Journal of Molecular Structure 1085 (2015) 97–103

Contents lists available at ScienceDirect

Journal of Molecular Structure

journal homepage: www.elsevier.com/locate/molstruc

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

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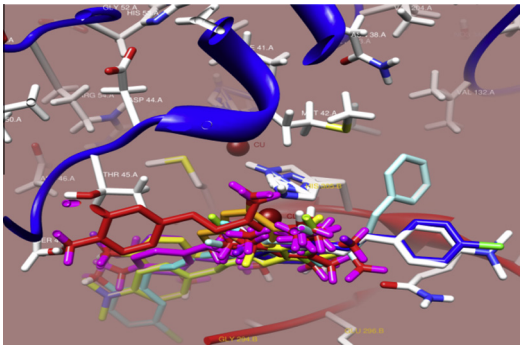
HIGHLIGHTS

- 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.



ARTICLE INFO

Article history:
Received 5 September 2014
Received in revised form 3 December 2014
Accepted 23 December 2014
Available online 31 December 2014

Keywords:
Chalcones
1D and 2D NMR spectroscopy
Tyrosinase inhibitors
Flavokawain A

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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http://dx.doi.org/10.1016/j.molstruc.2014.12.073
0022-2860/

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

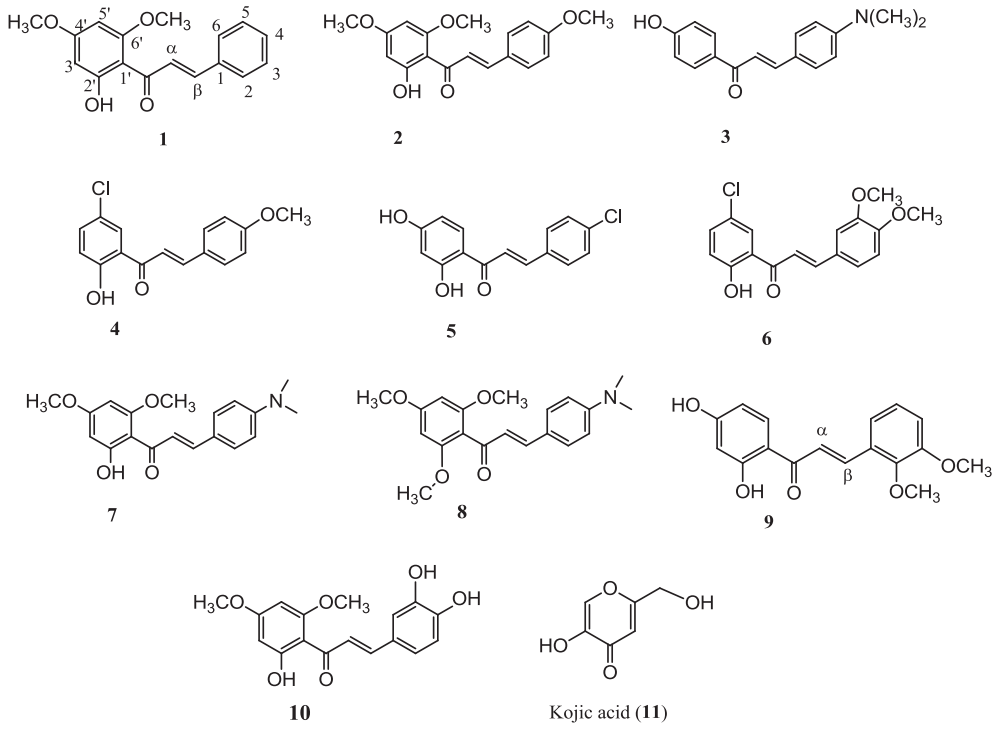

Fig. 1. The structures of compounds (**1–11**).

of L-DOPA solution (1.25 mM). About 0.9 mL of sodium acetate buffer solution (0.05 M, pH 6.8) was added and preincubated for 10 min at 25 °C. Then 0.05 mL of an aqueous solution of mushroom tyrosinase was added in the last mixture. The activity was expressed as the sample concentration that gave a 50% inhibition in the enzyme activity (IC_{50}). For the measurement of diphenolase inhibitory activity, 0.1 mL of a sample solution and 1.5 mL of 1.5 M, L-DOPA solution (0.1 M phosphate buffer, pH 6.8) were mixed with 0.4 mL H_2O, and preincubated at 25 °C for 5 min. Then, 0.5 mL of tyrosinase solution (125 U) was added and the reaction was monitored by using a UV microplate reader (SpectraMax, Plus 384, Molecular Devices, Inc., USA). The amount of dopachrome produced was monitored at 475 nm using UV microplate reader. For the measurement of monophenolase inhibitory activity, 0.1 mL of a sample solution and 1 mL of 2 M L-tyrosine solution were mixed with 0.9 mL of 0.1 M phosphate buffer (pH 6.8) and pre-incubated at 25 °C for 5 min. Then, 0.5 mL of tyrosinase solution (250 U) was added and the reaction was monitored at 475 nm for 20 min for detection of dopachrome formed. Kojic acid was used as positive control. Triplicate absorbance readings of control and test samples were averaged. The inhibition percentage of each sample was then calculated by using the following formula.

$$\text{Tyrosinase inhibition } (\%) = (A_{\text{control}}) - (A_{\text{sample}})/(A_{\text{control}}).$$

Statistical analysis

Statistical analysis were performed by using one-way analysis of variance (ANOVA), followed by Dunnett test post hoc for multi-group comparison test using GraphPadPrsim software version 5.0. Statistical significance of differences between groups was accepted at $P < 0.05$.

Docking protocol

In order to gain more insight at molecular level about binding mode of tyrosinase enzyme, computational studies involving docking was performed. So far there is no crystal bound structure available for tyrosinase target enzyme, hence experimentally resolved X-ray structure of apo-tyrosinase enzyme (PDBcode 1WX2 with resolution 1.82 Å) was retrieved from Protein Data Bank (PDB) [27]. Data set containing kojic acid (**11**) as a standard and nine chalcone derivatives were used for docking studies. All the compounds were docked in tyrosinase enzyme by using GOLD software [28]. First, all compounds were drawn and then each compound was converted into mol2 format by using babel program. Moreover, all compounds were filtered using OE Filter program from OpenEyes to adjust protonation state and followed by minimization with MMFF94 force field with gradient of 0.05 kcal/(molÅ) by Molecular Operating Environment [29–31]. Before docking protein structure of tyrosinase enzyme (PDB code 1WX2) was subjected to partial charge calculation and then followed by energy minimization with MMFF94 force field gradientof0.05 kcal/molÅ in MOE. Initially, by GOLD software, minimized structure of tyrosinase protein (PDB code 1WX2) was prepared by correcting protonation state (hydrogen atoms were added) and all water molecules were deleted. Copper ions play an important role for compound placement in active site [32]. After preparation of tyrosinase protein structure, active site containing Cu^{2+} ions metals were marked for docking as active site, where the compounds can dock. Docking was performed by keeping default parameters in which Lamarkican genetic algorithm was integrated. It provided full ligand flexibility in docking. One scoring function i.e. Gold score, was used as fitness score to quantify ligand binding interaction.

*General procedure for the synthesis of chalcones **1–10***

To a stirred solution of appropriate acetophenone (1 equiv), substituted benzaldehyde (1 equiv) in ethanol, KOH (20% w/v aqueous solution) were added. The mixture was stirred at room temperature for 48–72 h. The reaction mixture was cooled to 0 °C (ice-water bath) and acidified with HCl (10% v/v aqueous solution). In most cases, yellow precipitate were formed, filtered and washed with 10% aqueous HCl solution. The mixture was extracted

with ethyl acetate, the extracts were dried over Na_2SO_4 anhydrous and the solvent was evaporated to obtain final product as in solid or crystalline form hot MeOH. The crude products were purified with column chromatography by elution with ethyl acetate and hexane.

(E)-1-(2′-Hydroxy-4′,6′-dimethoxyphenyl)-3-phenylprop-2-en-1-one ***(1)***

Yield 82.4%. Yellow needles crystals: m.p. 102–104 °C. IR (KBr): 3060 (Ar C—H), 1642 (C=O), 1589, 1588, 1478, (C=C ring), 1202 (C—O) cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz): δ 14.3 (s, 1H, OH), 7.92 (d,1H, *J* = 15.0 Hz, Hβ), 7.80 (d,1H, *J* = 15.0 Hz, Hα), 7.64 (brd, 2H, H-2, 6), 7.48 (m, 3H, H-3, 4, 5), 6.98 (br,s, 1H, H-5'), 6.21 (br,s, 1H, H-3'), 3.93 (s, 3H, OMe, C-6′), 3.85 (s, 3H, OMe, C-4′). ^{13}C NMR ($CDCl_3$, 125 MHz): δ193.2 (C=O), 168.9 (C2′), 166.7 (C4′), 162.5 (C6′), 142.51 (Cβ), 141.0 (C4), 132.9 (C1), 130.2 (C2, C6), 128.1 (C3,C5), 126.6 (Cα), 108.14 (C1′), 93.6 (C3′), 90.94 (C5′), 55.53 (OCH_3), 55.7 (OCH_3). EI-MS *m/z* 284.23, (Molcular formula $C_{17}H_{16}O_4$).

(E)-1-(2′-Hydroxy-4′,6′-dimethoxyphenyl)-3-(4-methoxyphenylprop-2-en-1-one ***(2)***

Yield 82.4%. Yellow needles crystals. mp 110–112 °C. IR (KBr): 1654 (C=O), 3200 (Ar C—H str.), 1590, 1589, 1476, (C=C), 1202 (C—O str.), 850 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz): δ 13.6 (s, 1H, OH), 8.02 (d,1H, *J* = 15.5 Hz, Hβ), 7.81 (d,1H, *J* = 15.5 Hz, Hα), 7.73 (brd, 2H, H-2, 6), 7.49 (m, 2H, H-3, 5), 6.87 (br,s, 1H, H-5′), 6.02 (br,s, 1H, H-3′), 3.93 (s, 3H, OMe, C-6′), 3.85 (s, 3H, OMe, C-4′), 3.82 (s, 3H, OMe, C-4). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 192.6 (C=O), 168.7 (C2′), 166.2 (C4′), 163.1 (C6′), 160.9 (C4), 141.6 (Cβ), 130.1(C2, C6), 127.5 (C1), 125.4 (Cα), 114.3 (C3, C5), 93.56 (C3′), 91.4 (C5′), 55.79 (OCH_3), 55.53 (OCH_3), 55.35 (OCH_3). EI-MS *m/z* 298.33, (Molcular formula $C_{18}H_{18}O_5$).

*(E)-3-(4-Dimethylamino)phenyl)-1-(4′-hydroxyphenyl)prop-2-en-1-one (****3****)*

Yield: 66%. mp: 184–186 °C. IR (KBr) cm^{-1}: 3384 (OH), 1658 (C=O str.), 3081 (Ar C—H), 1590, 1482, 1434 (C=C), 1222 (C—O str.), 748 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz): δ 8.13–7.86 (m, 4H, C_6H_4Ar—H), 7.82 (d, *J* = 15.6 Hz, 1H, Hβ), 7.35 (d, *J* = 15.6 Hz, 1H, Hα), 6.87–6.93 (m, 4H, C_6H_4), 2.99 (s, 6H, 4-N(CH_3)$_2$), MS *m/z*: 268 $(M + 1)^+$. Anal. For $C_{17}H_{17}NO_2$.

*(E)-3-(4-Chlorophenyl)-1-(2′,4′-dihydroxyphenyl)prop-2-en-1-one (****5****)*

Yield: 65.4%; mp: 150–154 °C. IR (KBr): 3302 (OH), 1644 (C=O), 3042 (Ar C—H), 1592, 1582, 1440 (C=C), 1225 (C—O) cm^{-1}: ^1H NMR ($CDCl_3$): δ 12.7 (s, 1H, OH), 6.49–6.51 (m, 3H, C_6H_3), 7.34 (d, *J* = 15.6 Hz, 1H, Hα), 7.76–7.90 (m, 4H, C_6H_4), 8.05 (d, *J* = 15.6 Hz, 1H, Hβ), MS *m/z*: 275 $(M + 1)^+$. Anal. for $C_{15}H_{11}ClO_3$.

(E)-1-(2′,4′-Dihydroxy-phenyl)-3-(2,3-dimethoxyphenyl)-propenone ***(9)***

Yield 82.4 %. Yellow flat crystals: mp 184–186 °C. IR (KBr): 1652 (C=O), 2987 (Ar C—H str.), 1622, 1378, 1590 (C=C ring) cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz): δ 12.4 (s, 1H, OH), 8.15 (d,1H, *J* = 15.5 Hz, Hβ), 7.95 (br,d,1H, *J* = 9.0 Hz, H-6′), 7.81 (d,1H, *J* = 15.5 Hz, Hα), 6.41 (d, *J* = 2.5, 1H, H-3′), 7.12 (t, *J* = 8.0 Hz, each H- 5), 7.44 (d, *J* = 8.0 Hz, H-6), 7.15 (dd, *J* = 8.0 each, H-4), 6.30 (d, *J* = 2.5, 1H, H-5′), 3.88 (s, 3H, OMe, C-2), 3.87 (s, 3H, OMe, C-3). EI-MS *m/z* 284.23, (Molcular formula $C_{17}H_{16}O_4$).

*(2E)-3-[4-(Dimethylamino)phenyl]-1-(2′,4′,6′-trimethoxy-phenyl)-2-propen-1-one (****8****)*

Yield 73%. Yellowsolid, mp = 149–152 °C. IR (KBr): 3103, 2937, 2839, 1648, 1622, 1529, 1463, 1452, 1413, 1184, 968, 945, 815 (Ar) cm^{-1}. ^1H NMR (CDCl3): δ 7.41 (d, 2H, H2, H6), 7.27 (d, 1H, *J* = 15.0 Hz, Hβ), 6.78 (d, 1H, *J* = 15.0 Hz, Hα), 6.65 (d, 2H, H3, H5), 6.16 (*s*, 2H, H3′, H5′), 3.86 (s, 3H, OCH_3), 3.76 (*s*, 6H, OCH_3), 3.01 (6H, N—$(CH_3)_2$), ^{13}C NMR ($CDCl_3$): δ 194.95 (C=O), 162.19 (C4′), 158.80 (C2′, C6′), 152.05 (C4), 146.20 (Cβ), 130.47 (C2, C6), 124.67 (C1), 122.84 (Cα), 112.49 (C1′), 110.99 (C3, C5), 90.90 (C3′, C5′), 56.15 (*p*′-OCH_3), 55.70 (*p*′-OCH_3), 41.09 (*N*—$(CH_3)_2$).

Table 2
Crystal data and structure refinement for compound **9**.

Empirical formula	$C_{17}H_{16}O_5$
Formula weight	300.30
Temperature	100(2) K
Wavelength	0.71073 A
Crystal system, space group	Orthorhombic, *Pna2(1)*
Unit cell dimensions	*a* = 18.982(2) Å alpha = 90°
	b = 9.9786(12) Å beta = 90°
	c = 7.6015(9) Å gamma = 90°
Volume	1439.9(3) $Å^3$
Z, Calculated density	4, 1.385 Mg/m^3
Absorption coefficient	0.102 mm^{-1}
Crystal size	0.20 × 0.10 × 0.08 mm
Theta range for data collection	2.15–28.25°
Reflections collected/unique	16141/3549 [*R*(int) = 0.0383]
Completeness to theta = 28.25	99.8 %
Max. and min. transmission	0.9919 and 0.9798
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	3549 / 1 / 199
Goodness-of-fit on F^2	1.044
Final *R* indices [$I > 2\sigma(I)$]	*R*1 = 0.0326, *wR*2 = 0.0753
R indices (all data)	*R*1 = 0.0360, *wR*2 = 0.0774
Largest diff. peak and hole	0.236 and −0.183 e. $Å^{-3}$

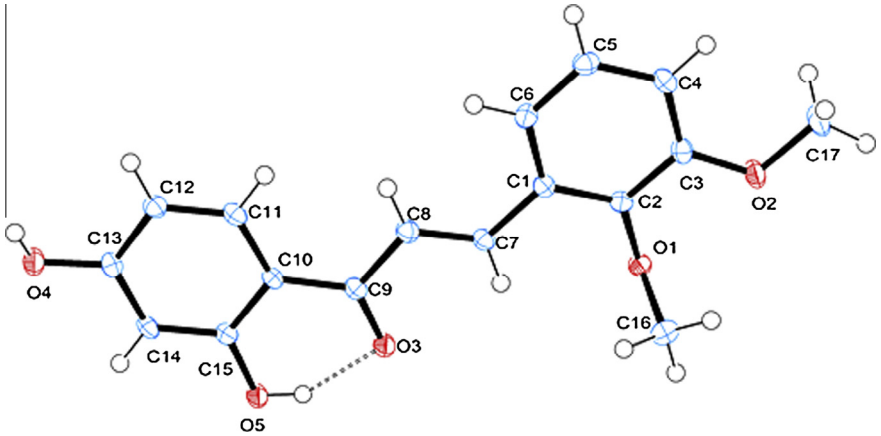

Fig. 2. The ORTEP diagram of compound **9**.

Results and discussion

Melanin an important pigment is widely distributed in bacteria, fungi, plants and animals, which play a vital protective role against skin photocarcinogenesis, however the abnormal production of melanin is a serious problem for human skin. Naturally occurring compounds flavonoids, stilbenes and resorcinols have been known as potential tyrosinase inhibitors [19]. Chalcones **1–10** were prepared via Claisen–Schmidt condensation reaction by using our designed compounds 2′ or 2′,4′-dihydroxy acetophenone with

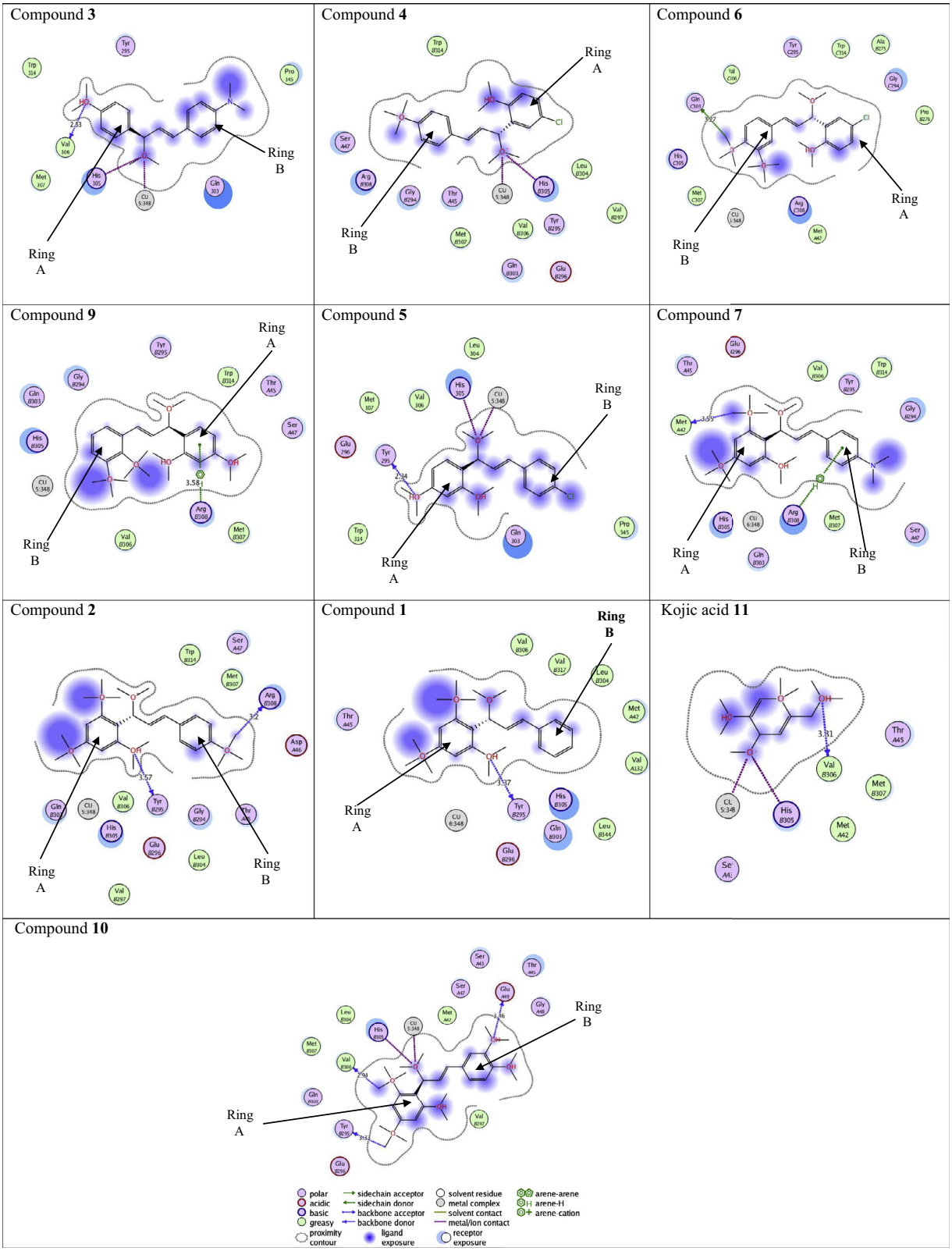

Fig. 3. 2D pictorial diagram showing worthy interactions of active binding site of tyrosinase enzyme (PDB code 1WX2) with all compounds.

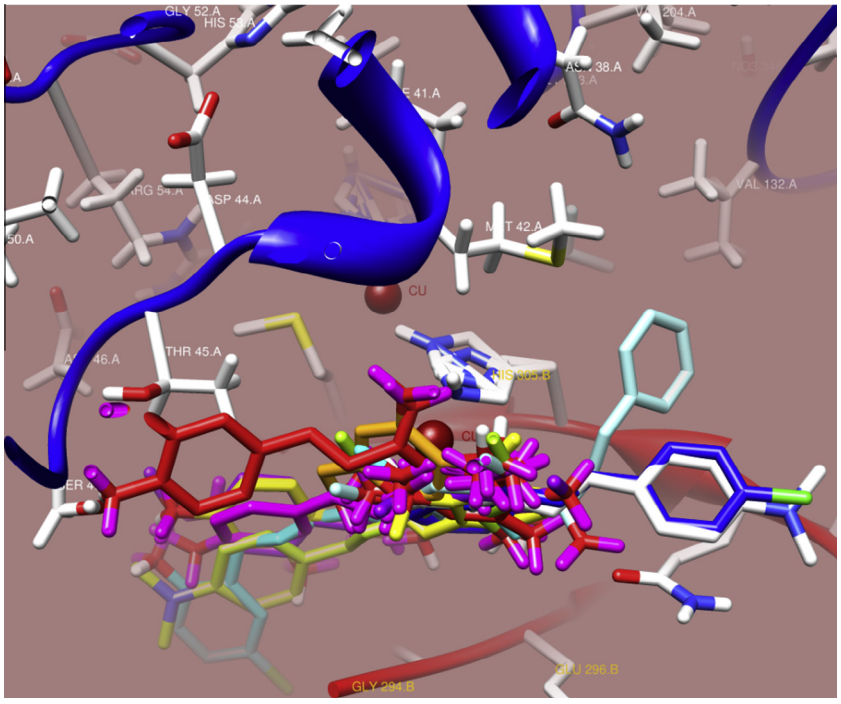

Fig. 4. Dock pose of all compounds in the active site of tyrosinase enzyme (PDB code 1WX2).

corresponding benzaldehyde in methanol. The compounds were characterized by detailed spectroscopic techniques and evaluation for tyrosinase inhibitions studies [20] (Fig. 1, Table 1).

Chalcones (**1–10**) were synthesized via a Claisen–Schmidt condensation reaction. A substituted methyl ketone and respective aldehydes were dissolved in methanol in addition of KOH pallets as discussed in experimental section. The synthetic compounds were characterized with help of IR, UV, EI-MS, ^{1}H NMR, ^{13}C NMR and single X-ray crystallographic techniques. The IR spectra of chalcones showed a peak at 1640–1648 (C=O), 1560–1588 (C=C–=O), 1460–1478 (C=C, Ar) and 1200–1000 (C–O) cm^{-1}. The ^{1}H NMR spectra of synthetic chalcones appeared at δ 12.0–14.4 (**1, 2, 4, 5, 6, 7, 9, 10**) were due the -chelated OH with carbonyl group. Two characteristics protons appeared at δ 8.0–7.89 (d, J = 15.5 Hz) and 7.8–7.5 (d, J = 15.5) were due the β and α (*E*) double bond protons, respectively. The aromatic protons appeared at δ 8.0–6.0 of both ring A & B in the ^{1}H NMR spectroscopy. All data of compounds were compared with the previously published data of chalcones [22,23]. While the structure of compound **9** was confirmed by single X-ray crystallography as shown in Fig. 2 and data presented in Table 2.

The chalcone derivatives **1–10** and kojic acid (**11**) (positive control) were examined for their inhibitory activity in *vitro* [21]. The finding results of compounds **1–3** exhibited significant percentage inhibition of tyrosinase with 90.61 ± 0.42–97.36 ± 0.36% as shown in Table 1. Compound **3** showed potential inhibition by 97.36 ± 0.36% with IC_{50} = 14.20 ± 012 μM, which is comparable with kojic acid (**11**) IC_{50} value of 12.01 ± 0.11 μM. Other chalcones derivatives, **4**-**7** and **9** exhibited a significant inhibition of tyrosinase with IC_{50} values 15.61 ± 0.31–14.23 ± 0.41 μM. However, the compounds **8** and **10** showed a lower inhibition (Table 1). Compounds **1**-**7** and **9** were further evaluated for their inhibition by oxidative hydroxylation of diphenolase activity. Compound **1**, **2**, **3** and **9** were identified as potent tyrosinase inhibitors based on their low IC_{50} values 14.38 ± 0.12, 14.26 ± 0.08, 14.20 ± 0.03 and 14.23 ± 0.41 μM, respectively (Table 1). These compounds were promising and comparable with the inhibitory effect of kojic acid (**11**) (positive control) with IC_{50} value at 12.01 ± 0.11 μM (Table 1). Among them, compounds **1**–**3** and **9** were found to be the most active as tyrosinase inhibitors, which showed the 90–97% inhibition comparable with standard kojic acid (**11**). Judging from the structures of **1**–**3** the compound **3** possessing 4-hydroxyl substituted group was found to be most active tyrosinase inhibitor possibly showed strong chelation with binuclear copper containing tyrosinase enzymes. While compounds flavokawain B (**1**) and flavokawain A (**2**) might be coupled or chelated with the binuclear copper active site. It is well established that substituted resorcinol moiety act as potential inhibitor of tyrosinase [19] and position of hydroxy group attached to the ring A is important for tyrosinase inhibition [10,21]. Tyrosinase is use mono-, di-, and trihydroxyphenols as substrates as it is evident in literature that the monohydroxyphenols (*p*-cresol and tyrosine), dihydroxyphenols (catechol, L-dopa, catechin, and chlorogenic acid), and trihydroxyphenols (pyrogallol), indicating the potential use of monohydroxyphenols [22]. However, in our findings position of hydroxyl group at 4′ (compound **3**) is slightly more selective than 2′-hydroxyl position (compound **2**, **3**, **4**, **9**) possibly electronically less crowded and chelated strongly with copper ions for tyrosinase inhibition [23–25]. These results showed that presence of hydroxy groups at 2′ or 2′, 4′ positions may also contributed significantly for tyrosinase inhibition.

Docking analysis and SARs

Docking results showed that most of the compounds establish ionic bridge between His 305 on enzymes and carbonyl oxygen atom of the ligands particularly with compounds **3**, **4**, **5**, **9** and kojic acid. Additionally, these compounds forms bond with copper ion exhibited batter IC_{50} value. Generally, all compounds establish one polar contact with either one of the amino acid including Glu 49, Tyr 295, Gln 303, Val 306 and Arg 308 by involving backbone and side chain atoms as acceptor. Fig. 3 summarizes all the interactions of each compound in the binding site of tyrosinase enzyme (PDB code 1WX2). Compound **3** contains a hydroxyl group

at ring A which forms strong hydrogen bonding with the backbone atom of Val 306 at a distance of 2.53 Å. This helps the compound to fit into the pocket. Copper ion is also co-ordinated with ketone group of compound at a distance of 1.82 Å (Fig. 3; panel 1), which correlates effectively with the experimental results and found to be the most active among all compounds. All compounds docked posed in active site of tyrosinase enzyme is depicted in Fig. 4.

Conclusions

Natural tyrosinase inhibitors could be better sources for depigmentation then synthetic one. Flavokawain B and A are natural chalcones could be a new natural tyrosinase inhibitors. Structure activity relationship of these chalcones strongly supported the finding that 4-hydroxy chalcone or resorcinols could be potential tyrosinase inhibitors and added a new compounds for depigmentation agents in food and cosmetic industry. It has been investigation that mono- and di-hydroxy groups at ring A, which are an important cluster to chelation with copper ion and enzyme. Molecular docking studies showed that most of the compounds showing ionic bridge between basic residue His 305 and oxygen atom of ligand. Besides these compounds (**1–10**), which forms a bond between copper ion and ligand atom showing improved IC_{50} values (Table 1). In generally, all compounds bearing at least one polar contact with either one of the key residue including Glu 49, Tyr 295, Gln 303, Val 306 and Arg 308. However, for their clinical studies on human, more detail studies are required for tyrosinase inhibition.

Acknowledgment

This project was supported by the Universiti Malaysia Pahang (Grant Nos. RDU 120373 and RDU 120389).

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