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Antidiabetic effects of trihydroxychalcone derivatives via activation of AMP-activated protein kinase

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ABSTRACT

In this study, trihydroxychalcone derivatives were synthesized and evaluated for their potential antidiabetic effects in a cell-culture system and a diabetic mouse model in vivo. Some of them increased fatty acid oxidation (FAO) in C2C12 myotubes without affecting the cell viability. In addition, some trihydroxychalcone derivatives, including chalcone **13**, facilitated AMP-activated protein kinase (AMPK) activation. Chalcone **13** administration to high-fat diet (HFD)-induced diabetic mice improved glucose tolerance, increased muscle FAO, and reduced fat accumulation in the liver and skeletal muscles. These results suggest that some chalcone derivatives, particularly chalcone **13**, might be potential therapeutic agents for the treatment of diabetes.

Introduction

Diabetes mellitus (DM) is a major chronic metabolic disorder. Insulin resistance, characterized by impaired insulin-dependent glucose uptake by the muscles and adipose tissues, usually precedes the development of type 2 DM. It is well known that obesity may induce insulin resistance; in addition, high skeletal muscle triglyceride levels are associated with insulin resistance [1–3]. As fatty acid oxidation (FAO) predominantly occurs in the skeletal muscles, the increase in energy expenditure owing to FAO in the skeletal muscles can be an effective way to reduce insulin resistance and treat diabetes. In fact, several studies have shown that the increase in FAO in skeletal muscles could reduce obesity-induced insulin resistance [4–6].

AMP-activated protein kinase (AMPK) is a master regulator of whole-body energy metabolism. AMPK is converted to the active form via phosphorylation at Thr172 in the α subunit when cellular energy levels are low. As AMPK activation improves insulin sensitivity and glucose homeostasis, AMPK is considered a promising target for the treatment of type 2 diabetes [7–9]. The active form of AMPK then phosphorylates and inactivates acetyl-CoA carboxylase (ACC) in the skeletal muscles. Because ACC facilitates the synthesis of malonyl CoA, an inhibitor of carnitine palmitoyltransferase 1 (CPT1), which is the major regulator of FAO, activation of AMPK finally results in an increase in FAO [10,11]. Metformin, a widely used drug for the treatment of diabetes, activates AMPK indirectly by increasing the AMP/ATP ratio [12,13].

Although AMPK is considered an important therapeutic target, AMPK agonists are not clinically used for the treatment of diabetes. 5-Aminoimidazole-4-carboxamide riboside (AICAR), a well-known AMPK activator, is phosphorylated and converted to AICAR monophosphate (ZMP) by adenosine kinase in the cells. ZMP is an AMP analog, which binds to AMPKγ subunit and stimulates the phosphorylation of Thr172 in the AMPK α subunit [14]. However, AICAR is not clinically used because of its short half-life and AMPK-independent effects [15,16]. Several AMPK agonists, which activate AMPK by directly binding to it, have been identified [17,18]; however, none has been used for the treatment of diabetes

Abbreviations: FAO, fatty acid oxidation; AMPK, AMP-activated protein kinase; AICAR, 5-aminoimidazole-4-carboxamide riboside; CPT1, carnitine palmitoyltransferase 1; ZMP, AICAR monophosphate; PTP1B, protein tyrosine phosphatase 1B; PPARγ, peroxisome proliferator-activated receptor gamma; DMC, 2′,4′-dihydroxy-6′-methoxy-3′,5′-dimethylchalcone; HFD, high-fat diet; mp, melting points; DMSO, dimethyl sulfoxide; TMS, tetramethylsilane; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; GM, *gastrocnemius* muscle; IP-GTT, intraperitoneal glucose tolerance test; FFA, free fatty acid; WAT, white adipose tissues.

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yet. Therefore, development of an AMPK agonist with a potential clinical use for the treatment of diabetes is urgently required.

Hydroxychalcone derivatives, fully conjugated aromatic ketones with a chalcone (1,3-diphenyl-2*E*-propenone) skeleton, have been shown to possess diverse biological activities, including anticancer, anti-inflammatory, and antioxidant activities [19–22]. In addition, they exhibit antidiabetic effects via inhibition of protein tyrosine phosphatase 1B (PTP1B) and α-glucosidase, as well as activation of peroxisome proliferator-activated receptor gamma (PPARγ) [23–25]. In a previous study, we showed that 2′,4′-dihydroxy-6′-methoxy-3′,5′-dimethylchalcone (DMC) isolated from *Cleistocalyx operculatus*, a plant widely distributed in Southern Asia, increased FAO via activation of AMPK in C2C12 myotubes and skeletal muscles. In addition, we demonstrated that DMC exhibited antidiabetic effects in vivo, where its administration for several weeks improved glucose tolerance in high-fat diet (HFD)-induced obese mice [26].

Although several trihydroxychalcone derivatives were shown to exhibit antiplatelet and anticytotoxic activities, their antidiabetic effects have not yet been thoroughly explored [27,28]. Therefore, it was necessary to prepare and characterize a library of structurally simplified and systematically designed DMC analogs to determine the structure-activity relationship and identify a promising antidiabetic drug candidate. In this study, we synthesized various trihydroxychalcone derivatives and evaluated their antidiabetic effects in a cell culture system and a diabetic mouse model in vivo. The effects of these derivatives on FAO in C2C12 myotubes and glucose tolerance in HFD-induced diabetic mice were investigated.

Materials and methods

Materials

2′,4′,6′-Trihydroxyacetophenone and 4′-hydroxybenzaldehyde were purchased from Alfa-Aesar. Benzaldehyde (**3**), 4-methoxybenzaldehyde (**5**), dimethyl sulfate, and chloromethyl methyl ether were purchased from Sigma-Aldrich Co. Ltd. (St. Louis, MO, USA). 2′-Hydroxy-4′,6′-di(methoxymethoxy)acetophenone (**1**) and 2′-hydroxy-4′,6′-dimethoxyacetophenone (**2**) were prepared by the reaction of 2′,4′,6′-trihydroxyacetophenone with chloromethyl methyl ether or dimethyl sulfate in the presence of K_2CO_3 in acetone at 50 °C, as previously described with a modification (Fig. 1) [29,30]. 4′-(Methoxymethoxy)benzaldehyde (**4**) was prepared, as previously described [31]. Chalcone derivatives **6–9**, **11**, and **12** were synthesized by Claisen–Schmidt condensation of the corresponding acetophenones **1** and **2** with benzaldehydes **3–5**, followed by appropriate deprotection, if necessary. AICAR was purchased from Sigma-Aldrich. DMC was isolated from *C. operculatus,* as previously described [32]. Antibodies against pAMPKα (T172) and AMPKα were purchased from Cell Signaling Technology (Danvers, MA, USA), whereas that against γ-tubulin was obtained from Sigma-Aldrich.

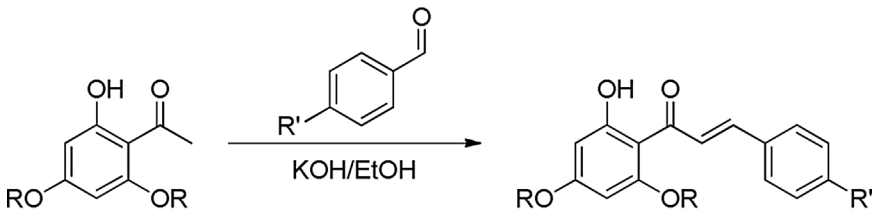
Apparatus

All reactions were carried out under an inert atmosphere of N_2. Analytical thin-layer chromatography (TLC) was performed using Merck Kieselgel 60 F254 pre-coated plates (0.25 mm) with a fluorescent indicator and visualized with UV light (254 and 365 nm) or by iodine vapor staining. Column chromatography was performed on silica gel (mesh, 70–230; 60 Å; BET surface area, ~500 m^2/g; pore volume, 0.75 cm^3/g). Gas chromatography (GC) analysis was performed using a Hewlett Packard gas chromatograph (HP-6890) equipped with a flame ionization detector to monitor the reaction progress. Melting points (mp) were measured by using a Bamstead Electrothermal (IA9100) apparatus and were uncorrected. The infrared spectra were recorded using Jasco FT/IR-4700 Fourier-transform infrared (FT-IR) spectrometer at wavelengths of 400–4000 cm^{-1} using KBr pellets. The absorption spectra were recorded using Jasco V-670 UV–vis spectrometer in the range of 200–900 nm. ^{1}H nuclear magnetic resonance (^{1}H NMR, 600 MHz) and ^{13}C NMR (150 MHz) spectra were recorded using dimethyl sulfoxide (DMSO)-d_6 as a solvent and tetramethylsilane (TMS) as an internal standard. Chemical shifts were expressed in δ units (ppm) by assigning the TMS resonance in the ^{1}H spectrum as 0 ppm and the DMSO-d_6 resonance in the ^{13}C spectrum as 39.5 ppm. All coupling constants, *J*, were expressed in hertz (Hz).

General procedure for preparation of chalcone derivatives 6–9, 11, and 12

Ethanol (200 mL) was added to acetophenone, benzaldehyde, and KOH (0.224 g, 4 mmol) in a round-bottomed flask under nitrogen atmosphere. After stirring for 72 h at room temperature, the reaction mixture was quenched with 1% aqueous HCl. The organic solution was extracted with EtOAc, washed with water and saturated aqueous NaCl, dried over $MgSO_4$, and concentrated under vacuum. The crude product was purified by recrystallization from ethanol to yield the corresponding chalcones.

2′-Hydroxy-4′,6′-di(methoxymethoxy)chalcone (**6**) was prepared by the reaction of **1** (1 g, 3.9 mmol) with **3** (0.44 mL, 4.3 mmol). The



1: R = MOM
2: R = CH_3

3: R' = H
4: R' = OMOM
5: R' = OCH_3

6: R = MOM, R' = H
7: R = MOM, R' = OMOM
8: R = MOM, R' = OCH_3
9: R = CH_3, R' = H
11: R = CH_3, R' = OMOM
12: R = CH_3, R' = OCH_3

Fig. 1. Synthesis of trihydroxychalcone derivatives **6 9**, **11**, and **12**.
Reactions of acetophenones with benzaldehydes were carried out in EtOH using KOH as a base at room temperature.