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Brief report

Tiliroside-derivatives enhance GLUT4 translocation via AMPK in muscle cells

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ABSTRACT

Tiliroside isolated from Chinese herb *Potentilla chinensis* showed therapeutic activities in diabetes. We synthesized 7 tiliroside-derivatives and examined their effects on surface GLUT4*myc* levels in muscle cells. Derivatives 2a and 3 increased surface GLUT4*myc* levels, and derivative 3 has the greatest potential. AMPK may be involved in tiliroside-derivatives-regulated GLUT4*myc* traffic.

1. Introduction

Type 2 diabetes mellitus (T2D) is a metabolic disorder characterized with insulin resistance in skeletal muscle, liver, and fat tissues [1]. Skeletal muscles take approximately 80% of dietary glucose via glucose transporter GLUT4 [2]. Reduction in insulin-stimulated glucose-uptake in skeletal muscles is shown in T2D [3–5]. Chinese herb *Potentilla chinensis* has been reported lowering blood glucose level of diabetic mice, and tiliroside is the main effective constituent [6]. Trans-tiliroside from *Rosa canina* potently reduced blood glucose levels after glucose loading in mice [7]. However, the direct effect of tiliroside on muscle has not been studies yet. In skeletal muscle tissue, GLUT4 is the major insulin-responsive glucose transporter. However, in cultured muscle cells GLUT1 and perhaps other GLUTs contribute to substantial amounts of glucose uptake [8]. In order to focus on the role of GLUT4, we took advantage of our muscle cells lines that express GLUT4*myc* with an exofacial *myc*-eptiope. The regulation of these compounds on GLUT4 was investigated by detection of cell surface GLUT4*myc* levels with anti-*myc* antibody. This paper deals

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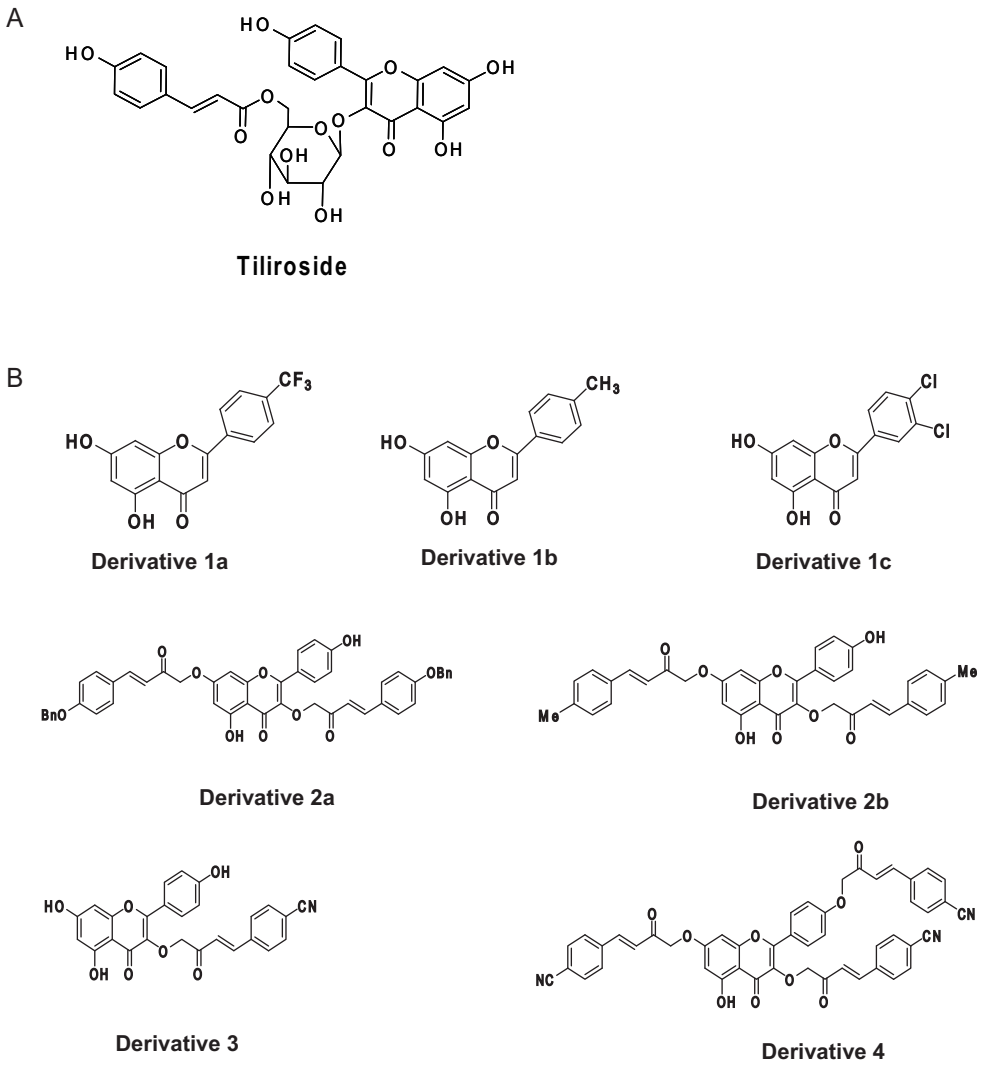

Fig. 1 – Chemical structures of tiliroside from Chinese herb Potentilla (A) and 7 tiliroside-derivatives (B).

with the effects of seven synthesized tiliroside-derivatives on GLUT4*myc* translocation in muscle cells.

2. Methods

2.1. *Synthesis of tiliroside derivatives*

Compounds **2a**, **2b**, **3** and **4** were synthesized through three steps. The first step was the Classin–Schimitt reaction from the substituted benzaldehyde to the α, β-unsaturated keto [9]. Then the α-hydrogen atom in this keto was substituted by bromine atom [10]. Finally, target compounds were prepared by the etherification reaction. Compound **1a** was synthesized in two steps starting from 2,4,6-trihydroxyacetophenone. Compounds **1b** and **1c** were synthesized from 2,4,6-trihydroxyacetophenone. After the peracylation of 2,4,6-trihydroxyacetophenone, the products were treated with KOH in pyridine at 50 °C to afford the intermediates, which were treated with 5% K_2CO_3 aq. at reflux obtaining **1b** and **1c**. The structures of all the compounds were determined by ^1H, ^{13}C NMR, and 2D NMR spectral data analysis, including COSY, HSQC, HMBC, and ROESY spectra.

2.2. *Cell culture and measurement of cell surface GLUT4myc density*

Myoblasts were cultured and differentiated into myotubes as described [8,11]. Cell surface GLUT4*myc* levels were measured by an antibody-coupled colorimetric assay [11,12].

2.3. *Cell lysates and immunoblotting*

Cells grown in 12-well plates were lysed with RIPA buffer (100 mM NaCl, 0.25% (w/v) sodium deoxycholate, 1.0% (w/v) NP40, 0.1% (w/v) SDS, 2 mM EDTA, 50 mM NaF, 10 nM okadaic acid, 1 mM sodium orthovanadate, protease inhibitor cocktail and 50 mM Tris–HCl, pH 7.2) on ice. Samples were electrophoresed on 7.5% SDS–PAGE. Immunoblots were developed with chemiluminescent reagent and autoradiographic film. Densitometric quantification of protein bands was performed using NIH Image J software.