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Enzymatic biosynthesis of novel bavachin glucosides via Bacillus UDP-glycosyltransferase

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ABSTRACT

A UDP-glycosyltransferase (YjiC) from *Bacillus licheniformis* was exploited for the glycosylation of bavachin. The *in vitro* glycosylation reaction generated three novel bavachin glucosides, which were structurally characterized as bavachin-4′-*O*-*β*-D-glucopyranoside (**1**), bavachin-7-*O*-*β*-D-glucopyranoside (**2**), and bavachin-4′,7-di-*O*-*β*-D-glucopyranoside (**3**) based on spectroscopic techniques. To enhance product yield, the reaction time, buffer pH, and UDP-glucose concentration were optimized. The water-solubility of compounds **1**, **2**, and **3** was approximately 5.18, 56.22, and 84.17 times higher than that of bavachin, respectively. In addition, compounds **1**-**3** displayed the highest stability at pH 8.8 and were stable up to 70 °C for 30 min. Furthermore, the biological activities of bavachin and compounds **1**-**3** were assayed. Bavachin showed moderate cytotoxicity against four human cancer cell lines while the three glycosylation products displayed weak activity. The results demonstrate that the UDP-glycosyltransferase (YjiC) has the capacity to synthesis bavachin glucosides and that glycosylation of bavachin enhances its water-solubility and stability.

1. Introduction

Flavonoids are a large group of plant secondary metabolites, that have received increasing research attention owing to their considerable biological benefits, including anti-tumor, antioxidant, anti-inflammatory, anti-diabetes, and antiviral activities (Xiao, 2017). In the plant, flavonoids exist in the form of flavonoid aglycones and their glycosides. The glycosylation of flavonoids significantly affects their solubility, stability and bioactivity (Ghimire et al., 2015).

Bavachin is a prenylation flavanone isolated from the seed of *Psoralea corylifolia* L., that reportedly has various biological activities. Current research indicates that bavachin might have therapeutic potential for type 2 diabetes by activating insulin signaling pathways (Lee et al., 2016), inhibit the synthesis of melanin on A375 cells by inhibiting the protein and mRNA expression of TYR, TRP-1, TRP-2, ERK1, ERK2 and JNK2 (Wang et al., 2016), promote osteoblasts proliferation and differentiation (Wang et al., 2001; Li et al., 2014), treat inflammatory diseases by inhibiting IL-6-induced STAT3 activation and phosphorylation (Lee et al., 2012). However, its low water solubility and poor absorption after oral administration limit the clinical use of bavachin (Gao et al., 2016).

Glycosylation is one of nature's prominent reactions in the synthesis or modification of biologically active compounds. *In vitro* enzymatic glycosylation is a common method for glycosylation, which utilizes glycosyltransferases to transfer sugar moieties from an activated donor to an acceptor molecule (Yang et al., 2004). In our previous work, we used YjiC to synthesize neobavaisoflavone glucosides (Ma et al., 2017), bavachinin glucoside (Dai et al., 2016), corylifol A glucosides (Li et al., 2017) and isobavachalcone glucosides (Li et al., 2015) *in vitro*. In this work, we studied *in vitro* glucosylation of bavachin using YjiC and we structurally characterized the bavachin glucosides (Fig. 1). We compared the water solubility, pH, and temperature stability of bavachin and the derived glucosides. Moreover, the anti-proliferative effects of bavachin and bavachin glucosides were investigated in four human tumor cell lines.

2. Results and discussion

2.1. In vitro glucosylation of bavachin

The substrate molecule, bavachin, has two phenolic hydroxyl groups at the C-7 and C-4′ positions, implying that these are two potential glycosylation sites. Bavachin was subjected to glucosylation reaction and the reaction products were analyzed by high-performance liquid chromatography (HPLC), which showed three different product peaks at 254 nm, with retention times of 11.5 min (**1**), 10.1 min (**2**),

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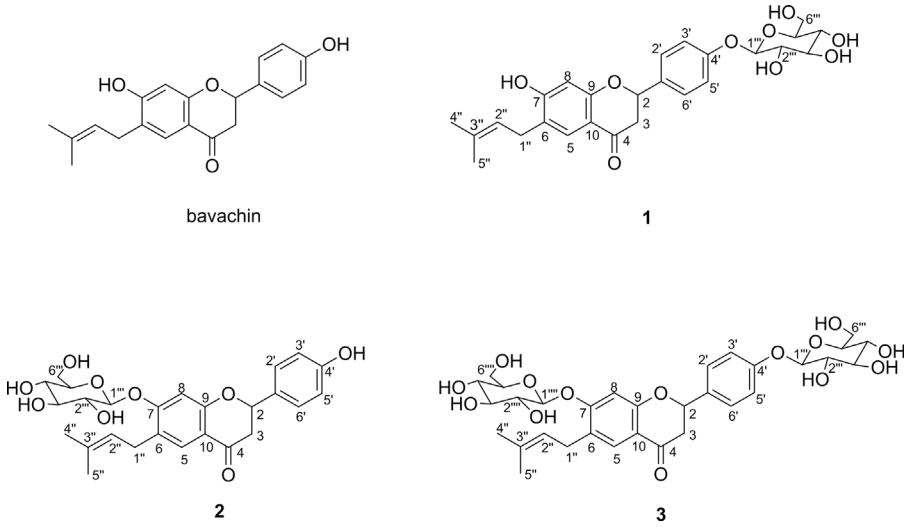

Fig. 1. Chemical structures of bavachin and compounds **1-3**.

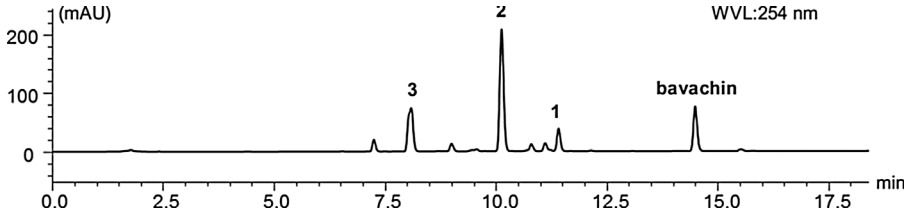

Fig. 2. HPLC analysis of *in vitro* glucosylation reaction mixture, monitored at 254 nm. 1, bavachin-4′-*O*-β-D-glucopyranoside; 2, bavachin-7-*O*-β-D-glucopyranoside; 3, bavachin-4′,7-di-*O*-β-D-glucopyranoside.

and 8.1 min (**3**) (Fig. 2). To further characterize the reaction products, a preparative-scale reaction was performed in a 50 ml reaction volume. The reaction products were purified by semi-prep HPLC to yield compounds **1**-**3**. The yields of compounds **1**-**3** were 1.1 mg, 6.3 mg, and 4.5 mg, respectively.

2.2. Identification of bavachin glucosides

Compound **1** was obtained as a pale yellow powder and its molecular formula $C_{26}H_{30}O_9$ was established by high resolution-electrospray ionization-mass spectrometry (HR-ESI–MS) at *m/z* 485.1814 $[M-H]^-$. The ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR) spectra of **1** were similar to those of the substrate, bavachin, except for the extra signals of a glucosyl moiety (Table 1). The ^{1}H- and ^{13}C NMR signals at δ_H 5.25 (1H, dd, J = 12.6, 5.4 Hz, H-2)/δ_C 79.0 (C-2), 3.06 (1H, dd, J = 16.2, 12.6 Hz, H-3α(ax)) and 2.63 (1H, dd, J = 16.2, 5.1 Hz, H-3β(eq))/δ_C 43.7 (C-3) indicated a flavanone skeleton. Signals of a prenyl group were also observed in the ^{1}H NMR spectrum at δ_H 1.70 (3H, s, H-5″), 1.64 (3H, s, H-4″), 5.25 (1H, t, J = 7.2 Hz, H-2″), and 3.14 (2H, d, J = 7.2 Hz, H-1″). The prenyl group was attached to C-6 on the basis of the HMBC correlations from the proton at δ_H 3.14 (2H, d, J = 7.2 Hz, H-1″) to δ_C 127.1 (C-5), 123.3 (C-6), and 161.8(C-7). The HMBC correlations from δ_H 4.87 (1H, d, J = 7.5 Hz, H-1‴) to δ_C 157.9 (C-4′) suggested that the glucosyl moiety was attached to C-4′ (Fig. 3). In addition, the glucosyl moiety was identified as a β conformation, based on the coupling constant of the anomeric proton at δ_H 4.87 (1H, d, J = 7.5 Hz, H-1‴). Therefore, compound **1** was identified as bavachin-4′-*O*-β-D-glucopyranoside, a novel compound (Fig. 1).

Compound **2** was obtained as a pale yellow powder and its molecular formula $C_{26}H_{30}O_9$ was deduced from HR-ESI–MS at *m/z* 485.1820 $[M-H]^-$. The ^{1}H- and ^{13}C NMR spectra of **2** (Table 1) resembled those of **1**, except for the location of the glucosyl moiety. The relative positions of the glucose moiety were confirmed by the HMBC correlations from δ_H 4.91 (1H, d, J = 7.2 Hz, H-1‴) to δ_C 161.9 (C-7) (Fig. 3). In addition, the glucose unit in **2** was identified as a β conformation, based on the coupling constant (J = 7.2 Hz) of the anomeric proton. Therefore, the structure of **2** was elucidated as bavachin-7-*O*-β-D-glucopyranoside, a new compound (Fig. 1).

Compound **3** was obtained as a yellow powder, with molecular formula $C_{32}H_{40}O_{14}$ as revealed by HR-ESI–MS at *m/z* 683.2106 $[M + Cl]^-$. The ^{1}H- and ^{13}C NMR spectra of **3** (Table 1) were similar to those of **1**, except for the sugar signals. The ^{1}H NMR spectral data of **3** revealed two anomeric H-atoms (δ_H 4.91 (J = 7.2), 4.88 (J = 7.2)) and corresponding C-atoms (δ_C 100.6, 100.7), evidencing the presence of two glucose moieties. The sugar linkage was determined by HMBC, which revealed a coupling between a carbon signal at δ_C 161.8 (C-7) and a proton signal at δ_H 4.91 (H-1‴), between a carbon signal at δ_C 158.0 (C-4′) and a proton signal at δ_H 4.88 (H-1⁗), showing that two glucose moieties were connected to C-7 and C-4′, respectively (Fig. 3). In addition, both sugars were identified as β-glucose on the basis of the coupling constant of the anomeric proton H-1‴(J = 7.2 Hz) and H-1⁗(J = 7.2 Hz). Therefore, compound **3** was elucidated to be a novel flavonoid glycoside, bavachin-4′,7-di-*O*-β-D-glucopyranoside (Fig. 1).

In this study, the two available reactive phenolic hydroxyl groups at C-7and C-4′ positions of bavachin have been successfully glucosylated using the UDP-glucosyltransferase YjiC. The results indicate that the UDP-glucosyltransferase YjiC have nonregioselectivity towards bavachin, which is consistent with the previous report (Pandey et al., 2014a, 2014b).

2.3. Effects of reaction conditions on the glucosylation of bavachin

The influences of time, concentration of UDP-Glc, and pH of Tris-Cl buffer were investigated to optimize the glucosylation reaction. The effects of variations in these reaction conditions on the products were analyzed by HPLC. First, the reaction time was varied (1, 3, 10, and 24 h), while other parameters were kept constant. HPLC analysis of the reaction mixture indicated that the concentrations of compounds **1** and **3** increased gradually with increasing time, with maximum concentrations being achieved at 24 h of incubation. In contrast, the concentration of compound **2** decreased gradually with increasing time (Fig. 4A). It is possible that compound **2** (bavachin-7-*O*-β-D-