Synthesis and Biological Evaluation of Laxiflorin J Derivatives as Potential Antitumor Agents

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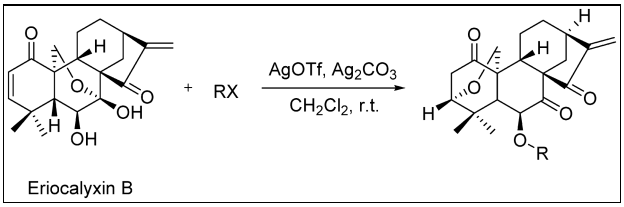

Laxiflorin J, isolated from the leaves of *Isodon eriocalyx var. laxiflora*, showed significant inhibitory activity toward T-24 cells. A series of laxiflorin J derivatives were synthesized and their *in vitro* activity was evaluated against BEL-7402, A-549, HT-29, HL-60, MOLT-4 tumor cell lines, with IC_{50} values ranging from 1.3 to 42.2 μg mL^{-1}.

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INTRODUCTION

The polycyclic diterpenes constitute a large class of natural products. Many of these compounds, especially the ent-kaurane diterpenoids, possess interesting biological activities such as antibacterial, antiparasitic, cytotoxic, antitumour, anti-HIV, anti-inflammatory, and antifungal activities [1–6]. Over the past 2 decades, we have devoted our efforts to the isolation, characterization and bioactivity of the ent-kaurane type diterpenes from *Isodon* species distributed in China [7]. A number of those isolated diterpenoids have been found to have potent antitumour activities with low toxicity. Typically, eriocalyxin B, laxiflorins (J-M), and oridonin were identified as promising candidates for new anticancer agents [7a,8–12]. Laxiflorin J (Fig. 1), a natural product first isolated in 2002 by our group from the leaves of *Isodon eriocalyx* var. *laxiflora* C. Y. Wu & H. W. Li shows significant inhibitory activity toward T-24 cells [13].

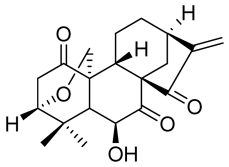
Biosynthetically, laxiflorin J might be derived from eriocalyxin B, a novel diterpenoid with α, β-unsaturated ketone moiety at C (-1), *via* an intramolecular 1,4-conjugate addition. This assumption was confirmed by the chemical transformation as indicated in Scheme 1.

As an analog of eriocalyxin B, laxiflorin J also owns the same unsaturated ketone moiety in the D-ring, which was assumed to be the active center. The antitumor mechanism might be the facile addition of soft nucleophiles, such as alkanethiols and L-cysteine, to the unsaturated ketone moiety in a 1,4-conjugate addition fashion, leading to the deactivation of SH-enzymes or coenzymes [14]. To date, only a few studies involving the acylation of the C(-6) OH group of eriocalyxin B have been conducted [15], unfortunately, loss of activity occurs with the formation of ester at the C(-6) OH group of laxiflorin J.

To get further insight toward the structure-activity relationship, we have designed and synthesized a number of ether derivatives of laxiflorin J by using eriocalyxin B as starting material. Their anticancer activities against BEL-7402, A-549, HT-29, HL-60, MOLT-4 tumor cell lines are reported herein.

RESULTS AND DISCUSSION

In this study, we mainly focused on the modification of the B ring system of laxiflorin J by attaching a lipophilic alkyl side chain to the OH group at C(-6). Concerning the etherification of laxiflorin J, we speculated that a Williamson reaction should lead to ether derivatives at C(-6). Unfortunately, the Williamson reaction



Laxiflorin J

Figure 1. Structure of laxiflorin J.

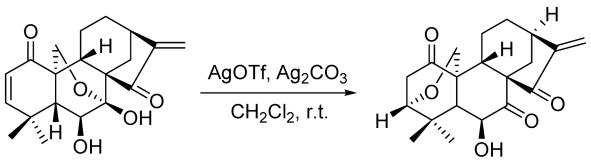
failed to afford the ether derivatives. After a careful survey of literatures, we decided to use a procedure described by Burk [16]. The Burk's protocol did provide the ether compounds, however in low yields (10%). We finally obtained the desired ethereal compounds in good yields (>70%) from eriocalyxin B by modification of Burk's procedure. The reagent 2,6-di-(*tert*-butyl)pyridine was replaced by Ag_2CO_3. The alkylation of eriocalyxin B with commercially available alkyl iodides were carried out in the presence of AgOTf and Ag_2CO_3 in CH_2Cl_2 under darkness for 3 d. This new combination afforded the desired product by an intermolecular alkylation of the C(-6) OH group and an intramolecular etherification with concomitant Michael addition toward the A-ring unsaturated ketone moiety of eriocalyxin B. The prepared ethereal derivatives are listed in Table 1.

The synthesized compounds as well as reference compound, namely, **VP-16**(etoposide), were evaluated against different human tumor cell lines (Table 2).

These *in vitro* data show that: (a), in most cases, the increase in lipophilicity by etherification could enhance cytotoxicity against tumor cell lines; (b), the lipophilic enhancement effects were affected by the length and the shape of the alkyl chain. The derivatives with longer side chain (5–9 C-atoms) were generally less active than those with shorter chain against BEL-7402, A549, HT-29 carcinoma cell lines. Compound **2** was identified to be more potent, with an IC_{50} value of 5.2μg mL-1 (against A549) and a 3-fold increase in efficacy with respect to the reference compound **VP-16**. Compound **2** (IC_{50} 6.3 μg mL^{-1}, against BEL-7402) showed twofold enhancement in potency with respect to the parent compound laxiflorin J.

In summary, a number of ethereal derivatives of laxiflorin J were synthesized by a modified etherification procedure. Their *in vitro* activity was evaluated against BEL-7402, A-549, HT-29, HL-60, MOLT-4 tumor cell lines, with IC_{50} values range from 1.3 to 42.2 μg mL^{-1}. The biological evaluation indicated that increase in

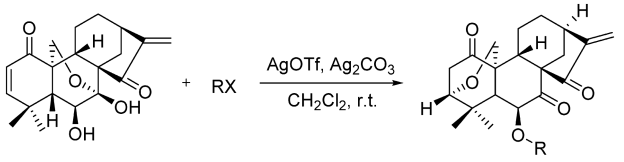
Scheme 1. Transformation of eriocalyxin B to laxiflorin J.



Eriocalyxin B Laxiflorin J

Table 1

Synthesis of analogs of laxiflorin J.



Eriocalyxin B

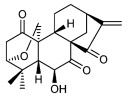
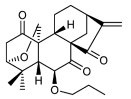
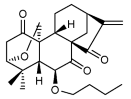
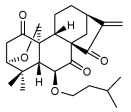
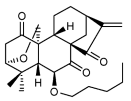
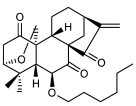
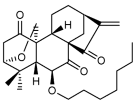
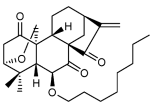
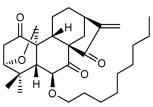
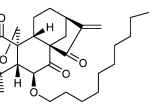
Entry	RX	Product	Yield (%)
1			81
2	Propyl iodide		75
3	Butyl iodide		73
4	*Iso*pentyl iodide		76
5	Pentyl iodide		78
6	Hexyl iodide		71
7	Heptyl iodide		77
8	Octyl iodide		79
9	Nonyl iodide		81
10	Decyl iodide		82

Table 2

In vitro cytotoxicity of compounds **1–10** effect on BEL-7402, A549, HT-29, HL60 and MOLT-4 cell lines.

	IC_{50} (μg/mL)				
Compound	BEL-7402	A549	HT-29	HL60	MOLT-4
1	11.8	9.6	10.2	5.3	1.3
2	6.3	5.2	7.4	6.2	3.2
3	11.1	7.9	9.4	3.4	2.2
4	7.7	7.4	6.7	3.2	2.5
5	9.5	7.9	11.0	4.7	3.0
6	18.2	16.4	18.2	7.2	5.2
7	17.2	14.5	14.3	7.6	6.2
8	28.2	23.0	35.2	9.5	8.6
9	39.3	19.8	42.2	5.3	15.5
10	26.6	16.4	30.7	1.5	3.5
VP-16	4.3	17.4	16.6	0.21	0.0089

lipophilicity by introduction of a short linear alkyl chain could enhance the cytotoxicity against tumor cell lines.

EXPERIMENTAL

General experimental. All regents were used as purchased from Acros, Aldrich and Fluka without further purification. Anhydrous CH_2Cl_2 was freshly distilled from CaH_2. For reactions carried out under Ar, the mixtures were degassed at high vacuum and purged with Ar at least three times. ^{1}H- and ^{13}C-NMR spectra were recorded at ambient temperature with a Bruker Avance 300 (300 MHz for ^{1}H and 75.5 MHz for ^{13}C) instrument in which TMS was used as internal standard for all measurements. MS data were recorded by using a VG Auto spec-3000 spectrometer or a Finnigan MAT 90 instrument. IR spectra were measured as KBr pellets by using a Bio-Rad FTS-135 spectrometer. UV spectra were obtained on a UV 2401 PC spectrometer. Optical rotations were measured by using a Horiba SEPA-300 polarimeter. Column chromatography (CC) was performed by using silica gel (200–300 mesh; Qingdao Marine Chemical, China). The starting material eriocalyxin B was isolated from the leaves of *Isodon eriocalyx var. laxiflora* according to the previously described method [17].

Synthesis of laxiflorin J from eriocalyxin B. To a solution of eriocalyxin B (1 equiv.) in dry CH_2Cl_2 was added AgOTf (3 equiv.) and Ag_2CO_3 (3.5 equiv.) at r. t. The mixture was stirred until all the starting material was consumed and then H_2O was added. Extraction of the aq. phase with AcOEt, drying (Na_2SO_4), removal of the solvent *in vacuo* and CC of the residue on silica gel [petroleum ether- AcOEt (8.5:1.5)] yielded laxiflorin J as white powder.

Compound 1 (laxiflorin J). $[\alpha]_D^{19} = -220.00$ (C = 0.075 in $CHCl_3$); ^{1}H-NMR (300 MHz,$CDCl_3$): δ = 6.10 (1H, s), 5.39 (1H, s), 4.69 (1H, d, J = 9.54 Hz), 4.57 (1H, d, J = 11.7 Hz), 4.12 (1H, d, J = 9.54 Hz), 3.73–3.71 (2H, br), 3.13 (1H, s), 2.83 (1H, d, J = 8.1 Hz), 2.71–2.68 (2H, m), 2.09 (1H, d, J = 11.7 Hz), 1.98–1.56 (6H, m), 1.50 (3H, s), 1.09 (3H, s) ppm; ^{13}C-NMR (75 MHz,$CDCl_3$): δ = 207.66, 206.83, 200.11, 146.99, 117.46, 77.00, 71.34, 61.50, 59.20, 51.96, 50.68, 41.83, 38.69, 37.70, 36.96, 36.80, 31.35, 29.26, 23.01, 20.51 ppm; IR (KBr): υ_{max} = 3441.45, 2926.88, 2882.34, 1748.40, 1644.38, 1454.49, 1107.72, 1065.01, 936.10 cm^{-1}; UV ($CHCl_3$) $\lambda_{max}(\log\epsilon)$= 241.60(3.85) nm; MS (EI): *m/z*: 344; MS (HR-ESI): *m/z*: calcd for $C_{20}H_{24}O_5Na$: 367.1521; found: 367.1525 $[M+Na]^+$.

General procedure for the synthesis of compounds 2–10. A solution of eriocalyxin B (1 equiv.) in dry CH_2Cl_2 was added to a suspension of powered, AgOTf (3 equiv.) and Ag_2CO_3 (3.5 equiv.) in dry CH_2Cl_2 at r. t.. A soln. of a iodoalkane (10 equiv.) in dry CH_2Cl_2 was then added, the flask was covered with an aluminum foil, and the mixture allowed to stir for 3 d at r. t. After filtration through celite, concentration *in vacuo* and CC afforded the pure products. All compounds were characterized by ^{1}H-NMR, ^{13}C-NMR, IR, MS, UV, and optical rotations.

Compound 2. $[\alpha]_D^{21} = -266.89$ (C = 0.148 in $CHCl_3$); ^{1}H-NMR (300 MHz,$CDCl_3$): δ = 6.05 (1H, s), 5.33 (1H, s), 4.68 (1H, d, J = 9.6 Hz), 4.11 (2H, d, J = 11.7 Hz), 3.80 (1H, d, J = 8.1 Hz), 3.69–3.67 (1H, m), 3.30 (1H, d, J = 8.1 Hz), 3.13 (1H, br), 2.81 (1H, d, J = 7.8 Hz), 2.69–2.66 (2H, m), 2.0 (1H, d, J = 11.7 Hz), 1.91–1.51 (8H, m), 1.36 (3H, s), 1.08 (3H, s), 0.99 (3H, t, J =7.2 Hz) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 207.75, 204.34, 200.29, 147.27, 116.75, 78.65, 77.43, 72.41, 61.79, 60.07, 52.04, 48.67, 41.71, 38.56, 37.86, 36.80, 36.65, 31.42, 29.33, 23.29, 22.75, 20.60, 10.74 ppm; IR (KBr): υ_{max} = 3430.45, 2958.48, 2930.53, 2879.98, 1745.25, 1726.26, 1705.53, 1645.22, 1460.67, 1122.06, 1060.01, 939.14 cm^{-1}; UV ($CHCl_3$) $\lambda_{max}(\log\epsilon)$= 241.40(3.81) nm; MS (EI): *m/z*:386; MS(HR-ESI): *m/z*: calcd for $C_{23}H_{31}O_5$: 387.2171; found: 387.2170 $[M+H]^+$.

Compound 3. $[\alpha]_D^{20} = -190.65$ (C = 0.132 in $CHCl_3$); ^{1}H-NMR (300 MHz,$CDCl_3$): δ = 6.05 (1H, s), 5.33 (1H, s), 4.68 (1H, d, J = 9.6 Hz), 4.11 (2H, d, J = 11.8 Hz), 3.83 (1H, d, J = 7.8 Hz), 3.68–3.67(1H, m), 3.30 (1H, d, J = 7.8 Hz), 3.10 (1H, br), 2.81 (1H, d, J = 7.5 Hz), 2.69–2.66 (2H, m), 2.03–1.36 (11H, m), 1.36 (3H, s), 1.08 (3H, s), 0.95 (3H, t, J = 7.5 Hz) ppm; ^{13}C-NMR (75 MHz,$CDCl_3$): δ = 207.79, 204.39, 200.36, 147.25, 116.80, 78.66, 77.45, 70.51, 61.78, 60.06, 52.02, 48.64, 41.71, 38.56, 37.86, 36.78, 36.63, 32.15, 31.40, 29.35, 22.75, 20.60, 19.35, 13.89 ppm; IR (KBr): υ_{max} = 3432.26, 2960.12, 2933.02, 2875.71, 1746.06, 1730.56, 1707.07, 1644.85, 1459.54, 1119.87, 1062.17, 936.39 cm^{-1}; UV ($CHCl_3$) λ_{max} ($\log\epsilon$)= 241.20 (3.72) nm; MS (EI): *m/z m/z* : 400; MS (HR-ESI): *m/z*: calcd for $C_{24}H_{32}O_5Na$: 423.2147; found: 423.2141 $[M+Na]^+$.

Compound 4. $[\alpha]_D^{21}$ = −208.12 (*C* = 0.197 in $CHCl_3$); ^{1}H-NMR (300 MHz,$CDCl_3$): δ = 6.05 (1H, s), 5.33 (1H, s), 4.68 (1H, d, *J* = 9.6 Hz), 4.12 (2H, d, *J* = 11.8 Hz), 3.86 (1H, d, *J* = 7.8 Hz), 3.69–3.67 (1H, m), 3.30 (1H, d, *J* = 7.8 Hz), 3.10 (1H, br), 2.81 (1H, d, *J* = 7.5 Hz), 2.69–2.66 (2H, m), 2.04–1.51 (10H, m), 1.36 (3H, s), 1.08 (3H, s), 0.91 (6H, dd, *J* = 7.3 Hz) ppm; ^{13}C-NMR (75 MHz,$CDCl_3$): δ = 207.73, 204.38, 200.33, 147.28, 116.74, 78.72, 77.44, 69.22, 61.80, 60.07, 52.03, 48.68, 41.72, 39.01, 38.57, 37.85, 36.81, 36.66, 31.90, 31.42, 29.36, 24.94, 22.67, 22.66, 20.60 ppm; IR (KBr): υ_{max} = 3432.22, 2956.07, 2930.18, 2871.82, 1749.85, 1729.35, 1644.20, 1459.35, 1115.84, 1063.74, 935.25 cm^{-1}; UV ($CHCl_3$) $\lambda_{max}(\log\epsilon)$= 241.60(3.69) nm; MS (EI): *m/z*: 414; MS (HR-ESI): *m/z*: calcd for $C_{25}H_{34}O_5Na$: 437.2303; found: 437.2303 $[M+Na]^+$.

Compound 5. $[\alpha]_D^{19}$ = −186.59 (*C* = 0.276 in $CHCl_3$); ^{1}H-NMR (300 MHz,$CDCl_3$): δ = 6.05 (1H, s), 5.33 (1H, s), 4.68 (1H, d, *J* = 9.6 Hz), 4.10 (2H, d, *J* = 11.8 Hz), 3.83 (1H, d, *J* = 7.8 Hz), 3.69–3.67(1H, m), 3.30 (1H, d, *J* = 7.8 Hz), 3.10 (1H, br), 2.81 (1H, d, *J* = 7.5 Hz), 2.70–2.66 (2H, m), 2.03–1.57 (13H, m), 1.37 (3H, s), 1.09 (3H, s), 0.87–0.85 (3H, d, *J*=7.5) ppm; ^{13}C-NMR (75 MHz,$CDCl_3$): δ = 207.70, 204.30, 200.25, 147.30, 116.68, 78.67, 77.42, 70.81, 61.80, 60.08, 52.05, 48.69, 41.71, 38.56, 37.86, 36.82, 36.66, 31.43, 29.67, 29.34, 28.29, 22.75, 22.44, 20.60, 14.05 ppm; IR (KBr): υ_{max} = 3423.31, 2954.84, 2925.59, 2855.33, 1751.71, 1728.01, 1643.93, 1459.47, 1115.95, 1063.35, 935.58 cm^{-1}; UV ($CHCl_3$) $\lambda_{max}(\log\epsilon)$= 241.00(3.67) nm; MS(EI): *m/z*: 414; MS (HR-ESI): *m/z*: calcd for $C_{25}H_{34}O_5Na$: 437.2303; found: 437.2312 $[M+Na]^+$.

Compound 6. $[\alpha]_D^{21}$ = −130.21 (*C* = 0.192 in $CHCl_3$); ^{1}H-NMR (300 MHz,$CDCl_3$): δ = 6.05 (1H, s), 5.33 (1H, s), 4.68 (1H, d, *J* = 9.6 Hz), 4.10 (2H, d, *J* = 11.6 Hz), 3.83 (1H, d, *J* = 7.8 Hz), 3.68–3.66 (1H, m), 3.29 (1H, d, *J* = 7.8 Hz), 3.10 (1H, br),2.81 (1H, d, *J* = 7.3 Hz), 2.69–2.63(2H, m), 2.02–1.46 (15H, m), 1.36 (3H, s), 1.08 (3H, s), 0.89–0.84 (3H, t, *J* = 7.5 Hz) ppm; ^{13}C-NMR (75 MHz,$CDCl_3$): δ = 207.74, 204.35, 200.32,147.27, 116.75, 78.68, 77.44, 70.87, 61.79, 60.07, 52.03, 48.66, 41.71, 38.57, 37.86, 36.80,36.65, 31.62, 31.42, 30.47, 30.01, 29.33, 27.04, 25.80, 22.75, 14.06 ppm; IR (KBr): υ_{max} = 3432.53, 2932.13, 2870.13, 1751.88, 1729.82, 1643.25, 1459.85, 1115.03, 1064.07, 935.91 cm^{-1}; UV ($CHCl_3$) $\lambda_{max}(\log\epsilon)$= 242.80(3.33)nm; MS (EI): *m/z*: 428; MS(HR-ESI): *m/z*: calcd for $C_{26}H_{36}O_5Na$: 451.2460; found: 451.2468 $[M+Na]^+$.

Compound 7. $[\alpha]_D^{21}$ = −210.35 (*C* = 0.145 in $CHCl_3$); ^{1}H-NMR (300 MHz,$CDCl_3$): δ = 6.05 (1H, s), 5.33 (1H, s), 4.68 (1H, d, *J* = 9.6 Hz), 4.10 (2H, d, *J* = 11.6 Hz), 3.83 (1H, d, *J* = 7.8 Hz), 3.68–3.67 (1H, m), 3.29 (1H, d, *J* = 7.8 Hz), 3.10 (1H, br),2.81 (1H, d, *J* = 7.3 Hz), 2.69–2.66(2H, m), 2.02–1.46 (11H, m), 1.36 (3H, s), 1.31–1.27 (6H, m), 1.08 (3H, s), 0.89–0.84 (3H, t, *J* = 7.5 Hz) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 207.74, 204.33, 200.29, 147.28, 116.73, 78.68, 77.44, 70.87, 61.80, 60.07, 52.04, 48.67, 41.72, 38.57, 37.86, 36.81, 36.66, 31.76, 31.42, 30.06, 29.34, 29.10, 26.11, 22.76, 22.58, 20.60, 14.06 ppm; IR (KBr): υ_{max} = 3432.43, 2930.37, 2857.33, 1751.49, 1728.82, 1644.21, 1457.93, 1115.94, 1064.17, 935.55 cm^{-1}; UV ($CHCl_3$) $\lambda_{max}(\log\epsilon)$= 241.40(3.76) nm; MS (EI): *m/z*: 442; MS (HR-ESI): *m/z*: calcd for $C_{27}H_{38}O_5Na$: 465.2616; found: 465.2605 $[M+Na]^+$.

Compound 8. $[\alpha]_D^{20}$ = −184.07 (*C* = 0.182 in $CHCl_3$); ^{1}H-NMR (300 MHz,$CDCl_3$): δ = 6.03 (1H, s), 5.32 (1H, s), 4.67 (1H, d, *J* = 9.6 Hz), 4.09 (2H, d, *J* = 12 Hz), 3.81 (1H, d, *J* = 7.8 Hz), 3.67–3.66 (1H, m), 3.30 (1H, d, *J* = 7.8 Hz), 3.10 (1H, br), 2.79 (1H, d, *J* = 7.5 Hz), 2.68–2.64 (2H, m), 2.06–1.37 (11H, m), 1.35 (3H, s), 1.33–1.25 (8H, m), 1.07 (3H, s), 0.87–0.83 (3H, t, *J* = 7.0 Hz) ppm; ^{13}C-NMR (75 MHz,$CDCl_3$): δ = 207.80, 204.39, 200.38, 147.26, 116.77, 78.67, 77.39, 70.86, 61.76, 60.05, 52.01, 48.63, 41.70, 38.57, 37.85, 36.77, 36.62, 31.80, 31.39, 30.04, 29.37, 29.21, 29.20, 26.13, 22.75, 22.63, 20.60, 14.09 ppm; IR (KBr): υ_{max} = 3432.90, 2928.95, 2856.15, 1751.67, 1729.45, 1643.25, 1459.51, 1115.82, 1064.24, 935.55 cm^{-1}; UV ($CHCl_3$) $\lambda_{max}(\log\epsilon)$= 241.20(3.64) nm; MS(EI): *m/z*: 456; MS (HR-ESI): *m/z*: calcd for $C_{28}H_{40}O_5Na$: 479.2773; found: 479.2772 $[M+Na]^+$.

Compound 9. $[\alpha]_D^{20}$ = −134.78 (*C* = 0.230 in $CHCl_3$); ^{1}H-NMR (300 MHz,$CDCl_3$): δ = 6.04 (1H, s), 5.32 (1H, s), 4.67 (1H, d, *J* = 9.6 Hz), 4.09 (2H, d, *J* = 12 Hz), 3.81 (1H, d, *J* = 7.8 Hz), 3.67–3.66(1H, m), 3.30 (1H, d, *J* = 7.8 Hz), 3.10 (1H, br), 2.79 (1H, d, *J* = 7.5 Hz), 2.68–2.65(2H, m), 2.06–1.37 (10H, m), 1.35 (3H, s), 1.33–1.25 (11H, m), 1.07 (3H, s), 0.87–0.83 (3H, t, *J* = 7.0 Hz) ppm; ^{13}C-NMR (75 MHz,$CDCl_3$): δ = 207.79, 204.39, 200.37, 147.26, 116.78, 78.68, 77.40, 70.87, 61.77, 60.06, 52.02, 48.63, 41.71, 38.57, 37.85, 36.79, 36.64, 31.86, 31.40, 30.06, 29.50, 29.51, 29.35, 29.25, 26.14, 22.76, 22.66, 20.60, 14.10 ppm; IR (KBr): υ_{max} = 3432.58, 2927.84, 2855.63, 1751.56, 1730.44, 1642.09, 1460.09, 1115.19, 1064.26, 935.91 cm^{-1}; UV ($CHCl_3$) $\lambda_{max}(\log\epsilon)$= 241.60 (3.39) nm; MS(EI): *m/z*: 470; MS (HR-ESI): *m/z*: calcd for $C_{29}H_{42}O_5Na$: 493.2929; found: 493.2917 $[M+Na]^+$.

Compound 10. $[\alpha]_D^{21}$ = −161.29 (*C* = 0.093 in $CHCl_3$); ^{1}H-NMR (300 MHz,$CDCl_3$): δ = 6.05 (1H, s), 5.33 (1H, s), 4.68 (1H, d, *J* = 9.6 Hz), 4.10 (2H, d, *J* = 11.6 Hz), 3.82 (1H, d, *J* = 7.6 Hz), 3.69–3.67 (1H, m), 3.30 (1H, d, *J* = 7.6 Hz), 3.10 (1H, br), 2.80 (1H, d, *J* = 7.5 Hz), 2.69–2.66(2H, m), 2.06–1.37 (11H, m), 1.36 (3H, s), 1.33–1.25 (12H, m), 1.08 (3H, s), 0.87–0.84 (3H, t, *J* = 7.5 Hz) ppm; ^{13}C-NMR (75 MHz,$CDCl_3$): δ = 207.74, 204.33, 200.29, 147.28, 116.73, 78.68, 77.43, 70.88, 61.80, 60.07, 52.04, 48.66, 41.72, 38.56, 37.86, 36.81, 36.66, 31.88, 31.42, 30.06, 29.54(3C), 29.43, 29.34, 26.14, 22.76, 22.66, 20.60, 14.08 ppm; IR (KBr): υ_{max} = 3432.91, 2926.04, 2854.53, 1751.64, 1688.81, 1459.64, 1116.20, 1064.46, 935.82 cm^{-1}; UV ($CHCl_3$) $\lambda_{max}(\log\epsilon)$= 241.60 (3.65) nm; MS(EI): *m/z*: 484; MS (HR-ESI): *m/z*: calcd for $C_{30}H_{44}O_5Na$: 507.3086; found: 507.3092 $[M+Na]^+$.

Inhibitory activities of compounds 1–10 against five tumor cell lines. Three tumor cell lines, including BEL-7402, A-549 and HT-29, were used to evaluate the cytotoxic activities of compounds 1–10. The growth inhibitory effect of compounds on the cell lines of BEL-7402, A-549 and HT-29 were measured by Sulforhodamine B (SRB) assay. Briefly, cells were seeded at the density of 6000 cells/well in 96-well plates and allowed to attach overnight. The cells were treated in triplicate with grade concentrations of compounds at 37°C for 72 h and were then fixed with 10% trichloroacetic acid and incubated at 4°C for 1 h. The culture plates were washed and dried, and SRB solution (0.4 wt %/vol in 1% acetic acid) was added and incubated for an additional 15 min. The culture plates were washed and dried again, the bound cell stains were solubilized with Tris buffer, and the optical density of each well was read on a plate reader at the wavelength of 515 nm. The growth inhibitory rate of treated cells was calculated by the formula of [1-(A515 treated/A515 control)]× 100%. The inhibitory activity was finally expressed in IC_{50} (the compound concentration

required for 50% growth inhibition of tumor cells), which was calculated by using Logit method. For HL-60 and MOLT-4 human cancer cell lines, the growth inhibition was tested by MTT assay (microculture tetrazolium 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide). Briefly, cells in 100 μL of culture medium were plated in each well of 96-well plates. Cells were treated in triplicate with grade concentrations of compounds at 37°C for 72 h. A 20-μL aliquot of MTT solution (5 mg/mL) was added directly to all the appropriate wells. The culture was then incubated for 4 h, and then 100 μL of "triplex solution"(10% SDS/5% isobutanol/12 mM HCl) was added. The plates were incubated at 37°C overnight and then measured by using a plate reader at 570 nM. The mean IC_{50} was determined from the results of three independent tests.

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