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Synthesis and in vitro cytotoxic activity evaluation of novel heterocycle bridged carbothioamide type isosteviol derivatives as antitumor agents

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ABSTRACT

Two series of novel carbothioamide-substituted pyrazole and isoxazolidine derivatives were facilely prepared by functional interconversions in ring D of the tetracyclic diterpene isosteviol. The in vitro cytotoxic activities against four human tumor cell lines were evaluated. Our results indicated that carbothioamide-substituted pyrazole derivatives exhibited noteworthy cytotoxic activities. Specifically, compound **12p** (IC_{50} = 6.51 μM) had the most potent cytotoxicity against Raji cell, which may be exploitable as a lead compound for the development of potent antitumor agents.

Cancer is one of the leading causes of mortality worldwide. Although many chemotherapeutic agents, such as 5-fluorouracil, taxol, etc. have been developed to treat this disease effectively, some side effects could happen simultaneously. Therefore, it is important and urgent to develop novel compounds as anticancer agents with higher bioactivities and lower toxicities.[1,2]

Isosteviol (ent-16-ketobeyeran-19-oic acid **1**) is a tetracyclic diterpenoid with a beyerane skeleton, obtained by acid hydrolysis of stevioside.[3,4] Recently, isosteviol derivatives have attracted scientific attention due to their remarkably broad spectrum of biological activities including antihypertension,[5] antihyperglycemia,[6] anti-inflammatory,[7] antioxidation and other biological activities.[8–15] Especially, isosteviol derivatives with D-ring modification exhibited higher cytotoxicity against cancer cells. It was shown in our previous work that the 15- and/or 16-functionalized isosteviol derivatives, obtained by means of group-conversion or structural modification, had good cytotoxic activities against B16-F10 melanoma cell.[16] Wu and co-workers built up a crucial fragment of exo-methylene cyclopentanone and a-methylenelactone in the ring D of isosteviol and obtained some compounds with significantly improved cytotoxicity.[17,18]

Carbothioamide-substituted pyrazole and isoxazolidine are important structural fragments of many bioactive compounds.[19–21] Kim and co-workers indicated recently that compound **A** (Fig. 1) showed potential ALK5 inhibitory activity as transforming growth factor-β type 1 receptor kinase inhibitors.[22] Synchronously, Mohamed et al. reported that the cytotoxicity could be greatly improved when carbothioamide-substituted pyrazole fragment was introduced to the ring-D of steroid (Fig. 1, Compound **B**).[23] In addition, Schwarz et al. carried out the introduction of isoxazolidine type subunit into the substituted benzoylpyrazole in order to improve the herbicidal bioactivities of the precursor compounds (Fig. 1, Compound **C**).[24] However, few reports have focused on the activity relationship of carbothioamide-substituted pyrazole and isoxazolidine isosteviol derivatives. Based on these findings and in continuation of our previous work, a series of novel compounds containing pyrazole and isoxazolidine ring fused with isosteviol structure have been designed and synthesized for the purpose of new antitumor agent discovery.

The carbothioamide-substituted isoxazolidine isosteviol derivatives **9a–9u** were prepared as shown in Scheme 1. Treatment of isosteviol obtained by acid hydrolysis of stevioside with CH_3CH_2Br and KOH in DMSO afforded the corresponding ethyl ester of isosteviol **2** in 96% yield. Compound **3** was stereoselectively synthesized via a one pot Tollens' reaction in 90% yield. Treatment of compound **3** with 4-methylphenylsulfonyl chloride in pyridine furnished compound **4** (75%), which was further converted to the ring opening product **5** in 96% yield via Grob fragmentation of compound **4** in the presence of NaOH in CH_3CN.[16]

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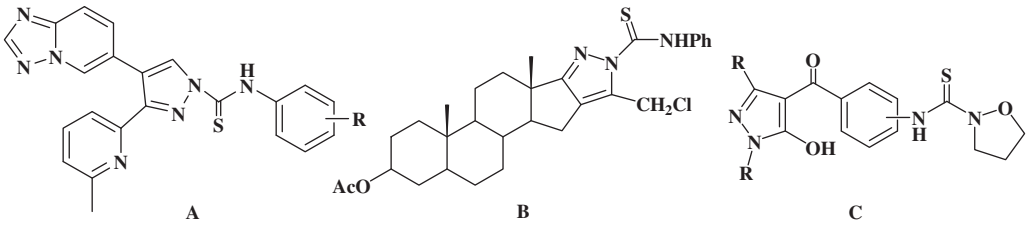
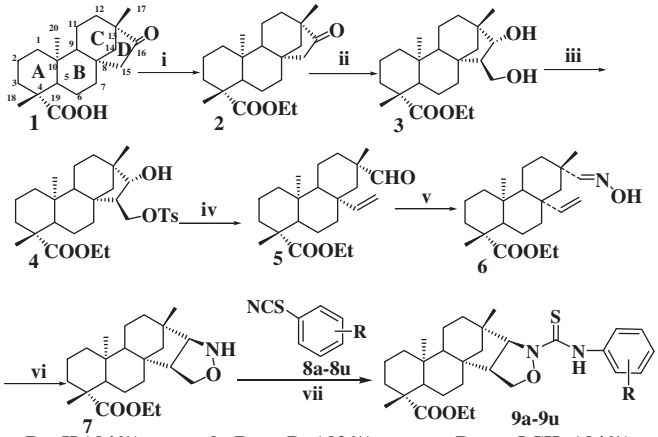


Figure 1. Chemical structures of compounds **A**–**C**.



a: R = H (94%) **h:** R = *o*-Br (92%) **o:** R = *m*-OCH_3 (94%)
b: R = *o*-F (88%) **i:** R = *m*-Br (89%) **p:** R = *p*-OCH_3 (91%)
c: R = *m*-F (81%) **j:** R = *p*-Br (96%) **q:** R = *o*-NO_2 (82%)
d: R = *p*-F (89%) **k:** R = *o*-CH_3 (90%) **r:** R = *m*-NO_2 (68%)
e: R = *o*-Cl (75%) **l:** R = *m*-CH_3 (86%) **s:** R = *p*-NO_2 (86%)
f: R = *m*-Cl (89%) **m:** R = *p*-CH_3 (79%) **t:** R = 2,6-Me_2 (76%)
g: R = *p*-Cl (93%) **n:** R = *o*-OCH_3 (98%) **u:** R = 2,4-Cl_2 (80%)

Scheme 1. Reagents and conditions: (i) EtBr, DMSO, KOH, rt, 3 h, 96%; (ii) HCHO, C_2H_5ONa, C_2H_5OH, 60 °C, 3 h, 90%; (iii) TsCl, Pyr, rt, 18 h, 75%; (iv) NaOH, CH_3CN, rt, 3 h, 96%; (v) $HONH_3Cl$, $NaHCO_3$, C_2H_5OH, 60 °C, 2 h, 97%; (vi) toluene, $BF_3{\cdot}OEt_2$, 80 °C, 1 h, 96%; (vii) **8a**–**8u**, NaH, anhydrous toluene, rt, (75–98%).

Oximation of the aldehyde **5** with hydroxylamine hydrochloride in the presence of sodium bicarbonate in ethanol gave only one of the two possible geometric isomers of the corresponding aldoxime **6** (97%), which went through a cyclization catalyzed by $BF_3{\cdot}OEt_2$ in boiling toluene to produce the condensed isoxazolidine derivative **7** in high yield (96%).[16] The intermediate **7** was treated with appropriately substituted phenyl isothiocyanates **8a–8u** in the presence of NaH in anhydrous toluene to afford target compounds **9a-9u** in yields of 75–98%. The structures of compounds **9a-9u** were characterized by IR, ^1H NMR, ^{13}C NMR and HRMS, respectively,[25] and the stereostructure of **9b** was confirmed by X-ray crystallographic analysis (Fig. 2).[26] The single crystal structure of compound **9b** indicated that the newly formed heterocycle took a β orientation at C-15 and C-16 on D-ring of isosteviol.

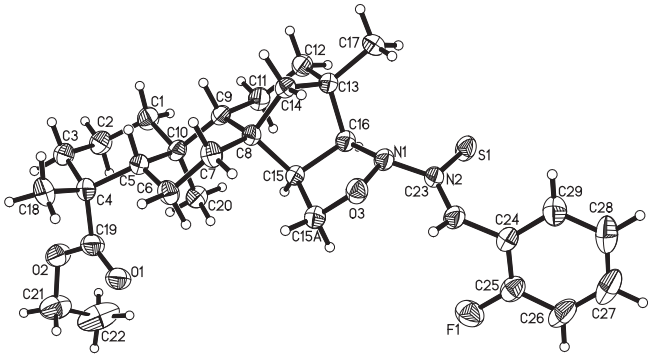


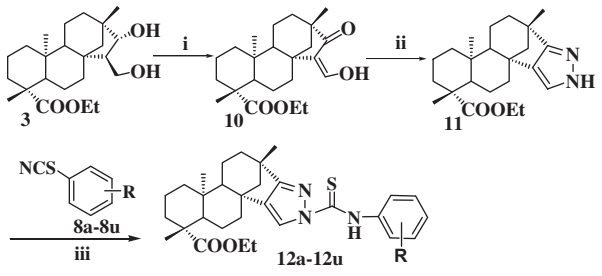
Figure 2. X-ray structure of compound **9b**.

Since the carbothioamide substituted pyrazole usually behaves as bioactive subunit in various natural products, we intended to introduce the special fragment to the skeleton of isosteviol in order to construct a novel family of bioactive molecules for the discovery of antitumor agent. Therefore, a series of carbothioamide-substituted pyrazole derivatives **12a–12u** were designed and synthesized via the following procedure (Scheme 2).

Treatment of compound **3** with TCC in CH_2Cl_2 afforded the corresponding product **10** in 62% yield. Condensation of **10** with hydrazine monohydrate in EtOH provided the pyrazole derivative **11** in 75% yield, which was further treated with appropriate substituted phenyl isothiocyanates **8a–8u** in the presence of NaH in anhydrous toluene to afford target compounds **12a–12u** in 28–92% yields. The structure of compounds **12a–12u** were characterized by IR, ^1H NMR, ^{13}C NMR and HRMS, respectively,[25] and the stereostructure of **12c** was confirmed by X-ray crystallographic analysis (Fig. 3).[26]

With the two series of novel carbothioamide-substituted isosteviol derivatives **9a–9u** and **12a–12u**, in hand, we began to investigate their antitumor activities against four human tumor cell lines. Expectedly, this successful strategy not only provided us several interesting compounds with noteworthy antitumor activities, but also provided valuable information of structure–activity relationship for new drug discovery.

The cytotoxic activities of these compounds were then evaluated in vitro against four cancer cell lines, including: gastric cancer (SGC 7901), lung cancer (A549), lympha cancer (Raji) and cervical cancer (Hela). Cisplatin was used as a positive control. The growth inhibition was determined according to the IC_{50} values in the presence of isosteviol derivatives **9a**–**9u** and **12a**–**12u** (Table 1).[27] The structure–activity relationship was also analyzed through the IC_{50} values.



a: R = H (92%) **h:** R = *o*-Br (68%) **o:** R = *m*-OCH_3 (58%)
b: R = *o*-F (83%) **i:** R = *m*-Br (50%) **p:** R = *p*-OCH_3 (62%)
c: R = *m*-F (38%) **j:** R = *p*-Br (64%) **q:** R = *o*-NO_2 (58%)
d: R = *p*-F (67%) **k:** R = *o*-CH_3 (68%) **r:** R = *m*-NO_2 (51%)
e: R = *o*-Cl (87%) **l:** R = *m*-CH_3 (54%) **s:** R = *p*-NO_2 (52%)
f: R = *m*-Cl (28%) **m:** R = *p*-CH_3 (78%) **t:** R = 2,6-Me_2 (69%)
g: R = *p*-Cl (53%) **n:** R = *o*-OCH_3 (73%) **u:** R = 2,4-Cl_2 (74%)

Scheme 2. Reagents and conditions: (i)TCC, CH_2Cl_2, rt, 1.5 h, 62%; (ii) $N_2H_4{\cdot}H_2O$, C_2H_5OH, reflux, 1 h, 75%; (iii) **8a**–**8u**, NaH, anhydrous toluene, rt, (28–92%).