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Novel hydroxypyridinone derivatives containing an oxime ether moiety: Synthesis, inhibition on mushroom tyrosinase and application in anti-browning of fresh-cut apples

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ABSTRACT

A range of hydroxypyridinone derivatives were synthesized starting from kojic acid. Among them, **10** and **11** were found to possess the strongest inhibitory effect on monophenolase activity of mushroom tyrosinase, having IC_{50} values of 2.04 and 1.60 μM, respectively. The IC_{50} values of **10** and **11** for the inhibition of diphenolase activity of mushroom tyrosinase were determined as 13.89 and 7.99 μM, respectively. Investigation of the inhibitory mechanism of these two compounds indicated that the inhibition was reversible and of a competitive-uncompetitive mixed type. The K_I and K_{IS} values of **10** were determined to be 24.84 and 32.54 μM, respectively, and the corresponding values for **11** being 18.07 and 21.34 μM, respectively. The effect of **11** on the browning process of fresh-cut apples was evaluated by measuring the color change and browning index. The results indicated that **11** had a significant effect on controlling the browning of fresh-cut apple slices.

1. Introduction

With increased fast-pace lifestyles of modern time, fresh-cut apples are popular due to the advantages of freshness, convenience and nutrition (Allende, Tomás-Barberán, & Gil, 2006; Chen, Xing, Wang, Zheng, & Wang, 2015). However, the shelf-life of fresh-cut products is greatly shortened as compared with intact fruits and vegetables, because fresh-cut leads to tissue wounding (Chung & Moon, 2009). Among the negative consequences of wounding in fresh-cut products, enzymatic browning is considered to be one of the main limitations on the shelf-life of fresh-cut products (Brecht, 1995). It is estimated that over 50% loss of fruit and vegetables is due to enzymatic browning (Mosneaguta, Alvarez, & Barringer, 2012). Enzymatic browning of fresh-cut produce which appears along the cutting side is known to be mainly caused by polyphenol oxidase (PPO) and peroxidase (POD) catalyzed oxidation (Tomás-Barberán & Espín, 2001).

Tyrosinase (EC 1.14.18.1), also known as PPO, is a copper-containing monooxygenase that is widely distributed in microorganisms, animals, and plants (Hamidian et al., 2013). Six histidine residues, located on a four helical bundle, coordinate two copper ions, which serve as the major cofactors in the active site (Zhao et al., 2016). As a key enzyme of melanogenesis, tyrosinase catalyzes the hydroxylation of L-tyrosinase (L-Tyr) to L-dihydroxyphenylalanine (L-DOPA) (monophenolase activity) and further the oxidation of L-DOPA to DOPA quinone (diphenolase activity). It is responsible for the undesirable enzymatic browning of fruits and vegetables that takes place during senescence or damage at the time of post-harvest handling (Liu, Shu, Liu, Huang, & Peng, 2016). On the one hand, the phenolic compounds in the food stuff are oxidized by PPO to form brown pigments, which can cause the browning of fruits and vegetables (Mayer, 2006). On the other hand, the oxidation products of phenolic compounds can react with other food components, including amines, peptides and proteins (Chen et al., 2015), which further reduces the food quality. Up to now, numerous tyrosinase inhibitors identified from both natural and synthetic origins have been reported (Chang, 2009; Kim & Uyama, 2005), but only a few are sufficiently potent for practical use due to poor inhibitory activity, solubility, instability, and safety concerns (Zheng, Zhang, Zhang, & Chen, 2016). Therefore, searching for novel and safe powerful tyrosinase inhibitors remains a challenge to the food and pharmaceutical industries.

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Kojic acid (**1**), 5-hydroxy-2-(hydroxymethyl)-4*H*-pyran-4-one, a metabolic product of many species of *Aspergillus* and *Penicillium* moulds., has been used as an anti-browning agent in foods that rapidly change color (Li et al., 2013). It was reported that application of kojic acid in the pre-storage of litchi fruit can delay pericarp browning and maintain the activities of antioxidative enzymes (Shah, Khan, & Ali, 2017). Application of kojic acid in combination with 4-hexylresocrinol and L-cysteine effectively reduced the browning in "Amasya" apple juice (İyidoğan & Bayındırlı, 2004). As kojic acid can inhibit tyrosinase by chelating the copper ion in the active site of tyrosinase, modification of kojic acid provides a potential route for superior tyrosinase inhibitors (Kwak, Choi, Park, & Lee, 2011; Lee, Park, Kim, Seo, & Kim, 2006; Noh et al., 2009). Hydroxypyridinones, are closely related to kojic acid, and have several potential applications in the medicine area. Hydroxypyridinones bind copper with superior affinity to that of kojic acid, thus they can in principle inhibit tyrosinase activity by binding copper ions coordinated in the active site of the enzyme. Indeed, in our previous work some hydroxypyridinones were demonstrated to possess stronger anti-tyrosinase activity than kojic acid (Li et al., 2013; Zhao et al., 2016). Hydroxypyridinone derivatives have also been demonstrated to have potential applications in the preservation of shrimp and surimi product (Chen et al., 2016; Dai, Zhang, Wei, Hider, & Zhou, 2016; Xu et al., 2014). In order to search for stronger tyrosinase inhibitors, in this study, a series of novel hydroxypyridinone derivatives containing an oxime ether moiety were synthesized. Their inhibitory effects on mushroom tyrosinase and on the browning process of fresh-cut apples were also investigated.

2. Materials and methods

2.1. Reagents

Tyrosinase from mushroom (lyophilized powder, ≥1000 unit/mg solid) was purchased from Sigma. Kojic acid, L-tyrosinase (L-Tyr) and L-3,4-dihydroxyphenylalanine (L-DOPA) were the products of Aladdin (Shanghai, China). All other reagents were of analytical grade.

2.2. General Synthesis

The products were identified by NMR, ESI-MS and HRMS. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance 500 spectrometer (Bruker Corp., Germany) with tetramethylsilane as an internal standard. Electrospray ionization (ESI) mass spectra were obtained by infusing samples into an LCQ Deca XP ion-trap instrument (ThermoFinnigan, San Jose, CA). High-resolution mass spectra (HRMS) were obtained on a QTOFMicro (Waters, U.S.) by direct infusing samples into the ESI source.

*Synthesis of 5-(benzyloxy)-2-(hydroxymethyl)pyridin-4(1H)-one (**3a**).* Compound **2** (10.0 g) was dissolved in ethanol (17 mL) and the mixture was heated to reflux, then ammonia solution (25–28%, 83 mL) was added. The reaction mixture was refluxed overnight until the reaction was complete. After the reaction mixture was cooled to room temperature, the precipitate was collected by filtration, washed twice with a small amount of diethyl ether and dried, providing the product compound **3a** as a white solid.

Compounds **2** and **3b–3f** were prepared according to our reported method (Li et al., 2013).

*General procedure for the synthesis of **4**.* Compound **3** (4 mmol) was dissolved in CH_2Cl_2 (20 mL) and activated manganese (IV) oxide (32 mmol) was added. The reaction mixture was stirred vigorously for 3 days at room 50 °C and monitored by TLC. After completion of the reaction, the mixture was filtered to remove the activated manganese (IV) oxide powder, and the filtrate was dried under vacuum to give the product **4**.

*5-(Benzyloxy)-4-oxo-1,4-dihydropyridine-2-carbaldehyde (**4a**):* Yield 61%. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 5.31 (s, 2H, CH_2), 7.34–7.49(m, 6H, Ph and C3-H in pyridinone ring), 8.42 (s, 1H, C6-H in pyridinone ring), 9.25 (s, 1H, CHO). ESI-MS: *m/z* 230 (MH^+).

*5-(Benzyloxy)-1-methyl-4-oxo-1,4-dihydropyridine-2-carbaldehyde (**4b**):* Yield 72%. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 2.49 (s, 3H, CH_3), 5.28 (s, 2H, CH_2), 7.00 (s, 2H, C3-H and C6-H in pyridinone), 7.29–7.45 (m, 5H, Ph), 9.75 (s, 1H, CHO). ESI-MS: *m/z* 244 (MH^+).

*5-(Benzyloxy)-1-ethyl-4-oxo-1,4-dihydropyridine-2-carbaldehyde (**4c**):* Yield 74%. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 1.27 (t, J = 7.0 Hz, 3H, CH_3), 4.24 (q, J = 7.0 Hz, 2H, CH_2), 5.25 (s, 2H, CH_2), 7.00 (s, 2H, C3-H and C6-H in pyridinone), 7.29–7.40 (m, 5H, Ph), 9.59 (s, 1H, CHO). ESI-MS: *m/z* 258 (MH^+).

*5-(Benzyloxy)-1-butyl-4-oxo-1,4-dihydropyridine-2-carbaldehyde (**4d**):* Yield 76%. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 0.86 (t, J = 7.5 Hz, 3H, CH_3), 1.13–1.21 (m, 2H, CH_2), 1.51–1.57 (m, 2H, CH_2), 4.18 (t, J = 7.5 Hz, 2H, CH_2), 5.25 (s, 2H, CH_2), 6.96 (1H, C3-H in pyridinone), 6.97 (s, 1H, C6-H in pyridinone), 7.30–7.40 (m, 5H, Ph), 9.58 (s, 1H, CHO). ESI-MS: *m/z* 286 (MH^+).

*5-(Benzyloxy)-1-hexyl-4-oxo-1,4-dihydropyridine-2-carbaldehyde (**4e**):* Yield 76%. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 0.87 (t, J = 7.0 Hz, 3H, CH_3), 1.23 (m, 6H, CH_2),1.56 (m, 2H, CH_2), 4.17 (t, J = 7.0 Hz, 2H, CH_2), 5.26 (s, 2H, CH_2), 6.97(s, 1H, C3-H in pyridinone ring), 6.99 (s, 1H, C6-H in pyridinone ring), 7.30–7.41(m, 5H, Ph), 9.58 (s, 1H, CHO). ESI-MS: *m/z* 314 (MH^+).

*5-(Benzyloxy)-1-octyl-4-oxo-1,4-dihydropyridine-2-carbaldehyde (**4f**):* Yield 78%. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 0.88 (t, J = 7.0 Hz, 3H, CH_3), 1.17–1.28 (m, 10H, $5CH_2$), 1.56 (m, 2H, CH_2), 4.17 (t, J = 7.0 Hz, 2H, CH_2), 5.26 (s, 2H, CH_2), 6.97 (s, 1H, C3-H in pyridinone), 6.98 (s, 1H, C6-H in pyridinone), 7.30–7.41 (m, 5H, Ph), 9.58 (s, 1H, CHO). ESI-MS: *m/z* 342 (MH^+).

*General Procedure for the Synthesis of **5**.* Compound **4** (2 mmol) was dissolved in EtOH/H_2O (10 mL/10 mL) and ethoxyamine hydrochloride (3 mmol) was added. The reaction mixture was stirred until all the solid was dissolved under ice bath. The ice bath was removed after NaOH solution (0.22 g in 10 mL of H_2O) was added dropwise, and the reaction mixture was stirred for 2 h at room temperature. After completion of the reaction, the mixture was concentrated to about half volume, and extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layers were concentrated, and the resulting residue was purified by silica gel column chromatography (CH_2Cl_2/CH_3OH, 15:1) to provide the product **5**.

*5-(Benzyloxy)-4-oxo-1,4-dihydropyridine-2-carbaldehyde O-ethyl oxime (**5a**):* Yield 71%. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 1.24 (t, J = 7.0 Hz, 3H, CH_3), 4.16 (q, J = 7.0 Hz, 2H, CH_2), 5.14 (s, 2H, CH_2), 7.31–7.46(m, 5H, Ph), 7.97 (s, 1H, CH). ESI-MS: *m/z* 273 (MH^+).

*5-(Benzyloxy)-1-methyl-4-oxo-1,4-dihydropyridine-2-carbaldehyde O-ethyl oxime (**5b**):* Yield 64%. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.31 (t, J = 7.0 Hz, 3H, CH_3), 3.68 (s, 3H, CH_3), 4.24 (q, J = 7.0 Hz, 2H, CH_2), 5.20 (s, 2H, CH_2), 6.66 (s, 1H, C3-H in pyridinone ring), 6.93 (s, 1H, C6-H in pyridinone ring), 7.29–7.42 (m, 5H, Ph), 7.91 (s, 1H, CH). ESI-MS: *m/z* 287 (MH^+).

*5-(Benzyloxy)-1-ethyl-4-oxo-1,4-dihydropyridine-2-carbaldehyde O-ethyl oxime (**5c**):* Yield 61%. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.26 (t, J = 7.0 Hz, 3H, CH_3), 1.30 (t, J = 7.0 Hz, 3H, CH_3), 4.00 (q, J = 7.0 Hz, 2H, CH_2), 4.23 (q, J = 7.0 Hz, 2H, CH_2), 5.19 (s, 2H, CH_2), 6.65 (s, 1H, C3-H in pyridinone ring), 6.93 (s, 1H, C6-H in pyridinone ring), 7.27–7.41 (m, 5H, Ph), 7.90 (s, 1H, CH). ESI-MS: *m/z* 301 (MH^+).

*5-(Benzyloxy)-1-butyl-4-oxo-1,4-dihydropyridine-2-carbaldehyde O-ethyl oxime (**5d**):* Yield 71%. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.87 (t, J = 7.5 Hz, 3H, CH_3), 1.12–1.22 (m, 2H, CH_2), 1.31 (t, J = 7.0 Hz, 3H, CH_3), 1.50–1.61 (m, 2H, CH_2), 3.93 (t, J = 7.5 Hz, 2H, CH_2), 4.23 (q, J = 7.0 Hz, 2H, CH_2), 5.21 (s, 2H, CH_2), 6.68 (s, 1H, C3-H in pyridinone ring), 6.89 (s, 1H, C6-H in pyridinone ring), 7.27–7.41 (m, 5H, Ph), 7.90 (s, 1H, CH). ESI-MS: *m/z* 329 (MH^+).

*5-(Benzyloxy)-1-hexyl-4-oxo-1,4-dihydropyridine-2-carbaldehyde O-ethyl oxime (**5e**):* Yield 63%. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.87 (t, J = 7.5 Hz, 3H, CH_3), 1.10–1.27 (m, 6H, $3CH_2$), 1.31 (t, J = 7.0 Hz,