Novel Methinic Functionalized and Dendritic C-Scorpionates
Article

Synthesis of Novel ent-Kaurane-Type Diterpenoid Derivatives Effective for Highly Aggressive Tumor Cells

Yu Hu 1, Xiao-Nian Li 2, Ze-Jin Ma 1, Pema-Tenzin Puno 2,*, Yong Zhao 1, Yan Zhao 1, Ye-Zhi Xiao 1, Wei Zhang 1 and Jing-Ping Liu 1,*

1 Department of Chemistry, Yunnan Normal University, Kunming 650092, China; xpcxyy@sina.com (Y.H.); mazejinmzj@sina.com (Z.-J.M.); zhaooy@126.com (Y.Z.); zhaoyyanr@163.com (Y.Z.); xyzlmm@sina.com (Y.-Z.X.); royzhangwei@sina.com (W.Z.)
2 State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Kunming 650201, China; lixiaonian@mail.kib.ac.cn
* Correspondence: punopematzenjin@mail.kib.ac.cn (P.-T.P.); Liujp72@163.com (J.-P.L.); Tel.: +86-871-65223616 (P.-T.P.); +86-871-65132144 (J.-P.L.)

Received: 19 November 2018; Accepted: 4 December 2018; Published: 5 December 2018

Abstract: We have designed and synthesized 6 ent-Kaurane-type diterpenoid derivatives containing α,β-unsaturated ketone moieties. In vitro, activity was evaluated against three human tumor cell lines and a rat myogenic cell line (HepG2, NSCLC-H292, SNU-1040, L6) by MTT assay. All the tested compounds exhibited comparable or higher activity than DDP and eriocalyxin B. Compounds 16, 17 and 18 are promising anti-tumor leads due to their cytotoxic potencies and higher selectivity, with SI values of 161.06, 47.80 and 128.20, respectively.

Keywords: ent-kaurane-type diterpenoid derivatives; antitumor; synthesis

1. Introduction

Highly aggressive tumors are a major cause of death worldwide, accounting for 8.8 million deaths each year, and are estimated to cause 9.6 million deaths in 2018. Globally, about 1 in 6 deaths is due to cancer. Approximately 70% of deaths from cancer occur in low- and middle-income countries, according to the World Health Organization (WHO) [1].

Plants are normally an important source of new drugs [2]. In recent years, Isodon genus members have been promising leads due to their extensive pharmacological and physiological effects, such as inhibition of hepatitis viral replication [3] and bacterial infections of the lung or gut [4], as well as its antimalaria [5], antiinflammatory [6], and anti-tumor properties [7]. Hitherto, over 1200 ent-Kaurane-type diterpenoids with highly compact polycyclic ring systems have been isolated from Isodon genus, notably the compounds oridonin [8]. Natural Diterpenoid Isoferritin A (IsoA) [9], longikaurin [10], xerophilusins [11] (Figure 1, 1–4), which have been studied for anti-tumor activity. A common structural unit presented by these diterpenoid compounds is the functionalized bridged C/D α,β-unsaturated ketone moiety. Unfortunately, the use of these natural diterpenoids for anti-tumor was hampered by its moderate potency and complex oxygenated diterpenoid scaffold. Therefore, it is very necessary to find compounds with simple structure and stronger activity.
In the preceding letter we reported the synthesis and biological evaluation of a series of eriocalyxin B derivatives (Figure 2, 5–7) and found that eriocalyxin B derivatives bearing one or two α,β-unsaturated ketone units are potent antitumor agents [12]. It is noteworthy that the 7-hydroxyl group and the hydrogen bond between 6-hydroxyl group and 15-carbonyl group are not necessary for antitumor activity.

2. Results and Discussion

2.1. Chemistry

Herein we report the synthesis of ent-Kaurane-type diterpenoid derivatives containing two α,β-unsaturated ketone moieties and their in vitro cytotoxic properties against three human tumor cell lines. Our synthesis began with reacting isobutyraldehyde with methyl vinyl ketone (MVK) in the presence of sulfuric acid which produced 4,4-dimethylcyclohex-2-enone (compound 8). Treatment of commercially available 1,3-cyclohexan-dione with ethanol in a reflux of benzene in the presence of NaH and allyl bromide produced compound 9 (86%). Compound 9 was treated sequentially with NaH and diethyl carbonate to directly produce β-ketoester. Treatment of β-ketoester with NaH and allyl bromide produced compound 10 (81%). The resulting compound 10 was reduced using NaBH₄ followed by acidification to produce compound 11. Following the well-established Ma protocol [13], the key intermediate 12 (52%) was obtained (Scheme 1).

Deprotonation of 8 with LiHMDS followed by a reaction with compound 12 led to compound 13 as the major product, along with isomer 14 in 82% combined yield (dr = 60:40). The structures of 13 and 14 were confirmed by NMR spectral and X-ray crystallographic analysis (Figure 3). Next, compound 13 was subjected to allylic oxidation with selenium dioxide (SeO₂, CH₂Cl₂, t-BuOOH) and IBX oxidation to form compounds 15 and 16, respectively. Compound 17 (or 18) was provided from the diastereoisomer 14 under the same conditions as 15 (or 16) (Scheme 2).
Scheme 1. Synthesis of compound 8 and compound 12.

Deprotonation of 8 with LiHMDS followed by a reaction with compound 12 led to compound 13 as the major product, along with isomer 14 in 82% combined yield (dr = 60:40). The structures of 13 and 14 were confirmed by NMR spectral and X-ray crystallographic analysis (Figure 3). Next, compound 13 was subjected to allylic oxidation with selenium dioxide (SeO2, CH2Cl2, t-BuOOH) and IBX oxidation to form compounds 15 and 16, respectively. Compound 17 (or 18) was provided from the diastereoisomer 14 under the same conditions as 15 (or 16) (Scheme 2).

Scheme 2. Synthesis of compounds 13, 14, 15, 16, 17 and 18.
2.2. Biological Evaluation

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. Cisplatin (DDP) and eriocalyxin B (5) were used as reference drugs. The results are summarized in Table 1. (IC50 value is defined as the concentrations corresponding to 50% growth inhibition). Remarkably, all the tested compounds exhibited comparable or even higher activity than their natural references eriocalyxin B and DDP. Although results are preliminary, we found a clear structure-activity relationship: the α,β-unsaturated ketone moiety is necessary for potency. Particularly for compound 16 (IC50 = 0.33; 1.69; 2.44 μM) and 18 (IC50 = 0.56; 1.35; 3.01 μM), which display two α,β-unsaturated ketone moieties, these had stronger in vitro potencies and cytotoxic activities to DDP. To obtain insight into the cytotoxic potential of these new compounds on normal cells, the effect of compounds 13, 14, 15, 16, 17, 18 and DDP (cisplatin) were evaluated in human liver carcinoma cell (HepG2) and rat myotubes (L6) cells. All the tested analogues exhibited higher selectivity than DDP. Especially, compound 16, which had more potency and selectivity than DDP and eriocalyxin B, with an SI (selective index, IC50 of normal cells/IC50 of tumor cells) value of 161.06. Interestingly, the two epimers 16, 18 showed no significant difference in their anticancer activities.

Table 1. In vitro cytotoxic activities of compounds 13–18 (IC50, μM) a,b.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>HepG2</th>
<th>NSCLC-H292</th>
<th>SNU-1040</th>
<th>L6</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>13.28</td>
<td>4.44</td>
<td>1.41</td>
<td>95.74</td>
</tr>
<tr>
<td>14</td>
<td>11.67</td>
<td>2.95</td>
<td>1.72</td>
<td>41.52</td>
</tr>
<tr>
<td>15</td>
<td>1.81</td>
<td>1.99</td>
<td>3.13</td>
<td>51.57</td>
</tr>
<tr>
<td>16</td>
<td>0.33</td>
<td>1.69</td>
<td>2.44</td>
<td>53.15</td>
</tr>
<tr>
<td>17</td>
<td>1.58</td>
<td>0.99</td>
<td>2.82</td>
<td>75.53</td>
</tr>
<tr>
<td>18</td>
<td>0.56</td>
<td>1.35</td>
<td>3.01</td>
<td>71.79</td>
</tr>
<tr>
<td>Eriocalyxin B</td>
<td>2.12</td>
<td>7.11</td>
<td>1.54</td>
<td>115.17</td>
</tr>
<tr>
<td>DDP</td>
<td>2.90</td>
<td>8.92</td>
<td>7.76</td>
<td>15.04</td>
</tr>
</tbody>
</table>

a: Cytotoxicity as IC50 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; b: Human 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).
3. Materials and Methods

3.1. Chemistry

All reagents were used as purchased from Energy Chemical (Shanghai, China), Tansoole (Shanghai, China), Acros (Beijing, China) and Aldrich (Shanghai, China) without further purification. Anhydrous CH2Cl2 was freshly distilled from CaH2, and THF was dried by Na. For reactions carried out under Ar, at least three times. The course of reaction was monitored by TLC. 1H NMR and 13C NMR spectra were recorded at ambient temperature with a Bruker Avance 300 (300 MHz for 1H and 75.5 MHz for 13C; 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 (Bremen, Germany). IR spectra were measured as KBr pellets by using a Bio-Rad FTS-135 spectrometer (California, CA, USA). All melting points are uncorrected. CC was performed by using silica gel (100–200 mesh; Qingdao Marine Chemical, Shandong, China).

4.4-Dimethylcyclohex-2-enone (8): To a solution of isobutyraldehyde (3 g, 41.6 mmol) and methyl vinyl ketone (2.91 g, 41.6 mmol) was added 1% mmol conc. H2SO4. The resulting mixture was stirred with a Dean-Stark apparatus in refluxing benzene (20 mL) for 5 h. The reaction mixture was neutralized by sat. NaHCO3 to pH = 7. Organic layer was separated and water was extracted with EtoAc (3 × 10 mL). The organic phases were combined, dried over MgSO4, and concentrated on a rotary evaporator. The resulting mixture was suspended in diethyl carbonate (10 mL) at 100 °C, was removed in vacuo and the residue was purified by flash column chromatography (eluting with ethyl acetate:petroleum = 1:10) to give compound 8 (92%) as pale yellow liquid: 1H NMR (400 MHz, CDCl3) δ = 6.67 (d, J = 6 Hz, 1H), 5.82 (d, J = 6 Hz, 1H), 2.45 (t, J = 6 Hz, 2H), 1.87 (t, J = 6 Hz, 2H), 1.17 (s, 6H) ppm; 13C NMR (100 M Hz, CDCl3) δ = 199.48, 159.79, 126.77, 36.03, 34.34, 32.77, 27.64 ppm; IR (KBr): v max = 3441.89, 2966.23, 2926.56, 1638.51 cm−1; MS (ESI): m/z: 125; HRMS (ESI) Calcd. for [(C8H12O) + H]+: 125.0966; found: 125.0961 [M + H]+.

3-Ethoxycyclohex-2-enone (9): To a solution of cyclohexane-1,3-dione (1.12 g, 10 mmol) in dry ethanol was added 1 % mmol TsOH. The resulting mixture was refluxed for overnight. Then the solvent was removed in vacuo and the residue was purified by flash column chromatography (eluting with EtoAc:petroleum = 1:20–1:10) to give compound 9 (86%) as colorless liquid: 1H NMR (300 MHz, CDCl3) δ = 4.74 (s, 1H), 3.36 (q, J = 6.9 Hz, 2H), 1.85 (t, J = 6.0 Hz, 2H), 1.74 (t, J = 6.0 Hz, 2H), 1.11 (p, J = 6.0 Hz, 2H), 0.80 (t, J = 6.9 Hz, 3H) ppm; 13C NMR (75.5 MHz, CDCl3) δ = 197.496, 176.357, 101.367, 62.996, 35.635, 27.897, 20.201, 12.970 ppm; IR (KBr): v max = 2979.73, 2937.66, 1725.23, 1655.39, 1605.02, 1186.77 cm−1; MS (ESI): m/z: 141; HRMS (ESI) Calcd. for [(C8H12O2) + H]+: 141.0916; found: 141.0906 [M + H]+.

Ethyl 1-allyl-4-ethoxy-2-oxocyclohex-3-enecarboxylate (10): NaH (0.6 g, 15 mmol, 60% in mineral oil) was suspended in diethyl carbonate (10 mL) at 100 °C. A solution of 9 (1.4 g, 10 mmol) in diethyl carbonate (2 mL) was added to the suspension by syringe and stirring was continued for 5 h at 100 °C. Upon cooling to ambient temperature, the reaction mixture was washed with brine (2 × 10 mL) and evaporated in vacuo without further purification. To a solution of crude product in dry THF was added NaH (0.4 g, 10 mmol, 60% in mineral oil) at room temperature. After 30 min, the mixture was added allyl bromide (1.2 g, 10 mmol) and refluxed for 3 h. The reaction mixture was poured into ice-water and extracted with ether. The ether layer was washed with water and dried over Na2SO4. Removal of solvent followed by CC gave compound 10 (81%) as colorless liquid: 1H NMR (300 MHz, CDCl3) δ = 5.77 (m, 1H), 5.35 (s, 1H), 5.09 (m, 2H), 4.17 (q, J = 6.9 Hz, 2H), 3.92 (q, J = 6.9 Hz, 2H), 2.74−2.32 (m, 5H), 1.97−1.91 (m, 1H), 1.19 (t, J = 6.9 Hz, 3H), 1.05 (t, J = 6.9 Hz, 3H) ppm; 13C NMR (75.5 MHz, CDCl3) δ = 194.98, 176.59, 171.13, 133.50, 118.42, 101.65, 64.24, 60.98, 55.33, 38.38, 37.95, 26.10, 13.93 ppm; IR (KBr): v max = 2979.73, 2937.66, 1725.23, 1655.39, 1605.02, 1186.77 cm−1; MS (ESI): m/z: 275; HRMS (ESI) Calcd. for [(C14H20O4) + Na]+: 275.1259; found: 275.1254 [M + Na]+.

Ethyl 1-allyl-4-oxocyclohex-2-enecarboxylate (11): To a solution of 10 (1 g, 3.97 mmol) in MeOH (5 mL) was added CeCl3·7H2O (1.48 g, 3.97 mmol). After 10 min of stirring, the reaction mixture was added NaBH4
(0.3 g, 7.94 mmol) about for 30 min. Then the mixture was acidified to pH 6 by the dropwise addition of concentrated HCl, and extracted with ethyl acetate and washed with brine. The combined organic layers were dried over Na₂SO₄ and the solvent was removed under reduced pressure. Purification of the residue by column chromatography afford compound 11 (84%) as pale yellow liquid: ¹H NMR (300 MHz, CDCl₃) δ = 6.88 (d, J = 10.2 Hz, 1H), 5.97 (d, J = 10.2 Hz, 1H), 5.66 (m, 1H), 5.10 (m, 2H), 4.15 (q, J = 7.2 Hz, 2H), 2.52–2.34 (m, 5H), 1.98–1.93 (m, 1H), 1.24 (t, J = 7.2 Hz, 3H) ppm; ¹³C NMR (75.5 MHz, CDCl₃) δ = 198.498, 172.728, 150.539, 131.920, 129.227, 61.435, 47.502, 42.884, 34.525, 30.238, 14.184 ppm; IR (KBr): υ_{max} = 2978.78, 2958.27, 1728.05, 1639.95, 1614.26 cm⁻¹; MS (ESI): m/z: 209; HRMS (ESI) Calcd. for [(C₁₂H₁₆O₃) + H⁺]: 209.1178; found: 209.1169 [M + H⁺].

Ethyl 6-methylene-4-oxobicyclo[3.2.1]oct-2-ene-1-carboxylate (12): To a solution of 11 (6 g, 28.85 mmol, 1.0 eq) in dry THF (200 mL) under Ar at −60 °C was added Et$_3$N (11.6 mL, 115.4 mmol, 4.0 eq). The mixture was stirred for 20 min at this temperature, and then TBSOTf (19.11 g, 72.13 mmol, 2.5 eq) was added dropwise. After full conversion was observed by TLC, the reaction mixture was quenched with saturated NaHCO₃ solution and extracted with EtOAc. The combined organic layers were dried over Na₂SO₄ and the solvent was removed under reduced pressure. The residue was passed through a short column of silica gel to provide the corresponding silyl enol ether as a colorless oil. The obtained silyl enol ether was dissolved in DMSO (200 mL) and Pd(OAc)$_2$ (650 mg, 2.89 mmol, 0.1 eq) was added, followed by 7 (11.6 mL, 115.4 mmol, 4.0 eq). The reaction was stirred under oxygen atmosphere (balloon with O₂) at 40 °C for 2 h. The mixture was warmed to room temperature, quenched with saturated NH₄Cl, and extracted with ethyl acetate and washed with brine. The combined organic layers were dried over Na₂SO₄, filtered, and concentrated under reduced pressure. Purification of the residue by flash column chromatography provide compound 13 (40%) as pale yellow liquid: ¹H NMR (300 MHz, CDCl₃) 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 = 9.5 Hz, 1H), 2.52 (d, J = 9.5 Hz, 1H), 2.25 (m, 2H), 1.25 (t, J = 7.2 Hz, 3H) ppm; ¹³C NMR (75.5 MHz, CDCl₃) δ = 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): υ_{max} = 2979.98, 1729.06, 1685.40, 1280.90, 1222.04, 1193.09 cm⁻¹; MS (ESI): m/z: 207; HRMS (ESI) Calcd. for [(C₁₂H₁₄O₃) + H⁺]: 207.1021; found: 207.1015 [M + H⁺].

3.1.1. General Method for Synthesis of 13 and 14

To a stirred solution of 8 (1 eq) and 12 (1 eq) in dry THF under Ar at −78 °C; was added LiHMDS (2 eq, 1.3 M in THF) over a period of 5 min and stirring at −78 °C was continued for 45 min. The resulting solution was warmed to room temperature, quenched with saturated NH₄Cl and extracted with Et₂O. The combined organic layers were dried over Na₂SO₄ and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography to provide 13 and 14.

Compound 13: The compound is obtained as crystal; m.p.: 112.0–114.6 °C; ¹H NMR (300 MHz, CDCl₃) δ = 6.56 (d, J = 6.0 Hz, 1H), 5.80 (d, J = 6.0 Hz, 1H), 5.10 (s, 1H), 5.01 (s, 1H), 4.15 (q, J = 6.0 Hz, 2H), 3.50 (m, 1H), 3.28 (s, 1H), 2.78 (s, 2H), 2.74–2.65 (m, 2H), 2.28 (m, 1H), 1.92–1.66 (m, 4H), 1.29 (t, J = 6.0 Hz, 3H), 1.26 (s, 3H), 1.25 (s, 3H) ppm; ¹³C NMR (75.5 MHz, CDCl₃) δ = 208.93, 198.35, 174.52, 158.35, 145.59, 126.50, 109.91, 61.00, 58.73, 52.97, 46.11, 43.09, 38.80, 38.36, 36.41, 36.11, 33.70, 30.64, 24.95, 14.21 ppm; IR (KBr): υ_{max} = 2962.26, 2901.21, 1722.91, 1710.24, 1672.31, 1229.31, 1194.50 cm⁻¹; MS (ESI): m/z: 353; HRMS (ESI) Calcd. for [(C₂₀H₂₆O₄) + Na⁺]: 353.1729; found: 353.1724 [M + Na⁺].

Compound 14: The compound is obtained as crystal; m.p.: 103.2–105.8 °C; ¹H NMR (300 MHz, CDCl₃) δ = 6.46(d, J = 6.0 Hz, 1H), 5.68 (d, J = 6.0 Hz, 1H), 5.04 (s, 1H), 4.88 (s,1H), 4.11 (q, J = 6.0 Hz, 2H), 3.30 (s, 1H), 2.65–2.39 (m, 6H), 1.96 (m, 1H), 1.78–1.22 (m, 3H), 1.01 (t, J = 6.0 Hz, 3H), 0.88 (s, 6H) ppm; ¹³C NMR (75.5 MHz, CDCl₃) δ = 206.74, 199.50, 175.14, 157.76, 146.00, 127.27, 109.41, 60.96, 59.66, 52.69, 45.89, 44.46, 43.35, 42.79, 37.86, 35.31, 34.03, 30.39, 24.74, 14.26 ppm; IR (KBr): υ_{max} = 2958.90, 1716.70, 1673.18, 1538.19, 1234.84, 1215.98 cm⁻¹; MS (ESI): m/z: 353; HRMS (ESI) Calcd. for [(C₂₀H₂₆O₄) + Na⁺]: 353.1729; found: 353.1732 [M + Na⁺].
3.1.2. General Method for Synthesis of 15 and 17

To a stirred solution of 13 (1.0 eq) in DCM, SeO₂ (0.9 eq) was added in one portion, and a solution of t-BuOOH (5.5 M in decane, 3 eq) 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 15.

Compound 15: The compound is obtained as pale yellow liquid; ¹H NMR (300 MHz, CDCl₃) δ = 6.52 (d, J = 6 Hz, 1H), 5.75 (d, J = 6 Hz, 1H), 5.40 (s, 1H), 5.30 (s, 1H), 4.58 (s, 1H), 4.16 (q, J = 6 Hz, 2H), 3.32 (m, 2H), 2.57 (m, 3H), 2.17~1.69 (m, 5H), 1.21 (t, J = 6 Hz, 3H), 1.08 (s, 6H) ppm; ¹³C NMR (75.5 MHz, CDCl₃) δ = 208.33, 198.08, 172.65, 158.66, 149.08, 126.53, 115.31, 79.98, 61.18, 59.47, 56.68, 46.35, 37.91, 36.43, 35.20, 33.54, 32.64, 30.62, 25.07, 14.28 ppm; IR (KBr):υmax = 3451.12, 2961.46, 2927.59, 2871.68, 1714.49, 1699.43, 1280.80, 1037.64 cm⁻¹; MS (ESI): m/z: 369; HRMS (ESI) Calcd. for [(C₂H₂O₆S) + Na⁺]: 369.1678; found: 369.1677 [M + Na⁺].

Compound 17: The compound is obtained as pale yellow liquid; ¹H NMR (300 MHz, CDCl₃) δ = 6.48 (d, J = 9 Hz, 1H), 5.68 (d, J = 9 Hz, 1H), 5.36 (s, 1H), 5.31 (s, 1H), 4.46 (s, 1H), 4.15 (q, J = 6 Hz, 2H), 3.37 (m, 1H), 2.45–2.39 (m, 5H), 1.98 (m, 2H), 1.78 (m, 1H), 1.68 (m, 1H), 1.24 (t, J = 6 Hz, 3H), 1.06 (s, 3H), 1.05 (s, 3H) ppm; ¹³C NMR (75.5 MHz, CDCl₃) δ = 206.21, 199.22, 173.03, 157.97, 150.47, 127.26, 114.27, 79.08, 61.06, 59.93, 57.98, 45.66, 43.62, 42.09, 37.51, 34.07, 31.78, 30.36, 24.80, 14.36 ppm; IR (KBr):υmax = 3374.71, 2985.31, 2963.32, 2945.34, 2928.06, 2873.40, 1726.97, 1705.28, 1670.93, 1633.80, 1616.71, 1228.42, 1203.67, 1085.27, 1045.22 cm⁻¹; MS (ESI): m/z: 369; HRMS (ESI) Calcd. for [(C₂H₂O₆S) + Na⁺]: 369.1678; found: 369.1682 [M + Na⁺].

3.1.3. General Method for Synthesis of 16 and 18

To a solution of 15 (1.0 eq) in EtOAc was added IBX (3.0 eq). The mixture was heated at 70 °C for 4 h 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 16.

Compound 16: The compound is obtained as pale yellow liquid; ¹H NMR (300 MHz, CDCl₃) δ = 6.57 (d, J = 9 Hz, 1H), 6.19 (s, 1H), 5.83 (d, J = 9 Hz, 1H), 5.59 (s, 1H), 4.27 (q, J = 6 Hz, 2H), 3.68 (m, 2H), 2.77–2.68 (m, 4H), 2.11 (d, 1H), 1.85 (m, 2H), 1.29 (t, J = 6 Hz, 3H), 1.16 (s, 6H) ppm; ¹³C NMR (75.5 MHz, CDCl₃) δ = 206.69, 198.72, 197.33, 168.85, 157.95, 142.16, 126.66, 120.53, 61.80, 61.61, 54.10, 46.06, 39.07, 37.43, 35.08, 33.91, 31.79, 30.32, 25.18, 14.19 ppm; IR (KBr):υmax = 3343.29, 2962.17, 2929.37, 2871.25, 1722.07, 1675.43, 1638.37, 1249.74, 1177.04, 1111.95 cm⁻¹; MS (ESI): m/z: 367; HRMS (ESI) Calcd. for [(C₂O₂H₄O₅S) + Na⁺]: 367.1521; found: 367.1528 [M + Na⁺].

Compound 18: The compound is obtained as pale yellow liquid; ¹H NMR (600 MHz, CDCl₃) δ = 6.58 (d, J = 9.9 Hz, 1H), 6.13 (s, 1H), 5.78 (d, J = 9.9 Hz, 1H), 5.58 (s, 1H), 4.25 (q, J = 7.1 Hz, 2H), 3.78 (s, 1H), 2.93 (d, J = 12.2 Hz, 1H), 2.66 (m, 3H), 2.45 (m, 1H), 2.13 (d, J = 17.2 Hz, 1H), 1.87 (m, 2H), 1.31 (t, J = 7.1 Hz, 3H), 1.18 (s, 3H), 1.14 (s, 3H) ppm; ¹³C NMR (150 MHz, CDCl₃) δ = 204.14, 200.19, 199.55, 169.71, 158.69, 142.90, 127.19, 119.93, 62.59, 61.80, 55.14, 45.85, 43.86, 40.88, 37.84, 34.18, 32.82, 30.26, 24.81, 14.18 ppm; IR (KBr):υmax = 3435.06, 2961.46, 2931.17, 2871.34, 1739.98, 1720.04, 1671.03, 1638.37, 1241.36, 1228.03, 1177.31, 1142.38 cm⁻¹; MS (ESI): m/z: 367; HRMS (ESI) Calcd. for [(C₂O₂H₄O₅S) + Na⁺]: 367.1521; found: 367.1515 [M + Na⁺].

3.2. Biological Evaluation

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 (DMEM). 10% 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 h. After attached to the culture bottle wall cells were divided cells into three groups, (1) control group; (2) positive control group; (3) experiment group. The administration concentrations of Cisplatin (DTT) and compounds were 0.1 μM–10 μM. After 24 h, 10% MTT was added and cells were culture for 3.5 h. Then the culture medium was discard and 150 μL DMSO was added. Eventually, after incubation for 10 min 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.

4. Conclusions

In conclusion, we have designed and synthesized several compounds bearing two α,β-unsaturated ketone moiety. Their in vitro activities were evaluated against HepG2, NSCLC-H292, SUM-1040 tumor cell lines and an L6 myoblast cell line, with IC_{50} values ranging from 0.33 to 115.17 μM. This study has revealed that compounds 16, 17 and 18 are promising anti-tumor leads due to the stronger cytotoxic potencies and higher selectivity they displayed than their natural counterparts.

Author Contributions: Synthesis and experimental work, Y.H., Z.-J.M., Y.-Z.X. and W.Z.; X-ray crystallographic analysis, X.-N.L.; Biological Evaluation, Y.Z. (Yong Zhao) and Y.Z. (Yan Zhao); Design and writing, P.-T.P. and J.-P.L.

Funding: This work was supported by a grant from the National Natural Science Foundation of China (21462053), the Science Foundation of Yunnan Province Office of Education (2014Z044), Program of the Undergraduate Innovation (20180809).

Conflicts of Interest: The authors declare no conflict of interest.

References


**Sample Availability:** Samples of the compounds 13–18 are available from the authors.