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# Preparation of tyrosinase inhibitors and antibrowning agents using green technology

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ABSTRACT

Chalcones and their derivatives have attracted great interests in recent years for their comprehensive biological activities. In this study, 2,4,2′,4′-tetrahydroxychalcone and its two derivatives, 1,3,5-tris-(2,4-dihydroxy-phenyl)pentane-1,5-dione (new compound) and 7,2′,4′-trihydroxyflavanone, were synthesized through one-pot green procedure catalyzed by boric acid in polyethylene glycol 400. Their structures were identified by ESI-MS and NMR spectral. Tyrosinase inhibitory activity and antibrowning test results showed that compounds **1**–**3** exhibited strong tyrosinase inhibitory activities and significant antibrowning effects on the fresh-cut lotus root slices at room temperature in 48 h. Among them, 0.01% 1,3,5-tris-(2,4-dihydroxy-phenyl)pentane-1,5-dione combined with 0.5% $V_C$ showed the best antibrowning ability. In brief, this study offers a protocol for one-pot green synthesis of high efficiency tyrosinase inhibitors which may be suitable as antibrowning agents for fresh-cut vegetables. More important, this study developed a new type of 1,5-dione derivative which may serve as new lead structures for novel tyrosinase inhibitors discovery.



## 1. Introduction

Browning is one of important factors which causes the loss of postharvest fruits and vegetables. Enzymatic browning caused by tyrosinase (EC 1.14.18.1) in fruits and vegetables during the commercial or domestic processing, handling and storage of foods not only impairs the colour attribute and sensory properties of these food products, but also leads to destroy essential amino acids, lower nutritional quality and impair digestibility, and eventually results in a significant decrease in nutritional and market values (Artés, Castañer, & Gil, 1998). Application of tyrosinase inhibitors is one of the common methods used to control enzymatic browning. Tyrosinase inhibitors get a lot of attention due to its capable to inhibit the activity of tyrosinase and possess the great potential market profits and enormous application prospects.

As one of the major classes of flavonoids, chalcones are both biosynthetic precursors of flavonoids and end products. Their derivatives have become of much interest in recent years because of a variety of biological activities such as antityrosinase (Khatib et al., 2004), antimicrobial (Ritter, Martins, Dias, & Pereira, 2014), anticancer (Jandial et al., 2014), antimalarial (Sulistyowaty, Nofianti, Suzana, & Budiati, 2014), anti-inflammatory (Yasuda et al., 2014) and antioxidants (El Sayed Aly, El Razek, Fodah, & Saleh, 2014). Chalcones are abundant in fruits, vegetables, spices, tea and soy based food stuff, and traditional herbal medicine. The main method for the synthesis of chalcones involves Clasein-Schmidt condensation between arylaldehydes and acetophenone in the presence of alkali metal hydroxides (Prasad, Rao, Rambabu, & Kumar, 2007). Over the past decade, many synthetic strategies have been developed to efficiently prepare chalcone derivatives, such as palladium-catalyzed Sonogashira coupling between aryl halides and propargyl alcohols (Müller, Ansorge, & Aktah, 2000), carbonylative Heck coupling (Hermange, Gøgsig, Lindhardt, Taaning, & Skrydstrup, 2011; Wu, Neumann, & Beller, 2010), and cross-coupling of ketones with arenes or aryl carboxylic acids (Shang et al., 2013; Wei, Tang, Cong, & Zeng, 2013). However, many

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of these methods suffered from the drawbacks such as harsh reaction conditions, toxic reagents and metal catalysts, strong acidic/basic conditions, prolonged reaction time, poor yields and low selectivity, oily products, and tedious extraction procedures. On the other hand, in most cases, the synthesis of polyhydroxy chalcones require protection of the hydroxyl groups and deprotection of the protective groups, this usually involves two or more than two additional steps and is inevitable to cost more time, increasing the use of toxic or hazardous reagents or catalysts. Several green modification methods have been developed to counter these problems. Kulkarni, Swami, and Zubaidha (2013) used calcium hydroxide as catalyst for synthesis of polyhydroxy chalcones. Sreedhar, Jayapal, Prasad, and Prasad (2010) applied PEG-400 as a recyclable solvent for synthesis of 4-hydroxy chalcones. Guan, Yin, Quan, and Quan (2004) used ethyl glycol as solution and boric acids as the catalyst for synthesis of hydroxylated chalcones and related derivatives. Tanemura, Suzuki, Nishida, and Horaguchiy (2005) synthesized high yields (40–98%) of 4-chloro and 4-meth oxychalcones by aldol condensation of ketones with aromatic aldehydes in water in the presence of polyethylene glycol 400 and sodium hydroxide. Sarda et al. (2009) developed a novel method for the synthesis of chalcone derivatives (yield: 80–92%) via Claisen–Schmidt is introduced using NaOH-$Al_2O_3$ under solvent free conditions. Kumar, Lamba, and Makrandi (2008) developed an efficient green procedure for the synthesis of 2′-hydroxychalcones using anhydrous barium hydroxide (C-200) as catalyst under grinding conditions. However, some of these green synthesis methods still suffered from poor yields, oily products, and tedious extraction procedures. More important, most of these synthetic methods were not used to synthesize polyhydroxy chalcones, whether they can be used for the synthesis of polyhydroxy chalcones needs more studies to confirm. Therefore, there is still a need to develop new methods for the synthesis of polyhydroxy chalcones by eco-friendly approaches, in short time, using inexpensive catalysts, and involving easy isolation procedures.

The aim of this study is to develop one-pot green procedure for the synthesis of 2,4,2′,4′-tetrahydroxychalcone and its derivatives by using an inexpensive, safe, simple, and eco-friendly catalyst, i.e. boric acid. Their tyrosinase inhibitory activities and antibrowning on fresh-cut lotus root slices were also investigated to examine their potential for practical application as antibrowning agents, especially for fresh-cut lotus root slices.

## 2. Materials and methods

### 2.1. Chemicals

2,4-Dihydroxybenzaldehyde and 2,4-dihydroxyacetophnone were purchased from Shanghai Darui Company (Shanghai, PR China). Mushroom tyrosinase (5771 units/mg), L-tyrosine, methanol-$d_6$ ($CD_3OD$), kojic acid, and 4-hexylresorcinol (4-HR) were purchased from Sigma Chemical Co (St. Louis, USA). Polyethylene glycol 400 (PEG 400), dimethyl sulfoxide (DMSO), ethanol (EtOH), methanol (MeOH), sodium dihydrogen orthophosphate ($NaH_2PO_4{\cdot}2H_2O$), anhydrous di-sodium hydrogen phosphate ($Na_2HPO_4$), boric acid, ascorbic acid ($V_C$), vitamin E, vitamin B1, calcium chloride ($CaCl_2$), and dichloromethane ($CH_2Cl_2$) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Suzhou, PR China). HPLC grade solvents were purchased from J&K Scientific (New Jersey, USA). Silica gel (200–300 mesh) for column chromatography and TLC plates (HSGF254) were purchased from Yantai Jiangyou Silicone Development Co., Ltd. (Yantai, PR China). Sephadex LH-20 was purchased from GE Healthcare Bio-Sciences AB (Uppsala, Sweden).

### 2.2. Materials

Lotus roots were purchased from a local supermarket at Wuxi, PR China. The Lotus roots were selected for uniformity of size and ground colour, no defects and mechanical damages. Before treatment, the lotus roots were stored in refrigerator at 4 °C.

### 2.3. General procedure for the synthesis of 2,4,2′,4′-tetrahydroxychalcone and its derivatives

The mixture of 2,4-dihydroxybenzaldehyde (0.036 mol), 2,4-dihydroxy-acetophnone (0.018 mol), and boric acid (0.025 mol) were dissolved in PEG-400 and then were stirred to react for 6 h at 130 °C. After the completion of reaction, the reaction mixture was extracted with ethyl acetate for three times. The collected organic layers were combined and concentrated in vacuum, and the residue was subjected to column chromatography on silica gel using dichloromethane-methanol (20:1) as eluent. The products were further purified by preparative HPLC (Waters 2545 equipped with a Waters 2767 sample manager and a waters 2487 UV detector, column YMC-Pack ODS-A C18 column, 5 μm, 20 × 250 mm) to give compounds **1** (Rt = 18.35 min, yield: 7.95%), **2** (Rt = 16.97 min, yield: 35.35%), and **3** (Rt = 12.50 min, yield: 8.02%). The gradient elution was as follows: initially, 40% B; 0–2 min, 50% B; 2–25 min, 80% B; 25–27 min, 100% B; 27–29 min, 100% B; 29–31 min, 40% B. The sample injection volume was 600 μL. The UV detector was set at 254 nm and 369 nm, and flow rate was set at 10 mL/min.

#### 2.3.1. 1,3,5-Tris-(2,4-dihydroxy-phenyl)pentane-1,5-dione (**1**):

Yellow powder; UV (MeOH) 230, 277, 313 nm; IR (KBr) $\nu_{max}$ 3407, 1631, 1513, 1445, 1225 $cm^{-1}$; $^1H$ NMR (Acetone-$d_6$, 400 MHz) $\delta$: 12.764 (2H, s, OH-2, 2′), 9.457 (2H, s, OH-4, 4′), 8.417 (1H, s, OH-2″), 8.030 (1H, s, OH-4″), 7.927 (2H, d, $J$ = 8.8 Hz, H-6, 6′), 7.014 (1H, d, $J$ = 8.0 Hz, H-6″), 6.416 (2H, dd, $J$ = 8.8, 2.4 Hz, H-5, 5′), 6.362 (1H, d, $J$ = 2.4 Hz, H-3″), 6.285 (2H, d, $J$ = 2.4 Hz, H-3, 3′), 6.236 (1H, dd, $J$ = 2.4 Hz, H-5″), 4.198 (1H, m, H-9), 3.515 (2H, dd, $J$ = 16.0, 7.2 Hz, H-8, 8′), 3.353 (2H, dd, $J$ = 16.0, 6.8 Hz, H-8, 8′); $^{13}C$ NMR (Acetone-$d_6$, 100 MHz) $\delta$: 205.125 (C=O, C-7, 7′), 166.425 (C, C-2, 2′), 165.549 (C, C-4, 4′), 157.861 (C, C-4″), 156.623 (C, C-2″), 133.899 (CH, C-6, 6′), 130.178 (CH, C-6″), 121.496 (C, C-1″), 114.372 (C, C-1, 1′), 108.757 (CH, C-5, 5′), 107.612 (CH, C-5″), 103.965 (CH, C-3″), 103.628 (CH, C-3, 3′), 43.227 ($CH_2$, C-8, 8′), 33.856 (CH, C-9). ESI-MS: $m/z$ 423.1 $[M\text{-}H]^-$ ($C_{23}H_{19}O_8$). HR-ESI-MS: $m/z$ 423.1068 $[M\text{-}H]^-$ (calcd for $C_{23}H_{19}O_8$, 423.1080).

#### 2.3.2. 2,4,2′,4′-tetrahydroxychalcone (**2**):

Yellow powder; $^1H$ NMR (Acetone-$d_6$, 400 MHz) $\delta$: 13.788 (1H, s, OH-2′), 9.533 (1H, s, OH-4), 9.317 (1H, s, OH-2), 9.004 (1H, s, OH-4′), 8.222 (1H, d, $J$ = 15.2 Hz, H-β), 8.023 (1H, d, $J$ = 9.2 Hz, H-6), 7.792 (1H, d, $J$ = 15.2 Hz, H-α), 7.691 (1H, d, $J$ = 8.4 Hz, H-6′), 6.519 (1H, d, $J$ = 2.0 Hz, H-3), 6.462 (1H, dd, $J$ = 9.2, 2.4 Hz, H-5), 6.457 (1H, dd, $J$ = 7.6, 2.0 Hz, H-5′), 6.358 (1H, d, $J$ = 2.4 Hz, H-3′); $^{13}C$ NMR (Acetone-$d_6$,100 MHz) $\delta$: 193.379 (C=O), 167.610 (C, C-4′), 165.440 (C, C-2′), 162.444 (C, C-4), 160.090 (C, C-2), 141.164 (CH, C-β), 133.023 (CH, C-6), 131.875 (CH, C-6′), 117.525 (CH, C-α), 115.362 (C, C-1′), 114.740 (C, C-1), 109.303 (CH, C-5), 108.672 (CH, C-5′), 103.892 (CH, C-3′), 103.783 (CH, C-3). ESI-MS: $m/z$ 271.0 $[M\text{-}H]^-$ ($C_{15}H_{11}O_5$).

#### 2.3.3. 7,2′,4′-Trihydroxyflavanone (**3**):

Pale yellow powder; $^1H$ NMR (Acetone-$d_6$, 400 MHz) δ: 9.465 (1H, br s, OH-7), 8.648 (1H, s, OH-4′), 8.413 (1H, br s, OH-2′), 7.738