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.

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. 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 and isosteviol 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-\(\alpha\)-teta-decanoylphorbol-13-acetate) and DMB\(A\) (7,12-dimethylbenz[\(\alpha\])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.

A series of tetracyclic diterpenoids bearing the \(\alpha\)-methylene lactone 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\textsubscript{50} value of 0.09 \(\mu\)M. The synthesis of these compounds has been achieved by functional interconversions.

Plenty of investigations have reported that \(\alpha\)-methylene lactone 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. Therefore, the synthesis of this structural moiety has received much attention, and the relationship between its activities and structure has also been studied. It appears that \(\alpha\)-methylene lactone may be regarded as alkylating agents by virtue of Michael addition with biological nucleophiles such as L-cysteine or thiol-containing enzymes (Enz-SH). 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 Maximiliana lappa exhibited potent cytotoxicity against HepG2, OVCAR-3 and HeLa cell lines with CD\textsubscript{50} values of 1.6, 2.0, 2.0 \(\mu\)g/mL, respectively. Kupchan et al. discovered that vernolepin (Fig. 1) bearing two \(\alpha\)-methylene lactone groups showed significant cytotoxicity activity against Walker intramuscular carcinosarcoma in vitro.

**Synthesis and evaluation of cytotoxic effects of novel \(\alpha\)-methylene lactone tetracyclic diterpenoids**

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**A B S T R A C T**

A series of tetracyclic diterpenoids bearing the \(\alpha\)-methylene lactone 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\textsubscript{50} value of 0.09 \(\mu\)M. © 2012 Elsevier Ltd. All rights reserved.

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and in vivo in rats. However, ent-kaurane diterpenoids possessing α-methylene-lactone 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, then reaction of 4 with selenium oxide and tert-butyl hydroperoxide led to 5 (Scheme 1). Oxidation of 5 with PDC provided compound 6. 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 which was successfully transformed into sulfone lactone 8 by Baeyer–Villiger oxidation with excessive mCPBA. Finally, desulfonation of 8 with DBU in THF under mild condition gave the desired compound 1a. Compound 1b was prepared from 1a by deprotection of methoxymethyl group with 10% HCl in THF. 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.

The synthetic approach employed to prepare 2a was outlined in Scheme 2. First, we attempted to reduce 4 with LiAlH4 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 10 with excessive acetic anhydride and K2CO3 in DMF led to compound 11. We next tried a phenylthio group as a stable protecting group of the α-methylene unit. Conjugate addition of p-thiocresol to enone 11 produced β-thioketone 12 which was successfully transformed into sulfone lactone 13 by Baeyer–Villiger oxidation with excessive mCPBA. Finally, desulfonation of 13 with DBU in THF under mild condition gave the desired compound 2a.


Scheme 1. Reagents and conditions: (a) MOMCl, DIPEA, DMF (90.0%); (b) SeO2, t-BuOOH, THF (85.0%); (c) PDC, DMF (75.0%); (d) p-thiocresol, Et3N, THF (65.6%); (e) 85% mCPBA, NaHCO3, CH2Cl2 (53.8%); (f) DBU, THF (69.9%); (g) 10% HCl, THF, H2O (84.0%); (h) R1R (for 1c, R = H; for 1d and 1f, R = Br; for 1e, R = Cl), K2CO3, DMF, KI (64.0–81.4%); (i) Ac2O, Et3N, THF, DMAP (65.2%).

Scheme 2. Reagents and conditions: (a) MOMCl, DIPEA, DMF (85.3%); (b) LiAlH4, THF, reflux (86.0%); (c) Ac2O, Et3N, THF, DMAP (77.6%); (d) SeO2, t-BuOOH, THF (75.6%); (e) PDC, DMF (72.0%); (f) 10% HCl, THF, H2O (82.3%); (g) p-thiocresol, Et3N, THF (87.6%); (h) 85% mCPBA, NaHCO3, CH2Cl2 (55.2%); (i) DBU, THF (70.6%); (j) 10% K2CO3, CH3OH, reflux (71.0%).
10 with acetic anhydride afforded 11. The following procedures for preparing 2a were exactly the same as for preparing 1a. Finally, 2a was converted to 2b with 10% KHCO₃ in refluxing CH₂OH. It was noteworthy that conjugate addition of p-thiocol to 13 could not get the β-thioketone, so we had to deprotect the MOM ether to reduce the steric hindrance.

Scheme 3 illustrated the synthesis of compounds 3a–d starting from isosteviol. First of all, we attempted to construct a hydroxymethyl in the α-position of 16-ketone with aqueous formaldehyde under base condition. Surprisingly, the 16-ketone was reduced at the same time. The mechanism of the reaction had been reported and was proposed as a one-spot ‘Aldo–Cannizzaro reaction’ process. After the diol 18 had been successfully achieved, we chose acetyl, a cleanly and conveniently deprotected group, to protect the primary alcohol selectively. Oxidation of 19 with PDC provided 20. However, the key intermediate lactone 22 couldn’t be prepared from 20 with excessive mCPBA until the deprotection of acetyl was completed. Treatment of 22 with p-toluenesulfonyl chloride in pyridine produced tosylate 23 which was heated under reflux in pyridine for 6 h to give the target compound 3a. Compound 3b was obtained via elimination of 24 which was accessed by removing the benzyl of 23 with 10% Pd- C. Eventually, two analogues (3c, d) of 3b have been synthesized.

In order to investigate whether the α-methylene lactone group is essential for the bioactivity of the compound; we changed the α-methylene group into the epoxy. Oxidation of 1f and 3a with excessive mCPBA afforded compounds 1h–1j of Scheme 4.

The structures of the target compounds were elucidated using spectroscopic techniques (IR, ¹H and ¹³C NMR, ESI/MS, HRMS).

The cytotoxic activities of compounds 1a–3e were determined in vitro against six cell lines: prostatic carcinoma (PC-3), colorectal carcinoma (HCT-116), breast carcinoma (MDA-MB-231), human erythroleukemic cell line (K562), hepatocellular carcinoma (HepG2) and gastric carcinoma (MGC-803). Doxorubicin hydrochloride was selected as a positive control. The IC₅₀ values were used to determine the growth inhibition in the presence of tetracyclic diterpenoids 1a–3e against PC-3, HCT-116, MDA-MB-231, K562, HepG2 and MGC-803 cancer cell lines. From the IC₅₀ values summarized in Table 1, the compounds 1c, 2a and 2b have

<table>
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<th>Compound</th>
<th>Anti-tumor activity in 48 h (IC₅₀, μM)</th>
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<tr>
<td></td>
<td>PC-3</td>
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<tr>
<td>1a</td>
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<td>65.35</td>
</tr>
<tr>
<td>3e</td>
<td>189.92</td>
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</tbody>
</table>

a Inhibition of cell growth by the listed compounds was determined using MTT assay.

b Data represent the mean value of three independent determinations.

d Dox = Doxorubicin hydrochloride.

References

Table 1


Scheme 3. Reagents and conditions: (a) HCHO (aq), NaOH, C₂H₅OH–H₂O, 75 °C, 3 h (56.1%); (b) BnBr, K₂CO₃, DMF, KI (70.4%); (c) Ac₂O, Et₃N, THF, DMAP, 45 min (85.2%); (d) PDC, DMF (83.1%); (e) 10% KOH, CH₃OH, rt (89.4%); (f) 85% mCPBA, NaHCO₃, CH₂Cl₂ (56.1%); (g) TsCl, pyridine, DMAP (53.7%); (h) 10% Pd-C, H₂, CH₂OH (92.3%); (i) pyridine, DMAP, reflux (66.2%); (j) RR (for 3c, R = I; for 3d, R = Br); K₂CO₃, DMF, KI (75.2%, 80.1%).

Scheme 4. Reagents and conditions: (a) 85% mCPBA, NaHCO₃, CH₂Cl₂ (51.3%, 55.7%).
shown significant cytotoxicities against all the six cell lines with the IC50 values ranging from 0.09 to 5.71 μM. Compounds 1a and 1c were found to be more effective than doxorubicin hydrochloride in HCT-116, MDA-MB-231, HepG2 and MGC803 cell lines. Esterification of 19-acyl with MOM ether or methyl improved the cytotoxicity (1a vs 1b, 1b vs 1c), while esterification of 19-acyl with propyl, allyl and benzyl group decreased the activity (1b vs 1d, 1b vs 1e, 1b vs 1f). Compound 1f showed selective inhibition against K562 (IC50 = 6.23 μM) and MGC803 (IC50 = 2.12 μM) cell lines. Compound 1g with the 13-hydrogen acylated exhibited slightly higher activity compared to compound 1f. On the contrary, removing the 19-acyl of 1a afforded 2b with better cytotoxicity against all the cell lines except HCT-116. Compared with 1h, compound 1f bearing the α-methylencelactone group displayed better activity, which indicated that α-methylencelactone group played an important role in their anticancer activities. However, the compounds (3a–e) with the isosteviol scaffold exhibited weak activities.

In summary, we have successfully synthesized three scaffolds bearing the α-methylencelactone moiety and evaluated their anticancer activities against six cell lines. We also proved that α-methylencelactone group was essential for the bioactivity of the compound, which was consistent with the previous literature.11 Compound 2b was found to be the most potent compound in HepG2 with IC50 value of 0.09 μM. Further researches on identifying their cellular targets are ongoing in our laboratory and the results will be reported in due course.

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References and notes