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# Chemo-Enzymatic Synthesis of Ester-Linked 2-Phenylindole-3-Carboxaldehyde-Monosaccharide Conjugate as Potential Prodrug

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**Abstract:** Chemo-enzymatic synthesis of ester-linked 2-phenylindole-3-carboxaldehyde-glucose conjugate (2-phenylindole-3-carboxyl-10''-O- $\beta$ -D-glucosyl ester) was achieved by using plant cell cultures as biocatalysts. The anticancer agent, 2-phenylindole-3-carboxaldehyde, induced apoptosis in cells, whereas 2-phenylindole-3-carboxyl-10''-O- $\beta$ -D-glucosyl ester showed no cytotoxicity and induced no apoptosis.

**Keywords:** chemo-enzymatic synthesis, 2-phenylindole-3-carboxyl-10''-O- $\beta$ -D-glucosyl ester, cytotoxicity, apoptosis

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## Introduction

2-Phenylindole-3-carboxaldehyde is one of the most potent anticancer agents as it acts as an inhibitor of tubulin polymerization. This antitumor compound presents disadvantages when used as a treatment as it has low water-solubility and exhibits toxicity toward normal tissues. Many efforts have been made to modify lipophilic anticancer agents, such as taxol, chemically in order to create a more soluble and more easily delivered drug.<sup>1–4</sup> Formation of prodrugs is important as it enable such lipophilic anticancer agents to be changed into soluble and non-cytotoxic compounds. The prodrugs that incorporate acids have attracted much attention as an ester linkage improves the solubility of lipophilic anticancer agents and can be hydrolyzed by hydrolytic enzymes to release lipophilic anticancer agents.<sup>1–4</sup> To date, little attention has been paid to chemo-enzymatic synthesis of ester-linked 2-phenylindole-3-carboxaldehyde-glycoside conjugates such as water-soluble 2-phenylindole-3-carboxaldehyde prodrugs, which employ enzymatic cleavage by hydrolytic enzymes, including esterases and glycosidases, as their mode of activation.

In this study, we report the chemo-enzymatic synthesis of ester-linked 2-phenylindole-3-carboxaldehyde-glycoside conjugate (ie, 2-phenylindole-3-carboxyl-10''-O- $\beta$ -D-glucosyl ester) and its effects on apoptosis inducing activity via DNA-damaging.

## Experimental

### General

2-Phenylindole-3-carboxaldehyde was purchased from Aldrich Chemical Co. The <sup>1</sup>H and <sup>13</sup>C nuclear magnetic resonance (NMR), H-H correlation spectroscopy (COSY), C-H COSY, and heteronuclear multiple-bond correlation (HMBC) spectra were recorded in CD<sub>3</sub>OD using a Varian XL-400 spectrometer (Varian Inc.). The chemical shifts were expressed in  $\delta$  (ppm) referring to tetramethylsilane. The fast atom bombardment mass spectrometry (FABMS) spectra were measured using a JEOL MStation JMS-700 spectrometer (JEOL Ltd.). High performance liquid chromatography (HPLC) was carried out on Crestpak C18S column (4.6  $\times$  150 mm, JASCO) [solvent: MeOH–H<sub>2</sub>O (2:3, v/v); detection: UV (228 nm); flow rate: 1.0 mL/min].

## Synthesis of 2-phenylindole-3-carboxyl-10''-O- $\beta$ -D-glucosyl ester

To a conical flask containing 0.25 mol of CrO<sub>3</sub> and water added 25 mL of sulfuric acid (oxidant reagent).<sup>5</sup> The oxidizing reagent was added dropwise to the starting material, 2-phenylindole-3-carboxaldehyde (1), in acetone. The reaction was quenched by adding isopropyl alcohol. The reaction mixture was purified by column chromatography on silica gel to produce 2-phenylindole-3-carboxylic acid.

A total amount of 1.5 mmol of 2-phenylindole-3-carboxylic acid (2) was administered to 10 flasks (0.15 mmol/flask) containing the cultured suspension cells. The flasks were incubated at 25 °C on a rotary shaker (120 rpm). After a five-day incubation period, the cells and medium were separated by filtration with suction. The filtered medium was extracted with ethyl acetate (EtOAc). The medium was further extracted with n-BuOH. The n-BuOH fraction was analyzed by HPLC. The cells were added MeOH, sonicated for 5 minutes, and extracted for 12 hours. The MeOH fraction was concentrated, and partitioned between H<sub>2</sub>O and EtOAc. The EtOAc fractions were combined, concentrated, and analyzed by HPLC. The H<sub>2</sub>O fraction was applied to a Diaion HP-20 column, which was washed with H<sub>2</sub>O and then eluted with MeOH. The MeOH eluate was subjected to preparative HPLC to give the final product of 2-phenylindole-3-carboxyl-10''-O- $\beta$ -D-glucosyl ester (3).

Spectral data of 2-phenylindole-3-carboxyl-10''-O- $\beta$ -D-glucosyl ester are as follows. 2-Phenylindole-3-carboxyl-10''-O- $\beta$ -D-glucosyl ester (3): <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>,  $\delta$  in ppm):  $\delta$  3.17–3.71 (6H, m, glucoside-H), 5.66 (1H, d, J = 8.0 Hz, glucoside anomer-H), 7.25–8.37 (9H, m, Phenyl-H); <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>,  $\delta$  in ppm):  $\delta$  60.6, 72.5, 76.9, 77.7, 79.1, 101.9, 121.4, 121.7, 122.6, 127.2, 127.7, 128.9, 130.0, 131.4, 135.6, 145.4, 163.0.

## Time course of biotransformation of 2-phenylindole-3-carboxylic acid

A total of 50 g (fr. wt) of the suspension cells of *Eucalyptus perriniana* was divided into eight flasks containing 100 mL of the MS medium. Substrate (0.1 mmol) was administered to each of flasks (1 mmol/L) and the mixtures were incubated on a rotary shaker at 25 °C. At 6-hour intervals, one of the flasks was taken out from the

rotary shaker, and the cells and medium were separated by filtration. The extraction and analysis procedures were same as described in Section 2.2. The product yields were determined based on the peak area from HPLC analysis and expressed as a percentage relative to the amount of total products from the completed reaction.

### Apoptosis induced by 2-phenylindole-3-carboxaldehyde and 2-phenylindole-3-carboxyl-10''-O- $\beta$ -D-glucosyl ester

Nuclear fragmentation was visualized using Diaminophenylindole (DAPI) Staining Protocol and excitation through UV radiation.<sup>6</sup> By applying this protocol, significant staining of DNA is obtained in dead cells.

## Results and Discussions

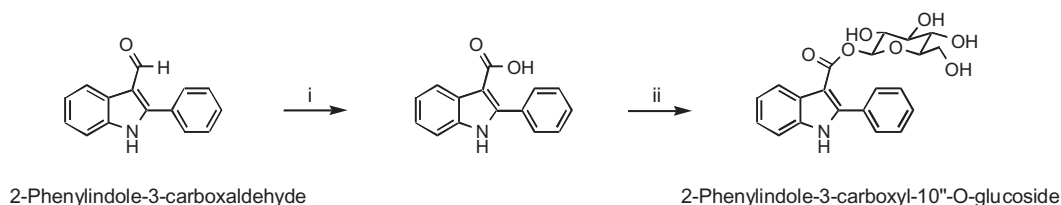
The 2-phenylindole-3-carboxaldehyde-prodrug (2-phenylindole-3-carboxyl-10''-O- $\beta$ -D-glucosyl ester) was synthesized from 2-phenylindole-3-carboxaldehyde (1) by chemo-enzymatic procedures as shown in Figure 1. The formyl group of 2-phenylindole-3-carboxaldehyde was oxidized with  $\text{CrO}_3$  dissolved in sulfuric acid. The reaction mixture was incubated in acetone. The reaction was stopped by adding isopropylalcohol. The reaction products were purified by column chromatography on silica gel to give 2-phenylindole-3-carboxylic acid (2, 51%).

Next, biotransformation of 2-phenylindole-3-carboxylic acid (2) by cultured plant cells was examined. Incubation of cultured *E. perriniana* cells with 2-phenylindole-3-carboxylic acid was performed at 25 °C on a rotary shaker (120 rpm). After a five-day incubation period, the cells were extracted using MeOH. After concentration of the MeOH fraction, the residue was partitioned between  $\text{H}_2\text{O}$  and EtOAc. The  $\text{H}_2\text{O}$  fraction was purified by a Diaion HP-20 column, which was washed with  $\text{H}_2\text{O}$  and then eluted with MeOH. The MeOH eluate which included

glycosides was subjected to preparative HPLC to give 2-phenylindole-3-carboxyl-10''-O- $\beta$ -D-glucosyl ester (3, 70%). No products were detected in the culture medium despite careful analysis on HPLC.

To assess the biotransformation of the culture over time, eight flasks containing cultured *E. perriniana* cells were assessed at 6 hour intervals. At the early stage of the incubation period, the substrate 2-phenylindole-3-carboxylic acid was smoothly converted into 2-phenylindole-3-carboxyl-10''-O- $\beta$ -D-glucosyl ester. After five days incubation, the amount of 2-phenylindole-3-carboxyl-10''-O- $\beta$ -D-glucosyl ester had not increased showing that the glycosylation reaction was equilibrated at that time.

The microtubule is essential for cellular functions such as mitosis and cell replication. Formation and depolymerization of microtubules are dynamic processes which can be interrupted by stabilization of microtubules and inhibition of polymerization. The taxanes stabilize the microtubule structures. On the other hand, indoles are attractive as inhibitors of tubulin polymerization. Alkylindole derivatives strongly inhibit the growth of breast cancer cells and their action can be rationalized by the cell cycle arrest in G2/M phase due to the inhibition of tubulin polymerization. As a result it can be concluded that such drugs induced cell apoptosis. The effect of 2-phenylindole-3-carboxaldehyde (1), 2-phenylindole-3-carboxylic acid (2), and 2-phenylindole-3-carboxyl-10''-O- $\beta$ -D-glucosyl ester (3) on cell death by apoptosis was investigated. Results show that apoptosis was induced only by 2-phenylindole-3-carboxaldehyde (1). Additionally it was shown that neither 2-phenylindole-3-carboxylic acid (2) nor 2-phenylindole-3-carboxyl-10''-O- $\beta$ -D-glucosyl ester (3) caused any cytotoxicity to induce apoptosis. It is important that the prodrugs show little or no cytotoxicity, as the purpose of producing prodrugs is to reduce the cytotoxicity of



**Figure 1.** Chemo-enzymatic synthesis of 2-phenylindole-3-carboxyl-10''-O- $\beta$ -D-glucosyl ester.

**Note:** Reagents and conditions: (i)  $\text{CrO}_3$ ,  $\text{H}_2\text{SO}_4$ ,  $\text{H}_2\text{O}$ , acetone; (ii) Plant cell cultures of *E. perriniana*.



the drugs. The anticancer prodrugs with glycosyl conjugation would exert cytotoxicity when hydrolyzed at the glycosyl portion and when the anticancer drugs are released.

Thus, a water-soluble 2-phenylindole-3-carboxaldehyde-prodrug (ie, 2-phenylindole-3-carboxyl-10''-O- $\beta$ -D-glucosyl ester) was synthesized by chemo-enzymatic procedures. The chemical glycosylation requires tedious steps including protection and deprotection of hydroxyl groups of sugar. Therefore the present synthetic procedure can be deemed superior to the chemical method. The cytotoxicity of 2-phenylindole-3-carboxyl-10''-O- $\beta$ -D-glucosyl ester was reduced, showing this glycoside derivative may act as potential 2-phenylindole-3-carboxaldehyde-prodrug. Further studies on in vivo therapeutic values of 2-phenylindole-3-carboxyl-10''-O- $\beta$ -D-glucosyl ester are now in progress.

### Author Contributions

KS, MH, HY, HH were responsible for data collection/entry/analysis and assistance with manuscript preparation. HH was responsible for the study design and preparation of the manuscript. All authors read and approved the final manuscript.

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