Synthesis and antitumor evaluation of neolaxiflorin B inspired compounds

This article was published in the following Dove Press journal:
Drug Design, Development and Therapy

Jing-Ping Liu¹
Ye-Zhi Xiao¹
Yu Hu¹
Xiao-Nian Li²
Ming-Jiang Wu³
Yong Zhao¹
Yan Zhao¹
Ze-Jin Ma¹
Jing Shen¹

¹Department of Chemistry, Yunnan Normal University, Kunming 650092, People's Republic of China; ²State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Kunming 650201, People's Republic of China; ³Criminal Science and Technology Key Lab of Yunnan Police College, Kunming 650223, People's Republic of China

Background: Neolaxiflorin B is derived from ent-kaurane like laxiflorin J and eriocalyxin B with a relatively low potency as an antitumor agent. During preliminary structure–activity relationship studies, the α,β -unsaturated ketone (enone) system is an important active group.

Methods: Seven neolaxiflorin B derivatives containing α,β -unsaturated ketone moieties were synthesized. In vitro, activity was evaluated against three human tumor cell lines and a rat myogenic cell line (HepG2, NSCLC-H292, SNU-1040, and L6, respectively) by MTT assay.

Results: Compound **15** appeared a promising antitumor lead due to its cytotoxic potency and relatively high selectivity, with an SI value of 13.14. Flow cytometry analysis was conducted to show that NSCLC-H292 cells were blocked in the G0/G1 phase in the presence of compound **15**, thus inhibiting the proliferation of tumor cells.

Conclusion: This study has revealed that compound **15** is a promising antitumor lead due to the cytotoxic potencies and the high selectivity it displayed when compared to natural counterparts.

Keywords: neolaxiflorin B inspired compounds, unsaturated ketone, antitumor activity

Introduction

In the past decade, over 1000 ent-kaurane diterpenoids have been isolated and identified from the *Isodon* genus.¹ According to recent reports, these diterpenoids possess a broad range of bioactivities, including anti-bacteria,² cytotoxic, anti-HIV, anti-inflammatory, and anti-fungal activity.³ Laxiflorin, as one of the members of the diterpenoids, is assumed to share the same biogenic precursor, ent-kaurene (1), with eriocalyxin B and longikaurin A (Figure 1).⁴ Biosynthetically, there is a possibility of mutual transformation among these diterpenoids.

According to the literature,⁵ neolaxiflorin B is derived from ent-kaurane like laxiflorin J and eriocalyxin B (Figure 2) with a relatively low potency as an antitumor agent. Although low in potency, its structure is novel, containing a unique scaffold, which we used as a basic template to synthesize novel natural product-like neolaxiflorin B derivatives to develop safe and effective antitumor agents.

During preliminary structure–activity relationship studies, the removal of the α,β -unsaturated ketone (enone) system in the D-ring decreased its antitumor activity.⁶ Meanwhile, mounting evidence also demonstrates that dienone compounds with double α,β -unsaturated ketone functionalities, such as eriocalyxin B (3), can undergo two successive alkylations at the β -positions by reacting with cellular thiols during biological cascade reactions.⁷ We, therefore, aimed to synthesize a neolaxiflorin B-like scaffold with double α,β -unsaturated ketone.

Correspondence: Jing-Ping Liu; Jing Shen
Department of Chemistry, Yunnan Normal University, No. 298, 121st Street, Yunnan, Kunming 650092, People's Republic of China
Tel/Fax +86 8 716 513 2144
Email LiuJP72@163.com; shenjingbox0225@hotmail.com

Figure 1 Structures of some *Isodon* diterpenoids and their biogenic precursor.

Figure 2 Structure of neolaxiflorin B.

Materials and methods

Materials and methods Information and methods

All reagents were used as purchased from Energy Chemical, Tansoole, Acros and Aldrich without further purification. Anhydrous CH_2Cl_2 was freshly distilled from CaH_2 , and THF was dried by Na. For those reactions that need to be performed in argon, at least three argon exchanges are required. The course of reaction was monitored by TLC. ^1H -NMR and ^{13}C -NMR spectra were recorded at ambient temperature with a Bruker Avance 300 (300 MHz for ^1H and 75.5 MHz for ^{13}C ; Bruker, Germany) or a Bruker Avance 400 (400 MHz for ^1H and 100 MHz for ^{13}C ; Bruker, Germany) 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. Characterization data for compounds are available free of charge via [supporting information](#) of associated content. All melting points are uncorrected.

CC was performed by using silica gel (100–200 mesh; Qingdao Marine Chemical, China)

4-(hydroxymethyl)-3-isopropylcyclopent-2-enone (6)

To a solution of **5** (0.248 g, 2 mmol, 1eq) in *n*-hexane (5 mL) at room temperature under argon was added $\text{Co}_2(\text{CO})_8$ (0.684 g, 2 mmol, 1eq). After 1 hr, the mixture was filtered through a short pad of neutral Al_2O_3 washed with *n*-hexane. The filtrate was added neutral Al_2O_3 (10 g) and concentrated under vacuum. To remove traces of oxygen, the residue is kept at 0.08 MPa for 15 mins, then the flask is flushed with argon. The procedure is repeated four times, further heated for 2 hrs at 50°C . The desired product **6** (70%) was achieved by silica gel column chromatography as a pale yellow liquid: ^1H NMR (300 MHz, CDCl_3) δ =5.83(s, 1H), 3.82–3.60 (m, 3H), 3.03 (s, 1H), 2.58 (m, 1H), 2.45–2.22 (m, 2H), 1.10 (dd, J =7.8 Hz, 6H) ppm; ^{13}C NMR (75.5 MHz, CDCl_3) δ =210.11, 189.49, 128.58, 62.56, 44.40, 39.32, 29.57, 21.37, 20.44 ppm; IR (KBr): ν_{max} =3388.32, 2964.75, 2874.24, 1718.07, 1676.32, 1603.50 cm^{-1} ; MS (ESI): m/z : 177; HRMS (ESI) Calcd for $[\text{C}_9\text{H}_{14}\text{O}_2+\text{Na}]^+$: 177.0891; found: 177.0888 $[\text{M}+\text{Na}]^+$.

Compound 7

To a solution of **6** (0.38 g, 2.47 mmol, 1eq) and imidazole (0.34 g, 4.94 mmol, 2eq) in CH_2Cl_2 (15 mL) at room temperature was added *tert*-Butyldimethylsilyl chloride (0.56 g, 3.71 mmol, 1.5eq). The solution was stirred overnight, and then the mixture was added water and extracted with CH_2Cl_2 . The combined organic layers were dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography to provide **7** (96%) as colorless liquid: ^1H NMR (300 MHz, CDCl_3) δ =5.93(s, 1H), 3.71(d, J =4.5 Hz, 2H), 3.07(m, 1H), 2.66–2.62(m, 1H), 2.51–2.43(m, 1H), 2.30–2.23(m, 1H), 1.17(d, J =6.9 Hz, 3H), 1.14(d, J =7.2 Hz, 3H), 0.83(s, 9H), 0.01(s, 6H) ppm; ^{13}C NMR (75.5 MHz, CDCl_3) δ =209.19, 188.59, 128.81, 63.50, 44.42, 39.51, 29.74, 25.83, 21.76, 20.74, 18.24, 18.11, –3.46, –5.48 ppm; IR (KBr): ν_{max} =2959.33, 2928.81, 2874.91, 2857.09, 1717.84, 1681.76, 1605.36, 1258.86, 1100.42 cm^{-1} ; MS (ESI): m/z : 291; HRMS (ESI) Calcd for $[\text{C}_{15}\text{H}_{28}\text{O}_2\text{Si}+\text{Na}]^+$: 291.1756; found: 291.1758 $[\text{M}+\text{Na}]^+$.

Compound 8

Compound **8** is obtained as pale yellow liquid: ^1H NMR (300 MHz, CDCl_3) δ =7.43(d, J =9.9 Hz, 1H), 5.81(d, J =9.9 Hz, 1H), 5.26(s, 1H), 5.05(s, 1H), 4.19(q, J =7.2 Hz, 2H), 3.47(br.,

1H), 2.86(d, $J=95.4$ Hz, 1H), 2.52(d, $J=95.4$ Hz, 1H), 2.25(m, 2H), 1.25(t, $J=7.2$ Hz, 3H)ppm; ^{13}C NMR (75.5 MHz, CDCl_3) $\delta=197.515$, 172.761, 152.359, 143.136, 126.641, 113.126, 61.584, 57.960, 51.617, 43.207, 41.109, 14.120 ppm; IR (KBr): $\nu_{\text{max}}=2979.98$, 1729.06, 1685.40, 1280.90, 1222.04, 1193.09 cm^{-1} ; MS (ESI): m/z : 207; HRMS (ESI) Calcd for $[\text{C}_{12}\text{H}_{14}\text{O}_3+\text{H}]^+$: 207.1021; found: 207.1015 $[\text{M}+\text{H}]^+$.

Compound 9

To a stirred solution of **7** (0.27 g, 1 mmol, 1eq) and **8** (0.23 g, 1.1 mmol, 1.1eq) in dry THF under Ar at -78°C was added LiHMDS (2 mL, 2eq, 1.0 M in THF) over a period of 5 mins and stirring at -78°C was continued for 45 mins. The resulting solution was warmed slowly to room temperature, quenched with saturated NH_4Cl and extracted with Et_2O . The combined organic layers were dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography (EtOAc /petroleum ether, 1:10) to provide **9** (77%) as a crystal and its epimer **10** (9%) as a white powder (dr=9:1). **9** is obtained as crystal; m.p.:124–129 $^\circ\text{C}$; ^1H NMR (400 MHz, CDCl_3) $\delta=5.99$ (s, 1H), 5.07(s, 1H), 5.06(s, 1H), 4.18(q, $J=7.2$ Hz, 2H), 3.84(dd, $J_1=10$ Hz, $J_2=3.2$ Hz, 1H), 3.72(dd, $J_1=9.6$ Hz, $J_2=4$ Hz, 1H), 3.26(d, $J=4.8$ Hz, 1H), 3.07(d, $J=9.2$ Hz, 1H), 2.82–2.80(m, 2H), 2.71–2.46(m, 4H), 2.37–2.14(m, 2H), 1.67(d, $J=16.8$ Hz, 1H), 1.26(t, $J=7.2$ Hz, 3H), 1.17(d, $J=6.8$ Hz, 3H), 1.14(d, $J=6.8$ Hz, 3H), 0.78(s, 9H), 0.00(s, 3H), -0.01 (s, 3H)ppm; ^{13}C NMR (100 MHz, CDCl_3) $\delta=209.37$, 208.47, 190.18, 174.31, 145.78, 128.72, 109.93, 62.43, 61.12, 59.07, 53.17, 51.83, 47.30, 44.44, 42.67, 35.93, 29.67, 25.82, 25.74(3C), 21.80, 20.58, 18.14, 14.34, -5.62 , -5.63 ppm; IR (KBr): $\nu_{\text{max}}=2957.43$, 2929.36, 2885.29, 2857.41, 1733.92, 1713.56, 1689.57, 1655.97, 1615.37 cm^{-1} ; MS (ESI): m/z : 497; HRMS (ESI) Calcd for $[\text{C}_{27}\text{H}_{42}\text{O}_5\text{Si}+\text{Na}]^+$: 497.2699; found: 497.2698 $[\text{M}+\text{Na}]^+$.

Compound 10

Compound **10** is obtained as white powder; m.p.:123–128 $^\circ\text{C}$; ^1H NMR (400 MHz, CDCl_3) $\delta=5.83$ (s, 1H), 5.07(s, 1H), 4.91(s, 1H), 4.13(q, $J=7.2$ Hz, 2H), 3.86(dd, $J_1=10$ Hz, $J_2=2.8$ Hz, 1H), 3.71(dd, $J_1=10$ Hz, $J_2=3.6$ Hz, 1H), 3.33(d, $J=4.8$ Hz, 1H), 3.20(d, $J=12$ Hz, 1H), 2.79–2.68(m, 6H), 2.33(s, 1H), 2.26–2.21(m, 1H), 1.80(d, $J=16.8$ Hz, 1H), 1.24(t, $J=7.2$ Hz, 3H), 1.12(d, $J=6.8$ Hz, 3H), 1.07(d, $J=6.8$ Hz, 3H), 0.78(s, 9H), 0.00(s, 6H) ppm; ^{13}C NMR (100 MHz, CDCl_3) $\delta=207.54$, 206.18, 187.32, 174.88, 146.31, 128.91, 109.49, 60.96, 60.67, 59.82, 53.36, 52.97, 51.22, 44.20, 42.95, 36.33, 35.09, 29.28, 25.75, 21.76, 20.37, 18.14, 14.27, -5.47 , -5.61

ppm; IR (KBr): $\nu_{\text{max}}=2952.56$, 2927.55, 2896.84, 2854.55, 1719.85, 1694.22, 1653.50, 1613.23, 1472.54 cm^{-1} ; MS (ESI): m/z : 497; HRMS (ESI) Calcd for $[\text{C}_{27}\text{H}_{42}\text{O}_5\text{Si}+\text{Na}]^+$: 497.2699; found: 497.2700 $[\text{M}+\text{Na}]^+$.

Compound 11

To a stirred solution of **9** (0.47 g, 1 mmol, 1.0eq) in DCM, SeO_2 (0.1 g, 0.9 mmol, 0.9eq) was added in one portion, and a solution of *t*-BuOOH (0.55 mL, 3 mmol, 5.5 M in decane, 3eq) was added dropwise. The solution was vigorously stirred at room temperature overnight, and then the mixture was filtered through a short pad of silica gel washed with EtOAc . The filtrate was concentrated under vacuum and purified by flash column chromatography to provide allylic alcohol **11** (71%) as colorless liquid: ^1H NMR (400 MHz, CDCl_3) $\delta=6.00$ (s, 1H), 5.42(s, 1H), 5.33(s, 1H), 4.61(s, 1H), 4.26(q, $J=8$ Hz, 2H), 3.85(m, 1H), 3.72m, 1H), 3.34(d, $J=4.0$ Hz, 1H), 3.09(d, $J=8$ Hz, 1H), 2.69–2.38(m, 4H), 2.15–2.02(m, 3H), 1.60(d, $J=16$ Hz, 1H), 1.28(t, $J=8$ Hz, 3H), 1.17(d, $J=4$ Hz, 3H), 1.13(d, $J=8$ Hz, 3H), 0.77(s, 9H), 0.00(s, 3H), -0.02 (s, 3H)ppm; ^{13}C NMR (100 MHz, CDCl_3) $\delta=207.54$, 206.18, 187.32, 174.88, 146.31, 128.91, 109.49, 60.96, 60.67, 59.82, 53.36, 52.97, 51.22, 44.20, 42.95, 36.33, 35.09, 29.28, 25.75, 21.76, 20.37, 18.14, 14.27, -5.47 , -5.61 ppm; IR (KBr): $\nu_{\text{max}}=3435.15$, 2958.67, 2929.86, 2900.27, 2857.55, 1732.39, 1719.69, 1704.16, 1613.47, 1471.93 cm^{-1} ; MS (ESI): m/z : 513; HRMS (ESI) Calcd for $[\text{C}_{27}\text{H}_{42}\text{O}_6\text{Si}+\text{Na}]^+$: 513.2648; found: 513.2655 $[\text{M}+\text{Na}]^+$.

Compound 12

To a solution of **11** (0.49 g, 1 mmol, 1.0eq) in EtOAc (10 mL) was added IBX (0.56 g, 2 mmol, 2.0eq). The mixture was heated at 70°C for 4 hrs and filtered through a short pad of silica gel washed with EtOAc . The solvent was removed under reduced pressure and the residue was purified by flash column chromatography to provide **12** (50%) as colorless liquid: ^1H NMR (400 MHz, CDCl_3) $\delta=6.13$ (s, 1H), 5.95(s, 1H), 5.53(s, 1H), 4.18(q, $J=8$ Hz, 2H), 3.70(m, 3H), 3.04(m, 1H), 2.79–2.54(m, 5H), 2.29(t, $J=4$ Hz, 1H), 2.07(m, 1H), 1.26(t, $J=8$ Hz, 3H), 1.16(t, $J=4$ Hz, 6H), 0.8(s, 9H), 0.05(s, 6H)ppm; ^{13}C NMR (100 MHz, CDCl_3) $\delta=206.98$, 204.92, 198.62, 186.14, 167.70, 141.98, 127.80, 118.79, 62.55, 60.67, 60.26, 53.62, 50.22, 48.93, 40.27, 37.66, 31.54, 28.67, 24.71, 20.51, 19.50, 17.13, 13.02, -6.69 , -6.74 ppm; IR (KBr): $\nu_{\text{max}}=2958.94$, 2931.02, 2903.23, 2857.93, 1724.34, 1613.69, 1471.01 cm^{-1} ; MS (ESI): m/z : 511; HRMS (ESI) Calcd for $[\text{C}_{27}\text{H}_{40}\text{O}_6\text{Si}+\text{Na}]^+$: 511.2492; found: 511.2496 $[\text{M}+\text{Na}]^+$.

General procedure for deprotection of silyl ether

HClO₄-SiO₂ (0.5 mmol/g, 50 mg) was added to a stirred solution of silyl ether substrate (1 mmol) in CH₂Cl₂ (5 mL) at room temperature. After complete conversion and filtration to remove the solid, saturated aqueous solution of NaHCO₃ was added and separated. The aqueous solution was extracted with CH₂Cl₂ (5 mL×3). The organic layer was combined, washed with brine (5 mL), dried over MgSO₄, and concentrated under reduced pressure. The residue was isolated through short column chromatography on silica gel, which was eluted with ethyl acetate-petroleum to give the target products.

Compound 13

Compound **13** is obtained as colorless liquid: ¹H NMR (400 MHz, CDCl₃) δ=6.04(s, 1H), 5.08(s, 1H), 5.07(s, 1H), 4.22(q, *J*=5.76 Hz, 2H), 3.90(dd, *J*₁=2.96 Hz, *J*₂=8.88 Hz, 1H), 3.68(dd, *J*₁=4.64 Hz, *J*₂=8.88 Hz, 1H), 3.28(d, *J*=4.12 Hz, 1H), 3.05–2.83(m, 4H), 2.66–2.50(m, 4H), 2.29–2.17(m, 3H), 1.29(t, *J*=5.76 Hz, 3H), 1.20(d, *J*=5.28 Hz, 3H), 1.18(d, *J*=5.64 Hz, 3H)ppm; ¹³C NMR (100 MHz, CDCl₃) δ=208.94, 208.07, 189.65, 175.40, 145.51, 128.99, 109.95, 63.68, 61.56, 58.77, 53.37, 52.65, 47.71, 44.56, 42.26, 35.70, 35.35, 29.86, 21.60, 20.59, 14.18ppm; IR (KBr): ν_{max} =3434.28, 3077.84, 2967.18, 2935.97, 2876.31, 1716.22, 1701.69, 1610.55, 1467.79 cm⁻¹; MS (ESI): *m/z*: 383; HRMS (ESI) Calcd for ([C₂₁H₂₈O₅i]+Na)⁺: 383.1834; found: 383.1837 [M+Na]⁺.

Compound 14

Compound **14** is obtained as colorless liquid: ¹H NMR (400 MHz, CDCl₃) δ=6.08(s, 1H), 5.46(s, 1H), 5.36(s, 1H), 4.66(s, 1H), 4.30(q, *J*=5.72 Hz, 2H), 4.12(q, *J*=5.72 Hz, 2H), 3.91(dd, *J*₁=2.96 Hz, *J*₂=8.88 Hz, 1H), 3.76(dd, *J*₁=4 Hz, *J*₂=8.84 Hz, 1H), 3.37(d, *J*=4.2 Hz, 1H), 3.11(d, *J*=7.4 Hz, 1H), 2.83–2.36(m, 5H), 2.15(m, 2H), 1.34(t, *J*=5.68 Hz, 3H), 1.21(d, *J*=5.32 Hz, 3H), 1.18(d, *J*=5.64 Hz, 3H)ppm; ¹³C NMR (100 MHz, CDCl₃) δ=208.42, 207.70, 189.86, 173.27, 149.67, 129.13, 114.97, 78.91, 63.00, 61.72, 60.42, 57.00, 52.22, 47.11, 40.61, 35.56, 32.16, 29.79, 21.63, 20.59, 14.20ppm; IR (KBr): ν_{max} =3434.70, 3080.00, 2967.55, 2933.39, 2877.04, 1716.14, 1608.85, 1467.55 cm⁻¹; MS (ESI): *m/z*: 399; HRMS (ESI) Calcd for ([C₂₁H₂₈O₆i]+Na)⁺: 399.1784; found: 399.1788 [M+Na]⁺.

Compound 15

Compound **15** is obtained as colorless liquid: ¹H NMR (400 MHz, CDCl₃) δ=6.16(s, 1H), 5.98(s, 1H), 5.55(s, 1H), 4.20(q,

J=5.72 Hz, 2H), 3.88(dd, *J*₁=3.2 Hz, *J*₂=8.52 Hz, 1H), 3.63(dd, *J*₁=5.04 Hz, *J*₂=8.52 Hz, 1H), 3.02–2.82(m, 3H), 2.63–2.38(m, 5H), 2.16(m, 2H), 1.30(t, *J*=5.72 Hz, 3H), 1.18(d, *J*=5.32 Hz, 3H), 1.16(d, *J*=5.64 Hz, 3H)ppm; ¹³C NMR (100 MHz, CDCl₃) δ=207.98, 206.55, 199.67, 186.67, 169.01, 143.03, 129.05, 119.95, 63.77, 61.78, 61.07, 54.58, 51.33, 50.18, 41.52, 38.92, 32.79, 29.82, 21.47, 20.57, 14.04ppm; IR (KBr): ν_{max} =3401.07, 2968.23, 2919.88, 2876.52, 2850.37, 1715.33, 1693.68, 1642.11, 1608.62, 1466.21 cm⁻¹; MS (ESI): *m/z*: 397; HRMS (ESI) Calcd for ([C₂₁H₂₆O₆i]+Na)⁺: 397.1627; found: 397.1629 [M+Na]⁺.

Cell culture and MTT test

Cells were purchased from the Wuxi Innovatbio Medicine Technology Co. Ltd (Wuxi, China) and were maintained at 37°C under the atmosphere of 5% CO₂. The cells of human non-small lung cancer (H292) and colon cancer cell line (SNU-1040) cells were cultured in RPMI-medium and others (rat myoblasts and HepG2) were cultured in dulbecco's modified eagle medium. Ten percent fetal bovine serum (Tianhang Biotechnology Co., Ltd., Zhejiang, China.) and 1% antibiotics (100 U/mL penicillin and 100 mg/mL streptomycin) was supplemented. Cells are inoculated in 96-well plate and cultured for 24 hrs. After attaching the culture bottle wall, the cells were divided into three groups, 1) control group; 2) positive control group; 3) experiment group. The administration concentrations of Cisplatin and compounds were 0.1 μM–10 μmol/L. After 24 hrs, 10% MTT was added and cells were cultured for 3.5 hrs. Then, the culture medium was discarded and 150 μL DMSO was added. Eventually, after incubation for 10 mins by shaking in the dark at room temperature, the absorbance was detected at 490 nm using a microplate reader, and the difference was analyzed by prism 7 software.

Flow cytometry

Cells were cultured in a six well plate and administrated with DTT and compound **15** for 48 hrs. After that, cells were digested and transferred into polyethylene pipe, followed by a centrifugation at 1000 for 5 mins. Then, 1 mL of precooled PBS was given to suspend the cells again. After another centrifugation, PBS was discarded and 70% ethanol was added to fix the cells overnight at 4°C. After rinsing with PBS, the cells were added to propidium iodide staining solution according to instructions. (Beyotime, China). The red fluorescence of the cells (1×10⁵) was detected at 488 nm wavelength by flow cytometer (BD ACCURI C6 PLUS).

Results and discussion

We constructed dienone functionality in the A and D-ring and deleted the complex spiral B-ring moiety of neolaxiflorin B. Compound **6** was obtained through the Pauson-Khand reaction. Starting material **5** was treated with 10 mol% Chlorobis (ethylene) rhodium (I) dimer ($[\text{RhCl}(\text{COD})]_2$) under CO at 1 atm in toluene at 100°C,⁸ but the desired product was not observed. Treatment of **5** with Molybdenum hexacarbonyl ($\text{Mo}(\text{CO})_6$) was also unsuccessful.⁹ We found that the conditions using classical dicobalt species Octacarbonyldicobalt ($\text{Co}_2(\text{CO})_8$) for the Pauson-Khand reaction, however, were successful. We evaluated the Pauson-Khand reaction conditions for the synthesis of compound **6** (Table 1). The optimum conditions for **6** were achieved using the following procedure: Compound **5** was mixed with stoichiometric $\text{Co}_2(\text{CO})_8$ in *n*-hexane under an inert argon atmosphere at rt. After 1 hr, neutral Al_2O_3 was added, and the solvent was removed using a rotary evaporator. The residue was incubated at 0.08 MPa for 15 mins at room temperature, then the flask was flushed with argon. The 0.08 MPa and argon flush steps were repeated four times and further heated at 50°C for 2 hrs. The desired product **6** was achieved by silica gel column chromatography.

A silylation reaction of compound **6** was used to produce compound **7**. Deprotonation of compound **7** with Lithium bis(trimethylsilyl)amide (LiHMDS), followed by reaction with compound **8**¹⁰ led to product **9** as the major product, along with the isomer **10** in 86% combined yield

Table 1 Pauson-Khand reaction of compound **5** for the synthesis of compound **6**

Catalyst	Solvent	Temp. (°C)	%yield
$[\text{RhCl}(\text{COD})]_2/\text{CO}$	Toluene	100	—
$\text{Mo}(\text{CO})_6$	Toluene	100	—
$\text{Co}_2(\text{CO})_8$	Toluene	100	—
$\text{Co}_2(\text{CO})_8$	DMF	100	—
$\text{Co}_2(\text{CO})_8$	MeOH	50	—
$\text{Co}_2(\text{CO})_8$	CH_2Cl_2	rt	—
$\text{Co}_2(\text{CO})_8$	<i>n</i> -hexane	rt	trace
$\text{Co}_2(\text{CO})_8$	<i>n</i> -hexane/ SiO_2/Ar	40	40
$\text{Co}_2(\text{CO})_8$	<i>n</i> -hexane/ $\text{Al}_2\text{O}_3/\text{Ar}$	rt-50	70

Scheme 1 Synthesis of compounds **7**, **9**, and **10**.

(dr =9:1) (Scheme 1). Compound **9** is a crystal that was confirmed by NMR spectral and X-ray crystallographic analysis (Figure 3), its epimer compound **10** is a powder.

Compound **9** was subjected to allylic oxidation with selenium dioxide (SeO_2), *tert*-butyl hydroperoxide (*t*-BuOOH) in

Figure 3 X-ray crystallographic analysis of compound **9**.

Scheme 2 Synthesis of compounds **11** and **12**.

Scheme 3 Synthesis of some alcohols.

CH_2Cl_2 , and 2-Iodoxybenzoic acid (IBX) oxidation to form compounds **11** and **12**, respectively (Scheme 2). Deprotection of the silyl ethers of compounds **9**, **11**, and **12** formed alcohol products in the presence of perchloric acid (HClO_4) (Scheme 3).

The cytotoxic properties of all newly synthesized compounds were evaluated in vitro against three human tumor cell lines (HepG2, NSCLC-H292, SNU-1040) and one rat myotubes cell line (L6) by MTT assay. Eriocalycin B (**5**) was used as reference drug. The results are summarized in Table 2. (IC_{50} value is defined as the concentrations corresponding to 50% growth inhibition).

Results are displayed in Table 2. From the table, compounds **9** ($\text{IC}_{50} = 1.01 \mu\text{M}$, NSCLC-H292), **10**

Table 2 In vitro cytotoxic activities of compounds **9–15** (IC_{50} , μM)^{a,b}

Compounds	Cell lines			
	HepG2	NSCLC-H292	SNU-1040	L6
9	>30	1.01	2.97	37.60
10	>30	3.67	5.25	17.55
11	1.58	5.73	7.08	48.58
12	2.00	1.08	2.85	80.60
13	3.48	3.48	1.23	27.61
14	3.95	3.05	1.08	23.98
15	1.76	1.66	3.17	216.93
EriocalyxinB	2.12	7.11	1.54	115.17

Notes: ^aCytotoxicity as IC_{50} values for each cell line, the concentration of compound that caused 50% reduction in absorbance at 570 nm relative to untreated cells using the MTT assay. ^bHuman liver carcinoma cell line (HepG2), non-small cell lung cancer cell line (NSCLC-H292), human colon cancer cell line (SNU-1040), normal skeletal muscle of rat myotubes (L6).

(IC₅₀ = 3.67 μ M, NSCLC-H292), **11** (IC₅₀ = 1.58 μ M, HepG2), **13** (IC₅₀ = 1.23 μ M, SNU-1040), and **14** (IC₅₀ = 1.08 μ M, SNU-1040) showed potency when comparing to the reference eriocalyxin B with only an enone in the A-ring. Meanwhile, these results indicated that the presence of hydroxyl groups at the 6 and 15 position had no effect on the activity. Episomers **9** and **10** did not show significant differences in cytotoxicity and selectivity, so we chose compound **9** that was more readily available for further modification. Remarkably, compounds **12** and **15** with a dienone in the A and C ring, showed stronger activity against all tumor cell lines compared to those with one enone (compounds **9**, **10**, **11**, **13**, and **14**). Deprotection of C-6 silyl ether of compound **12** produced compound **15**, which greatly decreased the cytotoxic activity against rat myoblast cell line (L6). To obtain insight into the cytotoxic potential of these new compounds on normal cells, the effect of compound **15** and eriocalyxin B was evaluated in human liver carcinoma cells (HepG2) and rat myotubes (L6) cells. The potency and selectivity of compound **15** were higher than that of eriocalyxin B. The SI value (selective index, IC₅₀ of normal cells/IC₅₀ of tumor cells) of compound **15** was 123.14, and the SI value of eriocalyxin B was 54.33.

After cytotoxic assessment experiment, we conducted flow cytometry analysis to further understand potential underlying cellular mechanisms of actions of compound **15** (Table 3).

We found that the percentage of NSCLC-H292 cells in G0/G1 phase increased significantly but those in G2/M decreased notably in the presence of DTT and compound **15**, indicating that cells were blocked in G0/G1 phase, thus inhibiting the proliferation of tumor cells.

Table 3 Percentage of G0/G1, S, and G2/M phase

Groups	G0/G1	S	G2/M
Control	23.63 $\pm$ 1.39	37.02 $\pm$ 1.37	18.90 $\pm$ 0.76
DTT 10 μ M	46.07 $\pm$ 0.86**	24.11 $\pm$ 0.64	14.20 $\pm$ 1.29
DTT 1 μ M	44.32 $\pm$ 0.87**	21.09 $\pm$ 1.75	11.12 $\pm$ 0.92*
DTT 0.1 μ M	40.67 $\pm$ 1.0**	23.28 $\pm$ 0.64	15.78 $\pm$ 1.55
15 10 μ M	43.98 $\pm$ 0.83**,#	23.44 $\pm$ 1.40	10.12 $\pm$ 0.72*
15 1 μ M	37.65 $\pm$ 1.88*,###	29.77 $\pm$ 0.71	11.65 $\pm$ 0.93*
15 0.1 μ M	33.6 $\pm$ 1.37*,###	31.91 $\pm$ 0.49	13.09 $\pm$ 0.56

Notes: Values are means $\pm$ S.E.M. Data were obtained from five separate experiments (n=5). Significance of the difference between groups was indicated as follows: *Compared with Control groups, # compound **15** compared with DTT. *P<0.05; **P<0.01; #P<0.05; ###P<0.01.

Conclusion

In conclusion, we have designed and synthesized several compounds bearing an α , β -unsaturated ketone moiety. The in vitro activities were evaluated against HepG2, NSCLC-H292, and SNU-1040 tumor cell lines and an L6 myoblast cell line. This study has revealed that compound **15** is a promising antitumor lead due to the cytotoxic potencies and the high selectivity it displayed when compared to natural counterparts. Further biological investigations are currently undergoing to investigate how compound **15** inhibits tumor cell proliferation in our laboratory and the results will be reported in due course.

Acknowledgment

This work was supported by grants from the National Natural Science Foundation of China (21462053, 21564018), the Science Foundation of Yunnan Province Office of Education (2014Z044), Program of the Undergraduate Innovation (2018059).

Disclosure

The authors report no conflicts of interest in this work.

References

1. Yan BC, Hu K, Sun HD, Puno PT. Recent advances in the synthesis of *Isodon* diterpenoids and schinortriterpenoids. *Chin J Org Chem*. 2018;38:2259. doi:10.6023/cjoc201806002
2. He ZA, Lu GZ, Zhu LL, Wang YX, Yan FL, Zhang JX. Antibacterial constituents from *Isodon excisoides*. *J Chin Chem Soc*. 2010;57:256–259. doi:10.1002/jccs.201000039
3. Liu M, Wang WG, Sun HD, Pu JX. Diterpenoids from *Isodon* species: an update. *Nat Prod Rep*. 2017;34:1090–1140. doi:10.1039/C7NP00027H
4. Lazarski KE, Moritz BJ, Thomson RJ. The total synthesis of *Isodon* diterpenes. *Angew Chem Int Ed*. 2014;53:10588–10599. doi:10.1002/anie.201404482
5. Wang WG, Du X, Li XN, et al. New bicyclo[3.1.0]hexane unit ent-kaurane diterpene and its seco-derivative from *Isodon eriocalyx* var. *laxiflora*. *Org Lett*. 2012;14:302–305. doi:10.1021/ol203061z
6. Zhao Y, Niu XM, Qian LP, Liu ZY, Zhao QS, Sun HD. Synthesis and cytotoxicity of some new eriocalyxin B derivatives. *Eur J Med Chem*. 2007;42:494–502. doi:10.1016/j.ejmech.2006.11.004
7. Niu XM, Li SH, Li ML, et al. Cytotoxic ent-kaurane diterpenoids from *Isodon eriocalyx* var. *laxiflora*. *Planta Med*. 2002;68:528–533. doi:10.1055/s-2002-32551
8. Lv C, Yan XH, Tu Q, et al. Isolation and asymmetric total synthesis of perforanoid A. *Angew Chem Int Ed*. 2016;55:7539. doi:10.1002/anie.201602783
9. Javier A, Marta RR, Juan CC. Mild and efficient molybdenum-mediated Pauson-Khand-type reaction. *Org Lett*. 2005;7:431–434. doi:10.1021/ol047678u
10. Hu Y, Li XN, Ma ZJ, et al. Synthesis of novel ent-kaurane-type diterpenoid derivatives effective for highly aggressive tumor cells. *Molecules*. 2018;23:3216. doi:10.3390/molecules23123216

Drug Design, Development and Therapy**Dovepress****Publish your work in this journal**

Drug Design, Development and Therapy is an international, peer-reviewed open-access journal that spans the spectrum of drug design and development through to clinical applications. Clinical outcomes, patient safety, and programs for the development and effective, safe, and sustained use of medicines are a feature of the journal, which has also

been accepted for indexing on PubMed Central. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <https://www.dovepress.com/drug-design-development-and-therapy-journal>