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Biological evaluation of coumarin derivatives as mushroom tyrosinase inhibitors

Jinbing Liu [a,*], Fengyan Wu [a], Lingjuan Chen [b], Liangzhong Zhao [a], Zibing Zhao [a], Min Wang [a], Sulan Lei [a]

[a] Department of Biology and Chemical Engineering, Shaoyang University, Shao Shui Xi Road, Shaoyang 422100, PR China
[b] College of Food Science and Engineering, Central South University of Forestry and Technology, Changsha, Hunan 410004, PR China

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ABSTRACT

A series of coumarin derivatives were synthesised and their inhibitory effects on the diphenolase activity of mushroom tyrosinase were evaluated. The results showed that some of the synthesised compounds exhibited significant inhibitory activities. Especially, 2-(1-(coumarin-3-yl)ethylidene)hydrazinecarbothioamide bearing thiose-micarbazide group exhibited the most potent tyrosinase inhibitory activity with IC_{50} value of 3.44 μM. The inhibition mechanism analysis of 2-(1-(coumarin-3-yl)-ethylidene)hydrazinecarbothioamide and 2-(1-(6-chlorocoumarin-3-yl)ethylidene)-hydrazinecarbothioamide demonstrated that the inhibitory effects of the compounds on the tyrosinase were irreversible. Preliminary structure activity relationships' (SARs) analysis suggested that further development of such compounds might be of interest.

1. Introduction

Tyrosinase (monophenol or *o*-diphenol, oxygen oxidoreductase, EC 1.14.18.1, syn. polyphenol oxidase), also known as polyphenol oxidase (PPO), is a copper-containing monooxygenase that is widely distributed in microorganisms, animals, and plants (Song et al., 2006). Tyrosinase catalyses by involving molecular oxygen in two distinct reactions: in the hydroxylation of monophenols too-diphenols (monophenolase) and in the oxidation of *o*-diphenols to *o*-quinones (diphenolase) (Chen, Liu, & Huang, 2003). Due to the high reactivity, quinines could polymerise spontaneously to form high molecular weight brown-pigments (melanins) or react with amino acids and proteins to enhance brown colour of the pigment produced (Matsuura, Ukeda, & Sawamura, 2006). Hyperpigmentations, such as *senile lentigo*, melasma, freckles, and pigmented acne scars are of particular concern to women. The treatment usually involves the use of medicines or medicinal cosmetics containing depigmenting agents or skin whitening agents (Tripathi et al., 1992). In clinical usage, tyrosinase inhibitors are used for treatments of dermatological disorders related to melanin hyperaccumulation and are essential in cosmetics for depigmentation (Schallreuter et al., 2009; Wood et al., 2009). For example, age spots and freckle were caused by the accumulation of an excessive level of epidermal pigmentation (Thanigaimalai et al., 2010).

Previous reports confirmed that tyrosinase was one of the main causes of most fruits and vegetables quality loss during post harvest handling and processing, leading to faster degradation and shorter shelf life (Yi et al., 2010). Tyrosinase has also been linked to Parkinson's and other neurodegenerative diseases (Zhu et al., 2011). In insects, tyrosinase is uniquely associated with three different biochemical processes, including sclerotisation of cuticle, defensive encapsulation and melanisation of foreign organism, and wound healing (Ashida & Brey, 1995). These processes provide potential targets for developing safer and effective tyrosinase inhibitors as insecticides and ultimately for insect control. Thus, the development of safe and effective tyrosinase inhibitors is of great concern in the medical, agricultural, and cosmetic industries. However, only a few such as kojic acid, arbutin, tropolone, and 1-phenyl-2-thiourea (PTU) (Fig. 1) are used as therapeutic agents and cosmetic products (Battaini, Monzani, Casella, Santagostini, & Pagliarin, 2000).

Coumarin derivatives are an important class of compounds, widely present in plants, including edible vegetables and fruits (Curini, Cravotto, Epifano, & Giannone, 2006; Rai et al., 2010). Coumarin derivatives are of great interest due to their diverse structural features and versatile biological properties, such as anti-inflammatory, antioxidant, vasorelaxant, cytotoxic, anti-HIV, antitubercular and antimicrobial (Belluti et al., 2010; Chimenti, Bizzarri, Bolasco, & Secci, 2010; Kostova, 2006; Neyts et al., 2009; Ostrov et al., 2007; Roussaki, Kontogiorgis, Hadjipavlou-Litina, Hamilakis, et al., 2010; Upadhyay & Mishra, 2010). In particular, their antibacterial, antifungal and anticancer activities make the compounds attractive for further derivatisation and screening as novel therapeutic agents (Khode, Maddi, Aragade, Palkar, & Ronad, 2009). The literature survey revealed that compounds with thiourea moieties have been reported to demonstrate a wide range of pharmacological activities, which include antibacterial, antifungal, anticonvulsant and tyrosinase inhibitory activity. Such as phenylthioureas, alkylthioureas and 1,3-bis-(5-methanesulfonylbu-

* Corresponding author. Tel./fax: +86 739 5431768.
E-mail address: xtliujb@yahoo.com.cn (J. Liu).

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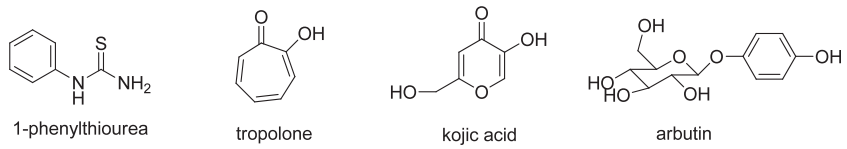

Fig. 1. Chemical structure of known tyrosinase inhibitors.

tyl)thiourea, displayed weak or moderate tyrosinase inhibitory activity (Ley & Bertram, 2001). More recently, our investigations also demonstrated that thiosemicarbazide derivatives exhibited potent inhibitory activities against mushroom tyrosinase (Liu, Yi, Wan, Ma, & Song, 2008). During recent years, extensive studies on the pharmacology of coumarin derivatives have been reported, but the tyrosinase inhibitor activities of this kind of compounds have hardly ever appeared in the literature. Stimulated by these results, in the present investigation, we synthesised a series of coumarin derivatives bearing thiosemicarbazide moieties or ester groups, their inhibitory activities against mushroom tyrosinase were evaluated using kojic acid as a comparing substance. Meanwhile, the structure–activity relationships of these compounds were also primarily discussed. The aim of the present study was the discovery of safe and efficient compounds as food additives or food preservatives, which can offer a clue to the design and synthesis of novel tyrosinase inhibitors.

2. Materials and methods

2.1. Chemical reagents and instruments

Melting points (m.p.) were determined with WRS-1B melting point apparatus and the thermometer was uncorrected. NMR spectra were recorded on Bruker 400 spectrometersat 25 °C in $CDCl_3$ or DMSO-d_6. All chemical shifts (δ) are quoted in parts per million downfield from TMS and coupling constants (*J*) are given in hertz. Abbreviations used in the splitting pattern were an follows: s = singlet, d = doublet, t = triplet, q = quintet, m = multiplet, LC–MS spectra were recorded using the LCMS-2010A. All reactions were monitored by TLC (Merck Kieselgel 60 F254) and the spots were visualised under UV light. Infrared (IR) spectra were recorded as potassium bromide pellets on VECTOR 22 spectrometer.

Tyrosinase, L-3, 4-dihydroxyphenylalanine (L-DOPA) and kojic acid were purchased from Sigma–Aldrich Chemical Co. Other chemicals were purchased from commercial suppliers and were dried and purified when necessary.

2.2. General procedures for the synthesis of substituted 3-acetylcoumarin

2.2.1. General

Piperidine (5 mol%) was added to the mixture of substituted salicylaldehyde (1 mmol) and ethylacetoacetate (1.1 mmol) in dry CH_3CN (10 ml), then the reaction mixture was stirred for about 4 h at the room temperature. The progress of the reaction was monitored by TLC. After completion of the reaction, formed precipitate was collected by filtration and washed with cold CH_3CN, then dried under vacuum to provide the substituted 3-acetylcoumarin.

2.2.2. 3-Acetylcoumarin (**1**)

Yield 76%. Yellow solid, mp 120.7–122.1 °C. IR (KBr): 3078, 3026, 1723, 1675, 1598, 1556, 1441, 1406, 1351, 1297, 1220, 1198, 1162, 1098, 967, 762 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 8.49 (s, 1H, C═CH), 7.65–7.63 (m, 2H, Ph-H), 7.37–7.32 (m, 2H, Ph-H), 2.71 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.41 (1C), 159.17 (1C), 155.27 (1C), 147.39 (1C), 134.33 (1C), 130.17 (1C), 124.92 (1C), 124.48 (1C), 118.20 (1C), 116.63 (1C), 30.49 (1C).

2.2.3. 6-Chloro-3-acetylcoumarin (**2**)

Yield 89.87%. Yellow solid, mp 213.1–213.9 °C. IR (KBr): 2946, 1726, 1611, 1469, 1412, 1355, 1278, 1134, 1082, 970, 829, 765 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 8.40(s, 1H, C═CH), 7.63 (dd, H, *J* = 8.5 Hz, 2.5 Hz, Ph-H), 7.60 (d, 1H, *J* = 2.5 Hz, Ph-H), 7.33 (d, 1H, *J* = 8.5 Hz, Ph-H), 2.72 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.41 (1C), 158.57 (1C), 152.23 (1C), 145.39 (1C), 141.08 (1C), 132.12 (1C), 128.65 (1C), 127.56 (1C), 126.94 (1C), 118.02 (1C), 16.14 (1C).

2.2.4. 6-Bromo-3-acetylcoumarin (**3**)

Yield 94.10%. Yellow solid, mp 229.1–229.9 °C. IR (KBr): 3039, 1732, 1675, 1608, 1550, 1415, 1351, 1233, 1201, 1063, 980, 832, 768 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 8.40 (s, 1H, C═CH), 7.78 (dd, H, *J* = 8.5 Hz, 2.5 Hz, Ph-H), 7.74 (d, 1H, *J* = 2.5 Hz, Ph-H), 7.27 (d, 1H, *J* = 8.5 Hz, Ph-H), 2.72 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.41 (1C), 158.62 (1C), 152.71 (1C), 145.32 (1C), 140.77 (1C), 134.69 (1C), 131.12 (1C), 126.65 (1C), 120.88 (1C), 118.67 (1C), 116.18 (1C), 16.16 (1C).

2.3. The general procedure for the synthesis of substituted 2-(1-(coumarin-3-yl) ethylidene)hydrazinecarbothioamide compound

2.3.1. General

The appropriate substituted 3-acetylcoumarin (10 mmol) was dissolved in anhydrous ethanol (20 ml), thiosemicarbazide (10 mmol) and acetic acid (0.5 ml) were added to the above system. The reaction mixture was refluxed for 6 h. The reaction was monitored by TLC. After completion of the reaction, the reaction mixture was cooled to room temperature. The appearing precipitate was filtered and recrystallised from 95% alcohol to obtain the corresponding substituted 2-(1-(coumarin-3-yl) ethylidene)hydrazinecarbothioamide compound.

2.3.2. 2-(1-(Coumarin-3-yl)ethylidene)hydrazinecarbothioamide (**4**)

Yield 85.38%. Yellow solid, mp 214.2–216.7 °C. IR (KBr): 3385, 3235, 3155, 1716, 1598, 1499, 1428, 1367, 1290, 1236, 1111, 1069, 970, 861, 765 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 10.61 (s, 1H, NH), 8.46 (s, 1H, C═CH), 8.39 (s, 1H, NH_2), 7.94 (s, 1H, NH_2), 7.76 (d, *J* = 8.5 Hz, H, Ph-H), 7.65 (t, 1H, *J* = 8.5 Hz, Ph-H), 7.44 (d, 1H, *J* = 8.5 Hz, Ph-H), 7.38 (t, 1H, *J* = 8.5 Hz, Ph-H), 2.38 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 179.72 (1C), 159.56 (1C), 154.07 (1C), 146.39 (1C), 142.55 (1C), 132.83 (1C), 129.56 (1C), 126.25 (1C), 125.20 (1C), 119.39 (1C), 116.41 (1C), 16.47 (1C).

2.3.3. 2-(1-(6-Chlorocoumarin-3-yl)ethylidene)hydrazinecarbothioamide (**5**)

Yield 87.68%. Yellow solid, mp 232.0–232.9 °C. IR (KBr): 3417, 3235, 3138, 1732, 1707, 1588, 1502, 1479, 1451, 1428, 1287, 1233, 1204, 1130, 1092, 954, 925, 871, 832, 772 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 10.49 (s, 1H, NH), 8.44 (s, 1H, NH_2), 8.41 (s, 1H, C═CH), 8.44 (s, 1H, NH_2), 7.82 (s, H, Ph-H), 7.68 (dd, 1H, *J* = 8.5 Hz, 2.5 Hz, Ph-H), 7.47 (d, 1H, *J* = 8.5 Hz, Ph-H), 2.45 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 179.31 (1C), 158.64 (1C),