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Structural modification of oridonin *via* DAST induced rearrangement†

Dong-Dong Luo,‡[a] Kai Peng,‡[a] Jia-Yu Yang,[a] Pawinee Piyachaturawat,[b] Witchuda Saengsawang,[b] Lei Ao,[c] Wan-Zhou Zhao,[c] Yu Tang *[a] and Sheng-Biao Wan*[a]

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A simple and efficient protocol was developed for the syntheses of oridonin analogues, *i.e.* 6,20-epoxy *ent*-kaurane diterpenoid analogues from oridonin *via* diethylaminosulfur trifluoride (DAST) promoted rearrangement, most of which exhibited superior anticancer activities compared with their precursor.

Introduction

Oridonin, an *ent*-kaurane diterpenoid extracted from *Isodon rubescens* (Chinese name "Donglingcao"), has received particular interest from the pharmaceutical community[1,2] due to its pharmacological utilities, *e.g.* unique and prominent anticancer activity[3] as well as its safety. It has been demonstrated that oridonin could significantly suppress cancer cell migration *via* regulation of non-muscle myosin IIA.[4] The anticancer mechanism of oridonin *in vitro* may involve multiple pathways,[5–13] for instance, oridonin can not only inhibit the proliferation of the breast cancer cell McF-7, but also induce apoptosis through the pathways of hampering the cell cycle and activation of mitochondria.[9,14–18] Targeting AML1-ETO (AE) fusion protein which plays a critical role in leukemogenesis shows potent antitumor activity with low adverse effects on t(8;21) leukemia *in vitro* and *in vivo*.[19] However, the clinical application of oridonin has been significantly impeded by its poor aqueous solubility, moderate potency, low bioavailability[20] and metabolic instability.[21] Thus it's highly desirable to synthesize the oridonin analogues *via* rational chemical modification of its structure for better pharmacological properties such as anticancer activity.

Oridonin belongs to the class of 7,20-epoxy *ent*-kaurane diterpenoid structures, which features 1,6,14-trihydroxy groups, 7-hemiacetal moiety in the B-ring and the α-methylene cyclopentanone in the D-ring. The strong hydrogen bonding interaction exists between 6β-OH and carbonyl group at C-15 (Fig. 1). It's difficult for 6β-OH and 7β-OH to participate in nucleophilic reaction due to the adjacent steric hindrance and the intramolecular hydrogen bonding interaction. The previous studies of oridonin on the structure–activity relationship (SAR) have proved that D-ring is crucial to anti-cancer activity,[22,23] and any modification of enone moiety[24] would lead to loss of anticancer activity.[25] As shown in Fig. 1, previous efforts on structural modification of oridonin were mainly focused on A ring,[2,26–28] 6-*O* positions[29] and 14-*O* positions[17,30,31] as well as B-ring opening *via* oxidative cleavage of C–C bond between C-6 and C-7.[31]

Diethylaminosulfur trifluoride (DAST) has been employed as a versatile fluorinating agent for various fluorination reactions,[32–34] *e.g.* fluorination of carbohydrate in which structural

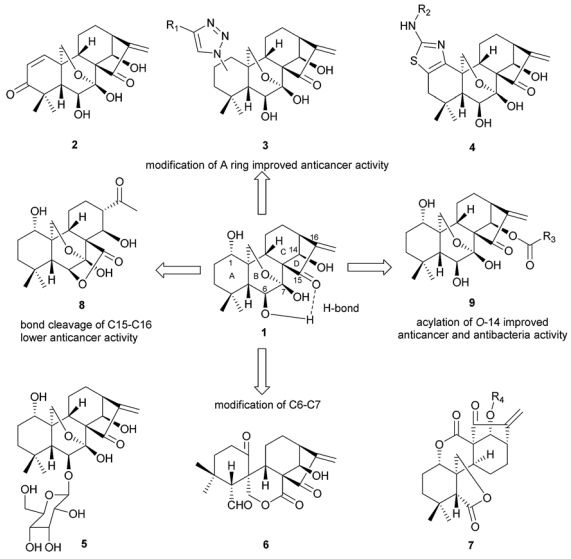

Fig. 1 Structures of oridonin and biologically active analogues.

[a]*Laboratory for Marine Drugs and Bioproducts of Qingdao National Laboratory for Marine Science and Technology, Key Laboratory of Marine Drugs, Ministry of Education, School of Medicine and Pharmacy, Ocean University of China, Yushan Road 5, Qingdao 266003, China. E-mail: biaowan@ouc.edu.cn; tangyu@ouc.edu.cn; Tel: +86-532-82031087*

[b]*Department of Physiology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand*

[c]*The Nanjing Han & Zaenker Cancer Institute (NHZCI), Nanjing OGpharma Co. Ltd., Nanjing 210036, China*

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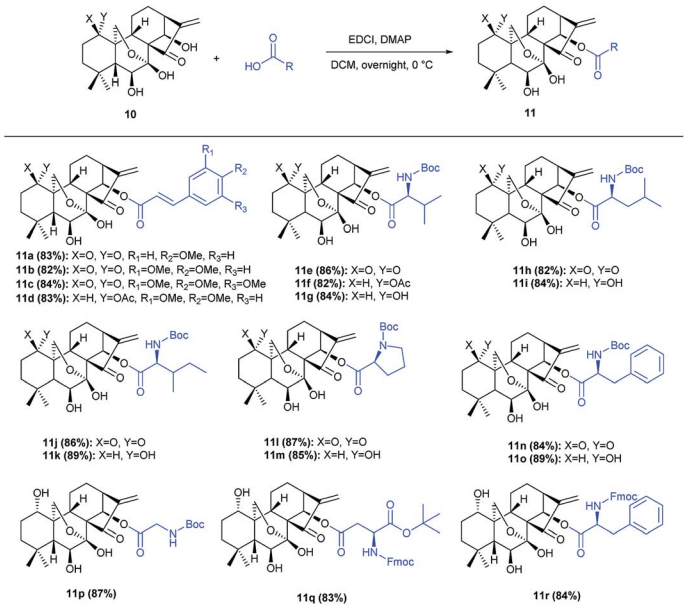
‡ These authors contributed equally to this work.



Scheme 1 Synthesis of 14-acyl oridonin analogues **11**.

rearrangements could be observed.[34–37] Therefore, it can be envisioned that the similar structural rearrangements of oridonin could occur in the presence of DAST, yielding new oridonin analogues. As a continuation of our research works on pharmaceutical molecules,[38,39] herein, we disclosed a simple and efficient method for preparation of novel 6,20-epoxy *ent*-kaurane diterpenoid analogues from oridonin, in which 6,20-*endo* ring was formed *via* DAST promoted rearrangement.

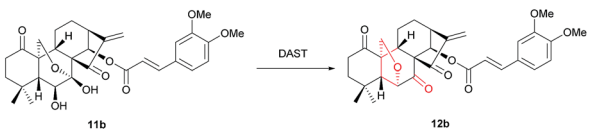
Results and discussion

Our currently ongoing research works indicated that new potent antitumor agents could be produced *via* installing protected amino acid residues or cinnamyl group at C-14 position of oridonin analogues. Thus, oridonin analogues **11a–11r** were prepared in good yields through esterification of oridonin, 1-acetyl-oridonin and 1-oxo-oridonin derivatives with 4-methoxy cinnamylic acid, 3,4-dimethoxy cinnamic acid, 3,4,5-trimethoxy cinnamic acid, Boc-Gly, Boc-L-Val, Boc-L-Leu, Boc-L-Ile, Boc-L-Pro, Boc-L-Phe, N-Fmoc-L-Asp-1-OtBu, Fmoc-L-Phe (Scheme 1), which would be exploited as substrates for rearrangement.

Initially, the feasibility of rearrangement was investigated using readily accessed 1-oxo-14-acyl oridonin analogue **11b** as the model substrate in the presence of DAST (1 equiv.) with dichloromethane (DCM) as solvent, and gratifyingly the desired product **12b** was furnished in 31% yield, the structure of which was determined unambiguously by X-ray crystallography (CCDC 1590323) (Table 1, entry 1). Afterwards, the impact of DAST amount on the reaction was examined, and the yields of **12b** could be increased to 40%, 57% and 80%, respectively with 3 equivalents, 5 equivalents and 10 equivalents of DAST employed (Table 1, entries 2–4). However, further increasing the amount of DAST led to inferior yield (Table 1, entry 5). The reaction temperature was also evaluated, and the reaction could be significantly accelerated at higher temperature, which, however, had a detrimental influence on the yield (Table 1, entry 6). Subsequently, various solvents were screened, and DCM was identified as the optimal one which furnished **12b** in highest yield (Table 1, entry 4). The employment of other solvents such as THF, MeCN, DMF and DMSO resulted in inferior yields (Table 1, entry 7–11), and notably, only a trace amount of **12b** was observed using acetone as solvent (Table 1, entry 11).

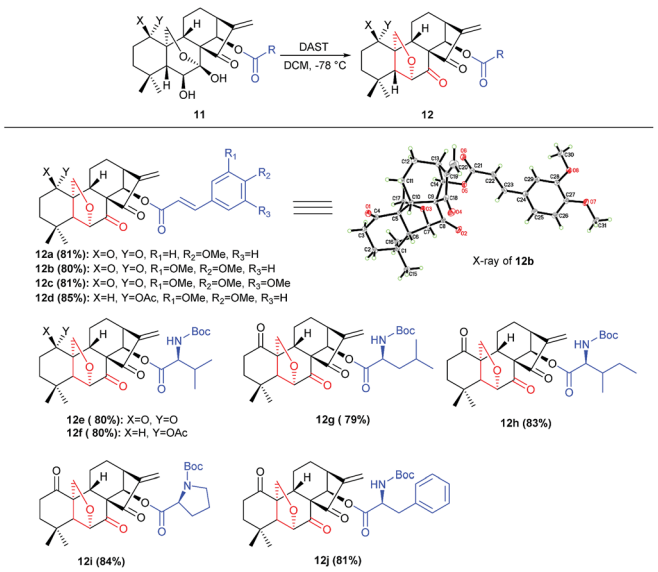
Other strategies for this rearrangement were also investigated for more efficient transformation and more environmentally benign conditions. Intriguingly, a trace amount of **12b** could be observed under Swern oxidation condition using DMSO, $(COCl)_2$ and i-Pr_2NEt (for details, see the ESI† control

Table 1 Optimization of the reaction conditions[a]

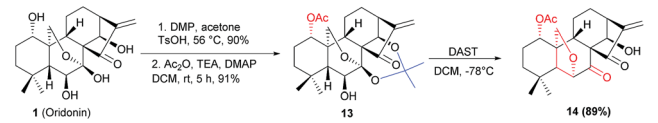


Entry	Reagent (equiv.)	Solvent	Yield[b] (%)
1	DAST (1)	DCM	31
2	DAST (3)	DCM	40
3	DAST (5)	DCM	57
4[c]	DAST (10)	DCM	80
5[c]	DAST (12)	DCM	77
6[d]	DAST (10)	DCM	68
7[e]	DAST (10)	THF	61
8[e]	DAST (10)	MeCN	54
9[e]	DAST (10)	DMF	61
10[f]	DAST (10)	DMSO	36
11[e]	DAST (10)	Acetone	Trace

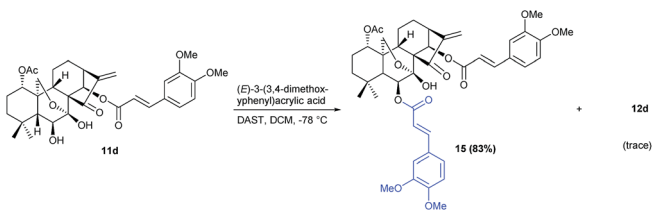
[a] Reaction condition: **11b** (0.09 mmol), solvent (5.0 mL), −78 °C for 10 min, then warmed up to room temperature for 8 h. [b] Yield of isolated products. [c] −78 °C for 10 min, then warmed up to rt for 2 h. [d] −78 °C for 10 min, then warmed up to 40 °C for 1 h. [e] −78 °C for 10 min, then warmed up to rt for 3 h. [f] Room temperature for 3 h.



Scheme 2 Synthesis of novel 6,20-epoxy *ent*-kaurane diterpenoid **12**.



Scheme 3 Synthesis of 6,20-epoxy-14-OH *ent*-kaurane diterpenoid **14**.



Scheme 4 Synthesis of 1-acetyl-6,14-diyl oridonin analogue **15**.

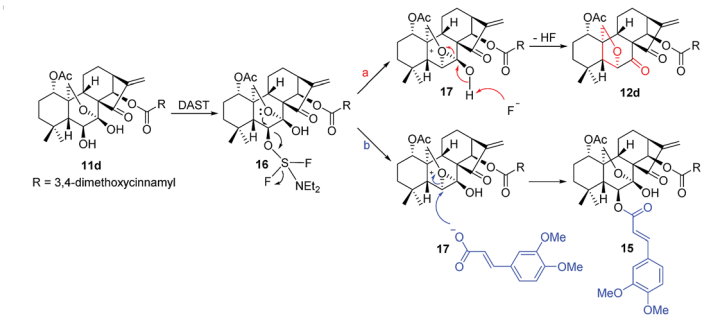
experiments). Afterwards, **11b** was subjected to Mitsunobu condition using 3,4,5-trimethoxybenzoic acid, PPh_3 and DIAD in anhydrous THF, whereas no **12b** was found. Finally, various Brønsted and Lewis acids, *e.g.* *p*-TsOH, CF_3SO_3H, HCl, $AlCl_3$ (for details see the ESI Table S-1†), were evaluated as catalyst for dehydroxylation, however, no **12b** could be detected, which suggested that the acidic additive could thoroughly block the transformation.

Under the optimal conditions, the generality of this protocol was investigated with sterically and electronically diverse substrates subjected to this rearrangement reaction. Gratifyingly, most of the substrates **11a–11r**, were well tolerated, furnishing a series of 6,20-epoxy-14-acyl *ent*-kaurane diterpenoids **12a–12j** in 79–84% yields (Scheme 2). Notably, the incorporated functionalities had a trivial impact on the transformation. Subsequently, aiming to prepare oridonin analogues carrying unprotected hydroxyl at C-14, the substrate **13** was synthesized *via* protection of 7,14-dihydroxyl of oridonin with 2,2-dimethoxypropane followed by acetylation with Ac_2O, and satisfyingly the correspondingly 6,20-epoxy-14-OH *ent*-kaurane diterpenoid **14** was delivered in 89% yield with **13** treated with DAST (Scheme 3).

Interestingly, when **11d** was treated with DAST in the presence of (*E*)-3-(3,4-dimethoxyphenyl)acrylic acid, the 1-acetyl-6,14-diacyl oridonin analogue **15** was isolated as the main product instead in 83% yield and only trace of **12d** was obtained (Scheme 4).

Based on these experimental results, a plausible mechanism was proposed (Scheme 5). Initially, **11d** reacts with DAST to produce the intermediate **16**, which undergoes an intramolecular nucleophilic substitution to furnish the bicyclic oxiranium ion intermediate **17**. Due to the basic nature of fluorine anion and comparative strong acidity of 7-OH, the hydroxyl group is readily deprotonated, followed by the opening of oxiranium ion moiety to yield **12d** (route a). In contrast, an alternative reaction pathway (route b) might operate in the presence of 3,4-dimethoxycinnamic acid, the oxiranium ion moiety can be preferentially attacked by the more nucleophilic carboxylate anion of 3,4-dimethoxycinnamic acid to give the product **15**.

The *in vitro* cytotoxicity of some products were determined by the methylthiazol tetrazolium (MTT) assay on the human hepatic carcinoma cell (HepG2), human multiple myeloma cell (RPMI-8226), human lung carcinoma (A549) cell lines with the commercial anticancer drug, paclitaxel (**PTX**), as the positive control. The results were summarized in Table 2. Notably, **11i** (IC_{50} = 0.98 μM) and **14** (IC_{50} = 2.07 μM) exhibited potent inhibitory activities against HepG2 cell line. Compound **11e** (IC_{50} = 7.60 μM) was found to be a potent cytotoxic agent against A549 cell lines (Fig. 2). In addition, to obtain the cytotoxicity of these new compounds on normal human cells, the



Scheme 5 A possible reaction mechanism.

Table 2 Cytotoxicity values of some compounds towards three selected tumor cell lines for 72 h[a]

Compounds	Cytotoxicity (IC_{50}, μM)		
	HepG2	RPMI-8226	A549
Oridonin	7.93 ± 1.25	9.84 ± 0.41	22.64 ± 1.28
PTX	0.19 ± 0.03	1.40 ± 0.50	0.44 ± 0.26
11e	13.81 ± 2.27	19.55 ± 2.07	7.60 ± 0.74
11g	10.75 ± 1.21	>100	21.14 ± 1.43
11h	16.07 ± 0.53	10.96 ± 1.06	19.35 ± 1.05
11i	0.98 ± 0.10	11.53 ± 1.33	19.82 ± 1.14
11j	15.28 ± 1.90	>100	18.13 ± 3.20
11k	15.28 ± 1.90	>100	18.13 ± 3.20
11l	8.71 ± 1.25	10.23 ± 0.40	15.08 ± 1.46
11m	13.53 ± 3.16	9.87 ± 0.97	14.01 ± 1.61
11o	14.62 ± 1.68	14.52 ± 0.90	14.81 ± 1.99
11p	45.00 ± 3.59	17.98 ± 1.38	72.22 ± 4.38
11q	11.37 ± 0.77	16.61 ± 2.04	21.08 ± 3.53
11r	23.68 ± 2.25	9.57 ± 0.92	23.80 ± 2.05
12a	13.96 ± 0.68	9.66 ± 1.50	23.56 ± 2.76
12b	16.07 ± 1.26	7.33 ± 1.42	16.73 ± 1.73
12d	18.13 ± 1.20	19.66 ± 1.8	11.03 ± 1.70
12e	9.42 ± 1.03	33.21 ± 3.87	18.06 ± 2.29
12g	11.00 ± 2.68	10.05 ± 1.31	13.34 ± 1.95
12h	9.37 ± 0.65	10.62 ± 0.95	14.13 ± 2.01
12i	16.58 ± 2.93	14.64 ± 1.75	12.85 ± 2.20
12j	21.60 ± 3.17	11.95 ± 1.34	17.58 ± 2.32
14	2.07 ± 0.29	9.54 ± 0.57	14.95 ± 3.57
15	9.02 ± 0.80	6.94 ± 0.41	10.31 ± 1.89

[a] IC_{50} values were presented as the mean ± SD (standard error of the mean) from three separated experiments.

effect of compounds **11e**, **11i**, **14** and oridonin was evaluated in human liver cancer cell line HepG2 and normal liver cell line L-O_2. Compared with LO_2 cells, oridonin was approximately 2-fold more selective in inhibiting the growth of HepG2 cells. The tested analogues **11i** (8.84-fold) and **14** (3.43-fold) exhibited higher selectivity than oridonin (see ESI Table S-2†). Particularly, compound **11i** seemed to be more selective than oridonin, with an SI (selectivity index, IC_{50} of normal cells/IC_{50} of tumor cells) value of 8.84. These results suggest that these 14-acyl oridonin analogues and novel 6,20-epoxy *ent*-kaurane diterpenoid analogues may serve as promising antitumor agents.

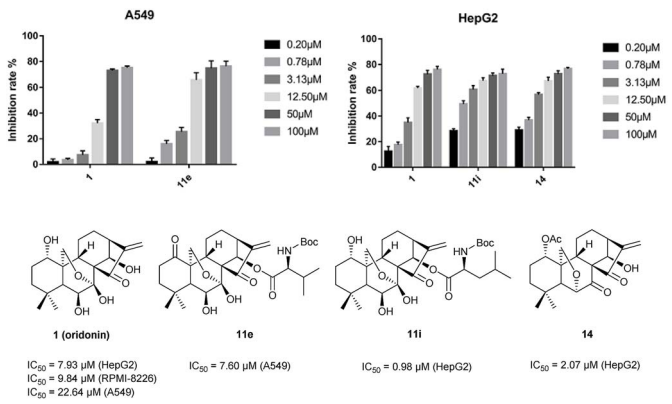


Fig. 2 Comparison of the activity and IC_{50} of **1**, **11e** in inhibiting A549 and **1**, **11i**, **14** in inhibiting HepG2.

Conclusions

In summary, we report a novel and concise protocol for syntheses of 6,20-epoxy *ent*-kaurane diterpenoid analogues from oridonin with α-methylene cyclopentanone core intact. The reaction features mild conditions and good yield. Remarkably, the prepared 14-acyl oridonin analogues **11e**, **11i** and 6,20-epoxy *ent*-kaurane diterpenoid analogue **14** exhibited superior anticancer activities compared to oridonin *in vitro*. Oridonin was endowed with more potent bioactivities *via* chemical modification, which will open a new avenue for development of novel anticancer agents. The further anticancer activity investigation of oridonin analogues is under the way.

Experimental

Chemistry section

Unless otherwise stated, all commercial reagents were used without additional purification and solvents were distilled prior to use. All reactions were carried out under nitrogen atmosphere. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ at 500 MHz, using $CDCl_3$ as a reference standard (δ = 7.26 ppm) for ^{1}H NMR and (δ = 77.00 ppm) for ^{13}C NMR or DMSO-d6 as a reference standard (δ = 2.50 ppm) for ^{1}H NMR and (δ = 39.52 ppm) for ^{13}C NMR. Melting points were measured with a Laboratory Device MEL-TEMP and were uncorrected. TLC was performed using commercially prepared silica gel plates (GF254), and visualization was effected at 254 nm and 365 nm. High resolution mass spectra (HRMS) were recorded on the Exact Mass Spectrometer equipped with ESI ionization source.

General procedure for the synthesis of compounds 11a–11r

To a stirring solution of oridonin (500 mg, 1.37 mmol) in acetone (40 mL) was added Jones reagent (0.6 mL) dropwise at ice-water bath. The resulting mixture was stirred at 0 °C for 20 min, and isopropyl alcohol was added to quench excess Jones reagent. Then the mixture was diluted with water and extracted with dichloromethane (3 × 10 mL). The extract was then washed with brine, dried over anhydrous $MgSO_4$, filtered, and evaporated to give a solid crude product. The crude residue was recrystallized from acetone–hexane to give compound **10a** as a white solid, 440 mg, 88% yield.

Treatment of oridonin (500 mg, 1.37 mmol) with 2,2-dimethoxypropane in the presence of *p*-TsOH in acetone afforded 7,14-(1-methylethylene)-dioxy-oridonin derivative (498 mg, 1.23 mmol) in 90% yield. Subsequently, this derivative (200 mg, 0.50 mmol) was treated with Ac_2O (0.05 mL, 0.50 mmol), Et_3N (1 mL) and DMAP (183 mg, 1.50 mmol) in 15 mL dichloromethane to yield the corresponding compound **13** (221 mg, 91% yield). Deprotection of compound **13** (200 mg, 0.44 mmol) with 2% HCl solution in 10 mL tetrahydrofuran gave the corresponding compound **10b** (155 mg, 87% yield).

Compound **10a** (100 mg, 0.25 mmol) was mixed with 4-methoxycinnamic acid (50 mg, 0.25 mmol), EDCI (143 mg, 0.75 mmol) and DMAP (92 mg, 0.75 mmol) in 10 mL anhydrous dichloromethane, and the resulting mixture was stirred under

nitrogen atmosphere at room temperature overnight. The reaction was poured into 1 M HCl solution, and the mixture was extracted with dichloromethane (3 × 5 mL). The organic layers were combined, washed with water and saturated NaCl solution, dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by column chromatography (SiO_2, DCM/MeOH) to give the compound **11a**. White solid, mp 136–137 °C. 121 mg, 83% yield. $R_f = 0.35$ (1: 25 MeOH in CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.60 (d, J = 15.9 Hz, 1H), 7.44 (d, J = 8.7 Hz, 2H), 6.88 (t, J = 5.8 Hz, 2H), 6.27 (s, 1H), 6.20 (d, J = 15.9 Hz, 1H), 5.92 (s, 1H), 5.62 (d, J = 0.9 Hz, 1H), 5.47 (d, J = 8.8 Hz, 1H), 4.32 (dd, J = 10.6, 1.0 Hz, 1H), 4.05 (dd, J = 10.6, 1.5 Hz, 1H), 3.83 (d, J = 6.1 Hz, 3H), 3.25 (d, J = 9.6 Hz, 1H), 2.62 (dt, J = 14.0, 8.8 Hz, 1H), 2.47 (ddd, J = 15.4, 10.9, 6.6 Hz, 1H), 2.38–2.24 (m, 3H), 2.08–2.01 (m, 1H), 1.99 (d, J = 8.6 Hz, 1H), 1.95–1.87 (m, 1H), 1.74 (ddd, J = 13.8, 8.8, 6.7 Hz, 2H), 1.68–1.60 (m, 1H), 1.37–1.30 (m, 1H), 1.21 (s, 3H), 1.01 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) *d* 211.78, 204.97, 165.43, 161.79, 149.32, 146.32, 130.08, 126.56, 122.20, 114.37, 114.06, 97.13, 75.54, 73.03, 64.93, 61.18, 60.35, 55.39, 50.77, 48.56, 41.32, 38.54, 35.83, 32.89, 30.53, 30.00, 29.69, 23.30, 19.05. HRMS (*m*/*z*) (ESI): calcd for $C_{30}H_{35}O_8$ 523.2326 $[M + H]^+$ found 523.2318.

All of the products **11b–11r** were synthesized according to above described procedure.

General procedure for the synthesis of compounds 12a–12k

Compound **11a** (94 mg, 0.18 mmol) was mixed with 0.24 mL DAST (diethylaminosulfur trifluoride) in anhydrous dichloromethane (10 mL), and the resulting mixture was stirred under nitrogen atmosphere at −78 °C for 10 min, then warmed up to room temperature and stirred for 2 h. The mixture was poured into water, and extracted with dichloromethane (3 × 5 mL). The organic layers were combined, washed with water and saturated NaCl solution, dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by column chromatography (petroleum ether/ethyl acetate) to give the compound **12a**. White solid, mp 119–120 °C. 73 mg, 81% yield. R_f = 0.46 (1 : 1 petroleum ether/ethyl acetate); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.58 (d, J = 15.9 Hz, 1H), 7.44 (d, J = 8.7 Hz, 2H), 6.86 (d, J = 8.7 Hz, 2H), 6.20 (d, J = 15.9 Hz, 1H), 6.16 (s, 1H), 5.72 (s, 1H), 5.50 (s, 1H), 4.38 (d, J = 9.7 Hz, 1H), 4.31–4.26 (m, 2H), 3.82 (s, 3H), 3.23 (d, J = 7.9 Hz, 1H), 2.74 (ddd, J = 14.6, 11.7, 6.3 Hz, 1H), 2.56–2.45 (m, 2H), 2.35 (dt, J = 14.6, 4.6 Hz, 1H), 2.18–2.10 (m, 2H), 1.96–1.81 (m, 3H), 1.77–1.69 (m, 1H), 1.66 (s, 1H), 1.19 (d, J = 15.1 Hz, 3H), 1.05 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 209.87, 202.56, 199.65, 166.04, 161.48, 147.69, 145.74, 129.98, 126.97, 119.22, 114.68, 114.21, 83.31, 77.27, 77.01, 76.76, 75.61, 70.10, 64.24, 61.58, 61.31, 55.34, 47.17, 42.05, 41.18, 36.37, 31.96, 31.33, 30.17, 29.68, 23.23, 19.17. HRMS (*m*/*z*) (ESI): calcd for $C_{30}H_{33}O_7$ 505.2221 $[M + H]^+$ found 505.2218.

All of the products **12b–12j** were synthesized according to above described procedure.

General procedure for the synthesis of compound 14

Compound **13** (100 mg, 0.22 mmol) was mixed with 0.30 mL DAST (diethylaminosulfur trifluoride) in anhydrous dichloromethane (10 mL) and the resulting mixture was stirred under nitrogen atmosphere at −78 °C for 10 min, then warmed up to room temperature and stirred for 2 h. The mixture was poured into water, and the mixture was extracted with dichloromethane thrice (3 × 5 mL). The organic layers were combined, washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography (6 : 1 petroleum ether/ethyl acetate) to give the compound **14**. Yellow solid, mp 115–116 °C. 77 mg, 89% yield. $R_f = 0.27$ (1 : 1 petroleum ether/ethyl acetate); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.13 (s, 1H), 5.53 (s, 1H), 4.88 (dd, J = 10.9, 6.0 Hz, 1H), 4.88 (dd, J = 10.9, 6.0 Hz, 1H), 4.74 (s, 2H), 4.58 (s, 1H), 4.17 (s, 1H), 4.12 (d, J = 10.0 Hz, 1H), 4.10 (q, J = 10.0 Hz, 2H), 4.09 (d, J = 10.0 Hz, 1H), 3.83 (dd, J = 43.1, 15.7 Hz, 1H), 3.11 (d, J = 8.7 Hz, 1H), 3.11 (d, J = 8.7 Hz, 1H), 2.44–2.35 (m, 1H), 2.45–2.33 (m, 1H), 2.19 (dd, J = 12.2, 6.1 Hz, 1H), 2.19 (dd, J = 12.2, 6.1 Hz, 1H), 1.99 (s, 3H), 1.89 (s, 1H), 1.64–1.57 (m, 2H), 1.49 (s, 1H), 1.41 (d, J = 8.7 Hz, 1H), 1.21 (s, 1H), 1.04 (s, 3H), 1.01 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 205.36, 203.22, 169.94, 149.04, 120.20, 82.53, 76.74, 73.59, 68.21, 64.94, 55.04, 52.97, 51.04, 43.79, 36.97, 32.26, 30.83, 29.68, 29.37, 24.60, 22.45, 21.40, 18.95. HRMS (*m*/*z*) (ESI): calcd for $C_{22}H_{29}O_6$ 389.1959 $[M + H]^+$ found 389.1959.

General procedure for the synthesis of compound 15

Compound **11d** (50 mg, 0.08 mmol) was mixed with (*E*)-3-(3,4-dimethoxyphenyl)acrylic acid (33 mg, 0.16 mmol), 0.11 mL DAST (diethylaminosulfur trifluoride) in anhydrous dichloromethane (10 mL) and the resulting mixture was stirred under nitrogen atmosphere at −78 °C for 10 min, then warmed up to room temperature and stirred overnight. The mixture was poured into water, and the mixture was extracted with dichloromethane thrice (3 × 5 mL). The organic layers were combined, washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography (6 : 1 petroleum ether/ethyl acetate) to give the compound **15**. White solid, mp 125–126 °C. 52 mg, 83% yield. $R_f = 0.63$ (1 : 1 petroleum ether/ethyl acetate); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.88 (d, J = 15.9 Hz, 1H), 7.59 (d, J = 15.9 Hz, 1H), 7.18 (d, J = 8.3 Hz, 1H), 7.15 (s, 1H), 7.04 (d, J = 8.3 Hz, 1H), 6.99 (s, 1H), 6.88 (d, J = 8.2 Hz, 1H), 6.81 (t, J = 9.1 Hz, 1H), 6.56 (d, J = 15.9 Hz, 1H), 6.20 (dd, J = 15.8, 6.4 Hz, 1H), 6.01 (d, J = 13.7 Hz, 2H), 5.36–5.31 (m, 2H), 5.02 (s, 1H), 4.73 (dd, J = 11.2, 5.5 Hz, 1H), 4.41 (d, J = 10.6 Hz, 1H), 4.28 (t, J = 8.9 Hz, 1H), 3.93 (d, J = 6.4 Hz, 6H), 3.89 (d, J = 5.4 Hz, 6H), 3.17 (d, J = 9.9 Hz, 1H), 2.61–2.53 (m, 1H), 2.36–2.31 (m, 1H), 2.04 (d, J = 7.4 Hz, 3H), 1.90–1.78 (m, 2H), 1.71 (d, J = 6.1 Hz, 1H), 1.56 (d, J = 12.9 Hz, 1H), 1.46 (t, J = 12.7 Hz, 1H), 1.40–1.30 (m, 3H), 1.21 (d, J = 8.9 Hz, 3H), 0.90 (d, J = 8.9 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 200.70, 169.93, 168.85, 165.90, 151.30, 151.20, 150.25, 149.16, 146.21, 146.05, 127.58, 127.10, 123.20, 117.17, 115.70, 114.89, 110.93, 110.86, 109.77, 109.31, 96.81, 75.94, 75.16, 74.05, 63.40, 60.14, 56.04, 52.18, 41.97, 40.46, 40.23, 37.70, 33.68, 31.65, 30.37, 29.68, 27.00, 25.17, 21.58, 21.38, 17.82. HRMS (*m*/*z*) (ESI): calcd for $C_{44}H_{51}NO_{13}$ 787.3324 $[M + H]^+$ found 787.3342.

In vitro cytotoxicity

The HepG2, RPMI-8226, A549, L-O_2 cell lines used in this study were all purchased from EnoGene company. RPMI-8226 and A549 cells were cultured in RPMI 1640 media containing 10% heat inactivated FBS and HepG2 and L-O_2 cells were cultured in DMEM media containing 10% heat inactivated FBS at 37 °C with 5% CO_2. In order to investigate the antitumor activity of some compounds, a commercial Paclitaxel (**PTX**) was used as a positive control drug.

10 000 cells of HepG2, RPMI-8226, A549 or L-O_2 were prepared into 200 μL cell suspension in each well of 96-well plates and the plates were incubated for 24 h at 37 °C with 5% CO_2. 100 μL medium with compounds was mixed into each well of 96-well plates, respectively. And the negative control group, the solvent control group, the positive control group were established, respectively. The plates were incubated for 72 h at 37 °C with 5% CO_2. Then 10 μL CCK-8 solution was mixed into each well of 96-well plates and the plates were incubated for 4 h. Optical absorbance at 450 nm was determined with microplate absorbance reader (Bio-Rad). IC_{50} values were calculated from the dose–response curves of the assay (Prism 7.0).

Conflicts of interest

There are no conflicts to declare.

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