tested in vitro against five human cancer lines, which contained human breast cancer cell line (MDA-MB-231), human breast cancer cell line (MCF-7), human bladder cancer cell line (T24), human bladder cancer cell line (J82), and human bladder cancer cell line (NTUB1). The results showed that jolkinolide B had medium antiproliferative activity with IC_{50} values ranging from 10.1 μM to 81.0 μM and its cytotoxicity against five kinds of cell lines was better than that of jolkinolide A (Table 1).

In summary, a concise and scalable approach for the synthesis of jolkinolides A and B has been developed starting from an easily available material in 10 and 11 steps with total yields over 10%, respectively. This will give a versatile access to various jolkinolides derivatives and related

Table 1 In Vitro[a] Cytotoxicity Data of Jolkinolides A and B

Compound	Antitumor activity in 48 h (IC_{50} μM)[b]				
	MDA-MB-231	MCF-7	T24	J82	NTUB1
jolkinolide A	90.96	43.02	37.75	78.81	88.43
jolkinolide B	12.00	70.50	10.11	81.02	23.00

[a] Inhibition of cell growth by the listed compounds was determined using MTT assay.
[b] Data represents the mean value of three independent determinations.

diterpenoids that are biologically active with pharmaceutical potential as antitumor and anti-inflammatory agents. Further structural modification and biological evaluation are ongoing, and the results will be reported in due course.

^{1}H and ^{13}C NMR spectra were recorded at 300 MHz and 75 MHz, respectively, relative to internal TMS standard. LRMS and HRMS were recorded in ESI mode. IR spectra were recorded either on neat samples (KBr disks) or as thin films. The melting points of the compounds were measured on a Kofler-type Reichert Thermovar micro hot stage microscope and were uncorrected. Petroleum ether (PE) used was the fraction boiling in the 30–60 °C range.

***ent*-Kaur-16-en-13,19-diol (7)**
To a suspension of $LiAlH_4$ (6.70 g, 176.64 mmol) in anhyd THF was added dropwise a solution of steviol (**6**, 22.50 g, 70.65 mmol) in anhyd THF at 0 °C. The mixture was heated to reflux for 6 h, then quenched with sat. NH_4Cl solution, and extracted with EtOAc. The combined organic layers were washed with brine, dried (anhyd Na_2SO_4), filtered, and evaporated to dryness to give a residue that was purified by flash chromatography (silica gel, PE–EtOAc, 2:1) to give **7** (19.40 g, 63.72 mmol, 90%) as a white amorphous solid; mp 231–233 °C.

IR (KBr): 3418, 3110, 3002, 1661, 1400, 1275, 1071, 764, 750, 570 cm^{-1}.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 4.86 (s, 1 H), 4.66 (s, 1 H), 3.50 (m, 1 H), 3.31 (m, 1 H), 2.48 (s, 2 H), 2.01 (s, 2 H), 1.89–1.85 (d, *J* = 10.74 Hz, 1 H), 1.75–1.62 (m, 4 H), 1.57–1.21 (m, 7 H), 1.14–1.10 (d, *J* = 10.83 Hz, 1 H), 0.92 (s, 3 H), 0.89–0.85 (m, 5 H), 0.82 (s, 3 H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 156.7, 102.8, 79.1, 63.1, 56.8, 54.8, 47.8, 46.6, 43.0, 42.0, 41.5, 40.8, 39.7, 35.7, 28.0, 20.4, 20.2, 18.3, 18.2, 13.4.

MS (ESI): *m*/*z* = 327 $[M + Na]^+$.

HRMS: *m*/*z* $[M + Na]^+$ calcd for $C_{20}H_{32}O_2Na$: 327.2300; found: 327.2309.

(*ent*-13-Hydroxykaur-16-en-19-oxy)methyl Xanthate (8)
To a solution of **7** (2.00 g, 6.57 mmol) in THF (10 mL) was added 60% NaH (0.47 g, 19.71 mmol) with stirring at 0 °C. The mixture was heated to reflux for 2 h, then cooled to r.t., CS_2 (0.44 mL, 7.23 mmol) was added and the mixture was heated to reflux for 1 h. After cooling to r.t., MeI (0.44 mL, 7.23 mmol) was added, the mixture was stirred at r.t. for 1 h, quenched with sat. NH_4Cl solution, and extracted with EtOAc. The combined organic layers were washed with brine, dried (anhyd Na_2SO_4), concentrated, and then purified by chromatography (silica gel, PE–EtOAc, 10:1) to give **8** (2.30 g, 5.83 mmol, 89%) as a white amorphous solid; mp 103–105 °C.

IR (KBr): 3470, 3302, 2982, 2925, 2849, 2359, 1708, 1695, 1444, 1230, 1070, 1057, 960, 874, 749 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.98 (s, 1 H), 4.82 (s, 1 H), 4.76–4.76 (d, *J* = 11.1 Hz, 1 H), 4.41–4.38 (d, *J* = 10.9 Hz, 1 H), 2.56 (s, 3 H), 2.18–2.10 (m, 3 H), 1.88–1.81 (m, 5 H), 1.77–1.73 (m, 3 H), 1.63–1.42 (m, 9 H), 1.26–1.22 (d, *J* = 9.5 Hz, 1 H), 1.01 (s, 6 H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 216.0, 155.9, 103.0, 80.2, 77.4, 56.8, 54.7, 47.4, 46.9, 41.6, 41.5, 40.1, 39.2, 38.9, 37.7, 36.4, 27.8, 20.4, 20.2, 18.8, 18.2, 17.8.

MS (ESI): *m*/*z* = 417 $[M + Na]^+$.

HRMS: *m*/*z* $[M + Na]^+$ calcd for $C_{22}H_{34}O_2S_2Na$: 417.1898; found: 417.1907.

***ent*-13-Hydroxykaur-16-ene (9)**
To a solution of **8** (2.30 g, 5.83 mmol) in toluene (50 mL) was added a catalytic amount of AIBN (0.30 g, 1.81 mmol). The mixture was purged with N_2 for 15 min, then Bu_3SnH (3.70 mL, 13.35 mmol) was added over 3 h via syringe pump with stirring at 90–110 °C. The mixture was cooled to r.t., sat. NaF solution was added and the mixture was stirred overnight and then extracted with EtOAc. The combined organic layers were washed with brine, dried (anhyd Na_2SO_4), filtered, and evaporated to dryness to give a residue that was purified by flash chromatography (silica gel, PE–EtOAc, 15:1) to give **9** (1.40 g, 4.85 mmol, 83%) as a white amorphous solid; mp 117–119 °C.

IR (KBr): 3111, 2994, 2946, 2992, 2846, 2842, 2360, 2354, 1400, 1367, 1112, 1088, 877 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.98 (s, 1 H), 4.82 (s, 1 H), 2.19–2.11 (m, 3 H), 1.85–1.76 (m, 3 H), 1.66–1.65 (m, 2 H), 1.62–1.59 (m, 2 H), 1.58–1.49 (m, 6 H), 1.45–1.42 (m, 2 H), 1.39–1.30 (m, 2 H), 1.28–1.13 (m, 7 H), 1.03 (s, 3 H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 156.3, 102.7, 80.4, 56.2, 54.7, 47.6, 47.1, 42.0, 41.7, 41.3, 40.3, 39.4, 39.1, 33.6, 33.3, 23.4, 21.2, 20.2, 18.6, 17.5.

MS (ESI): *m*/*z* = 311 $[M + Na]^+$.

HRMS: *m*/*z* $[M + Na]^+$ calcd for $C_{20}H_{32}ONa$: 311.2351; found: 311.2358.

***ent*-Kaur-16-ene-13,15-diol (10)**
To a solution of **9** (2.60 g, 9.01 mmol) in THF (30 mL) were added SeO_2 (0.60 g, 5.41 mmol) and *t*-BuOOH (1 mL) and the mixture was stirred at r.t. for 12 h. The mixture was diluted with water and extracted with EtOAc. The combined organic layers were washed with brine, dried (anhyd Na_2SO_4), filtered, and evaporated to dryness to give a residue that was purified by flash chromatography (silica gel, PE–EtOAc, 2:1) to give **10** (2.20 g, 7.23 mmol, 80%) as a white amorphous solid; mp 244–246 °C.

IR (KBr): 3551, 3474, 3227, 3115, 2932, 1370, 1363, 1004, 562 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.30 (s, 1 H), 5.26 (s, 1 H), 3.82 (s, 1 H), 2.10–2.06 (d, *J* = 11.1 Hz, 1 H), 1.88–1.76 (m, 4 H), 1.75–1.49 (m, 5 H), 1.44–1.37 (m, 4 H), 1.31–1.25 (m, 3 H), 1.18–1.11 (m, 2 H), 1.02 (s, 3 H), 0.95–0.92 (m, 1 H), 0.87 (s, 3 H), 0.82 (s, 3 H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 161.6, 107.1, 80.8, 78.1, 56.2, 53.5, 45.6, 43.4, 42.0, 40.8, 40.5, 39.1, 35.5, 33.9, 33.3, 21.9, 19.9, 19.5, 18.6, 17.8.

MS (ESI): *m*/*z* = 327 $[M + Na]^+$.

HRMS: *m*/*z* $[M + Na]^+$ calcd for $C_{20}H_{32}O_2Na$: 327.2300; found: 327.2308.

***ent*-8-Carboxy-13-oxopodocarpane (11)**
A solution of **10** (1.50 g, 4.96 mmol) in MeOH (20 mL) was subjected to O_3 for 2 h and then Et_3N (1.35 mL) was added at –78 °C. The mixture was stirred and allowed to warm to r.t. over 3 h and then neutralized with 10% HCl and extracted with EtOAc. The combined organic layers were washed with brine, dried (anhyd Na_2SO_4), filtered, and evaporated to dryness to give a residue that

was purified by flash chromatography (silica gel, PE–EtOAc, 2:1) to give **11** (1.00 g, 3.42 mmol, 69%) as a white amorphous solid; mp 145–147 °C.

IR (KBr): 3553, 3110, 2967, 1734, 1616, 1399, 1071, 986, 623 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 2.63–2.59 (d, *J* = 11.3 Hz, 1 H), 2.04–1.95 (m, 1 H), 1.90–1.58 (m, 5 H), 1.47–1.39 (m, 3 H), 1.36–1.25 (m, 6 H), 1.16–0.92 (m, 5 H), 0.87 (s, 3 H), 0.82 (s, 3 H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 200.3, 165.5, 125.2, 54.0, 51.6, 41.9, 39.5, 39.0, 36.5, 35.4, 33.8, 33.4, 22.4, 22.0, 20.1, 18.7, 15.4.

MS (ESI): *m*/*z* = 315 $[M + Na]^+$.

HRMS: *m*/*z* $[M + Na]^+$ calcd for $C_{18}H_{28}O_3Na$: 315.1936; found: 315.1941.

***ent*-Podocarp-8(14)-en-13-one (5)**

To a solution of **11** (0.70 g, 2.39 mmol) in benzene (10 mL) were added $Cu(OAc)_2$ (0.14 g, 0.72 mmol), pyridine (1.44 mL, 18.81 mmol), and $Pb(OAc)_4$ (1.27 g, 2.87 mmol). The mixture was purged with N_2 for 15 min, it was stirred in the dark for 1 h and then heated to reflux for 6 h, 10% HCl was added, and the mixture was extracted with EtOAc. The combined organic layers were washed with brine, dried (anhyd Na_2SO_4), and evaporated to dryness to give a residue that was purified by flash chromatography (silica gel, PE–EtOAc, 10:1) to give **5** (0.40 g, 1.62 mmol, 68%) as a colorless oil liquid.

IR (KBr): 3320, 2923, 1678, 1596, 1463, 1384, 1283, 924, 893, 731 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 5.88 (s, 1 H), 2.51 (d, *J* = 15.3 Hz, 1 H), 2.41 (d, *J* = 15.2 Hz, 1 H), 2.31–2.10 (m, 2 H), 2.04–1.92 (m, 3 H), 1.76–1.70 (m, 2 H), 1.62–1.39 (m, 4 H), 1.28–1.06 (m, 3 H), 0.93 (s, 3 H), 0.88 (s, 3 H), 0.82 (s, 3 H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 200.3, 165.5, 125.2, 54.0, 51.6, 41.9, 39.5, 39.0, 36.5, 35.4, 33.7, 33.4, 22.4, 22.0, 20.1, 18.7, 15.4.

MS (ESI): *m*/*z* = 269 $[M + Na]^+$.

HRMS: *m*/*z* $[M]^+$ calcd for $C_{17}H_{26}O$: 246.1984; found: 246.1987.

***ent*-8β,14β-Epoxy-13-podocarpanone (4)**

To a solution of **5** (1.50 g, 6.09 mmol) in MeOH (20 mL) were added dropwise 4 M NaOH (4.50 mL) and 30% H_2O_2 (4.50 mL) with stirring at 0 °C. The mixture was stirred at r.t. for 8 h, the majority of the solvent was evaporated, and then 10% HCl (10 mL) was added and the mixture was extracted with EtOAc. The combined organic layers were washed with sat. Na_2SO_3 solution and brine, dried (anhyd Na_2SO_4), filtered, and evaporated to dryness to give a residue that was purified by flash chromatography (silica gel, PE–EtOAc, 20:1) to give **4** (1.30 g, 4.95 mmol, 81%) as a white amorphous solid; mp 98–101 °C.

IR (KBr): 3383, 2948, 2923, 1699, 1673, 1595, 1384, 1258, 763, 750 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 3.17 (s, 1 H), 2.41–2.37 (m, 1 H), 2.27–2.15 (m, 3 H), 2.08–1.87 (m, 4 H), 1.87–1.76 (m, 3 H), 1.73–1.51 (m, 5 H), 0.90 (s, 3 H), 0.83 (s, 3 H), 0.81 (s, 3 H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 208.9, 67.2, 63.7, 54.4, 48.3, 41.8, 39.8, 39.7, 35.1, 33.8, 33.7, 33.2, 21.7, 21.4, 18.5, 16.7, 16.1.

MS (ESI): *m*/*z* = 263 $[M + H]^+$.

HRMS: *m*/*z* $[M + Na]^+$ calcd for $C_{17}H_{26}O_2Na$: 285.1831; found: 285.1837.

***ent*-8β,14β-Epoxy-12-hydroxypodocarp-11(12)-en-13-one (3)**

O_2 was bubbled into a mixture of **4** (0.30 g, 1.14 mmol) and *t*-BuOK (0.46 g, 4.18 mmol) in anhyd *t*-BuOH (10 mL). The mixture was stirred at 40 °C for 40 min, then the majority of the solvent was evaporated, 10% HCl was added and the mixture was extracted with EtOAc. The combined organic layers were washed with brine, dried (anhyd Na_2SO_4), and evaporated to dryness to give a residue that was purified by flash chromatography (silica gel, PE–EtOAc, 15:1) to give **3** (0.26 g, 0.94 mmol, 82%) as a white amorphous solid; mp185–187 °C.

IR (KBr): 3563, 3006, 2920, 2849, 1767, 1698, 1275, 1260, 1204, 1122, 756, 749 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 5.76 (s, 1 H), 3.37 (s, 1 H), 2.63 (d, *J* = 5.0 Hz, 1 H), 2.11–2.07 (m, 1 H), 1.87–1.80 (m, 1 H), 1.73–1.68 (m, 1 H), 1.57–1.45 (m, 6 H), 1.32–1.15 (m, 3 H), 0.94 (s, 3 H), 0.87 (s, 3 H), 0.73 (s, 3 H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 189.4, 145.1, 110.2, 63.2, 53.6, 52.7, 41.5, 41.3, 39.7, 33.5, 32.8, 29.7, 21.8, 20.8, 20.3, 18.3, 14.5.

MS (ESI): *m*/*z* = 277 $[M + H]^+$.

HRMS: *m*/*z* $[M - H]^+$ calcd for $C_{17}H_{23}O_3$: 275.1647; found: 275.1655.

Jolkinolide A (1)

To a solution of **3** (0.20 g, 0.72 mmol) and acid **13** (0.30 g, 1.59 mmol) in CH_2Cl_2 (10 mL) were added DCC (0.74 g, 3.62 mmol) and DMAP (0.09 g, 0.71 mmol) and the mixture was stirred at r.t. for 10 h. The majority of the solvent was evaporated from the mixture was evaporated, the residue was dissolved with EtOAc, filtered, washed with 1 M HCl and $NaHCO_3$, and evaporated to dryness to give a residue that was purified by flash chromatography (silica gel, PE–EtOAc, 3:1) to give **12** (0.30 g, 0.64 mmol, 88%) as an oil liquid.

To a stirred solution of **12** (0.23 g, 0.49 mmol) in anhyd THF at 0 °C was added 60% NaH (0.20 g, 4.91 mmol). When the addition was complete, the mixture was warmed to r.t. for 3 h, then quenched with sat. NH_4Cl solution, and extracted with EtOAc. The combined organic layers were washed with brine, dried (anhyd Na_2SO_4), filtered, and evaporated to dryness to give a residue that was purified by flash chromatography (silica gel, PE–EtOAc, 15:1) to give jolkinolide A (**1**) (0.13 g, 0.41 mmol, 84%) as a white amorphous solid; mp 218–219 °C [Lit.[5] mp 202–203 °C; Lit.[11c] mp ~220 °C (dec.)]; $[\alpha]_D^{20}$ +78 (*c* 0.05, $CHCl_3$) {Lit.[11c] $[\alpha]_D^{25}$ +130 (*c* 0.7, $CHCl_3$)}.

IR (KBr): 3353, 2922, 2851, 1764, 1289, 1248, 781, 748 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 5.47 (d, *J* = 5.3 Hz, 1 H), 3.70 (s, 1 H), 2.64 (d, *J* = 5.2 Hz, 1 H), 2.13 (m, 1 H), 2.08 (s, 3 H), 1.82–1.76 (m, 1 H), 1.72–1.66 (m, 1 H), 1.65–1.19 (m, 8 H), 0.97 (s, 3 H), 0.87 (s, 3 H), 0.73 (s, 3 H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.5, 147.5, 144.9, 125.2, 103.9, 61.1, 54.4, 53.5, 51.8, 41.5, 41.3, 39.8, 34.1, 33.5, 33.4, 21.8, 20.8, 18.4, 14.4, 8.5.

MS (ESI): *m*/*z* = 315 $[M + H]^+$.

HRMS: *m*/*z* $[M + Na]^+$ calcd for $C_{20}H_{26}O_3Na$: 337.1780; found: 337.1788.

Jolkinolide B (2)

To a solution of jolkinolide A (**1**) (23mg, 73 μmol) in CH_2Cl_2 (3 mL) was added MCPBA (50 mg, 292 μmol). The mixture was stirred at r.t. for 30 h, then ice water was added and the mixture was extracted with EtOAc. The combined organic layers were washed with brine, dried (anhyd Na_2SO_4), filtered, and evaporated to dryness to give a residue that was purified by flash chromatography (silica gel, PE–EtOAc, 5:1) to give jolkinolide B (**2**) (20 mg, 61 μmol, 83%) as a white amorphous solid; mp 216–217 °C [Lit.[5] mp 221–222 °C; Lit.[11c] mp ~215 °C (dec.)]; $[\alpha]_D^{25}$ +240 (*c* 0.03, $CHCl_3$) {Lit.[11c] $[\alpha]_D^{25}$ +220 (*c* 0.4, $CHCl_3$)}.

IR (KBr): 3359, 2967, 2924, 1777, 1595, 1275, 1260, 764, 747, 750 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 4.03 (s, 1 H), 3.67 (s, 1 H), 2.28 (s, 1 H), 2.08 (s, 3 H), 2.01–1.92 (m, 3 H), 1.83–1.10 (m, 8 H), 0.92 (s, 3 H), 0.88 (s, 3 H), 0.82 (s, 3 H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 169.5, 148.6, 130.2, 85.2, 66.0, 60.9, 55.3, 53.5, 48.0, 41.3, 39.1, 35.6, 34.6, 33.5, 33.1, 21.9, 20.9, 18.4, 15.4, 8.7.

MS (ESI): m/z = 353 $[M + Na]^+$.

HRMS: m/z $[M + Na]^+$ calcd for $C_{20}H_{26}O_4Na$: 353.1729; found: 353.1737.

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(12) Cytotoxity assay in vitro: the human cancer cell lines were provided by Prof. Yangchang Wu research group (China Medical University) and maintained in a humidified atmosphere at 37 °C in 5% CO_2. The cells were cultured in high glucose RPMI-1640 media containing 10% FBS, 100 IU/mL penicillin, and 100 μg/mL streptomycin. Cell cytotoxicity was determined by MTT assay. Briefly, cells were seeded in 96-well-plate (5000 cells/well) and incubated for 48 h. Then, the tested compounds with various concentrations were added to the wells and 48 h later the MTT solution (0.5 mg/mL) was added and incubated for 1 h. DMSO (100 μL) was added to each well to dissolve the reduced MTT crystals. The absorbance of each well was measured at 570 nm with a microplate reader.

