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Caged xanthones displaying protein tyrosine phosphatase 1B (PTP1B) inhibition from *Cratoxylum cochinchinense*

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ABSTRACT

Four new caged xanthones (**1**–**4**) and two known compounds (**5**, **6**) were isolated from the roots of *Cratoxylum cochinchinense*, a polyphenol rich plant, collected in China. The structures of the isolated compounds (**1**–**6**) were characterized by obtaining their detailed spectroscopic data. In particular, compounds **1** and **6** were fully identified by X-ray crystallographic data. The isolated compounds (**1**–**6**) were evaluated against protein tyrosine phosphatase 1B (PTP1B), which plays an important role in diabetes, obesity, and cancer. Among these compounds, **3**, **4**, and **6** displayed significant inhibition with IC_{50} values of 76.3, 43.2, and 6.6 μM, respectively. A detailed kinetic study was conducted by determining K_m, V_{max}, and the ratio of K_{ik} and K_{iv}, which revealed that all the compounds behaved as competitive inhibitors.

1. Introduction

Cratoxylum cochinchinense Lour. is a renowned polyphenol-rich plant that belongs to the family Guttiferea and is widely distributed throughout southeast Asian countries, especially China [1]. The plant has been extensively used in folk medicine to treat cough, fever, diarrhea, and ulcer; it has also found use as a food spice and tea products [2]. *C. cochinchinense* has diverse chemical constituents such as xanthones, flavonoids, tocotrienols, triterpenoids, and caged xanthones [3–10]. The reported biological functions, namely antimalarial, antibacterial, cytotoxic and anti-HIV effects, are related to the xanthones, which are the most abundant secondary metabolites in this plant [11–13]. Among them the caged xanthones have been regarded as minor metabolites, and to date only three caged xanthones, cochinchinoxanthone, cochinchinone C, and cochinchinone D have been reported from *C. cochinchinense* [9,10,14]. In this study, we isolated four new caged xanthones, which were potential inhibitors of protein tyrosine phosphatase 1B (PTP 1B).

PTP1B belongs to the family of protein tyrosine phosphatase (PTPs) enzymes, and catalyzes the de-phosphorylation of protein tyrosine and regulates the cellular levels of phosphorylation [15]. This enzyme family is actively involved in controlling various cellular processes like differentiation, proliferation, migration, apoptosis, and responses to the immune system [16–18]. PTP1B has a prominent role in the down regulation of insulin and leptin signaling pathways by dephosphorylating the insulin receptor (IR) and the insulin receptor substrate (IRS), thereby modulating the glucose and lipid metabolism, which results in the development of diabetes and obesity [19]. Recently, over-expression of this enzyme has also been linked with breast cancer and tumorigenesis [20,21]. Moreover, PTP1B knockout mice have proven to be highly resistant to obesity and hypersensitive to insulin, as observed in a glucose tolerance test [22]. Thus, deletion of PTP1B or its inhibition by bioactive molecules results in enhanced insulin and leptin sensitivity; hence, PTP1B is an effective target and its inhibition is a promising strategy for the treatment of type 2 diabetes (T2DM) and the prevention of obesity.

Caged xanthones are known to be a class of compounds that display potent PTP1B inhibition [23]. In the course of searching for new PTP1B inhibitors, we discovered that the extract of *C. cochinchinense* showed potent PTP1B inhibition.

The present study aimed to investigate the caged xanthones that occur in *C. cochinchinense* and their structures were established in terms of spectroscopic data including X-ray crystallographic analysis. All isolated compounds were examined for their PTP1B inhibitory activities, followed by an investigation of their inhibitory mechanisms, which were ascertained using double reciprocal plots.

2. Experimental section

2.1. General experimental procedures

1D and 2D NMR spectra were recorded on a Bruker (AM 500 MHz) spectrometer (Billerica, MA), using either $CDCl_3$ or

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acetone-D6 as solvent and tetramethylsilane (TMS) as an internal standard. X-ray data were collected on a Bruker instrument. Melting points were measured on a Thomas Scientific Capillary Melting Point Apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Varian 640-IR (Varian, Inc. USA) infrared Fourier transform spectrophotometer (KBr). UV spectra were measured using a Beckman DU650 spectrophotometer. Electron ionization (EI) and EI high-resolution (HR) mass spectra were obtained on a JEOL JMS-700 instrument (JEOL, Tokyo, Japan). A UPLC system coupled with Q-TOF/MS (from Waters Corp. Milford, MA, USA) was used for ESIMS and HRESIMS analysis. Optical rotations were measured on a Perkin-Elmer 343 polarimeter (Perkin-Elmer, Bridgeport, USA). Column chromatography was performed using silica gel (230–400 mesh; Merck Co., Darmstadt, Germany), YMC-gel ODS-A (S-75 μm; YMC), and Sephadex LH-20 (GE Healthcare Bioscience AB, Uppsala, Sweden). Enzymatic assays were carried out on a SpectraMax M3 Multi-Mode microplate reader (Molecular Devices, USA). All chemicals were analytical grade.

2.2. Plant material

The *C. cochinchinense* roots were collected from the Yunfu area, Guangdong province, China, in February 2015 and identified by Huagu Ye, a senior scientist at the Chinese Academy of Science, Guangdong. A voucher specimen (No. 22719) of this raw material was deposited at the Herbarium of the South China Botanical Garden, the Chinese Academy of Science, Guangdong, China.

2.3. Extraction and isolation

Bark from the dried root of *C. cochinchinense* (1.4 kg) was extracted with methanol (15 L × 3) at room temperature for 7 days. The combined extract was evaporated to give a reddish black residue of (195 g), which was suspended in H_2O (2.5 L) and further portioned with hexane (3 L × 3) and ethyl acetate (3 L × 3). The ethyl acetate extract (25 g) was subjected to silica gel CC (10 × 40 cm, 230–240 mesh, 750 g) using a gradient of hexane to ethyl acetate (50:1 → 1:2, *v/v*), which provided 10 fractions (A-J). Fraction F (1.3 g) was chromatographed over MPLC, with reversed silica gel CC (130 g) and eluted with a gradient flow of MeOH in H_2O (0 to 100%, *v/v*) at a rate of 10 mL/min, to afford four sub-fractions (F1-F4). Sub-fraction F2 (220 mg) enriched with compound **5** and **6** was further repeatedly separated through a Sephadex LH-20 column, eluting with a solvent system of MeOH: H_2O (90:10, *v/v*) to afford compound **5** (80 mg) and **6** (30 mg). Fraction H (0.5 g) was fractionated through MPLC using reversed silica gel CC (40 g) with gradient elution of increasing MeOH in H_2O (30–100, *v/v*) to afford six sub-fractions (H1-H6). Sub-fractions H2 and H4 enriched with compound **1** and **4** were purified by Recycling preparative HPLC (YMC C_{18} column) having isocratic elution of MeOH-H_2O (80:20, *v/v*) to yield compound **1** (20 mg) and **4** (7.8 mg), respectively. Fraction J (1.2 g) was separated through MPLC using reversed silica gel CC (100 g) eluted with increasing MeOH in H_2O (40–100%, *v/v*), using a flow rate of 10 mL/min to afford sub-fractions (J1-J5). Sub-fractions J2 and J4, which were combined (80 mg) on the basis of the detection of similar patterns using TLC analysis, were enriched with compound **2** and **3**. These two sub-fractions were further purified over Recycling preparative HPLC (YMC C_{18} column) through isocratic elution with MeOH-H_2O (70:30, *v/v*) to afford compound **2** (16 mg) and **3** (15 mg), respectively. All the isolated compounds were identified on the basis of their spectroscopic data.

2.3.1. Cochinchinoxanthone A (**1**)

White powder, mp 168–170 °C; $[\alpha]_D^{25}$ +10 (*c* 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 230 (4.09), 275 (3.82), 345 (3.44) nm; IR (KBr) ν_{max} 3462, 1741, 1656 cm^{-1}; EIMS, *m/z* 412 $[M]^+$; HREIMS *m/z* 412.1882 (calcd for $C_{24}H_{28}O_6$, 412.1886); for ^1H NMR and ^{13}C NMR (500 MHz, $CDCl_3$) spectroscopic data, see Table 1.

2.3.2. Cochinchinoxanthone B (**2**)

White powder, mp 170–173 °C; $[\alpha]_D^{25}$ -59 (*c* 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 230 (4.19), 275 (4.01), 355 (3.64) nm; IR (KBr) ν_{max} 3377, 1741, 1647 cm^{-1}; EIMS, *m/z* 428 $[M]^+$; HREIMS *m/z* 428.1833 (calcd for $C_{24}H_{28}O_7$, 428.1834); for ^1H NMR and ^{13}C NMR (500 MHz, $CDCl_3$) spectroscopic data, see Table 1.

2.3.3. Cochinchinoxanthone C (**3**)

White powder, mp 141–143 °C; $[\alpha]_D^{25}$ +36 (*c* 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 230 (4.10), 285 (4.02), 315 (3.49) nm; IR (KBr) ν_{max} 3373, 1741, 1639 cm^{-1}; EIMS, *m/z* 428 $[M]^+$; HREIMS *m/z* 428.1837 (calcd for $C_{24}H_{28}O_7$, 428.1835); for ^1H NMR and ^{13}C NMR (500 MHz, $CDCl_3$) spectroscopic data, see Table 1.

2.3.4. Cochinchinoxanthone D (**4**)

White powder, mp 138–140 °C; $[\alpha]_D^{25}$ +8 (*c* 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 230 (4.11), 275 (3.73), 355 (3.32) nm; IR (KBr) ν_{max} 3479, 1749, 1647 cm^{-1}; EIMS, *m/z* 442 $[M]^+$; HREIMS *m/z* 442.1837 (calcd for $C_{25}H_{30}O_7$, 442.1992); for ^1H NMR and ^{13}C NMR (500 MHz, $CDCl_3$) spectroscopic data, see Table 1.

2.4. X-ray crystallographic analysis

Colorless crystals of **1** and yellow crystals of **5** and **6** were obtained from acetone–Et_2O–hexane (1:1:1, V/V/V). Crystal data for **1, 5,** and **6** were collected at 100 K on a Bruker SMART APEX II ULTRA diffractometer equipped with an APEX II CCD using Cu Kα radiation (λ = 0.71073 Å) generated by a rotating anode. Cell refinement and data reduction were performed with the software package APEX2. The structures were solved by direct methods using the SHEXLXTL package, expanded using different Fourier techniques, and refined by the program and full-matrix least-squares calculations. The non-hydrogen atoms were refined anisotropically, and hydrogen atoms were fixed at the calculated position. Crystallographic data for **1** and **6** were deposited with the Cambridge Crystallographic Data Center as CCDC1565983 and CCDC1565984, respectively. These data can be obtained free of charge from the Cambridge Crystallographic Data Center www.ccdc.cam.ac.uk (or e-mail: deposit@ccdc.cam.ac.uk).

2.4.1. Crystallographic data for **1**

$C_{24}H_{28}O_6$, MW = 412.46, monoclinic, space group P121/c1, Z = 4, a = 12.7157(2) Å, b = 14.5945(3) Å, c = 11.1324(2) Å; α = 90°, β = 95.1700(10), γ = 90°, V = 2057.54(6) $Å^3$, T = 173.15 K, μ (Cu Kα) = 0.095 (mm^{-1}), ρ_{calc} = 1.332 mg/m^{-3}, F(0 0 0) = 880, 33,844 reflections measure, 4037 unique (R_{int} = 0.0332), final R indices for I > 2σ(I), R1 = 0.0377, w*R*2 = 0.0916, R indices for all data R1 = 0.0444, w*R*2 = 0.0976, completeness to 2θ (25.242) 9.99%, maximum transition 0.978, minimum transmission 0.974.

2.4.2. Crystallographic data for **6**

$C_{23}H_{26}O_7$, MW = 414.44, monoclinic, space group P1211, Z = 2, a = 7.2242(4) Å, b = 13.4166(7) Å, c = 10.9509(6) Å; α = 90°, β = 107.986(3), γ = 90°, V = 1009.54(10) $Å^3$, T = 173.15 K, μ (Cu Kα) = 0.101 (mm^{-1}), ρ_{calc} = 1.363 mg/m^{-3}, F(0 0 0) = 440, 9627 reflections measure, 3731 unique (R_{int} = 0.0316), final R indices for I > 2σ(I), R1 = 0.0453, w*R*2 = 0.1144, R indices for all data R1 = 0.0550, w*R*2 = 0.1209, completeness to 2θ (25.242) 9.99%, maximum transition 0.983, minimum transmission 0.996.