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Mushroom tyrosinase inhibitors from *Aloe barbadensis* Miller

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ABSTRACT

Two new chromones, 5-((*S*)-2′-oxo-4′-hydroxypentyl)-2-(β-glucopyranosyl-oxy-methyl) chromone (**1**) and 5-((*S*)-2′-oxo-4′-hydroxypentyl)-2-methoxychromone (**2**), together with four known analogues, 8-C-glucosyl-7-O-methyl-(*S*)-aloesol (**3**), isoaloeresin D (**4**), 8-C-glucosyl-(*R*)-aloesol (**5**), and aloesin (**6**) were isolated from the aqueous extract of *Aloe barbadensis* Miller. Their structures were determined on the basis of spectroscopic evidences (1-D and 2-D NMR, HRMS, UV, and IR data), chemical methods and the literature data. The Mosher's method was applied to establish the absolute configuration of compounds **1** and **2**. The inhibitory effects of these chromones on the activity of mushroom tyrosinase were examined, and compound **6** was identified as a noncompetitive tyrosinase inhibitor with an IC_{50} value of 108.62 $\mu g \cdot mL^{-1}$.

1. Introduction

Aloe barbadensis Mill, a member of Asphodelaceae (Liliaceae) family, is a short-stemmed succulent herb widely distributed in Europe, Asia and southern parts of North America [1]. Of over 300 Aloe species, *A. barbadensis* has long been used in traditional medicine for the treatment of various diseases, and also used as raw materials of cosmetics and health foods [2,3]. Among its previously investigated chemical constituents [4], chromones and their derivatives have been reported to have multiple biological properties, for example, anticancer [5], antibacterial [6], antioxidant [7], anti-inflammatory [8] and mushroom tyrosinase inhibitory activity [9]. In a search for bioactive components from *A. barbadensis*, we found that the aqueous extract of this plant exhibited mushroom tyrosinase inhibitory activity. Phytochemical investigation has led to the isolation of two new chromones (**1** and **2**) and four known analogues including 8-C-glucosyl-7-O-methyl-(*S*)-aloesol (**3**), isoaloeresin D (**4**), 8-C-glucosyl-(*R*)-aloesol (**5**), and aloesin (**6**) from the aqueous extract of *A. barbadensis*. Herein, the isolation, structural elucidation and mushroom tyrosinase inhibitory activity assay of six isolates are described.

2. Experimental

2.1. Generals

Melting points were determined using a WRS-2A melting point apparatus and are uncorrected. Optical rotations were measured on a Perkin Elmer digital polarimeter. Ultraviolet (UV) spectra were recorded using a Shimadzu UV2457 spectrophotometer and infrared (IR) spectra were obtained with a Bruker Tensor 37 spectrophotometer. High-resolution mass spectra (HRMS) were acquired on a Shimadzu LCMS-IT-TOF, and MS data were measured on an Agilent 1200 series LC-MS/MS system consisting of a quaternary pump, a vacuum degasser, an autosampler, a thermostat column and a multimode electrospray ionization/APCI spray chamber. 1-D (^1H, ^{13}C, DEPT) and 2-D (COSY, HMBC, HSQC) NMR spectra were recorded on a Bruker AVANCE 400 spectrometer and chemical shifts (δ) were given in ppm and were referenced to the CD_3OD signals (δ_H 3.30, δ_C 49.0). AB-8 macroporous resins (manufactured by Nankai University Chemical Industry Factory, Tianjin, China) were used for column chromatography. Thin layer chromatography (TLC) analysis was carried out on silica gel plates (Marine Chemical Ltd., Qingdao, China). Preparative medium pressure liquid chromatography (MPLC) was carried out on an Eyela

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instrument (Tokyo, Japan) consisted of a VSP-3050 pump, UV-9000 UV–vis detector and DC-1500 fraction collector, using an Eyela column (300×20 mm i.d.) filled with Chromatorex SMB ODS (20–40 μm, Fuji Silisia Ltd., Nagoya, Japan). Analytical HPLC was carried out on a LC-20AT Shimadzu liquid chromatograph with a Nucleodur 100–5 C_{18} column (250×4.6 mm, 5 μm), connected with an SPD-M20A diode array detector (DAD) and an Alltech 3300 evaporative light scattering detector (ELSD). Absorbance of mushroom tyrosinase assay was measured on a FlexStation 3 microplate reader (Molecular Devices, USA) and analyzed using a SoftMax Pro 5 software (Molecular Devices, USA).

2.2. Plant material

The dried *A. barbadensis* powder was purchased from Yunnan Yuanjiang Evergreen Biological Co., Ltd. (Yuxi, China), and authenticated by Prof. Xin-jun Xu, School of Pharmaceutical Sciences, Sun Yat-Sen University, P. R. China. A voucher specimen (Batch no: 20120301) was deposited in School of Pharmaceutical Sciences, Sun Yat-Sen University, Guangzhou, China.

2.3. Extraction and isolation

The dried *A. barbadensis* powder (about 50 g) was extracted five times with H_2O under ultrasonication at ambient temperature and filtered. The filtrate was concentrated under the reduced pressure to give a crude aqueous extract (about 20 g). The aqueous extract was then subjected to AB-8 resin column chromatography eluted with a gradient of EtOH–H_2O (15:85 to 55:45, v/v) to give 3 major fractions 1–3. Fraction 2 was further purified on RP-C_{18} MPLC using CH_3OH–H_2O (26:74, v/v; flow rate: 10 mL·min^{-1}) as mobile phase to afford new compounds **1** (58.1 mg) and **2** (66.5 mg). Fraction 1 was then separated on a RP-C_{18} MPLC eluted with CH_3OH–H_2O (26:74, v/v; flow rate: 10 mL·min^{-1}) to obtain compounds **6** (70.2 mg), **3** (90.2 mg) and **5** (45.0 mg). Compound **4** (2.8 g) was obtained from fraction 3 using RP-C_{18} MPLC with the mobile phase of CH_3OH–H_2O (33:67, v/v; flow rate: 20 mL·min^{-1}).

Compound (**1**, Fig. 1): 5-((*S*)-2′-oxo-4′-hydroxypentyl)-2-(β-glucopyranosyl-oxy-methyl)chromone; slightly white amorphous powder; mp 167.4 °C; $[\alpha]_{20}^{D}$ −43.12° (c 1.09 , H_2O); UV (MeOH) λ_{max} nm (log ε): 225 (4.32), 249 (3.96), 302 (3.86); IR bands (KBr) ν_{max} cm^{-1}: 3401, 2966, 1712, 1652, 1605, 1480; HRMS (ESI) calcd. for $C_{21}H_{26}O_{10}$ $[M-H]^-$ 437.1453, found 437.1448; ^{1}H and ^{13}C NMR spectral data see Table 1.

Compound (**2**, Fig. 1): 5-((*S*)-2′-oxo-4′-hydroxypentyl)-2-methoxychromone; yellowish amorphous powder; mp 121.0 °C; $[\alpha]_{20}^{D}$ −22.09° (c 0.86, MeOH); UV (MeOH) λ_{max} nm (log ε): 225 (4.10), 248 (3.76), 302 (3.68); IR bands (KBr) ν_{max} cm^{-1}: 3347, 2963, 1718, 1643, 1603, 1480; HRMS (ESI) calcd. for $C_{15}H_{16}O_5$ $[M-H]^-$ 275.0925, found 275.0917; ^{1}H and ^{13}C NMR spectral data see Table 1.

2.4. Hydrolysis of compound **1**

About 1.0 mg of **1** was dissolved in 1.0 mL of 20% aqueous HCl solution, and left at 70 °C for about 4 h with constant stirring. The hydrolysate was dried in vacuum at 45 °C and re-dissolved by methanol–water (1:1, v/v) solution, which was filtered through a membrane filter and injected for a reversed-phase HPLC–DAD–ELSD analysis. The HPLC system using CH_3CN (B) and H_2O (A) as mobile phase was run with a gradient program at 1 mL·min^{-1} (15%B–20%B, 0–10 min; 20%B, 10–20 min), flow rate of mobile phase, UV detection wavelength, drift tube temperature, nitrogen flow-rate and gain were set at 1.0 mL·min^{-1}, 254 nm, 85 °C, 2.0 L·min^{-1} and 8. HPLC analysis of the hydrolysate from compound **1** revealed the presence of its aglycone (R_t = 12.94 min) and glucopyranose (R_t = 2.50 min); their retention time was identical with that of compound **2** and standand glucopyranose. The hydrolysate and standand glucopyranose were also spotted on an analytical silica gel TLC plate [the plate was developed with n-BuOH–HOAc–H_2O (3:1:1, v/v/v), sprayed aniline-oxalic acid solution for visualization]. The hydrolysate from compound **1** exhibited a dark yellow spot (R_f = 0.51) which was identical with that observed for standand glucopyranose.

2.5. Preparation of (*R*) and (*S*)-MTPA Esters (**2a** and **2b**) of **2**

Compound **2** (7.0 mg) was dissolved in 500 μL of dry pyridine and stirred at room temperature (rt) for 10 min. For preparation of the (*R*)-MTPA ester (**2a**) of **2**, 50 μL of (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPA-Cl) was added to the reaction vial, and the mixture was stirred at rt for 16 h. Completion of the reaction was monitored by LC/MS. The reaction mixture was dried in vacuo and redissolved in CH_3OH, and purification by analytical ODS HPLC using 85% CH_3CN in H_2O provided (*R*)-MTPA ester of compound **2** (**2a**, 6.5 mg).

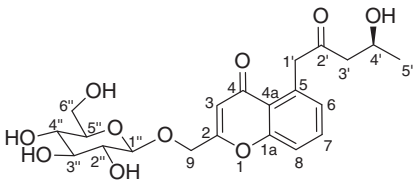
Compound **2a**: white, amorphous solid; ^{1}H NMR data (400 MHz, CD_3OD) δ 7.69 (t, *J* = 7.9, H = 7), 7.55 (d, *J* = 7.0, H-8), 7.06 (d, *J* = 7.4, H-6), 6.22 (s, H-3), 5.63 (m, H-4′), 5.36 (s, H-9), 4.26 (d, *J* = 17.0, H-1a′), 4.02 (d, *J* = 17.1, H-1b′), 3.28 (s, O-CH_3), 3.20 (s, O-CH_3), 3.13 (dd, *J* = 7.9, 17.5, H-3a′), 3.00 (dd, *J* = 4.5, 17.7, H-3b′), 1.43 (d, *J* = 6.2, H-5′); ESI-MS *m/z* 709.1 $[M+H]^+$.

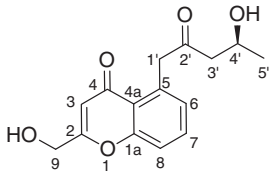
In an analogous way, (*S*)-MTPA ester (**2b**) of compound **2** was obtained from (*S*)-(+)-MTPA-Cl similarly to **2a**. (*S*)-MTPA ester of compound **2** was purified on an ODS analytical column using 85% CH_3CN in H_2O as eluent to obtain **2b** (7.0 mg).

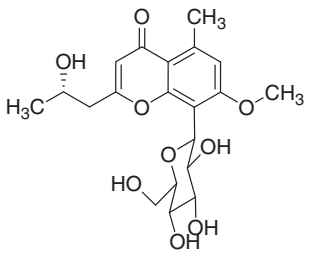
Compound **2b**: white, amorphous solid; ^{1}H NMR data (400 MHz, CD_3OD) δ 7.71 (t, *J* = 7.9, H = 7), 7.55 (d, *J* = 8.0, H-8), 7.16 (d, *J* = 7.3, H-6), 6.26 (s, H-3), 5.60 (m, H-4′), 5.36 (q, *J* = 14.12, 14.12, 14.15, H-9), 4.36 (d, *J* = 17.1, H-1a′), 4.17 (d, *J* = 17.1, H-1b′), 3.60 (s, O-CH_3), 3.47 (s, O-CH_3), 3.18 (dd, *J* = 8.36, 17.8, H-3a′), 3.00 (dd, *J* = 4.2, 17.8, H-3b′), 1.33 (d, *J* = 6.3, H-5′); ESI-MS *m/z* 731.0 $[M+Na]^+$.

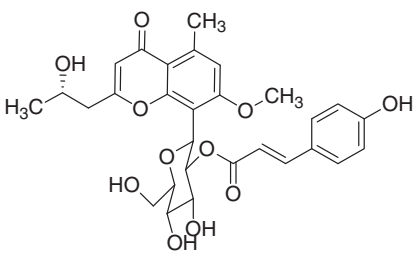
2.6. Determination of mushroom tyrosinase inhibition activity

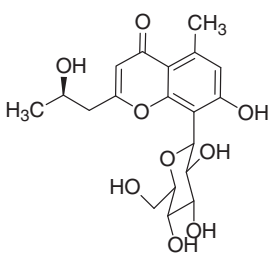
The mushroom tyrosinase inhibition activity of all tested compounds, using L-DOPA as substrate, was measured according to the method of Lin et al. [10] with slight modification. Mushroom tyrosinase and L-DOPA used for the bioassay were each manufactured at Worthington Biochemical Corp. (Lakewood, NJ, USA) and Boston Biomedical Inc. (Boston, MA, USA). Phosphate used for preparing buffer was purchased from Tianjin Damao Chemical Reagent Factory (Tianjin, China). Mushroom tyrosinase, L-DOPA and tested samples were prepared by dissolving in 1/15 mol·L^{-1} Na_2HPO_4–NaH_2PO_4











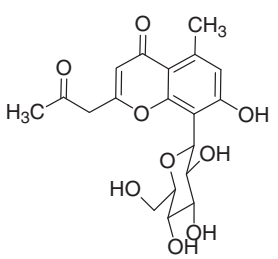


Fig. 1. The structures of compounds **1**–**6**.

buffer (pH 6.8). Reaction mixtures containing 50 μL of 2 mmol·L^{-1} of L-DOPA, 50 μL of phosphate buffer and 50 μL of different concentrations of tested compounds were added in 96 well microtiter plates, following by adding 50 μL of 0.2 mg·mL^{-1} of mushroom tyrosinase. Then, the absorbance variations accompanying the oxidation of the substrate (L-DOPA) were recorded using a FlexStation 3 microplate reader at 475 nm under a constant temperature of 37 °C. Average velocity (v) of the oxidation of the substrate (L-DOPA) was determined from the linear slope of curve. Arbutin was used as positive control. Inhibitory effects of tested samples on the enzyme activity were represented as % of relative enzyme activity = $v_t/v \times 100$, where v_t = average velocity of the oxidation of the substrate with tested sample and v = average velocity of the oxidation of the substrate without tested sample.

2.7. Determination of the inhibition type of compound **6** *on mushroom tyrosinase*

The kinetic behavior of mushroom tyrosinase during the oxidation of L-DOPA by compound **6** was studied according to the method of Cho et al. [11]. Mushroom tyrosinase (50 μL; 0.2 mg·mL^{-1}) was incubated with 50 μL of various concentrations of enzyme substrate and 50 μL of phosphate buffer, and then 50 μL of different concentrations of tested sample were simultaneously added to the reaction mixtures. The absorbance variations from these studies were used to generate Lineweaver–Burke plots to determine the inhibition type of aloesin on mushroom tyrosinase, and concentrations of compound **6** for curves 0–5 were 0, 100, 200, 300, 400, 500 μg·mL^{-1}, respectively. Kinetic parameter (K_m) of the tyrosinase activity was calculated by linear regression from Lineweaver–Burk plots. Inhibition constant (K_i) was calculated by Dixon graphical chart with $1/v$ versus the concentration of compound **6**, and concentrations of L-DOPA for curves 1–5 were 0.5, 0.7, 1.0, 1.4, 2.0 mmol·L^{-1}, respectively.

3. Results and discussion

Compound **1** was obtained as slightly white amorphous powder. The HRESIMS displayed the molecular ion peak at m/z 437.1448 $[M-H]^-$ (calcd. 437.1453) corresponding to the molecular formula $C_{21}H_{26}O_{10}$, which was consistent with the NMR data. The IR spectrum exhibited absorption bands for hydroxyl groups (3401 cm^{-1}), carbonyl group (1712 cm^{-1}), and aromatic moiety (1652, 1605 and 1480 cm^{-1}). The UV maximum absorption (λ_{max} 225, 248 and 303 nm) displayed a chromone ring feature [12]. Analysis of 1D-NMR spectra data of **1** (Table 1) further displayed the presence of a chromone skeleton [one olefinic proton singlet at δ_H 6.47 (s, H-3); three aromatic protons at δ_H 7.18 (d, J = 7.2, H-6), 7.69 (t, J = 7.9, H-7) and 7.51 (d, J = 8.4, H-8); four quaternary sp^2 carbons at δ_C 122.7 (C-4a), 159.2 (C-1a), 137.3 (C-5) and 166.7 (C-2), showing no HSQC correlation with any protons; a conjugated carbonyl carbon at δ_C 182.1(C-4)] [13], a β-linked glucosyl moiety [an anomeric proton at δ_H 4.48 (d, J = 7.7, H-1″); δ_C 104.0, 74.9, 77.9, 71.4, 77.7 and 62.6], a 2-oxo-4-hydroxypentyl group [a methyl (δ_C 23.6, C-5′); two methylenes (δ_C 50.7 and 52.6, C-1′ and C-3′); a hydroxyl-bearing methine (δ_C 65.2, C-4′) and a carboxyl (δ_C 210.1, C-2′)] [14], and an oxygenated

Table 1
^{13}C (100 MHz) and ^{1}H NMR (400 MHz) data of compound **1**[a] and **2**[b].

Carbon	1		2	
	δ_C	δ_H mult. (*J* in Hz)	δ_C	δ_H mult. (*J* in Hz)
2	166.7		170.4	
3	111.0	6.47, s	109.5	6.41, s
4	182.1		182.0	
5	137.3		137.7	
6	130.8	7.18, d (7.2)	130.6	7.24, d (7.3)
7	135.1	7.69, t (7.9)	134.8	7.74, t (7.9)
8	119.1	7.51, d (8.4)	119.0	7.56, d (8.5)
1a	159.2		159.3	
4a	122.7		122.9	
9	67.8	4.82, d (15.0)	61.5	4.58, s
		4.69, d (15.0)		
1′	50.7	4.36, d (17.3)	50.7	4.45, d (17.3)
		4.26, d[c](17.3)		4.34, d[c](17.3)
2′	210.1		209.0	
3′	52.6	2.84, m	52.8	2.90, m
4′	65.2	4.29, m[c]	65.3	4.36, m[c]
5′	23.6	1.24, d (6.2)	23.7	1.32, d (6.4)
1″	104.0	4.48, d (7.7)		
2″	74.9	3.26, m		
3″	77.9	3.36, m		
4″	71.4	3.31, m		
5″	77.7	3.29, m		
6″	62.6	3.87, d (11.8)		
		3.68, m		

[a] Recorded in (CD_3OD+D_2O), *J* in Hz, δ in ppm.
[b] Recorded in (CD_3OD), *J* in Hz, δ in ppm.
[c] Overlapping signals.

methylene (δ_C 67.8, C-9). The aforementioned data suggested that compound **1** was a chromone glucoside derivative. 2D-NMR experiments established the structure of compound **1**(Fig. 2). The HMBC correlations from H-1′ to C-4a, C-6, and C-5, and from H-6 to C-1′ indicated that 2-oxo-4-hydroxypentyl was attached at C-5 of chromone ring. The oxygenated methylene was connected to C-2 of chromone ring by HMBC correlations of H-9/C-2 and C-3, and H-3/C-9. The attachment of the glucosyl moiety to C-9 was made by the HMBC correlations of H-9/C-1″ (anomeric carbon) and H-1″ (anomeric proton)/C-9. Comparison of acid hydrolysis products of compound **1** with standard glucopyranosyl confirmed the presence of glucopyranosyl in **1**. Thus, compound **1** was identified as 5-(2′-oxo-4′-hydroxypentyl)-2-(β-glucopyranosyl-oxy-methyl) chromone.

Compound **2**, yellowish amorphous powder, had a molecular formula $C_{15}H_{16}O_5$ as determined by HRESIMS (*m/z* found 275.0917 $[M-H]^-$, calcd. 275.0925). The IR, UV, ^{1}H and ^{13}C NMR spectra of **2** (Table 1) were similar to those of **1**, except for the absence of the signals for glucosyl moiety in **2**, indicating that **2** was a de-glucosyl derivative of **1**. The molecular formula of compound **2** showed 162 mass units less than that of compound **1**, which supported the assumption made above.

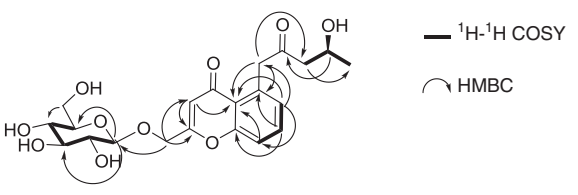
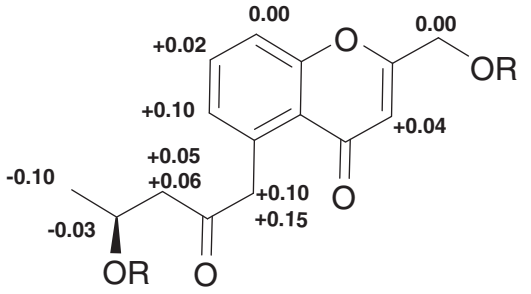


Fig. 2. Key ^{1}H–^{1}H COSY and HMBC correlations for compound **1**.



2a,R=(*R*)-MTPA
2b,R=(*S*)-MTPA

Fig. 3. $\Delta\delta_{SR}$ values ($\Delta\delta_{SR}=\delta_S-\delta_R$) obtained for (*S*)- and (*R*)-MTPA esters of **2**.

The extensive analyses of 1D- and 2D-NMR data of compound **2** and acid hydrolysis of compound **1** suggested that **2** should be the aglycone of compound **1**. The absolute configuration of the hydroxy-bearing carbon (C-4′) of the 2-oxo-4-hydroxypentyl chain in **2** was established by the Mosher's method [15,16]. Compound **2** (the aglycone of compound **1**) was treated with (*R*)-(−)- and (*S*)-(+)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride (MTPA-Cl) in dry pyridine separately to yield (*R*)- and (*S*)-MTPA ester derivatives **2a** and **2b**, respectively. All proton signals of the two Mosher diastereoisomeric esters were assigned by a HSQC experiment, and ^{1}H NMR chemical shift values of the (*S*)-MTPA esters (**2b**) were subtracted from the corresponding values of the (*R*)-MTPA esters (**2a**) (Fig. 3). Positive $\Delta\delta_{SR}$ ($\delta_S-\delta_R$) values were found for H-3a′ and H-3b′ (+0.05 and +0.06, respectively), while a negative $\Delta\delta_{SR}$ value was found for H-5′CH_3 (−0.10). Following the MTPA rules, these data indicated the *S*-configuration for the hydroxy-bearing carbon (C-4′) in compound **2**. Thus, the structure of compound **2** was elucidated as 5-((*S*)-2′-oxo-4′-hydroxypentyl)-2-methoxychromone, and the *S*-configuration of the hydroxy-bearing carbon (C-4′) in **1** (glucoside derivative of compound **2**) could also be established.

Four known compounds (**3**–**6**) were identified by comparing their physical and spectral data with literature data. They were 8-C-glucosyl-7-O-methyl-(*S*)-aloesol (**3**) [12], isoaloeresin D (**4**) [12], 8-C-glucosyl-(*R*)-aloesol (**5**) [17] and aloesin (**6**) [12].

Preliminary screening with mushroom tyrosinase revealed that the aqueous extract of *A. barbadensis* inhibited L-DOPA

Table 2
Mushroom tyrosinase inhibition of compounds **1**–**6**.

Compounds	Relative enzyme activity[a] (%)
1	N
2	N
3	N
4	N
5	91.33
6	27.30
Aqueous extract	91.49
Arbutin[b]	86.62

N, no inhibition.
[a] The relative enzyme activity of tested samples were measured at the concentration of 500 $\mu g \cdot mL^{-1}$ on the mushroom tyrosinase.
[b] Positive control.

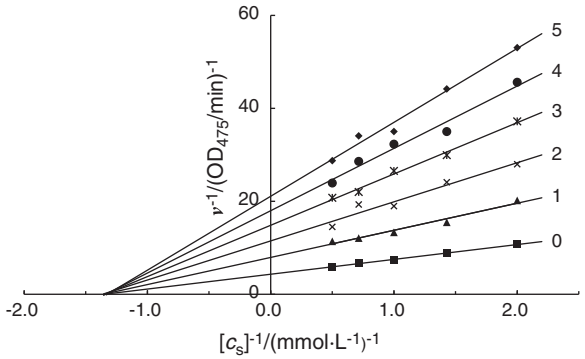


Fig. 4. Lineweaver–Burk plots for inhibition of compound **6** on mushroom tyrosinase for the catalysis of L-DOPA.

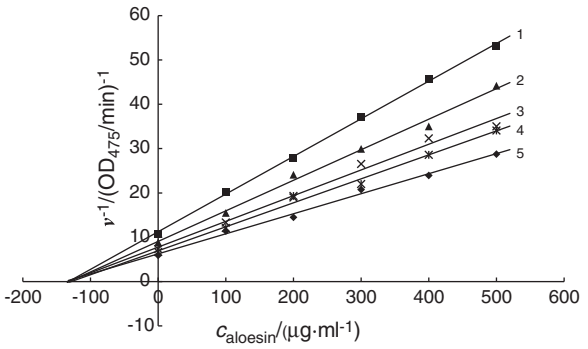


Fig. 5. Dixon plots for inhibition of compound **6** on mushroom tyrosinase.

oxidation (relative enzyme activity was 91.49%). Consequently, six chromones isolated from the aqueous extract were tested on mushroom tyrosinase assay in which L-DOPA was the substrate, and arbutin was the positive control (Table 2). Compound **6** strongly inhibited the enzyme activity and was more potent than positive control (arbutin); compound **5** showed slightly inhibitory activity and compounds **1**–**4** had no activity on mushroom tyrosinase. Some possible structure–activity relationship could be inferred from tyrosinase inhibitory assay results: (1) compounds **5** and **6**, the chromone derivatives with a 7-hydroxy at the chromone ring, showed inhibitory effect on mushroom tyrosinase; (2) when the 7-hydroxyl group at the chromone ring was absent or methylated, the compounds, such as compounds **1**–**4**, became inactive against mushroom tyrosinase; (3) compound **6** with 2-oxopropyl group at C-2 showed remarkable enzyme inhibitory activity compared with compound **5** with 2-hydroxypropyl group at C-2, and it inhibited the enzyme activity to a greater degree than the positive control (arbutin); (4) these studies suggested that both 7-hydroxyl group and 2-oxopropyl group of the chromone affected the tyrosinase inhibitory activity, with the 2-oxopropyl group playing a more important role, possibly. Additionally, the inhibition kinetics of compound **6** analyzed by the Lineweaver–Burk plots (Fig. 4 and Table 3) indicated that compound **6** was a noncompetitive inhibitor of mushroom tyrosinase with an IC_{50} value of 108.62 $\mu g \cdot mL^{-1}$ because the vertical axis intercept ($1/V_m$) enlarged with increasing of the inhibitor's concentration but with a common the horizontal axis intercept ($-(1/K_m)$), then the K_m was equal to 0.78 ± 0.03 $mmol \cdot L^{-1}$; and the equilibrium constant for inhibitor binding with the free enzyme (K_i) obtained from the horizontal axis intercept ($-K_i$) in Dixon plots (Fig. 5) was 130.88 ± 3.90 $\mu g \cdot mL^{-1}$.

In summary, two new chromones (**1**–**2**), together with four known analogues (**3**–**6**) were isolated from the aqueous extract of *A. barbadensis* Miller. All the isolates were tested on mushroom tyrosinase assay, and compound **6** appeared to be the key tyrosinase inhibitor in the aqueous extract of *A. barbadensis* Miller and has great potential to be developed into an anti-browning agent for food products and skin whitening agent for cosmetics.

Table 3
Inhibition constants of compound **6** for mushroom tyrosinate.

IC_{50}^{*} ($\mu g \cdot mL^{-1}$)	108.62
K_m ($mmol \cdot L^{-1}$)	0.78 ± 0.03
K_i ($\mu g \cdot mL^{-1}$)	130.88 ± 3.90
Inhibition	Reversible
Inhibition type	Noncompetitive

IC_{50}^{*}, the inhibitor concentrations leading to 50% activity lost.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.fitote.2012.09.028.

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