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Design and synthesis of chalcone derivatives as potent tyrosinase inhibitors and their structural activity relationship

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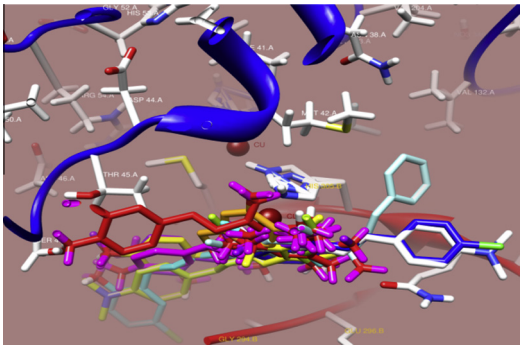
HIGHLIGHTS

- Chalcones derivatives were prepared through the Claisen–Schmidt condensation reaction.
- Compounds were characterized by detailed spectroscopic techniques and single-crystal X-ray structural analysis.
- Flavokawain B (**1**), flavokawain A (**2**) and compound **3** were found to be potential tyrosinase inhibitors.
- Detailed molecular docking and SARs studies were correlated well with the tyrosinase inhibition studies *in vitro*.

GRAPHICAL ABSTRACT

In this study, a series of chalcones (**1–10**) have been synthesized and examined for their tryrosinase inhibitory activity. The results showed that flavokawain B (**1**), flavokawain A (**2**) and compound **3** were found to be potential tyrosinase inhibitors, indicating IC_{50} 14.20–14.38 μM values. This demonstrates that 4-substituted phenolic compound especially at ring A exhibited significant tyrosinase inhibition. Additionally, molecular docking results showed a strong binding affinity for compounds **1–3** through chelation between copper metal and ligands.



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ABSTRACT

Browning of fruits and vegetables is a serious issue in the food industry, as it damages the organoleptic properties of the final products. Overproduction of melanin causes aesthetic problems such as melisma, freckles and lentigo. In this study, a series of chalcones (**1–10**) have been synthesized and examined for their tryrosinase inhibitory activity. The results showed that flavokawain B (**1**), flavokawain A (**2**) and compound **3** were found to be potential tyrosinase inhibitors, indicating IC_{50} 14.20–14.38 μM values. This demonstrates that 4-substituted phenolic compound especially at ring A exhibited significant tyrosinase inhibition. Additionally, molecular docking results showed a strong binding affinity for compounds **1–3** through chelation between copper metal and ligands. The detailed molecular docking and SARs studies correlate well with the tyrosinase inhibition studies *in vitro*. The structures of these compounds were elucidated by the 1D and 2D NMR spectroscopy, mass spectrometry and single X-ray crystallographic

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Flavokawain B
Molecular docking studies

techniques. These findings could lead to design and discover of new tyrosinase inhibitors to control the melanine overproduction and overcome the economic loss of food industry.

Introduction

Skin health and appearance is a major concerned issue for people globally. As early as last century, pharmaceuticals and traditional remedies have become incrementally popular for this purpose. Skin disorders are often related to deleterious effect of ultraviolet radiations. The failure of melanogenesis regulation may causes overproduction of melanin and resulting hyperpigmentation trauma such as post-inflammatory melanoderma, melasma and solar lentigines [1,2]. Tyrosinase (EC 1.14.18.1) is a copper-containing enzyme, which plays a key role in melanin biosynthesis. Tyrosinase catalyzes the aerobic hydroxylation of L-tyrosine to L-DOPA and the subsequent oxidation of L-dopa to L-dopaquinone, which lead to the accumulation of melanin and hyperpigmentation [3]. In addition, unfavorable enzymatic browning of plant-derived foods by tyrosinase causes a decrease in nutritional values and economic loss in food industry. Tyrosinase inhibitors are used for the treatment of melasma, postinflammatory hyperpigmentation and Addison's disease [4].

However, these inhibitors suffer from toxicity and lack of efficacy. Even, the most popular drug hydroquinone is used as a tyrosinase inhibitor [5] causes DNA damage [6] and carcinogenic effect [7]. The use of hydroquinone strong depigmentation with adverse effect reported in long term of usage [8,9]. Therefore, the use of hydroquinone in cosmetic products was banned in the European Union and is under scrutiny by the United States Food and Drug Administration (FDA).

Tyrosinase inhibition has been indicating as an important target for inhibition of melanin production [5,10]. Most of the enzyme inhibitors, such as kojic acid, tropolone and arbutin are structurally similar to L-DOPA and tyrosine, which act competitively for tyrosinase [11–13]. Naturally derived compounds have a major contribution for the development effective inhibitors for treatment with low side effects. Chalcones form a group of polyphenols, which are widely distributed in edible plants and possess diverse pharmacological activities including anti-inflammatory, antipyretic, analgesic, bactericidal, insecticidal, anti-fungal, anticancer and antioxidant [14,15]. Flavokawain B (**1**) and flavokawain A (**2**) were isolated from *Piper methysticum* [16] exhibited a strong cytotoxicity againist various cancer cell lines [17,18]. The main purpose of this study is to discover efficient tyrosinase inhibitors. This may contribute in the development of effective inhibitors to control the melanin overproduction and improve the quality of fruits, vegetables and plant-derived foods.

Material and methods

General

Melting points were determined on a melting point apparatus, XSP-12 500X and were uncorrected. UV spectra were recorded on UV–visible spectrophotometer CARY 100 in MeOH. IR spectra were recorded on a Perkin–Elmer RXI Fourier Transform Infrared spectrometer (FTIR) as KBr disks. Mass spectra were measured on a Finnigan MAT SSQ 710 spectrometer by electron impact at 70 eV. NMR spectra were recorded in $CDCl_3$ or acetone-d_6 using a Varian 500 MHz NMR spectrometer. Column chromatography was performed on silica gel (60 Merck 9385 (230–400 mesh, ASTM).

Single-crystal X-ray crystallography

The molecular structure of the of (*E*)-1-(2′,4′-dihydroxy-phenyl)-3-(2,3-dimethoxyphenyl)-propenone (**9**) was determined by single X-ray crystal diffraction. The data were collected at 100 K by using a Bruker APEXII with a CCD area-detector X-ray diffractometer. The structure was solved by direct method with SHELXS97 program and refined on F^2 by full-matrix least-squares methods with anisotropic non-hydrogen atoms. The compound crystallizes in the orthorhombic *Pna2(1)/c* space group with the crystallographic detail presented in Table 1. The 1,3-diaryl-2-propen-1-one molecule (excluding the hydroxyl and methoxy substituents) is a planar molecule with r.m.s deviation of 0.018 Å (Fig. 1). The molecule adopts a *trans* configuration at the C=C bond with a distance of 1.3372(19) Å. One of the hydroxyl group is intramolecularly hydrogen bonded with the carbonyl substituent (O_5—$H_5\cdots O_3$ 2.5059(15) Å), while the other hydroxyl group is intermolecular hydrogen bonded to the methoxy oxygen of an adjacent molecule (O_4—$H_4\cdots O_1$ = 2.7849(14) Å, symmetry code: $-0.5+x$, $0.5-y$, $-1+z$) giving rise to a polymeric chain structure. The crystal packing of the compound is formed *via* π-π stacking interaction [distance between ring centroids, Cg(1)—Cg(2)^{i} :3.8495(9) Å, symmetry operator *i*: *x*, *y*, $1+z$ and dihedral angle between planes: 3.53(7)°]. The full crystallographic data of compound **9** has been deposited at the Cambridge Crystallographic Data Center (CCDC) as supplementary publication (number is CCDC938805). Union Road, Cambridge CB2 1EZ, UK [Fax: þ44 1223 336033. email: deposit@ccdc.cam.ac.uk or at www.ccdc.cam.ac.uk].

Chemicals

The following reagents were purchased commercially: mushroom tyrosinase (EC 1.14.18.1), dimethyl sulfoxide (DMSO), L-3,4-dihydroxyphenylalanine (L-DOPA), Dulbecco's Modified Eagle's Medium (DMEM), Kojic acid from Sigma Chemical Co. (St. Louis, MO, USA).

Tyrosinase enzymatic assay

Tyrosinase inhibition assays were performed according to a modified method described by Kubo [26]. Mushroom tyrosinase (EC 1.14.18.1, Sigma Product T3824 with an activity of 3320 U/mg) was used in this bioassay protocol [26]. The solutions compounds **1–10** were prepared (200 μg/mL) in DMSO and mixed with 0.5 mL

Table 1
Percentage Inhibitions and IC_{50} values of compounds (**1–10**) and Kojic acid (**11**).

Compound	Percentage of inhibition (%)	IC_{50} (μM)
1	90.61 ± 0.42	14.38 ± 0.12
2	95.20 ± 0.47	14.26 ± 0.08
3	97.36 ± 0.36	14.20 ± 0.03
4	84.85 ± 0.17	15.61 ± 0.31
5	63.68 ± 0.56	20.59 ± 0.33
6	75.23 ± 0.31	15.64 ± 0.01
7	82.31 ± 1.72	15.01 ± 0.42
8	29.51 ± 2.80	NT
9	87.20 ± 0.69	14.23 ± 0.41
10	20.29 ± 0.97	NT
Kojic acid (**11**)	99.21 ± 0.16	12.01 ± 0.11