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Synthesis and evaluation of cytotoxic effects of novel α-methylenelactone tetracyclic diterpenoids

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ABSTRACT

A series of tetracyclic diterpenoids bearing the α-methylenelactone group have been synthesized and screened for their in vitro anti-tumor activities against six human cancer cell lines. The results showed that compounds **1c**, **2a** and **2b** exhibited significant cytotoxicity superior to the positive control doxorubicin hydrochloride against MDA-MB-231, K562 and HepG2 cell lines. In particular, compound **2b** was identified as the most promising anticancer agent against HepG2 cells with IC_{50} value of 0.09 μM.

Cancer is one of the leading causes of death worldwide and causes serious problems in human life. Therefore, various categories of anti-tumor agents have been developed. However, some side effects could happen simultaneously and the resistance to available chemo-therapeutic agents was rising. Hence, it is urgent to develop novel compounds as anticancer agents with higher bioactivities and lower toxicities.[1,2]

Natural products have always been interesting sources for developing novel leading compounds. Stevioside (Fig. 1) is the primary sweet component in the leaves of *Stevia rebaudiana* Bertoni which is a plant native to South America.[3,4] Stevioside consists of three molecules of glucose and steviol as its aglycone. A large number of researches have suggested that stevioside tastes 300 times sweeter than sucrose and can be used as a non-caloric sweetener in South America, Japan, and China. Moreover, stevioside along with its metabolic components steviol[5] and isosteviol[6] possesses multiple pharmacological activities including anti-hyperglycemic, anti-inflammatory, anti-tumor and anti-diarrheal. It has been shown that these three compounds strongly inhibited the cancer formation induced by TPA (12-*o*-tetra-decanoylphorbol-13-acetate) and DMBA (7,12-dimethylbenz[α]anthracene) in a two-stage carcinogenesis test in mouse. In addition, isosteviol inhibited both mammalian DNA polymerases and human DNA topoisomerase II. Taken together, these compounds could be served as promising chemopreventive agents against chemical carcinogenesis.[7,8] In order to develop potential anticancer agents of higher cytotoxicity, some structural modifications have been done. In our previous work, we built up a crucial fragment of *exo*-methylene cyclopentanone in the ring D of steviol and isosteviol and obtained some compounds with significantly improved cytotoxicity.[9] Tao and coworkers. synthesized various 15- and 16-substituted isosteviol derivatives by means of functional interconversions, then obtained some compounds with promising activities against B16-F10 melanoma cells.[10]

Plenty of investigations have reported that α-methylenelactone is a crucial building block of many natural products and exhibits wide-ranging biological activities such as anti-tumor, anti-inflammatory, antimicrobial and so on.[11] Therefore, the synthesis of this structural moiety has received much attention,[12,13] and the relationship between its activities and structure has also been studied. It appears that α-methylenelactone may be regarded as alkylating agents by virtue of Michael addition with biological nucleophiles such as L-cysteine or thiol-containing enzymes (Enz-SH).[14] Many sesquiterpene lactones isolated from different kinds of natural plants have been reported to display interesting biological activities. For instance, costunolide (Fig. 1) isolated from the root of *Saussurea lappa* exhibited potent cytotoxicity against HepG2, OVCAR-3 and HeLa cell lines with CD_{50} values of 1.6, 2.0, 2.0 μg/mL, respectively.[15] Kupchan et al. discovered that vernolepin (Fig. 1) bearing two α-methylenelactone groups showed significant cytotoxicity activity against Walker intramuscular carcinosarcoma in vitro

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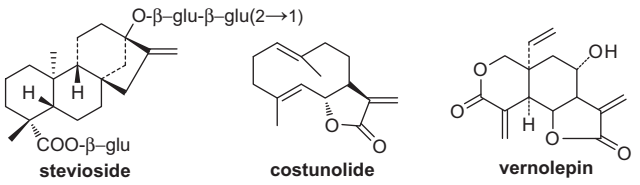
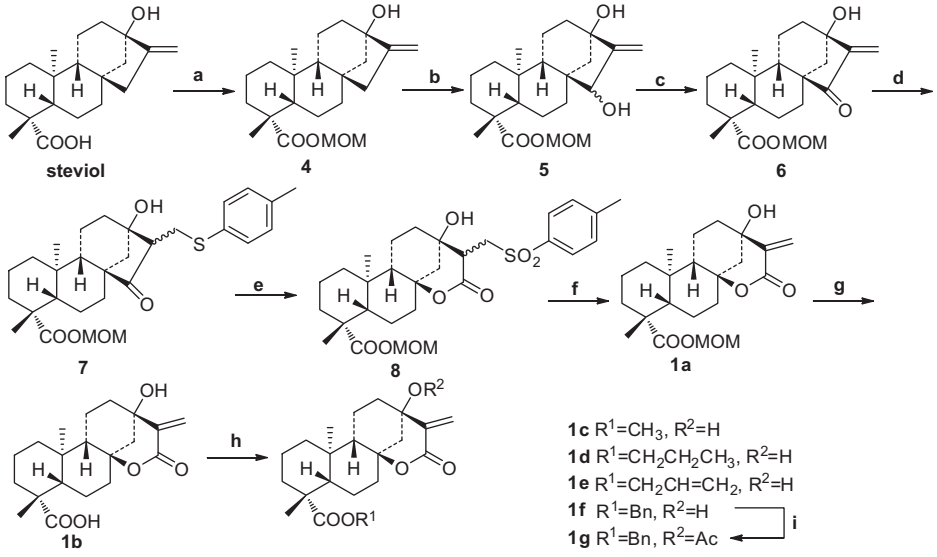


Figure 1. Chemical structures of stevioside, costunolide and vernolepin.

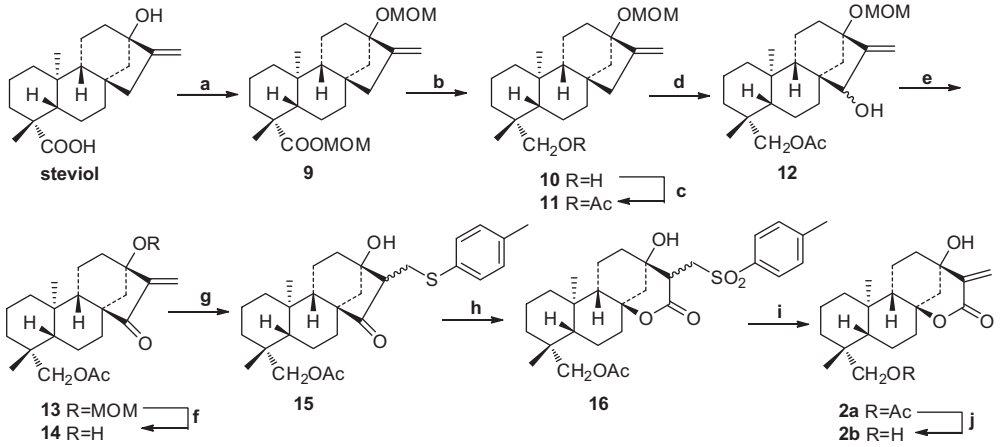
and in vivo in rats.[16] However, *ent*-kaurane diterpenoids possessing α-methylenelactone group are rare in the natural products discovered recently. Therefore, we tried to introduce this critical moiety into steviol and isosteviol and obtained three scaffolds of *ent*-kaurene diterpenoids. Some derivatives were also synthesized and screened for their anticancer activities against six cancer cell lines in vitro by MTT method.

The synthetic route towards the target compounds was described as follows: first, treatment of steviol with chloromethyl methyl ether and *N*,*N*-diisopropylethylamine afforded **4** in 1 h,[17] then reaction of **4** with selenium oxide and *tert*-butyl hydroperoxide led to **5** (Scheme 1).[18] Oxidation of **5** with PDC provided compound **6**.[19] We next tried a phenylthio group as a stable protecting group of the α-methylene unit. Conjugate addition of *p*-thiocresol to enone **6** produced β-thioketone **7**[20] which was successfully transformed into sulfone lactone **8** by Baeyer–Villiger oxidation with excessive *m*CPBA.[21,22] Finally, desulfonylation of **8** with DBU in THF under mild condition gave the desired compound **1a**.[23] Compound **1b** was prepared from **1a** by deprotection of methoxymethyl group with 10% HCl in THF.[24] Esterification of **1b** with different kinds of halohyrocarbons afforded **1c–f**. Compound **1g** could be obtained by acylation of **1f** with acetic anhydride in the presence of DMAP.[25]

The synthetic approach employed to prepare **2a** was outlined in Scheme 2. First, we attempted to reduce **4** with $LiAlH_4$ in anhydrous THF under refluxing condition. Although the reaction could proceed smoothly, the yield was very low due to the poor liposolubility of the product. Therefore, we had to protect the 13-hydroxy of steviol with excessive MOM ether as well. By doing this, compound **10** could be obtained in a good yield (86%). Acylation of



Scheme 1. Reagents and conditions: (a) MOMCl, DIPEA, DMF (90.0%); (b) SeO_2, *t*-BuOOH, THF (85.0%); (c) PDC, DMF (75.0%); (d) *p*-thiocresol, Et_3N, THF (65.6%); (e) 85% *m*CPBA, $NaHCO_3$, CH_2Cl_2 (53.8%); (f) DBU, THF (69.9%); (g) 10% HCl, THF, H_2O (84.0%); (h) R^1R (for **1c**, R = I; for **1d** and **1f**, R = Br; for **1e**, R = Cl), K_2CO_3, DMF, KI (64.0–81.4%); (i) Ac_2O, Et_3N, THF, DMAP (65.2%).



Scheme 2. Reagents and conditions: (a) MOMCl, DIPEA, DMF (85.3%); (b) $LiAlH_4$, THF, reflux (86.0%); (c) Ac_2O, Et_3N, THF, DMAP (77.6%); (d) SeO_2, *t*-BuOOH, THF (75.6%); (e) PDC, DMF (72.0%); (f) 10% HCl, THF, H_2O (82.3%); (g) *p*-thiocresol, Et_3N, THF (87.6%); (h) 85% *m*CPBA, $NaHCO_3$, CH_2Cl_2 (55.2%); (i) DBU, THF (70.6%); (j) 10% $KHCO_3$, CH_3OH, reflux (71.0%).