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Synthesis and biological evaluation of novel *exo*-methylene cyclopentanone tetracyclic diterpenoids as antitumor agents

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ABSTRACT

The structure of *exo*-methylene cyclopentanone, which exists in nature tetracyclic diterpenoids products, has been proved to be an innate group for the treatment of cancer and inflammation. In this letter, four different scaffolds of tetracyclic diterpenoids including the structure *exo*-methylene cyclopentanone were synthesized from steviol and isosteviol and evaluated in vitro for their antitumor activity against three human cancer lines. Compounds **1a**, **1b**, **2b** and **3b** showed significant cytotoxicity, particularly, tetracyclic diterpenoids **2b**, **3b** were identified as the most potent and selective anticancer agents superior to adriamycin with IC_{50} values of 0.9 μM and 1.5 μM, against Hep-G2 and MDA-MB-231 cell lines, respectively.

Tetracyclic diterpenoids, especially *ent*-kaurane diterpenoids with an enone system in ring D, constitute an important class of natural products which exhibit interesting pharmacological activeties.[1,2] Natural and synthetic *ent*-kaurane diterpenoids show broad spectrum of biological activities such as anti-inflammatory, anti-tuberculosis, and anticancer.[3,4] Different mechanisms have been proposed such as the inhibition of the neutrophile response due to the inhibition of cytosolic Ca^{2+}, as well as the inhibition of prostaglandin E_2 production through the suppression of nuclear factor-κB (NF-κB) activation.[5] The latter mechanism seems to be more relevant to the anti-inflammatory effect of *ent*-kaurane, and it make these compounds good candidates for NF-κB activity modulation through interference with the steps leading up to the release of IκB kinase (IKK).

The inhibitory activity of *ent*-kaurane toward NF-κB has been accounted for by invoking the presence of reactive centers and various studies have focused on the *exo*-methylene cyclopentanone group. This reactive group interacted with biological nucleophiles such as the mercapto group of the cysteine residue in the DNA-binding domain of the NF-κB subunit through an irreversible Micheal-type addition.[6,7] A large number of *ent*-kaurane diterpenoids isolated from different natural plants have been reported to display interesting biological activities. Fujita et al. and Xu et al.[8–10] reported that the natural *ent*-kaurane diterpenoids oridonin and its derivatives with an enone system in ring D had significant cytotoxicity against BGC-7901, BEL-7402 and SW-480 cell lines in vitro (Fig. 1). Zhang et al.[11] discovered that *ent*-kaurane diterpenoids semipinnated brake isolated from Pteris semipinnata L had significant cytotoxicity superior to 5-FU. Inflexusin and rabdoserrin D, isolated from Isodon phyllostachys by Li et al.[12], were demonstrated good cytotoxicity against K569 cell. These natural *ent*-kaurane diterpenoids all contain the structure of *exo*-methylene cyclopentanone in ring D.

For the reason above we try to build up a crucial structure fragment of *exo*-methylene cyclopentanone in the ring D of steviol (the aglycone of stevioside, which shares the same mother ring with oridonin but has little antitumor or anti-inflammatory activity)

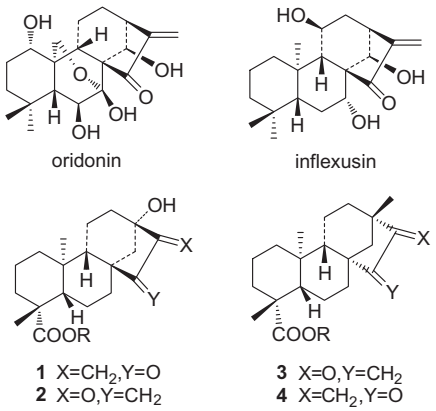

Figure 1. Typical natural tetracyclic diterpenoids and target compounds.

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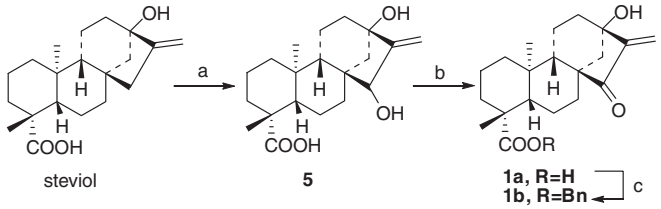
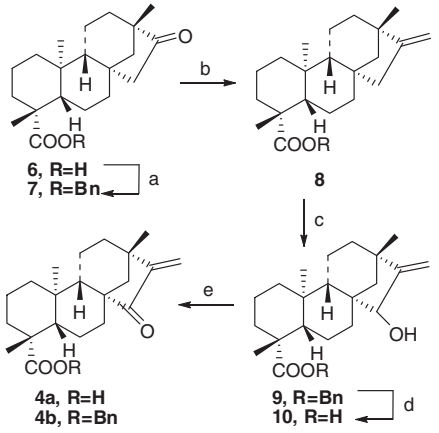


Scheme 1. Reagents and conditions: (a) SeO_2, *t*-BuOOH, THF (59.5%); (b) PDC, DMF (71.5% for **1a**); (c) BnBr, K_2CO_3, DMF, KI (78.7%).

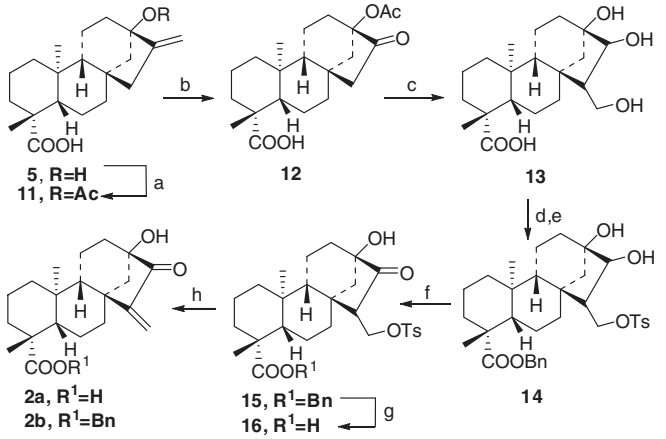
and isosteviol (steviol's rearrangement isomer) to obtain a series of novel anticancer compounds.[13,15] Previous work proved that the modification of steviol and isosteviol was effective for increasing its cytotoxicity. Tao et al.[14] reported that hydroxyl groups in ring D of isosteviol may play an important role in binding to some receptors. Terai et al. demonstrated that steviol and its derivatives show a positive response in forward mutation assay using *Salmonella typhimurium* TM677 in the presence of metabolic activation system.[15] Herein, we report the facile synthesis and biological activities of four different scaffolds of tetracyclic diterpenoids, in which the compound **1** and **4** have a structure 16-*exo*-methylene-15-cyclopentanone while the compound **2** and **3** have a structure 15-*exo*-methylene-16-cyclopentanone. (Fig. 1)

Our approaches to synthesize the title compounds are described below. The synthesis of **1a** and **1b** is shown in Scheme 1. Starting from steviol, treatment with SeO_2 and *t*-BuOOH led to 16-hydroxy steviol **5**. Oxidation of 16-hydroxy steviol **5** with PDC gave the desired compound **1a**.[16,17] Esterification of **1a** with benzyl bromide provided **1b** successfully. Preparation of the corresponding compound of isosteviol with 15-carbonyl-16-*exo*-methylene structure is illustrated in Scheme 2. Benzyl bromide was added to isosteviol **6** followed by Wittig reaction and SeO_2 oxidation to give **9**.[18,19] However, the synthesis of compounds **4a** and **4b** was unsuccessful under the same conditions as above. Furthermore, Swern oxidation of **9** and **10** resulted in the formation of the target compound **4** in good yield.[20–22]

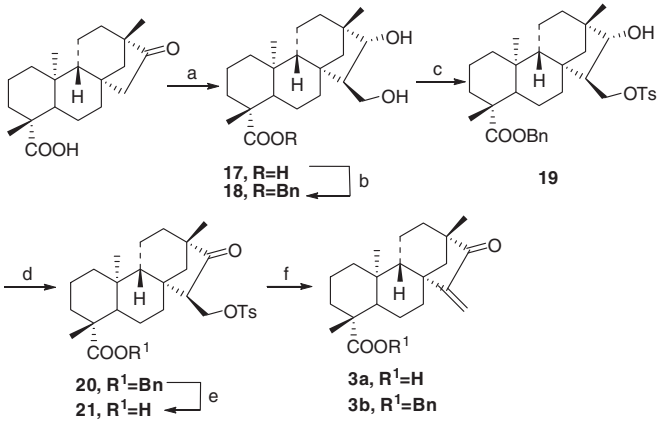
The synthetic approaches employed to prepare another two skeletons are outlined in Scheme 3 and 4. Normal way was to construct a hydroxymethyl in the α-position of 15-ketone, and then to access 16-ketone-15-ene structure by eliminating monomolecular H_2O.[23–26] Any attempt to obtain 16-ketone-15-hydroxymethyl failed to meet our expectation. Therefore, one abnormal Cannizzaro-type reaction was adopted, the α-position of 16-ketone was hydroxymethylated and 16-carbonyl was reduced in one-step.[14,27]



Scheme 2. Reagents and conditions: (a) BnBr, K_2CO_3, DMF, KI; (b) $CH_3P^+Ph_3I^-$, *n*-BuLi, THF, reflux (88.1% for two-steps); (c) SeO_2, *t*-BuOOH, THF (85.9%); (d) 10% Pd-C, 85% HCOOH (84.6%); (e) $(COCl)_2$, DMSO, TEA (72.2%).



Scheme 3. Reagents and conditions: (a) Ac_2O, DMAP, TEA, THF; (b) O_3/O_2, CH_2Cl_2, Ph_3P (85.6% for two-steps); (c) HCHO (aq), NaOH, C_2H_5OH–H_2O, 75 °C, 3 h (78.8%); (d) BnBr, K_2CO_3, DMF, KI; (e) TsCl, pyridine, DMAP, rt (53.7% for two-steps); (f) PDC, DMF (63.6%); (g) 10% Pd-C, H_2, C_2H_5OH (85.7%); (h) pyridine, DMAP, reflux (53.6%).



Scheme 4. Reagents and conditions: (a) HCHO (aq), NaOH, C_2H_5OH–H_2O, 75 °C, 3 h (56.1%); (b) BnBr, K_2CO_3, DMF, KI (70.4%); (c) TsCl, pyridine, DMAP, rt (68.8%); (d) PDC, DMF (74.0%); (e) 10% Pd–C, H_2, C_2H_5OH, (84.1%); (f) pyridine, DMAP, reflux (73.6%).

Scheme 3 illustrates the synthesis of compounds **2a** and **2b**. Before hydroxymethylation of the α-position in 15-ketone, we needed to synthesize *ent*-13-acetoxyl-16-oxo-17-demethylkaurane-19-acid **12** by protecting 13-hydroxy and oxidizing the double bond with Ac_2O and O_3, respectively.[28,29] After the diol **13** had been successfully achieved, we attempted to selectively oxidize 15-position secondary alcohol with sodium hypochlorite and acetic anhydride as reference,[23,24] but the anticipated compound had not been obtained. So we had to protect the primary alcohol of **13** selectively before oxidation of 15-hydroxy group. We used TsCl instead of tritylchloride to protect the primary alcohol of **13**, on account of a large steric of tritylchloride.[30–32] Oxidation of **14** with PDC gave 15-oxo product **15**. Finally, elimination of the TsOH provided the target compound **2b**. Compound **2a** was obtained through elimination of **16**, which was accessed by removing the benzyl of **15** with 10% Pd-C. Using the same methodology of the conversion of **12** to **2a** and **2b**, target compounds **3a** and **3b** were synthesized starting from isosteviol **7** as shown in Scheme 4.

The structure of the target compounds was elucidated using spectroscopic techniques (IR, ^1H and ^{13}C NMR, ESI/MS).

Steviol, isosteviol and novel tetracyclic diterpenoids **1**–**4** were assayed for their in vitro cytotoxicity against three human cancer cell lines: breast carcinoma (MDA-MB-231), hepatocellular carci-

Table 1
In vitro cytotoxicity data of tetracyclic diterpenoids **1–4**

Compound	Antitumor activity in 48 h (IC_{50}, μM)[a]		
	MDA-MB-231	Hep-G2	MGC-803
Steviol	ND	ND	ND
1a	ND	3.69	3.38
1b	2.51	2.70	1.80
2a	ND	ND	ND
2b	1.24	0.95	2.41
Isosteviol	ND	ND	ND
3a	ND	ND	ND
3b	1.58	ND	2.22
4a	ND	ND	ND
4b	ND	ND	ND
Adriamycin	2.26	2.08	2.53
Oridonin	2.38	3.66	3.57

ND: not detectable.
[a] Data expressed in terms of IC_{50} value were obtained by dose dependent response.

noma (Hep-G2) and gastric carcinoma (MGC-803). Adriamycin and oridonin were selected as positive control. The IC_{50} values were used to determine the growth inhibition in the presence of tetracyclic diterpenoids **1–4** against MDA-MB-231, Hep-G2 and MGC-803 cancer cell lines. From the IC_{50} values summarized in Table 1, the compound **1a**, **1b, 2b** and **3b** have shown significant cytotoxicity.

The compound **1a** with 16-methylene-15-carbonyl group of steviol is moderately cytotoxic against Hep-G2 and MGC-803 cell without selectivity. Esterification of the acid in 19-position is beneficial for the activity (compound **1a** vs **1b**, **3a** vs **3b**). The compound **1b** improved cytotoxicity against all cancer cell lines when compared to compound **1a**. Exchange of the methylene and carbonyl led to the compound **2a** with almost no activity, but compound **2b** expresses the most potent anticancer agent superior to adriamycin and oridonin with IC_{50} value 1.24 μM, 0.9 μM and 2.41 μM against MDA-MB-231, Hep-G2 and MGC-803, respectively. Further, Compound **3b** has displayed significant cytotoxicity against MDA-MB-231 and MGC-803 with IC_{50} values of 1.58 μM and 2.22 μM, respectively. Isosteviol derivatives **4a** and **4b** have no activity against any cell lines.

In summary, we prepared four different scaffolds of tetracyclic diterpenoids that inhibited the growth of MDA-MB-231, Hep-G2, and MGC-803 cancer cell lines at micromolar concentration. Compounds **1a**, **1b**, **2b**, and **3b** have significant cytotoxicity against part of cancer cell lines. In isosteviol series, compounds **3a**, **4a**, and **4b** exhibit poor cytotoxicity. The preliminary anticancer activity study of both steviol and isosteviol series reveals that the *exo*-methylene cyclopentanone moiety is beneficial for anticancer activity. Further, initial antitumor activity results of tetracyclic diterpenoids **1–4** have generated further interest to optimize their activity and investigate specific biological targets of these compounds at molecular level.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.11.055.

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