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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 μg·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 (^{1}H, ^{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