การศึกษาความเป็นพิษต่อเซลล์ของอนุพันธ์โรทีนอยด์
Cytotoxicity Studies of Rotenoid Derivatives

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บทคัดย่อ

สารประกอบ 6-Deoxyclitoriacetal เป็นสารสกัดจากรากแห้งของหนอนตายหยาก การสังเคราะห์และศึกษาความเป็นพิษต่อเซลล์ของอนุพันธ์ 6-Deoxyclitoriacetal ที่ดัดแปลงโครงสร้างด้วยหมู่ฟังก์ชันที่แตกต่างกันที่คาร์บอนตำแหน่งที่ 11 เพื่อศึกษาความสัมพันธ์ของโครงสร้างกับการออกฤทธิ์ทางชีวภาพ 6-Deoxyclitoriacetal นั้นมีความเป็นพิษต่อเซลล์ถูกดัดแปลงโครงสร้างเพื่อเพิ่มประสิทธิภาพในการเป็นยารักษาโรคมะเร็งอีกชนิดหนึ่ง ชุดอนุพันธ์ของ 6-deoxyclitoriacetal กับเบส ไพริมีดีนให้ค่าความเป็นพิษต่อเซลล์ในการยับยั้งเซลล์มะเร็ง 3 ชนิด แสดงด้วยค่า IC_{50} ในช่วง 0.02 ถึง 25.39 µg/ml หมู่ฟังก์ชันที่สามารถเกิดอันตรกิริยาระหว่างโมเลกุล เช่น พันธะไพ และพันธะไฮโดรเจนกับดีเอ็นเอสามารถเพิ่มฤทธิ์ความเป็นพิษต่อเซลล์

คำสำคัญ : หนอนตายหยาก ความเป็นพิษต่อเซลล์ ไพริมีดีน เซลล์มะเร็ง ดีเอ็นเอ

Abstract

6-Deoxyclitoriacetal was extracted from the dried roots of *Stemona collinse* Craib. The synthesis and cytotoxicity of 6-deoxyclitoriacetal derivatives with different functional groups at the C11-OH position for structure-activity relationship study was described. Known cytotoxic substances such as 6-deoxyclitoriacetal may be modified to enhance its effectiveness as an anti-cancer drug candidate. The synthetic analogues bearing pyrimidine base moieties showed cytotoxic activities against three cancer cell lines, with IC_{50} values ranging from 0.02 to 25.39 µg/ml. The functional groups that have intermolecular interactions, such as π-π interactions and hydrogen bonding, with DNA can improve cytotoxicity.

Keywords : *Stemona collinse* Craib., cytotoxicity, cancer cell lines, pyrimidine, DNA

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Introduction

6-Deoxyclitoriacetal (Figure 1a) is a rotenoid. Rotenoid, a four fused ring A, B, C and D (Figure 1b), possess various bioactivities such as antimicrobial, antiviral action, insecticide and antifeedant properties (Barnes *et al*., 1966; Chau *et al*., 1967; Kole *et al*., 1992).

One of interesting activities of rotenoid compounds is anticancer activity (Cassady *et al*., 1990; Newman *et al*., 2003). 6-Deoxyclitoriacetal from the roots of *Clitoria macrophylla* was known to show strong cytotoxic activity against culture P388 lymphocytic leukemia cell (Shiengthong *et al*., 1974). Rotenoids from *Amorpha fruticosa* were found to be inhibitors of human cancer cell line (Roengsumran *et al*., 2003). In Thailand, the rotenoid compounds were isolated from the dried roots of *Stemona collinsae* Craib. 6-Deoxyclitoriacetal has been known to have cytotoxic activity against various types of human carcinoma (Fang & Casida, 1998; Lin *et al*., 1992). Therefore, in order to enhance its cytotoxic activity, the structure-activity relationship on cytotoxicity was investigated. The hypothesis that might attribute to cytotoxic activity of a compound was proposed and proved. That is, the molecule should possess three characteristics: (i) the molecule has a bent shape, (ii) the molecule has a planar part to intercalate with DNA and (iii) the molecule has functional groups that have intermolecular interactions to stabilize the intercalation (Sangthong *et al*., 2011; Chaires *et al*., 1990; Li *et al*., 2009).

In order to enhance the cytotoxicity of 6-deoxyclitoriacetal and develop more potent analogues, derivatives of 6-deoxyclitoriacetal have been synthesized and evaluated for their cytotoxicity. In this work, we describe the synthesis and cytotoxicity of 6-deoxyclitoriacetal derivatives with different functional groups at the C11-OH position and study the structure-activity relationship on their cytotoxicity.

Materials and Methods

2.1 Materials

The ground dried roots of *Stemona collinsae* Craib. were purchased from Vejpongosot Pharmacy Co., Ltd. All chemical substances and solvents were purchased from Merck and Aldrich.

2.2 Extraction and isolation

The ground dried roots of *Stemona collinsae* Craib. were extracted with dichloromethane. The dichloromethane fraction was concentrated to obtain dark brown crude extract and then separated on silica gel column using hexane: ethyl acetate: dichloromethane = 2:1:2 (v/v) as eluent (Roengsumran *et al*., 2003). The fractions showing similar spots were combined and then concentrated to dryness. The methanol was used to recrystallize 6-deoxyclitoriacetal from the other rotenoid compounds. The obtained product was characterized by spectroscopic methods.

2.3 Synthesis of 6-deoxyclitoriacetal analogues

In order to study the effect of functional groups on pyrimidine base to the cytotoxicity, three pyrimidine bases were chosen to synthesize with 6-deoxyclitoriacetal. They composed of cytosine, thymine and uracil,

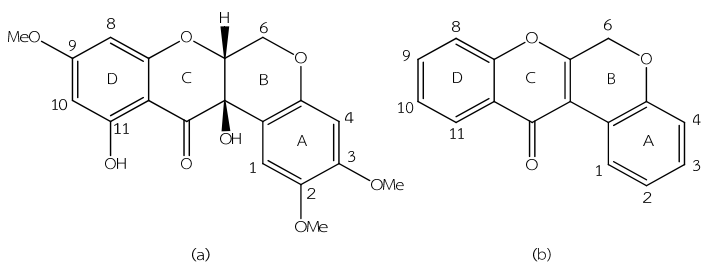

Figure 1. Chemical structures of the (a) 6-deoxyclitoriacetal (b) rotenoid core.

respectively. The 6-deoxyclitoriacetal was added with tosylate group (compound A) before removed it with each pyrimidine base (A1-A3). To the reaction mixture of the cytosine and sodium hydride in DMF was refluxed for 3 hours and then added the compound A in DMF. The reaction mixture was poured into ice bath and extracted with dichloromethane. The combined extract was dried over anhydrous magnesium sulphate and the solution was filtered (Woo *et al*., 2007). Solvent was removed under reduced pressure and the crude product was purified by silica gel column chromatography eluting with hexane: ethyl acetate: dichloromethane (1:2:2, v/v), leading to compound A1. The other compounds (compound A2-A3) were prepared as the same method. The reaction scheme for the modification of 6-deoxyclitoriacetal is shown schematically in Figure 2.

6-Deoxyclitoriacetal and all derivatives were tested for cytotoxic activity against three cell lines including human oral carcinoma (KB), breast cancer (MCF-7) and human small lung (NCI-H187) for the evaluation of growth inhibition. Cell line growth was monitored using the MTT assay as reported and compared with doxorubicin as a positive control.

Results and Discussion

In general, the major target for many anticancer drugs is deoxyribonucleic acid (DNA). It is a long polymer made from repeating units called nucleotides. Pyrimidine base is one of the nucleotide components. Pyrimidine includes the base cytosine, thymine and uracil, which are components of DNA and RNA. Pyrimidine analog was used as a drug in the treatment of cancer (Li *et al*., 2009). It belongs to the family of drugs called antimetabolites. Pyrimidine is a nitrogenous heterocyclic base. The nitrogen atoms in heterocyclic base may increase the stability of the complex through forming hydrogen bonds with DNA. Therefore, in this work the structure of 6-deoxyclitoriacetal was modified with certain pyrimidine base. In order to study the effect of functional groups on pyrimidine base to the cytotoxicity, three pyrimidine bases were chosen to synthesize with 6-deoxyclitoriacetal namely cytosine, thymine and uracil. Compound A is a tosylate derivative of 6-deoxyclitoriacetal. Compounds A1 to A3 are the products of replacing the tosylate derivative with the pyrimidine base mentioned above. All synthesized compounds and 6-deoxyclitoriacetal were tested for cytotoxicity against KB (Human mouth carcinoma), MCF7

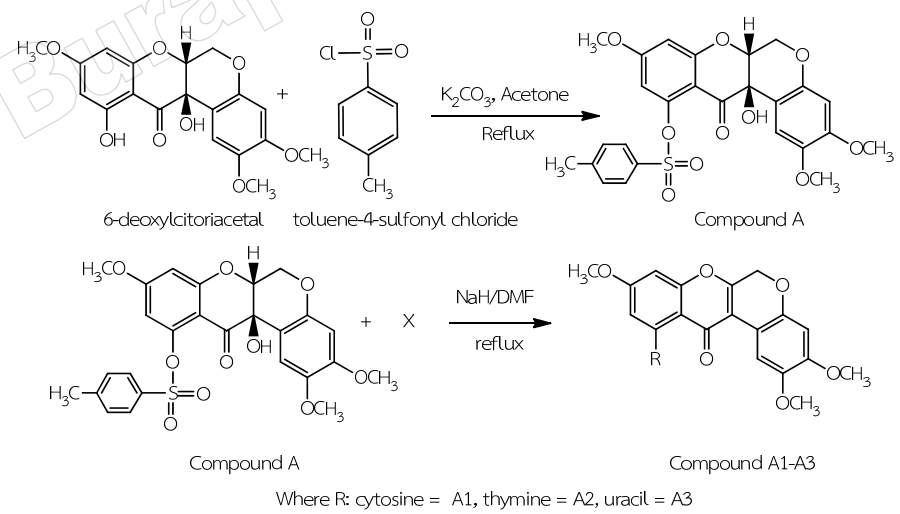

Figure 2. Schematic chart showing the synthesis route of the 4 derivatives from 6-deoxyclitoriacetal (where X: pyridine base; cytosine = A1; thymine = A2; uracil = A3, respectively)

(Breast cancer) and NCI-H187 (Human small lung cancer) cell lines. Doxorubicin and were used as the positive control for this cytotoxic activity assay. The cytotoxic activities of 6-deoxyclitoriacetal and their 6-deoxyclitoriacetal analogues were reported in IC_{50} value. The IC_{50} is the half maximal (50%) inhibitory concentration (IC) of a substance. In general, higher IC_{50} means lower cytotoxic activity. The cytotoxic activity results of all compounds were tabulated in Table 1.

Table 1 Cytotoxicity for compounds A, A1-A3

Compound	IC_{50} (μg/ml)		
	KB	MCF7	NCI-H187
Dox	0.33	0.82	0.04
6-deoxyclitoriacetal	0.08	0.26	0.04
A	0.02	inactive	0.02
A1	18.65	inactive	11.61
A2	25.39	inactive	11.61
A3	1.45	inactive	0.26

Dox = Doxorubicin

The cytotoxic activities of each compound were indicated as follows. Compound A has tosylate group at C11. The side chain has toluene-4-sulfonic acid which is composed of two carbonyl groups. It adopts a bent shape at C6a-C12a. This compound exhibited very strong cytotoxicity against two cancer cell lines. Compound A was very active against KB cell line with the IC_{50} value of 0.02 μg/ml and against NCI-H187 cell line with the IC50 value of 0.02 μg/ml. Unfortunately, it was inactive against MCF7 cell line. Compound A1 was synthesized by adding the pyrimidine base, cytosine, to replace the tosylate group. The structure has a double bond at C6a-C12a. Therefore, it adopts only a planar structure. This compound exhibited moderate cytotoxicity against two cancer cell lines; KB cell line with the IC_{50} value of 18.65 μg/ml and against NCI-H187 cell line with the IC_{50} value of 11.61 μg/ml but inactive against MCF7 cell line. Compound A2 was synthesized by adding the pyrimidine base, thymine, instead the tosylate group. The structure at C6a-C12a position was similar to that of A1. Therefore, it also adopts a planar structure. This compound exhibited moderate cytotoxicity against two cancer cell lines; KB cell line with the IC_{50} value of 25.39 μg/ml and NCI-H187 cell line with the IC_{50} value of 11.61 μg/ml. However, it was inactive against MCF7 cell line. Compound A3 was synthesized by adding the pyrimidine base, uracil, instead the tosylate group. It also has a planar structure at C6a-C12a position which is similar to that of compound A1 and compound A2. Compound A3 has better cytotoxicity than A1 and A2. It exhibited strong cytotoxicity against KB and NCI-H187 cancer cell lines with the IC_{50} value of 1.45 and 0.26 μg/ml, respectively. As a result, compound A showed the most potential cytotoxic activity against KB and NCI-H187 cell lines with the IC_{50} value of 0.02 and 0.02 μg/ml, respectively. This is very possibly because the molecule adopts a bent shape and bears two carbonyl groups at C11 position. They can increase the stability of the substance-DNA complex by forming strongly hydrogen bonds with DNA. Whereas, compound A1 to compound A3 was planar structure, they had different cytotoxicity against KB and NCI-H187 cell lines, but showed the same activities in MCF7 and inactive against MCF7 cell lines. For KB cancer cell line, compound A3 showed more potential cytotoxic activity than compound A1 and A2. Since, compound A3 has the nitrogen atoms and two carbonyl groups in heterocyclic compounds; they can help to stabilize the DNA complex through the forming hydrogen bonds with DNA. However, compound A1 was more active than compound A2 in KB cell line because compound A1 has the carbonyl group and the amino group, while compound A2 presented the methyl group leading to the steric effect. This might disturb the binding of molecule with the base pair in DNA. In addition, the cytotoxic activities of compound A1 and compound A2

showed the comparative cytotoxic activities in NCI-H187, while compound A3 showed the good cytotoxic activity against this cancer cell line.

Conclusion

6-Deoxyclitoriacetal is a rotenoid extracted from the dried roots of *Stemona collinse* Craib. 6-Deoxyclitoriacetal has good cytotoxic activity against various types of human carcinoma. In order to enhance its cytotoxic activity, the structure-activity relationship on cytotoxicity was investigated. The hypothesis that might attribute to cytotoxic activity of a compound was proposed and proved. In order to study the effect of the functional groups on the DNA-binding ability, the 6-deoxyclitoriacetal analogues were prepared and then tested for their cytotoxicity. All compounds were tested the cytotoxicity against three cancer cells. Among the 6-deoxyclitoriacetal – pyrimidine base analogues (A and A1 to A3), uracil derivative showed the strongest cytotoxicity. This is consistent with the commercial anticancer drug containing uracil pharmacophore. The results have confirmed the hypothesis, *i.e*., the molecule should possess three characteristics: (i) the molecule has a bent shape, (ii) the molecule has a planar part to intercalate with DNA and (iii) the molecule has functional groups that have intermolecular interactions to stabilize the intercalation.

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