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D-ring modified novel isosteviol derivatives: Design, synthesis and cytotoxic activity evaluation

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ABSTRACT

A series of polyhydric, amino alcohol and tricyclic derivatives were facilely synthesized by D-ring modification of isosteviol. These compounds were screened for their cytotoxic activities against four human tumor cell lines in vitro. Among them, the 15-α-aminomethyl-16-β-hydroxyl isosteviol **23** exhibits significant cytotoxicity superior to the positive control (cisplatin) against EC9706, PC-3 and HCT-116 cell lines.

The investigation aimed at discovering anti-carcinogenic compounds has attracted considerable interest in recent years since cancer is a leading cause of death worldwide. Natural products have always played a major role in anticancer medicine[1–3] and the unique metabolites produced by diterpenoids have increasingly become major players in antitumor drug discovery.[4–6] Isosteviol is a tetracyclic diterpenoid with a beyerane skeleton, obtained by acid hydrolysis of stevioside.[7] More and more researches in recent years showed that isosteviol as well as its derivatives possess a remarkably broad spectrum of biological activities.[8–15]

Previous reports proved that modifications on D-ring of the isosteviol skeleton could change its biological activities or lead to new activities.[16] Zhang and co-workers[17] reported that *exo*-methylene cyclopentanone sub-structure in the D-ring of tetracyclic diterpenoids could conduce noteworthy cytotoxicity superior to adriamycin. Nguyen's group[18] found two new symmetric dimers of *ent*-kaurane diterpenoid with the connectivity at ring-D from *Croton tonkinensis* showed a potent anti-tumor activity. Our previous study also showed that the C-15 and C-16 functionalized isosteviol had good cytotoxic activities against B16-F10 melanoma cells.[19] In addition, some work also indicated that biological activities of terpenoids would be improved by the introduction of hydroxyl groups through chemical synthesis[20] or microbial transformations.[21–23]

It is also well known that amino alcohols are important structural fragments of many bioactive compounds,[24–26] such as Naltrexone (Fig. 1, Compound (**A**)) and their derivatives.[27] Taneja et al. reported that the cytotoxicity was greatly improved when amino alcohol fragment was introduced to the ring-A of boswellic acids (Fig. 1, Compound (**B**)).[28] However, few reports have focused on the activity relationship of amino alcohol substituted isosteviol. Moreover, compounds with tricyclic skeleton are widely found in natural products, and some of them are proved to be good antitumor agents.[29] Isosteviol, an *ent*-beyerane type tetracyclic diterpene, can be converted to tricyclic compounds by D-ring opening reaction and showed excellent inhibition constants.[30] On the basis of these results, we designed and synthesized a series of novel polyhydric, amino alcohol and tricyclic derivatives via D-ring modifications of isosteviol for the purpose of discovering new antitumor active compounds.

Initial synthetic efforts were focused on novel polyhydric isosteviol derivatives and the synthetic routes are depicted in Scheme 1.

Esterification of compound **1** with CH_3CH_2Br and KOH in DMSO afforded the corresponding isosteviol ethyl ester **2** in high yield,

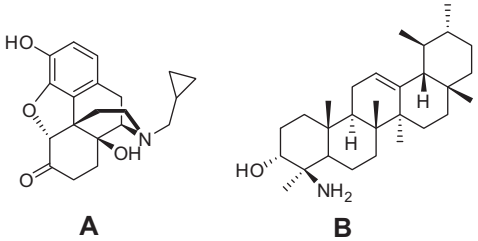

Figure 1. Structure of Compound **A** and **B**.

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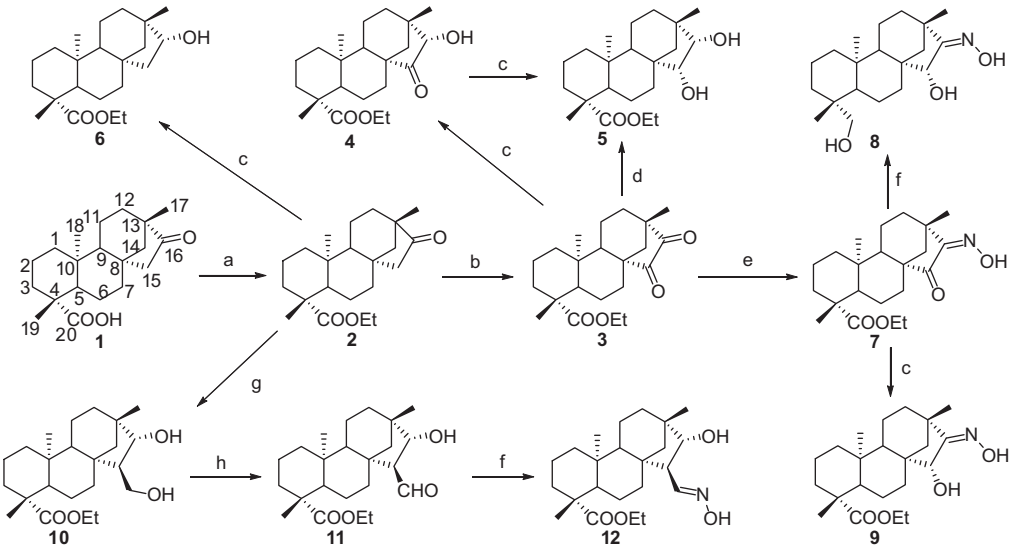

Scheme 1. Reagents and conditions: (a) EtBr, KOH, DMSO, rt, 3 h (95%); (b) Ac_2O, SeO_2, reflux, 6 h (83%); (c) 1.2 equiv. $NaBH_4$, MeOH, 0 °C; (d) 3.0 equiv $NaBH_4$, MeOH, 0 °C to rt, 2 h (90%); (e) $NH_2OH{\cdot}HCl$, $NaHCO_3$, EtOH, reflux; (f) 2.5 equiv. $LiAlH_4$, THF, reflux (80%); (g) HCHO, C_2H_5ONa, C_2H_5OH, 60 °C, 3 h (92%); (h) TEMPO, NBS, CH_2Cl_2/H_2O, TBAB, reflux (90%).

whereas only very low yields of **2** were obtained while using the classical esterification methods. Compound **2** was converted to **6** easily under $NaBH_4$/EtOH conditions.[31] The 15,16-diketone **3** was obtained as an orange crystal by the oxidation of **2** with selenium dioxide in acetic anhydride under reflux condition in high yield. Reduction of **3** with 1.2 equiv. or 3 equiv of $NaBH_4$ in methanol in ice bath gave uniquely the mono reduced product **4** or the 1,3-diol **5**, respectively. But a mixture of **4** and **5** were obtained when the amount of $NaBH_4$ was from 1.2 to 3 equiv. Compound **5** could also be prepared by the reduction of **4** with $NaBH_4$. The absolute configurations of the two newly formed chiral centers at C-15 and C-16 in compound **5** were identified by X-ray crystallographic analysis and the two hydroxyl groups located at *endo* position unambiguously (Fig. 2).[33] The steric effects of C10-CH_3, C13-CH_3 and ring carbon may be the reason to direct the approach of H^- onto the two carbonyls easily from the *exo* positions.

It is well known that oximido groups are usually considered as bioactive elements in biological studies. Therefore, one of the two carbonyls in compound **3** was designed to be converted to oximido following the introduction of a hydroxy unit by reduction of the other carbonyl group for the purpose of investigating the structure–activity relationship. After treatment of **3** with hydroxylamine hydrochloride and $NaHCO_3$ in ethanol under reflux

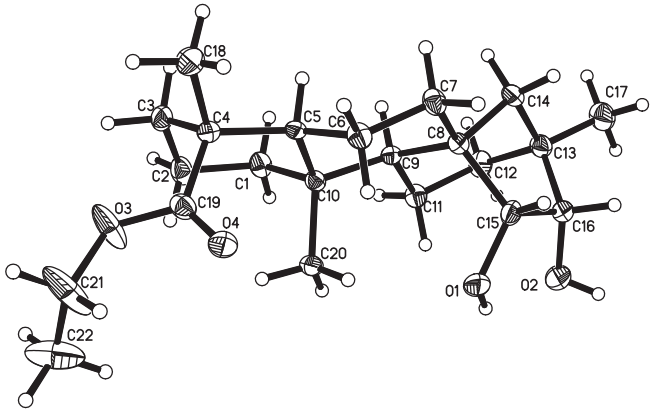

Figure 2. X-ray structure of compound **5**.

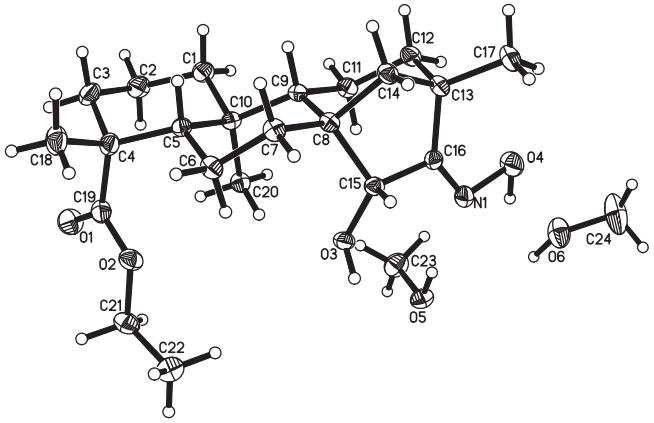

Figure 3. X-ray structure of compound **9**.

condition, only the 15-ketone-16-oxime derivative **7** was obtained as a yellow crystal. But attempts to obtain the 15-oximed derivative failed to meet our expectation. Then we decided to convert the carbonyl group at C-15 and the ester group at C-20 of compound **7** to hydroxyl groups in order to construct multi hydroxyl derivatives. When excess amount of $LiAlH_4$ in THF was employed as reducing agent, compound **8** was obtained as a white solid, whereas $NaBH_4$ could only reduce the C-15 carbonyl to give compound **9** as the unique product in high yield. The NOESY spectrum of compound **8** suggests that the C-15 hydroxyl group was at *endo* configuration. The absolute configuration of compound **9** was confirmed by the X-ray structure (Fig. 3),[33] indicating an *endo* direction of the hydroxyl group at C-15.

Moreover, in our previous work, compound **10** could be successfully achieved through a one-pot 'Aldol-Cannizzaro reaction' process,[32] which promoted us to probe 1,3-diol and its derivatives for the evaluation of their antitumor activities. Firstly, a clean and convenient TEMPO catalyzed oxidization of **10** gave **11** in 90% yield. Followed oximation reaction of **11** with hydroxylamine hydrochloride in the presence of $NaHCO_3$ in ethanol, compound **12** was furnished (87%).

The following work was focused on the introduction of amino and related functional groups onto the D-ring of isosteviol. A series