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The conversion of oridonin to spirolactone-type or enmein-type diterpenoid: Synthesis and biological evaluation of *ent*-6,7-*seco*-oridonin derivatives as novel potential anticancer agents

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ABSTRACT

Starting from commercial available natural product oridonin (**1**), a practical synthesis of *ent*-6,7-*seco*-oridonin derivatives (**2**, **3**, **5**, and **9**) was accomplished and their biological activities were evaluated. The conversion of spirolactone-type diterpenoid to enmein-type was first completed. The results demonstrated that all synthesized *ent*-6,7-*seco*-oridonin derivatives could markedly inhibit the proliferation of cancer cells. Compared with Taxol, the most cytotoxic compound **5** has similar potency in A549 cell and slightly less cytotoxicity in Bel-7402 cell. Compound **5** was also more potent than parent compound oridonin in mice with MGC-803 gastric cancer *in vivo*. Then a series of novel 14-*O*-derivatives of **5** were further designed and synthesized, which showed better activity than **5** and similar activity as Taxol *in vitro*. The structure–activity relationships of oridonin derivatives were also discussed in the present investigations.

1. Introduction

Natural products (NP) have been the major sources of chemical diversity for starting materials while driving pharmaceutical discovery over the past century. A total of 19 NP based drugs were approved for marketing worldwide in between the year 2005–2010, among which 7 were classified as NPs, 10 as semi-synthetic NPs, and 2 as NP derived drugs [1]. But the limited source of plants as well as the difficulty of chemical synthesis and modification restricted the development of natural medicines.

Meanwhile, in the last few years, a growing number of publications have reported the use of kaurane diterpenes for the synthesis of novel antimicrobial, cytotoxic, and trypanocidal agents, and this synthetic approach is still far from being fully exploited by the current interest in the chemistry of natural products [2,3]. Oridonin (**1**) is a widely distributed *ent*-kaurene in the *Rabdosia* plants and has recently attracted much attention because of its anti-tumor activity with novel mechanism of inhibition effect on NF-κB activation [4–6], induction of G_2/M phase arrest and apoptosis [7]. However, in many cases, the potency of **1** is relatively weak. Therefore it is needed to search for novel derivatives of **1** via structural modification and bioassay-guided systematic drug design and synthesis.

6,7-*seco*-Oridonin derivatives (especially enmein-type and spirolactone-type diterpenoids, Fig. 1) have been shown to have stronger cytotoxicity than oridonin [8–11] but they were much more difficult to be isolated from natural plants source. So the conversion of commercial available oridonin to 6,7-*seco*-oridonin derivatives may provide ample materials for intensive study. Furthermore, in previous studies we found that a series of novel 1-*O*- and 14-*O*-derivatives of **1** exhibited stronger cytotoxicity against six cancer cell lines (BGC-7901, SW-480, HL-60, Bel-7402, A549, and B16) *in vitro* and some of them have stronger anti-tumor activity than parent compound **1** and positive control cyclophosphamide in mice with H22 liver tumor *in vivo* [12,13]. It has been believed that the 6,7-dihydroxyl-15-oxo-16-methylene moiety of those compounds is indispensable for the biological activity of most natural *ent*-kaurene diterpenoids [14,15]. In this regard, it is interesting to examine whether the 6,7-*seco*-15-oxo-16-methylene derivatives of *ent*-kaurene diterpenoids with enmein- or spirolactone-type structures (Fig. 2) would remain or further

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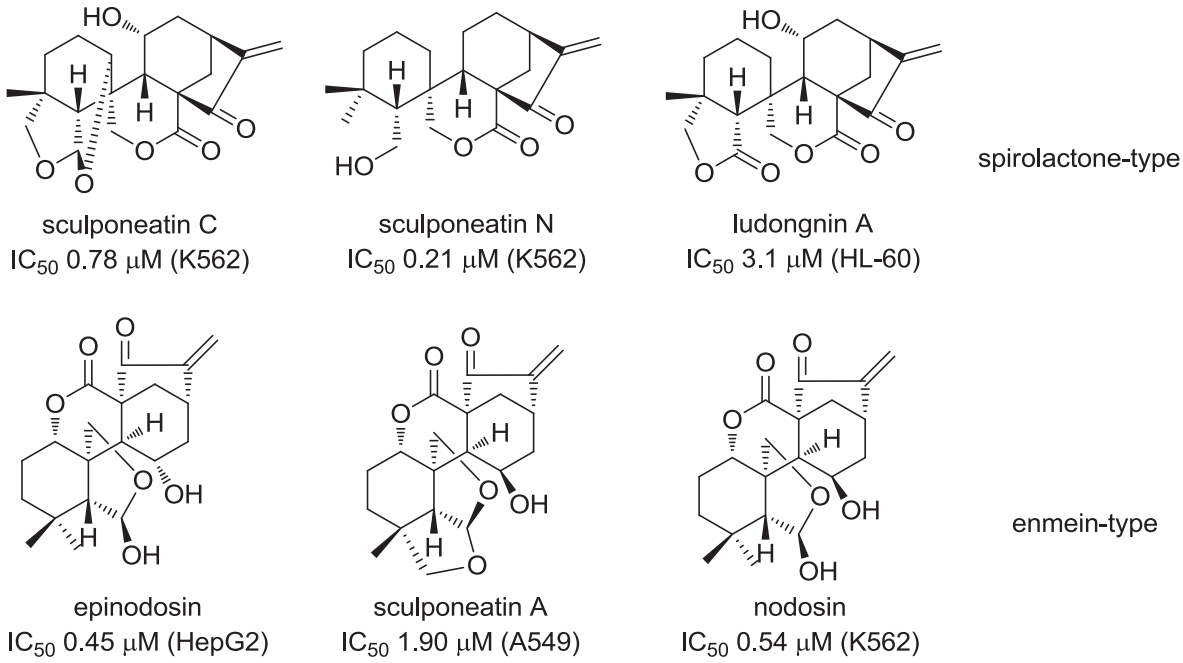


Fig. 1. Some enmein-type or spirolactone-type diterpenoids isolated from natural plants and their cytotoxicity.

improve anti-tumor activity of oridonin. So the synthesis and biological evaluation of *ent*-6,7-*seco*-oridonin derivatives were accomplished, and the conversion of spirolactone-type diterpenoid to enmein-type was first completed. Based on the observed pharmacological data, structure–activity relationships have been established.

2. Results and discussion

2.1. Chemistry

The conversion of kaurene-type to 6,7-*seco*-kaurene-type compounds were achieved by periodate or lead tetraacetate oxidation [14,16,17]. The products were either enmein-type or spirolactone-type. When the starting material has a hydroxyl group at C-1, the former was obtained; otherwise, the latter formed.

The synthesis of enmein-type derivatives is outlined in Schemes 1 and 2. Treatment of **1** with sodium periodate in water gave ester **2** in 91% yield. Oxidation of **1** and **2** with Jones reagent at 0 °C afforded corresponding ketones **3** and **4** in yields of 87% and 98%, respectively. Treatment of **1** with 2,2-dimethoxypropane in the presence of TsOH in acetone provided ketal **6** in 85% yield. Compound **6** upon reaction with Ac_2O/DMAP/TEA led to acetylated compound **7** in the yield of 95%. Deprotection of **7** with 10% HCl/THF (1:1) gave the corresponding alcohol **8** in almost quantitative yield [18]. Interestingly, compounds **4** and **8** without a hydroxyl group at C-1 could be converted to spirolactone aldehydes **5** and **9** instead of enmein-type compounds by oxidation using lead tetraacetate in the presence of sodium carbonate in THF in yields of 95% and 97%, respectively [19].

Hydrolyzed the spirolactone-type compound **9** with 1 mol/l NaOH, and then enmein-type compound **2** was got again (Scheme 3). We assumed that the conversion mechanism of kaurene-type to enmein-type or spirolactone-type diterpenoid was shown in Scheme 4. The above convenient conversion made enmein-type or spirolactone-type diterpenoids available for our further research to find new chemical entities for anticancer agents.

Since 14-*O*-derivatives of **1** exhibited stronger cytotoxicity than parent compound **1** [12,13], we continued to explore whether 14-*O*-derivatives of **5** could also improve anticancer potency of compound **5**. So a series of novel 14-*O*-derivatives of **5** were further designed and synthesized. As shown in Scheme 5, treatment of **5** with corresponding acids in the presence of DMAP/EDC in DCM gave corresponding 14-*O*-derivatives (compounds **10**–**20**).

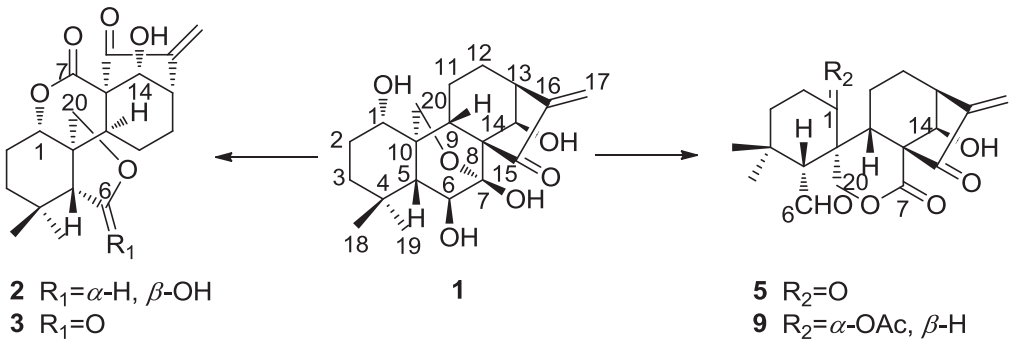
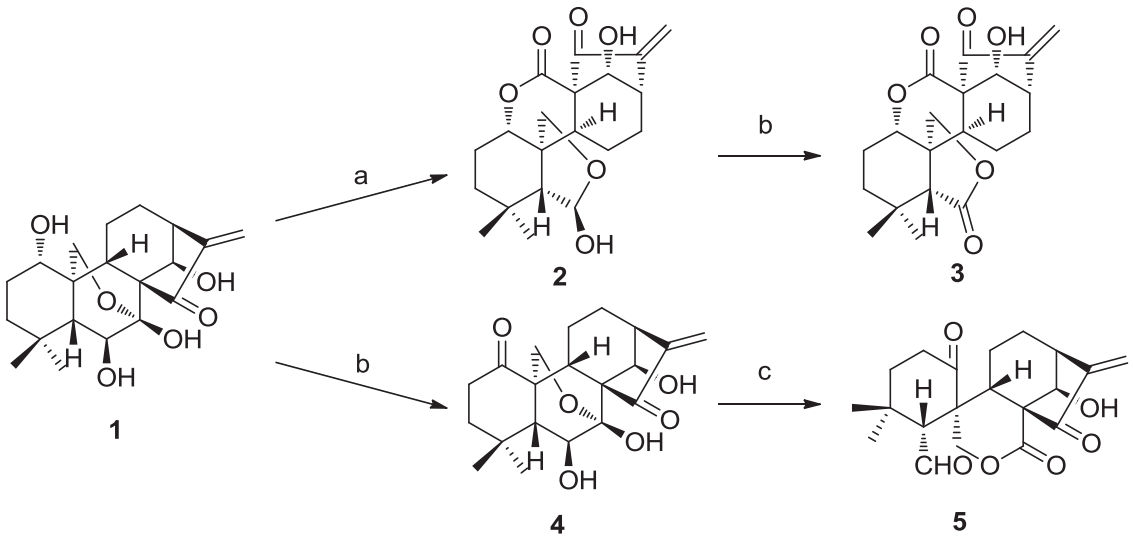


Fig. 2. Structures of oridonin (**1**), enmein-type derivatives (**2**, **3**), spirolactone-type derivatives (**5**, **9**).



Scheme 1. Synthesis of *ent*-6,7-*seco*-oridonin derivatives **2**, **3**, and **5**. Reagents and conditions: (a) $NaIO_4$, H_2O, rt; (b) Jones reagent, acetone, isopropanol, 0 °C; (c) $Pb(OAc)_4$, Na_2CO_3, THF, rt.

2.2. Biological evaluation of 6,7-seco-oridonin derivatives in vitro

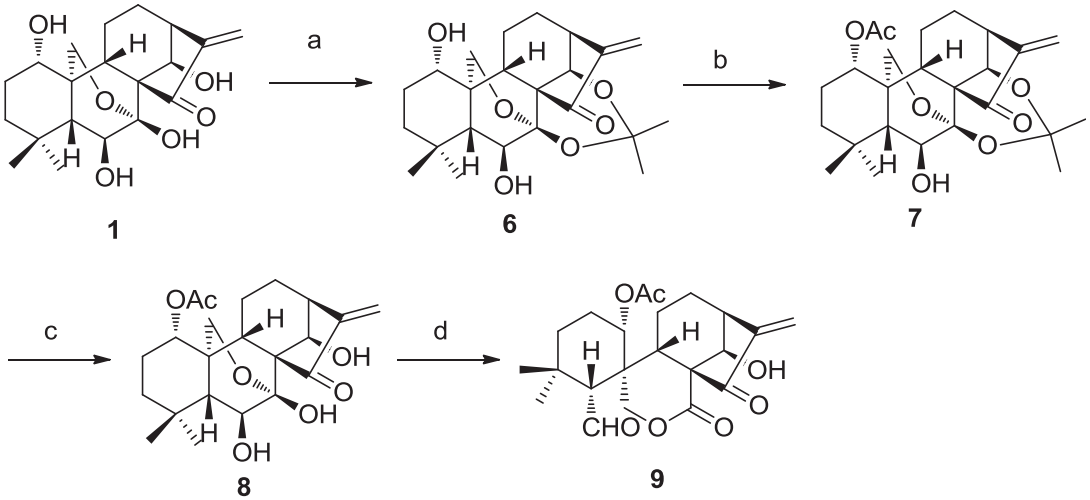
The synthesized enmein- and spirolactone-type derivatives (**2**, **3**, **5**, and **9**) were firstly evaluated for their cytotoxicity against six cancer cell lines (K562, MCF-7, CaEs-17, Bel-7402, Hela and A549) *in vitro*. As shown in Table 1, noticeably, in comparison with oridonin, these compounds exhibited significant cytotoxicity, especially against K562 cell with IC_{50} values varied from 2.18 to 9.56 μM. The most cytotoxic compound **5** showed stronger cytotoxicity against five cancer cell lines than oridonin, among which it is about 2-fold more potent in K562, CaEs-17 and Bel-7402 cells, 8-fold more potent in Hela cell and 6-fold more potent in A549 cell. It has similar cytotoxicity as Taxol in A549 cell and slightly less than Taxol in Bel-7402 cell. The results have revealed that 1-oxo-6,7-*seco*-oridonin **5** had more cytotoxic activity than the other derivatives of oridonin. So compound **5** was regarded as the most promising compound.

2.3. The anti-tumor activity of 6,7-seco-oridonin derivative 5 in vivo

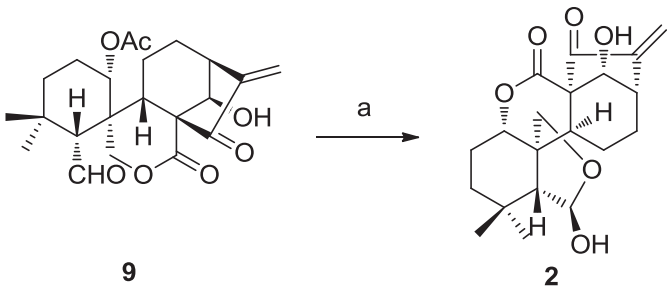
Based on the *in vitro* evaluated results, we further tested anti-tumor activity of compound **5** *in vivo* by performing the assay in mice with MGC-803 gastric cancer. As shown in Table 2, compound **5** has stronger anti-tumor activity than parent compound oridonin in mice with MGC-803 gastric cancer. Thus the structure of **5** was selected for further modification to find novel candidate for cancer therapeutic agents [20,21].

2.4. Cytotoxicity of 14-O-derivatives of compound 5

The synthesized novel 14-*O*-derivatives of **5** were further tested against four cancer cell lines (K562, MGC-803, CaEs-17 and Bel-7402). Taxol was selected as positive control. These results were summarized in Table 3 and presented as the concentration of drug



Scheme 2. Synthesis of *ent*-6,7-*seco*-oridonin derivative **9**. Reagents and conditions: (a) 2,2-Dimethoxypropane, acetone, TsOH, 56 °C; (b) Ac_2O, TEA, DMAP, rt; (c) 10% HCl, THF, rt; (d) $Pb(OAc)_4$, Na_2CO_3, THF, rt.



Scheme 3. The conversion of spirolactone-type derivative **9** to enmein-type derivative **2**. Reagents and conditions: (a) 10% NaOH, MeOH, rt.

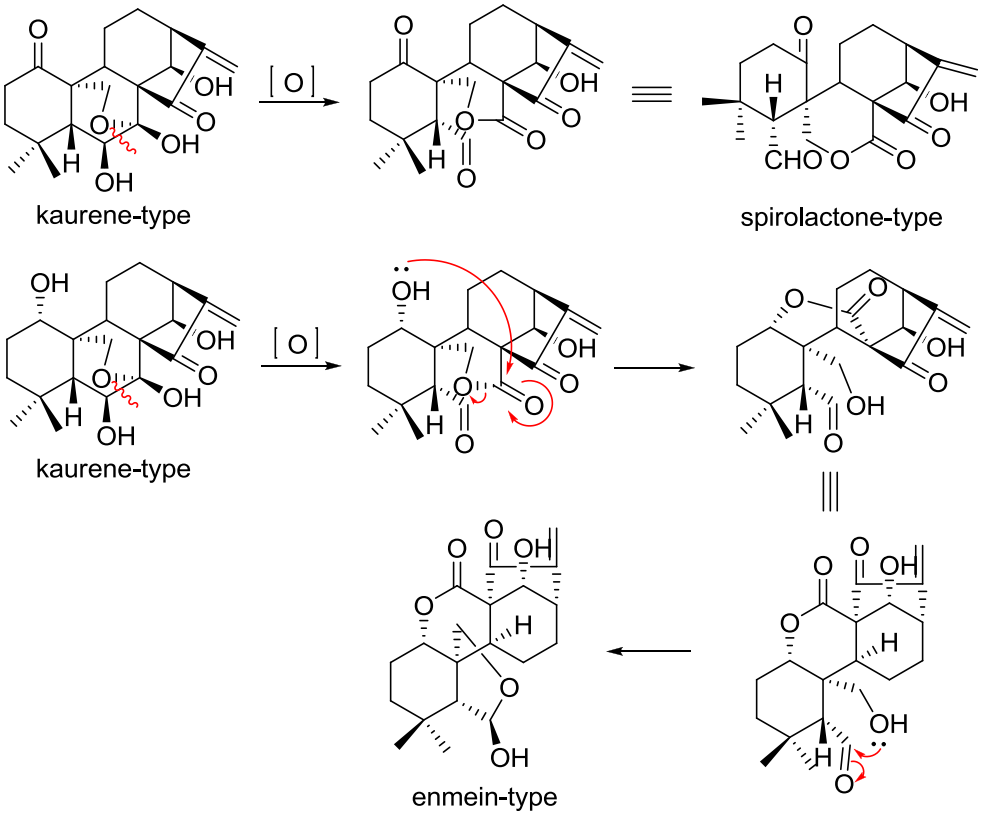
inhibiting 50% cell growth (IC_{50}). To our delight, most of the 14-*O*-derivatives exhibited stronger cytotoxicity than parent compound **5** and similar cytotoxicity as Taxol in the four cancer cell lines.

2.5. Structure–activity relationship (SAR)

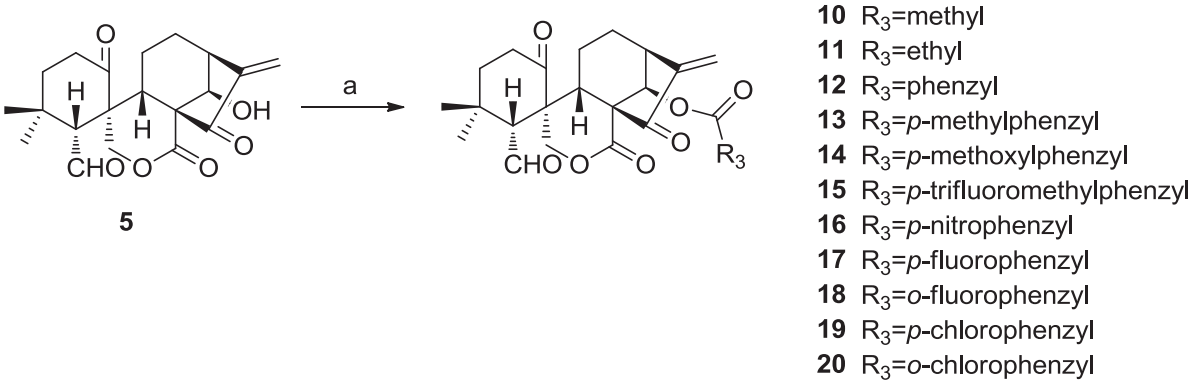
The present results demonstrated that all synthesized *ent*-6,7-*seco*-oridonin derivatives could markedly inhibit the proliferation of cancer cells. The 6,7-*seco*-15-oxo-16-methylene derivatives of *ent*-kaurene diterpenoids with enmein-type structure (compounds **2** and **3**) or spirolactone-type structure (compounds **5** and **9**) can improve the anti-tumor activity of oridonin. Among which, 1-oxo-6,7-*seco*-oridonin **5** might be more cytotoxic than oridonin and its spirolactone-type analogue 1-*O*-acetyl-6,7-*seco*-oridonin **9**. Furthermore, the SAR showed that in the derivatives of *ent*-6,7-*seco*-oridonin **5**, when R_3 were alkyl groups substitution (compounds **10** and **11**), the IC_{50} values slightly changed with enhancing the length of carbon chain. As for substitution of aromatic groups (compounds **12**–**20**), it could be found that the cytotoxicity activity was more potent, especially when aromatic ring was substituted by electron donating groups (compounds **13** and **14**). These derivatives showed better activity than that with electron withdrawing groups (compounds **15** and **16**). Changing the position of fluorine on benzene ring (compounds **17** and **18**), the IC_{50} values in four cancer cell lines were almost no change. While substituted with chlorine at *ortho*-position (compound **20**), the cytotoxicity was stronger than that at *para*-position (compound **19**). Nevertheless, a broader range of substitution patterns of *ent*-6,7-*seco*-oridonin would have to be synthesized and tested before comprehensive SAR could be obtained.

3. Conclusion

Starting from commercial available natural product oridonin, a practical synthesis of *ent*-6,7-*seco*-oridonin derivatives can be accomplished and the conversion of oridonin to spirolactone-type or enmein-type diterpenoid as well as spirolactone-type diterpenoid to enmein-type are accessible. The present study demonstrated that all synthesized *ent*-6,7-*seco*-oridonin derivatives could markedly inhibit the proliferation of cancer cells. Among which, 1-oxo-6,7-*seco*-oridonin **5** exhibited stronger cytotoxicity than oridonin, and it was also more potent than oridonin in mice with MGC-803 gastric cancer *in vivo*. Furthermore, most of the 14-*O*-derivatives of compound **5** showed stronger cytotoxicity than parent compound **5** and similar cytotoxicity as Taxol in the four cancer cell lines (K562, MGC-803, CaEs-17 and Bel-7402). Based on the evaluated results, the SAR was discussed, which showed that the 6,7-*seco*-15-oxo-16-methylene derivatives of *ent*-kaurene diterpenoid with enmein- or spirolactone-type structures could improve the anti-tumor activity of oridonin. As the results, the potential of these derivatives as cancer therapeutic agents invite us



Scheme 4. The mechanism of conversion: from kaurene-type to spirolactone-type or enmein-type diterpenoid.



Scheme 5. Synthesis of 14-*O*-derivatives of *ent*-6,7-*seco*-oridonin derivative **5**. Reagents and conditions: (a) Corresponding acids, EDC, DMAP, DCM, rt.

to continue exploring more and more this family of interesting and promising compounds. And it would also provide valuable information in the field of structure modification of natural lead compounds and anticancer drugs development.

4. Experimental

4.1. Chemistry

All commercially available solvents and reagents were used without further purification. Melting points were taken on XT-4 micro melting point apparatus and uncorrected. Optical rotations were measured using a JASCO P-1020 digital polarimeter, and Infrared (IR) spectra (KBr) were recorded on a Nicolet Impact 410 instrument (KBr pellet). ^{1}H and ^{13}C NMR spectra were recorded with a Bruker AV-300 or ACF 500 spectrometer in the indicated solvents (TMS as internal standard): the values of the chemical shifts are expressed in δ values (ppm) and the coupling constants (J) in Hz. Mass spectra were obtained using FTMS-2000.

4.1.1. *ent*-6β,14α-Dihydroxy-1,7-epoxy-7,15-dioxo-6,20-hemiketal-6,7-seco-16-kaurene (**2**)

Compound **1** (364 mg, 1 mmol) was dissolved in water (10 ml). To this solution was added sodium periodate (3.16 g, 14.8 mmol). The mixture was stirred at room temperature for 24 h and then extracted with dichloromethane. The dichloromethane layer was washed with brine, dried over anhydrous Na_2SO_4, filtered, and evaporated to give compound **2** (360 mg, 99%) as a white powder: mp 183–185 °C; IR (KBr) υ_{max} 3450, 1754, 1645 cm^{-1}; $[\alpha]_D^{20}$ −80.3 (*c* 0.44 $CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 6.17 (1H, d, J = 3.3 Hz, 6-OH), 5.92 (1H, s, 17-CH_2), 5.52 (1H, s, 17-CH_2), 5.38 (1H, d, J = 2.1 Hz, 14-OH), 5.17 (1H, d, J = 2.1 Hz, 6-CH), 4.67 (1H, s, 14-CH), 4.65 (1H, m, 1-CH), 3.87, 3.58 (each 1H, dd, $J_A = J_B$ = 9.1 Hz, 20-CH_2), 2.95 (1H, d, J = 9.1 Hz, 13-CH), 2.49 (2H, m, 12-CH_2), 1.67 (2H, m, 2-CH_2), 1.36 (2H, m, 11-CH_2), 1.26 (1H, m, 9-CH), 0.93 (3H, s, 18-CH_3), 0.88 (3H, s, 19-CH_3); ^{13}C NMR (DMSO-d_6) δ 200.30, 166.88, 150.42, 118.59, 100.73, 75.16, 72.81, 71.28, 62.18, 54.99, 53.78, 49.86, 47.67, 43.07, 36.44, 32.67, 30.70, 29.55, 23.22, 22.82, 18.75; ESIMS m/z 747.4 $[2M + Na]^+$; HR-MS (ESI, M + H) m/z: calcd for $C_{20}H_{27}O_6$: 363.1802, found 363.1799.

4.1.2. *ent*-14α-Hydroxy-1,7-epoxy-6,7,15-trioxo-6,20-epoxy-6,7-seco-16-kaurene (**3**)

Compound **2** (72 mg, 0.2 mmol) was dissolved in acetone. To this solution Jones reagent was added dropwise until a red color persisted. The mixture was stirred at 0 °C for 15 min and isopropanol was added. Then the mixture was diluted with water and extracted with dichloromethane. The extract was washed with brine, dried over anhydrous Na_2SO_4, filtered, and evaporated to give compound **3** (63 mg, 87%) as a white powder: mp 248–250 °C; IR (KBr) υ_{max} 3508, 1785, 1751, 1645 cm^{-1}; $[\alpha]_D^{20}$ −68.5 (*c* 0.23 $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 5.95 (1H, s, 17-CH_2), 5.57 (1H, s, 17-CH_2), 5.57 (1H, d, J = 3.5 Hz, 14-OH), 4.77 (1H, m, 14-CH), 4.72 (1H, m, 1-CH), 4.51, 3.69 (each 1H, dd, $J_A = J_B$ = 9.9 Hz, 20-CH_2), 3.00 (1H, d, J = 9.6 Hz, 13-CH), 1.08 (3H, s, 18-CH_3), 0.95 (3H, s, 19-CH_3); ^{13}C NMR (DMSO-d_6) δ 200.25, 175.45, 166.31, 151.48, 149.84, 119.45, 73.78, 71.03, 70.98, 61.66, 50.19, 47.42, 45.77, 42.87, 35.50, 32.66, 31.83, 29.09, 23.42, 22.87,18.15; ESIMS m/z 361.1 $[M + H]^+$; HR-MS (ESI, M + H) m/z: calcd for $C_{20}H_{25}O_6$: 361.1646, found 361.1647.

4.1.3. *ent*-6β,7β,14β-Trihydroxy-1,15-dioxo-7,20-epoxy-16-kaurene (**4**)

Following the procedure described for preparation of compound **3**, compound **4** was prepared from **1** as a white solid (87.8%): mp 215–217 °C; IR (KBr) υ_{max} 3387, 1702, 1643 cm^{-1}; $[\alpha]_D^{20}$ 115.1 (*c* 0.27 $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 6.25 (1H, s, 17-CH_2), 5.84 (1H, d, J = 12.0 Hz, 6-OH), 5.65 (1H, s, 17-CH_2), 5.30 (1H, s, 14-OH), 4.89 (1H, s, 14-CH), 4.29, 4.02 (each 1H, dd, $J_A = J_B$ = 10.5 Hz, 20-CH_2), 3.79 (1H, m, 6-CH), 3.07 (1H, d, J = 9.3 Hz, 13-CH), 1.19 (3H, s, 18-CH_3), 0.99 (3H, s, 19-CH_3); ^{13}C NMR (DMSO-d_6) δ 212.75, 206.79, 151.49, 121.03, 98.15, 72.31, 72.11, 64.07, 60.86, 59.68, 48.20, 47.92, 42.78, 38.10, 35.40, 32.55, 30.55, 29.48, 23.40, 18.44,; ESIMS m/z 363.2 $[M + H]^+$.

Table 1
IC_{50} Values of *ent*-6,7-*seco*-oridonin derivatives (**2**, **3**, **5**, **9**) for human tumor cell lines.[a]

Compd.	K562	MCF-7	CaEs-17	Bel-7402	Hela	A549
Taxol[b]	0.41 ± 0.02	4.77 ± 0.36	0.43 ± 0.03	1.89 ± 0.09	0.89 ± 0.06	3.46 ± 0.23
Oridonin	4.76 ± 0.32	14.60 ± 1.29	11.03 ± 1.02	7.48 ± 0.53	32.70 ± 2.98	26.15 ± 1.78
2	8.11 ± 0.76	68.20 ± 4.37	30.84 ± 2.09	32.96 ± 2.19	60.70 ± 4.77	50.86 ± 4.26
3	2.64 ± 0.19	20.30 ± 1.98	23.67 ± 1.78	15.98 ± 1.08	39.70 ± 2.98	43.10 ± 3.17
5	2.18 ± 0.17	24.00 ± 1.27	5.85 ± 0.27	5.03 ± 0.37	4.63 ± 0.29	4.58 ± 0.25
9	9.56 ± 0.53	76.70 ± 6.32	72.55 ± 5.38	75.44 ± 4.96	79.35 ± 6.28	25.99 ± 1.33

[a] Results are expressed as IC_{50} values in μM. Cell lines: K562 human leukemia cell line; MCF-7 human breast cancer cell line; CaEs-17 human esophageal cancer cell line; Bel-7402 human hepatoma cell line; Hela human cervical cancer; A549 human lung carcinoma.
[b] Taxol was used a positive control. Data are means of three experiments.

Table 2
The anti-tumor activity of oridonin derivative **5** in mice with MGC-803 gastric cancer.

Compd.	Injection	Number of mice		Weight of mice (g)		Weight of tumor $X \pm SD$ (g)	Ratio of inhibition (%)	*P*-Value
		Start	End	Start	End			
Normal saline	ip	8	8	15.26 ± 0.92	23.16 ± 1.10	1.17 ± 0.13		
Taxol[a]	iv	8	8	15.43 ± 0.93	24.10 ± 1.58	0.39 ± 0.30	66.56%	< 0.01
5	ip	8	8	15.63 ± 1.19	19.81 ± 1.45	0.80 ± 0.18	31.41%	< 0.01
Oridonin	ip	8	8	15.16 ± 0.99	20.06 ± 1.88	0.86 ± 0.30	26.28%	< 0.05

[a] Taxol was used as a positive control.

4.1.4. *ent*-1,6,7,15-Tetraoxo-7,20-epoxy-6,7-seco-16-kaurene (**5**)

Compound **4** (100 mg, 0.28 mmol) was dissolved in THF (10 ml), and sodium carbonate (240 mg, 2.26 mmol) was added. Lead tetraacetate (425 mg, 1 mmol) was added as three portions with intervals of 5 min. The reaction mixture was stirred at room temperature for 15 min, and then insoluble matter was filtered. The mixture was diluted with water and extracted with dichloromethane. The extract was washed with brine, dried over anhydrous Na_2SO_4, filtered, and evaporated to give compound **5** (95 mg, 95%) as a white powder: mp 153–155 °C; IR (KBr) υ_{max} 3444, 1711, 1646 cm^{-1}; $[\alpha]_D^{20}$ 74.1 (*c* 0.20 $CHCl_3$); ^1H NMR ($CDCl_3$) δ 9.84 (1H, s, 6-CHO), 6.21 (1H, s, 17-CH_2), 5.62 (1H, s, 17-CH_2), 5.41 (1H, s, 14-OH), 4.98 (1H, s, 14-CH), 4.98, 4.77 (each 1H, dd, $J_A = J_B = 11.7$ Hz, 20-CH_2), 3.11 (1H, d, $J = 9.3$ Hz, 13-CH), 1.22 (3H, s, 18-CH_3), 1.08 (3H, s, 19-CH_3); ^{13}C NMR (DMSO-d_6) δ 210.23, 204.46, 199.83, 169.68, 149.67, 120.53, 71.87, 67.52, 61.22, 59.88, 52.37, 44.46, 42.94, 36.61, 36.57, 33.61, 30.53, 29.35, 24.81, 17.96; ESIMS *m*/*z* 361.2 $[M + H]^+$; HR-MS (ESI, M + H) *m*/*z*: calcd for $C_{20}H_{25}O_6$: 361.1646, found 361.1646.

4.1.5. *ent*-1α,6β-Dihydroxy-7,14-isopropylidene ketal-15-oxo-7,20-epoxy-16-kaurene (**6**)

Compound **1** (100 mg, 0.27 mmol) was dissolved in acetone (10 ml). TsOH and 2,2-dimethoxypropane (1 ml) were added to this solution. The mixture was stirred at 56 °C for 15 min, and diluted with water and extracted with dichloromethane. The extract was washed with saturated $NaHCO_3$ solution and brine, dried over anhydrous Na_2SO_4, filtered, and evaporated to afford compound **6** (94 mg, 85%) as a white powder: mp 204–206 °C; IR (KBr) υ_{max} 3414, 1713, 1641 cm^{-1}; $[\alpha]_D^{20}$ −13.2 (*c* 0.14 $CHCl_3$); ^1H NMR ($CDCl_3$) δ 6.15 (1H, s, 17-CH_2), 5.77 (1H, d, $J = 11.7$ Hz, 6-OH), 5.56 (1H, s, 17-CH_2), 4.79 (1H, s, 14-CH), 4.24, 4.04 (each 1H, dd, $J_A = J_B = 9.9$ Hz, 20-CH_2), 3.90 (1H, m, 1-CH), 3.47 (1H, m, 6-CH), 3.06 (1H, d, $J = 9.0$ Hz, 13-CH), 1.65 (6H, s, $-CH_3$), 1.15 (3H, s, $-CH_3$), 1.12 (3H, s, $-CH_3$); ^{13}C NMR (DMSO-d_6) δ 206.28, 151.46, 119.68, 100.10, 94.60, 72.74, 72.40, 70.04, 62.76, 58.78, 56.33, 50.52, 40.47, 38.73, 33.39, 30.38, 29.20, 25.75, 22.38, 19.62; ESIMS *m*/*z* 405.2 $[M + H]^+$.

Table 3
IC_{50} Values of 14-*O*-derivatives (**10**–**20**) of *ent*-6,7-*seco*-oridonin **5** for human tumor cell lines.[a]

Compd.	K562	MGC-803	CaEs-17	Bel-7402
Taxol[b]	0.41 ± 0.02	0.85 ± 0.06	0.43 ± 0.03	1.89 ± 0.09
Oridonin	4.76 ± 0.32	5.69 ± 0.39	11.03 ± 1.02	7.48 ± 0.53
10	1.39 ± 0.17	1.66 ± 0.15	1.35 ± 0.27	1.73 ± 0.65
11	1.31 ± 0.12	1.78 ± 0.89	1.40 ± 0.31	1.65 ± 0.82
12	1.48 ± 0.22	1.92 ± 0.70	1.71 ± 0.08	1.85 ± 0.53
13	1.27 ± 0.34	2.24 ± 0.18	1.05 ± 0.90	1.54 ± 0.90
14	1.65 ± 0.39	1.87 ± 0.95	2.98 ± 0.16	2.80 ± 0.72
15	2.82 ± 0.11	4.93 ± 0.24	8.96 ± 0.23	3.99 ± 0.33
16	1.33 ± 0.52	3.33 ± 0.38	2.68 ± 0.13	3.30 ± 1.00
17	2.70 ± 0.42	3.96 ± 0.19	8.31 ± 0.46	3.75 ± 0.33
18	2.86 ± 0.24	3.94 ± 0.67	8.55 ± 0.50	3.95 ± 1.08
19	1.38 ± 0.27	3.12 ± 0.16	2.55 ± 0.32	3.84 ± 0.91
20	1.22 ± 0.48	2.66 ± 0.44	2.01 ± 0.07	2.89 ± 0.34

[a] Results are expressed as IC_{50} values in μM. Cell lines: MGC-803 human gastric cancer cell line.
[b] Taxol was used a positive control. Data are means of three experiments.

4.1.6. *ent*-1α-*O*-Acetyl-6β-hydroxy-7,14-isopropylidene ketal-15-oxo-7,20-epoxy-16-kaurene (**7**)

Compound **6** (90 mg, 0.22 mmol) was dissolved in dichloromethane, DMAP and TEA (0.2 ml) were added. The mixture was stirred at room temperature for 2.5 h, and diluted with water and extracted with dichloromethane. The extract was washed with saturated $NaHCO_3$ solution and brine, dried over anhydrous Na_2SO_4, filtered, and evaporated to give compound **7** (94 mg, 95%) as a white powder: mp 112–114 °C; IR (KBr) υ_{max} 3367, 1737, 1643 cm^{-1}; $[\alpha]_D^{20}$ −3.0 (*c* 0.47 $CHCl_3$); ^1H NMR ($CDCl_3$) δ 6.16 (1H, s, 17-CH_2), 6.12 (1H, d, $J = 9.0$ Hz, 6-OH), 5.80 (1H, s, 14-CH), 5.51 (1H, s, 17-CH_2), 4.64 (1H, m, 1-CH), 4.27, 4.20 (each 1H, dd, $J_A = J_B = 10.8$ Hz, 20-CH_2), 3.80 (1H, m, 6-CH), 3.20 (1H, d, $J = 9.9$ Hz, 13-CH), 2.06 (3H, s, $-CH_3$), 2.00 (3H, s, $-CH_3$), 1.13 (6H, s, $-CH_3$); ^{13}C NMR (DMSO-d_6) δ 207.37, 169.73, 169.24, 150.67, 119.68, 95.76, 74.71, 74.01, 73.31, 62.40, 61.67, 59.22, 52.24, 41.48, 37.56, 33.34, 32.41, 30.08, 24.94, 21.53, 21.34, 17.89; ESIMS *m*/*z* 447.2 $[M + H]^+$.

4.1.7. *ent*-1α-*O*-Acetyl-6β,7β,14β-trihydroxy-15-oxo-7,20-epoxy-16-kaurene (**8**)

Ketal deprotection of **7** (50 mg, 0.11 mmol) was added to 10 ml of 10% HCl/THF (1:1) and the solution was stirred at room temperature for 1 h. Then the mixture was diluted with water and extracted with dichloromethane. The extract was washed with brine, dried over anhydrous Na_2SO_4, filtered, and evaporated to give compound **8** (45 mg, 99%) as a white powder: mp 222–224 °C; IR (KBr) υ_{max} 3449, 1721, 1646 cm^{-1}; $[\alpha]_D^{20}$ −16.3 (*c* 0.14 $CHCl_3$); ^1H NMR ($CDCl_3$) δ 6.34 (1H, d, $J = 11.4$ Hz, 6-OH), 6.18 (1H, s, 17-CH_2), 5.56 (1H, s, 17-CH_2), 4.86 (1H, s, 14-CH), 4.45 (1H, m, 1-CH), 4.26, 4.18 (each 1H, dd, $J_A = J_B = 10.2$ Hz, 20-CH_2), 3.80 (1H, m, 6-CH), 3.06 (1H, d, $J = 9.3$ Hz, 13-CH), 1.98 (3H, s, $-CH_3$), 1.15 (6H, s, $-CH_3$); ^{13}C NMR (DMSO-d_6) δ 208.60, 169.67, 151.80, 119.62, 96.98, 74.86, 73.11, 72.59, 62.56, 61.32, 59.42, 51.52, 42.76, 39.20, 37.06, 33.34, 32.46, 29.83, 24.94, 21.64, 21.36, 17.51; ESIMS *m*/*z* 407.2 $[M + H]^+$.

4.1.8. *ent*-1α-*O*-Acetyl-14β-hydroxy-6,7,15-trioxo-7,20-epoxy-6,7-seco-16-kaurene (**9**)

Following the procedure described for preparation of compound **5**, compound **9** was prepared from compound **8** as a white solid (97%): mp 154–156 °C; IR (KBr) υ_{max} 3422, 1740, 1712 cm^{-1}; $[\alpha]_D^{20}$ 36.9 (*c* 0.37 $CHCl_3$); ^1H NMR ($CDCl_3$) δ 9.76 (1H, d, $J = 4.2$ Hz, 6-CHO), 6.23 (1H, s, 17-CH_2), 5.71 (1H, s, 14-OH), 5.63 (1H, s, 17-CH_2), 5.22, 5.12 (each 1H, dd, $J_A = J_B = 12.3$ Hz, 20-CH_2), 4.66 (1H, m, 1-CH), 4.40 (1H, s, 14-CH), 3.07 (1H, d, $J = 9.0$ Hz, 13-CH), 2.03 (3H, s, $-CH_3$), 1.19 (3H, s, $-CH_3$), 1.04 (3H, s, $-CH_3$); ^{13}C NMR (DMSO-d_6) δ 205.37, 199.34, 171.86, 169.40, 149.60, 120.99, 74.76, 71.47, 67.26, 60.82, 60.12, 44.18, 42.93, 42.61, 38.44, 33.73, 32.08, 29.40, 24.34, 23.32, 21.05, 16.89; ESIMS *m*/*z* 405.1 $[M + H]^+$; HR-MS (ESI, M + H) *m*/*z*: calcd for $C_{22}H_{29}O_7$: 405.1908, found 405.1911.

4.1.9. ent-1,6,7,15-Tetraoxo-7,20-epoxy-(14β-O-acetyl)-6,7-seco-16-kaurene (**10**)

Compound **5** (72 mg, 0.2 mmol) was dissolved in dichloromethane, then EDC, DMAP and acetic acid (15 mg, 0.24 mmol) were added. The reaction mixture was stirred at room temperature for about 8 h. Then the mixture was washed with 10% HCl. The organic layer was washed with brine, dried over anhydrous Na_2SO_4. After flash chromatography (dichloromethane/methanol 300:1), **10** was got as white solid (48 mg, 69%): mp 96–97 °C; IR (KBr) υ_{max} 2962, 1771, 1732, 1648, 1463, 1373, 1176 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.85 (1H, s, −CHO), 6.21 (1H, s, 17-CH_2), 5.72 (1H, s, 14-CH), 5.62 (1H, s, 17-CH_2), 4.95, 4.73 (each 1H, dd, $J_A = J_B$ = 11.7 Hz, 20-CH_2), 3.14 (1H, d, J = 9.3 Hz, 13-CH), 1.22 (3H, s, $-CH_3$), 1.08 (3H, s, $-CH_3$), 1.06 (3H, s, $-CH_3$); ^{13}C NMR (DMSO-d_6) δ 210.23, 204.13, 198.18, 169.78, 166.22, 146.94, 120.62, 73.79, 67.37, 61.12, 59.77, 51.99, 45.11, 41.36, 36.68, 36.14, 33.15, 29.95, 29.07, 28.92, 23.86, 17.89; ESIMS m/z 403.1 $[M + H]^+$, 420.2 $[M + NH_4]^+$, 437.5 $[M + Cl]^-$; HR-MS (ESI, M + H) m/z: calcd for $C_{22}H_{27}O_7$: 403.1751, found 403.1759.

4.1.10. ent-1,6,7,15-Tetraoxo-7,20-epoxy-(14β-O-propionyl)-6,7-seco-16-kaurene (**11**)

Following the procedure described for preparation of compound **10**, compound **11** was prepared from compound **5** as a white solid (43 mg, 62%): mp 132–133 °C; IR (KBr) υ_{max} 2960, 1765, 1725, 1647, 1467, 1374, 1244 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.85 (1H, s, −CHO), 6.20 (1H, s, 17-CH_2), 5.74 (1H, s, 14-CH), 5.63 (1H, s, 17-CH_2), 4.97, 4.72 (each 1H, dd, $J_A = J_B$ = 11.4 Hz, 20-CH_2), 3.10 (1H, d, J = 8.7 Hz, 13-CH), 1.24 (3H, s, $-CH_3$), 1.08 (3H, s, $-CH_3$), 0.86 (3H, s, $-CH_3$); ^{13}C NMR (DMSO-d_6) δ 210.23, 204.08, 198.16, 173.02, 166.14, 146.96, 120.52, 73.59, 67.34, 61.11, 59.78, 51.97, 45.09, 41.36, 36.66, 36.12, 33.14, 29.92, 29.06, 26.75, 23.89, 17.88, 8.59; ESIMS m/z 417.2 $[M + H]^+$, 434.2 $[M + NH_4]^+$, 451.4 $[M + Cl]^-$; HR-MS (ESI, M + H) m/z: calcd for $C_{23}H_{29}O_7$: 417.1908, found 417.1903.

4.1.11. ent-1,6,7,15-Tetraoxo-7,20-epoxy-(14β-O-benzoyl)-6,7-seco-16-kaurene (**12**)

Following the procedure described for preparation of compound **10**, compound **12** was prepared from compound **5** as a white solid (45 mg, 62%): mp 102–104 °C; IR (KBr) υ_{max} 2959, 1768, 1717, 1646, 1452, 1274, 762, 713 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.82 (1H, s, −CHO), 7.79 (2H, d, J = 5.7 Hz, Ar−H), 7.63 (1H, t, J = 5.2 Hz, Ar−H), 7.49 (2H, t, J = 5.2 Hz, Ar-H), 6.22 (1H, s, 17-CH_2), 6.16 (1H, s, 14-CH), 5.78 (1H, s, 17-CH_2), 4.94, 4.55 (each 1H, dd, $J_A = J_B$ = 7.5 Hz, 20-CH_2), 3.33 (1H, d, J = 7.5 Hz, 13-CH), 1.19 (3H, s, $-CH_3$), 1.13 (3H, s, $-CH_3$); ^{13}C NMR (DMSO-d_6) δ 210.18, 204.16, 198.15, 166.02, 164.81, 147.11, 133.46, 129.12, 128.65, 120.95, 74.47, 67.38, 60.76, 59.89, 52.14, 45.19, 41.51, 36.59, 36.31, 33.22, 30.03, 29.14, 23.95, 17.95; ESIMS m/z 465.1 $[M + H]^+$, 482.2 $[M + NH_4]^+$, 499.3 $[M + Cl]^-$; HR-MS (ESI, M + H) m/z: calcd for $C_{27}H_{29}O_7$: 465.1908, found 465.1906.

4.1.12. ent-1,6,7,15-Tetraoxo-7,20-epoxy-(14β-O-p-methylbenzoyl)-6,7-seco-16-kaurene (**13**)

Following the procedure described for preparation of compound **10**, compound **13** was prepared from compound **5** as a white solid (41 mg, 62%): mp 208–210 °C; IR (KBr) υ_{max} 2957, 1763, 1718, 1612, 1465, 1289, 748 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.82 (1H, s, −CHO), 7.68 (2H, d, J = 3.4 Hz, Ar−H), 7.29 (2H, d, J = 3.4 Hz, Ar−H), 7.45 (1H, t, J = 1.8 Hz, Ar−H), 6.19 (1H, s, 17-CH_2), 6.15 (1H, s, 14-CH), 5.77 (1H, s, 17-CH_2), 4.92, 4.53 (each 1H, dd, $J_A = J_B$ = 7.5 Hz, 20-CH_2), 3.31 (1H, d, J = 7.5 Hz, 13-CH), 2.36 (3H, s, $-CH_3$), 1.19 (3H, s, $-CH_3$), 1.13 (3H, s, $-CH_3$); ^{13}C NMR (DMSO-d_6) δ 210.22, 204.16, 198.21, 166.01, 164.85, 147.13, 143.90, 129.21, 126.56, 120.87, 74.34, 67.39, 60.87, 59.89, 52.13, 45.19, 41.54, 36.64, 36.27, 33.24, 30.06, 29.15, 24.02, 21.15, 17.98; ESIMS m/z 479.2 $[M + H]^+$, 496.3 $[M + NH_4]^+$, 513.6 $[M + Cl]^-$; HR-MS (ESI, M + H) m/z: calcd for $C_{28}H_{31}O_7$: 479.2064, found 479.2059.

4.1.13. ent-1,6,7,15-Tetraoxo-7,20-epoxy-(14β-O-p-methoxybenzoyl)-6,7-seco-16-kaurene (**14**)

Following the procedure described for preparation of compound **10**, compound **14** was prepared from compound **5** as a white solid (23 mg, 35%): mp 108–110 °C; IR (KBr) υ_{max} 2958, 1768, 1714, 1606, 1512, 1466, 769, 695 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.81 (1H, s, −CHO), 7.78 (2H, d, J = 8.4 Hz, Ar−H), 6.82 (2H, d, J = 8.4 Hz, Ar−H), 6.19 (1H, s, 17-CH_2), 6.15 (1H, s, 14-CH), 5.77 (1H, s, 17-CH_2), 4.94, 4.55 (each 1H, dd, $J_A = J_B$ = 8.4 Hz, 20-CH_2), 3.74 (3H, s, $-OCH_3$), 3.32 (1H, d, J = 7.2 Hz, 13-CH), 1.17 (3H, s, $-CH_3$), 1.14 (3H, s, $-CH_3$); ^{13}C NMR (DMSO-d_6) δ 210.26, 204.17, 198.26, 166.04, 163.35, 147.16, 132.11, 131.32, 114.01, 74.21, 67.40, 60.97, 59.91, 55.52, 52.12, 45.17, 41.58, 36.68, 33.24, 30.04, 29.12, 24.05, 17.99; ESIMS m/z 495.0 $[M + H]^+$, 512.1 $[M + NH_4]^+$, 529.2 $[M + Cl]^-$; HR-MS (ESI, M + H) m/z: calcd for $C_{28}H_{31}O_8$: 495.2013, found 495.2011.

4.1.14. ent-1,6,7,15-Tetraoxo-7,20-epoxy-(14β-O-p-trifloromethylbenzoyl)-6,7-seco-16-kaurene (**15**)

Following the procedure described for preparation of compound **10**, compound **15** was prepared from compound **5** as a white solid (40 mg, 37%): mp 252–254 °C; IR (KBr) υ_{max} 2968, 1761, 1732, 1710, 1412, 1383, 1325, 1291, 1261, 1281, 1127, 1101, 1065, 1051, 1018, 768, 701 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.85(1H, s, −CHO), 8.04 (1H, d, J = 8.1 Hz, Ar−H), 7.80 (1H, d, J = 8.1 Hz, Ar−H), 7.65 (1H, d, J = 8.1 Hz, Ar−H), 7.17 (1H, d, J = 8.1 Hz, Ar−H), 6.29 (1H, s, 17-CH_2), 6.22 (1H, s, 14-CH), 5.61 (1H, s, 17-CH_2), 4.88, 4.50 (each 1H, dd, $J_A = J_B$ = 12.5 Hz, 20-CH_2), 3.32 (1H, d, J = 7.5 Hz, 13-CH), 2.62 (1H, m, 12-CH_2), 2.10 (1H, m, 12-CH_2), 1.91 (2H, m, 11-CH_2), 1.72 (1H, m, 9-CH), 1.69 (2H, m, 2-CH_2), 1.57 (2H, m, 3-CH_2), 1.27 (1H, m, 5-CH_2), 1.25 (3H, s, 18-CH_3), 1.24 (3H, s, 19-CH_3); ^{13}C NMR (DMSO-d_6) δ 210.14, 204.16, 198.08, 166.10, 163.78, 133.12, 132.76, 130.14, 129.95, 125.81, 121.32, 75.00, 67.45, 60.49, 59.97, 52.29, 45.32, 41.49, 36.52, 33.27, 30.36, 29.27, 23.85, 17.94; ESIMS m/z 533.1 $[M + H]^+$, 550.3 $[M + NH_4]^+$; HR-MS (ESI, M + H) m/z: calcd for $C_{28}H_{28}F_3O_7$: 533.1782, found 533.1784.

4.1.15. ent-1,6,7,15-Tetraoxo-7,20-epoxy-(14β-O-p-nitrobenzoyl)-6,7-seco-16-kaurene (**16**)

Following the procedure described for preparation of compound **10**, compound **16** was prepared from compound **5** as a white solid (62 mg, 83%): mp 268–270 °C; IR (KBr) υ_{max} 2956, 1758, 1729, 1647, 1533, 1467, 846 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.84 (1H, s, −CHO), 8.18 (2H, d, J = 8.4 Hz, Ar−H), 8.02 (2H, d, J = 8.4 Hz, Ar−H), 6.19 (1H, s, 17-CH_2), 6.15 (1H, s, 14-CH), 5.75 (1H, s, 17-CH_2), 4.95, 4.53 (each 1H, dd, $J_A = J_B$ = 8.1 Hz, 20-CH_2), 3.25 (1H, d, J = 7.2 Hz, 13-CH), 1.16 (3H, s, $-CH_3$), 1.13 (3H, s, $-CH_3$); ^{13}C NMR (DMSO-d_6) δ 210.15, 204.22, 198.07, 166.11, 163.40, 150.31, 147.07, 134.78, 130.53, 123.94, 121.53, 75.22, 67.46, 60.37, 59.98, 52.31, 45.35, 41.44, 36.48, 33.28, 30.21, 29.30, 23.84, 17.92; ESIMS m/z 510.1 $[M + H]^+$, 527.3 $[M + NH_4]^+$, 544.3 $[M + Cl]^-$; HR-MS (ESI, M + NH_4) m/z: calcd for $C_{27}H_{31}N_2O_9$: 527.2024, found 527.2021.

4.1.16. ent-1,6,7,15-Tetraoxo-7,20-epoxy-(14β-O-p-florobenzoyl)-6,7-seco-16-kaurene (**17**)

Following the procedure described for preparation of compound **10**, compound **17** was prepared from compound **5** as a white solid (12 mg, 12%): mp 244–246 °C; IR (KBr) υ_{max} 2963, 1763, 1724, 1604, 1505, 1283, 1261, 1151, 1093, 1050, 1016, 854, 802, 761, 686, 612 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.90 (1H, s, −CHO), 7.97 (2H, m, Ar−H), 7.07 (2H, m, Ar−H), 6.31 (1H, s, 17-CH_2), 6.16 (1H, s, 14-CH), 5.63 (1H, s, 17-CH_2), 4.88, 4.50 (each 1H, dd, $J_A = J_B$ = 12.5 Hz, 20-CH_2), 3.34 (1H, d, J = 7.5 Hz, 13-CH), 2.62 (1H, m, 12-CH_2), 2.10 (1H, m, 12-CH_2), 1.91 (2H, m, 11-CH_2), 1.72 (1H, m, 9-CH), 1.69 (2H, m, 2-CH_2), 1.57 (2H, m, 3-CH_2), 1.45 (1H, m, 5-CH_2), 1.31 (3H, s, 18-CH_3), 1.27 (3H, s, 19-CH_3); ^{13}C NMR (DMSO-d_6) δ 210.12, 204.03, 198.11, 166.15, 163.87, 147.10, 132.05, 127.31, 125.90, 120.98, 115.72, 74.61, 67.41,

60.70, 59.92, 52.20, 45.23, 41.53, 36.56, 33.21, 30.34, 29.16, 23.90, 17.94; ESIMS *m/z* 483.1 $[M + H]^+$, 500.1 $[M + NH_4]^+$; HR-MS (ESI, M + H) *m/z*: calcd for $C_{27}H_{28}FO_7$: 483.1814, found 483.1811.

4.1.17. ent-1,6,7,15-Tetraoxo-7,20-epoxy-(14β-O-o-florobenzoyl)-6, 7-seco-16-kaurene (**18**)

Following the procedure described for preparation of compound **10**, compound **18** was prepared from compound **5** as a white solid (13 mg, 13%): mp 206–208 °C; IR (KBr) υ_{max} 2924, 2853, 1701, 1686, 1655, 1615, 1561, 1466, 1384, 1257, 1090, 775, 649 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.87 (1H, s, –CHO), 1.25 (3H, s, 19-CH_3), 1.26 (3H, s, 18-CH_3), 1.48 (1H, m, 5-CH_2), 1.68 (2H, m, 3-CH_2), 1.86 (2H, m, 2-CH_2), 7.85 (1H, m, Ar–H), 7.50 (1H, m, Ar–H), 7.15 (1H, m, Ar–H), 7.07 (1H, m, Ar–H), 6.29 (1H, s, 17-CH_2), 6.19 (1H, s, 14-CH), 5.62 (1H, s, 17-CH_2), 4.90, 4.50 (each 1H, dd, $J_A = J_B$ = 12.3 Hz, 20-CH_2), 3.33 (1H, d, J = 7.5 Hz, 13-CH), 2.64 (1H, m, 12-CH_2), 2.53 (1H, m, 12-CH_2), 2.46 (2H, m, 11-CH_2), 1.89 (1H, m, 9-CH); ^{13}C NMR (DMSO-d_6) δ 210.16, 204.13, 198.13, 166.07, 163.66, 162.76, 160.81, 147.08, 131.68, 125.35, 121.23, 120.61, 115.64, 74.90, 67.44, 60.58, 59.93, 52.23, 45.26, 41.49, 36.46, 33.26, 30.14, 29.19, 23.93, 17.94; ESIMS *m/z* 483.1 $[M + H]^+$, 500.3 $[M + NH_4]^+$; HR-MS (ESI, M + H) *m/z*: calcd for $C_{27}H_{28}FO_7$: 483.1814, found 483.1807.

4.1.18. ent-1,6,7,15-Tetraoxo-7,20-epoxy-(14β-O-p-chlorobenzoyl)-6, 7-seco-16-kaurene (**19**)

Following the procedure described for preparation of compound **10**, compound **19** was prepared from compound **5** as a white solid (46 mg, 62%): mp 234–236 °C; IR (KBr) υ_{max} 2937, 1760, 1726, 1643, 1593, 1485, 849 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.82 (1H, s, –CHO), 7.65 (1H, t, J = 1.8 Hz, Ar–H), 7.55 (2H, m, J = 3.0 Hz, Ar–H), 7.45 (1H, t, J = 1.8 Hz, Ar–H), 6.26 (1H, s, 17-CH_2), 6.12 (1H, s, 14-CH), 5.76 (1H, s, 17-CH_2),1.13 (3H, s, –CH_3), 4.94, 4.58 (each 1H, dd, $J_A = J_B$ = 7.5 Hz, 20-CH_2), 3.31 (1H, d, J = 7.5 Hz, 13-CH), 1.19 (3H, s, –CH_3); ^{13}C NMR (DMSO-d_6) δ 210.12, 204.12, 198.08, 166.00, 163.98, 147.07, 138.42, 130.91, 128.91, 128.16, 121.13, 74.70, 67.39, 60.59, 59.90, 52.19, 45.24, 41.46, 36.41, 33.22, 30.11, 29.19, 23.90, 17.91; ESIMS *m/z* 499.2 $[M + H]^+$, 516.2 $[M + NH_4]^+$, 533.6 $[M + Cl]^-$; HR-MS (ESI, M + NH_4) *m/z*: calcd for $C_{27}H_{31}ClNO_7$: 516.1784, found 516.1785.

4.1.19. ent-1,6,7,15-Tetraoxo-7,20-epoxy-(14β-O-o-chlorobenzoyl)-6, 7-seco-16-kaurene (**20**)

Following the procedure described for preparation of compound **10**, compound **20** was prepared from compound **5** as a white solid (41 mg, 63%): mp 217–219 °C; IR (KBr) υ_{max} 2956, 1760, 1725, 1643, 1591, 1470, 752 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.87 (1H, s, –CHO), 7.78 (1H, d, J = 7.8 Hz, Ar–H), 7.24 (2H, m, Ar–H), 7.16 (1H, m, Ar–H), 6.81 (2H, d, J = 8.4 Hz, Ar–H), 6.23 (1H, s, 17-CH_2), 6.12 (1H, s, 14-CH), 5.54 (1H, s, 17-CH_2), 4.93, 4.55 (each 1H, dd, $J_A = J_B$ = 8.4 Hz, 20-CH_2), 3.34 (1H, d, J = 6.9 Hz, 13-CH), 1.16 (3H, s, –CH_3), 1.12 (3H, s, –CH_3); ^{13}C NMR (DMSO-d_6) δ 210.15, 204.12, 197.98, 166.10, 163.58, 147.11, 133.43, 132.23, 131.24, 130.79, 129.07, 127.25, 121.10, 74.92, 67.46, 60.59, 59.95, 52.26, 45.37, 41.50, 36.49, 33.21, 30.12, 29.21, 23.78, 17.90; ESIMS *m/z* 499.0 $[M + H]^+$, 516.2 $[M + NH_4]^+$, 533.4 $[M + Cl]^-$; HR-MS (ESI, M + NH_4) *m/z*: calcd for $C_{27}H_{31}ClNO_7$: 516.1784, found 516.1782.

4.2. Cytotoxic activity in vitro

The MTT assay was employed *in vitro* cytotoxicity assay, which was performed in 96-well plates. K562 cell at the log phase of their growth cycle (5×10^4 cell/ml) were added to each well (100 μl/well), then treated in four replicates at various concentrations of the samples (0.39–100 μg/ml), and incubated for 24 h at 37 °C in a humidified atmosphere of 5% CO_2. After 72 h, 20 μl of MTT solution (5 mg/ml) per well was added to each cultured medium, which was incubated for further 4 h. Then, DMSO was added to each well (150 μl/well). After 10 min at room temperature, the OD of each well was measured on a Microplate Reader (BIO-RAD instruments Inc NO.550) at a wavelength of 490 nm. In these experiments, the negative reference agents was 0.1% DMSO, and Taxol was used as the positive reference substance with concentration of 10 μg/ml. The same method was used in cytotoxic testing against MCF-7, CaEs-17, Bel-7402, Hela, A549 and MGC-803 cell lines.

4.3. Anti-tumor activity in vivo

Institute of Cancer Research (ICR) female mice with body weight of 12–16 g was transplanted with MGC-803 cell subcutaneously into the right oxter according to protocols of tumor transplant research. After 7 d of tumor transplantation, mice in MGC-803 group was weighed and divided into four groups at random. The groups with oridonin and **5** were administered intraperitoneally 10 mg/kg in a vehicle of 1% DMSO/2% poloxamer/97% saline, respectively. The positive control group was treated with Taxol (10 mg/kg) through intravenous injection in a vehicle of 1% DMSO/2% poloxamer/97% saline. The negative control group received 0.9% normal saline through intraperitoneal injection. All of the test compounds were given through injections after 7 d of tumor transplantation (or inoculation). Treatments were done at a frequency of intravenous or intraperitoneal injection one dose per day for a total of 25 consecutive days. After the treatments, all of the mice were killed and weighed simultaneously, and then tumors were segregated and weighed. Ratio of inhibition of tumor (%) = (1 − average tumor weight of treated group/average tumor weight of control group) × 100%.

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References

[1] B.B. Mishra, V.K. Tiwari, Eur. J. Med. Chem. 46 (2011) 4769–4807.
[2] P.A. García, A.B. Oliveira, R. Batista, Molecules 12 (2007) 455–483.
[3] L. Wang, D.-H. Li, C.-L. Wang, Y.-H. Zhang, J.-Y. Xu, Mini-Rev. Med. Chem. 11 (2011) 910–919.
[4] J.-Y. Yang, J.-Y. Xu, X.-M. Wu, W.-Y. Hua, Chin. J. Mode. Appl. Pharm. 24 (2007) 277–282.
[5] Y. Aoyagi, Y. Adachi, K. Ozawa, C. Yokomizo, M.Y. Gui, Y.R. Jin, X.W. Li, N. Ohno, K. Takeya, Bioorg. Med. Chem. 19 (2011) 2450–2457.
[6] C.-H. Leung, S.P. Grill, W. Lam, Q.-B. Han, H.-D. Sun, Y.-C. Cheng, Mol. Pharmacol. 68 (2005) 286–297.
[7] N. Kang, J.-H. Zhang, F. Qiu, S. Chen, S. Tashiro, S. Onodera, T. Ikejiama, J. Nat. Prod. 73 (2010) 1058–1063.
[8] X. Luo, J.-X. Pu, W.-L. Xiao, Y. Zhao, X.-M. Gao, X.-N. Li, H.-B. Zhang, Y.-Y. Wang, Y. Li, H.-D. Sun, J. Nat. Prod. 73 (2010) 1112–1116.
[9] L.-M. Li, G.-Y. Li, J.-X. Pu, W.-L. Xiao, L.-S. Ding, H.-D. Sun, J. Nat. Prod. 72 (2009) 1851–1856.
[10] X.-N. Li, J.-X. Pu, X. Du, L.-G. Lou, L.-M. Li, S.-X. Huang, B. Zhao, M. Zhang, F. He, X. Luo, W.-L. Xiao, H.-D. Sun, J. Nat. Prod. 73 (2010) 1803–1809.
[11] X. Li, J.-X. Pu, Z.-Y. Weng, Y. Zhao, Y. Zhao, W.-L. Xiao, H.-D. Sun, Chem. Biodivers. 7 (2010) 2888–2896.
[12] J.-Y. Xu, J.-Y. Yang, Q. Ran, L. Wang, J. Liu, Z.-X. Wang, X.-M. Wu, W.-Y. Hua, S.-T. Yuan, L.-Y. Zhang, M.-Q. Shen, Y.-F. Ding, Bioorg. Med. Chem. Lett. 18 (2008) 4741–4744.
[13] L. Wang, Q. Ran, D.-H. Li, H.-Q. Yao, Y.-H. Zhang, S.-T. Yuan, L.-Y. Zhang, M.-Q. Shen, J.-Y. Xu, Chin. J. Nat. Med. 9 (2011) 161–166.
[14] K. Fuji, M. Node, M. Sai, E. Fujita, T. Shingu, W.H. Watson, D.A. Grossie, V. Zabel, Chem. Pharm. Bull. 37 (1989) 1465–1469.
[15] E. Fujita, Y. Nagao, K. Kaneko, S. Nakazawa, H. Kuroda, Chem. Pharm. Bull. 24 (1976) 2118–2127.

[16] M. Node, M. Sai, K. Fuji, E. Fujita, S. Takeda, N. Unemi, Chem. Pharm. Bull. 31 (1983) 1433–1436.
[17] Y. Zhao, X.-M. Niu, L.-P. Qian, Z.-Y. Liu, Q.-S. Zhao, H.-D. Sun, Eur. J. Med. Chem. 42 (2007) 494–502.
[18] A. Ramani, N. Birlirakis, A.F. Barrero, S. Arseniyadis, Eur. J. Org. Chem. 4 (2005) 683–700.
[19] T. Miura, K. Kanemoto, S. Natsume, K. Atsumi, H. Fushimi, T. Yoshida, K. Ajito, Bioorg. Med. Chem. 16 (2008) 10129–10156.
[20] J.-Y. Xu, L. Wang, Y.-H. Zhang, H.-Q. Yao, D.-H. Li, C.-L. Wang, Q. Liu, Chin. Pat. Appl. 201010509348.5 (2010).
[21] J.-Y. Xu, D.-H. Li, H. Cai, L. Wang, S.-T. Xu, F. Sun, H.-Q. Yao, J.-Y. Jiang, Y.-J. Sun, Chin. Pat. Appl. 201110254258.0 (2011).