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Four new cinnamoyl-phloroglucinols from the leaves of *Xanthostemon chrysanthus*

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ABSTRACT

Four new cinnamoyl-phloroglucinols (**1–4**) were isolated from the leaves of *Xanthostemon chrysanthus*. Compounds **1** and **2** represent the first example of natural phloroglucinols with an oxazole unit. Their structures were elucidated on the basis of NMR spectroscopic data and single crystal X-ray diffraction. Compound **3** showed moderate cytotoxic activity against MDA-MB-231 and SGC-7901 cells with IC_{50} values of 25.26 ± 0.35 μM and 31.2 ± 0.94 μM, respectively.

1. Introduction

The plant *Xanthostemon chrysanthus*, belonging to family Myrtaceae, is widely distributed in northern Australia and southeast Asia [1]. It is known by the common name 'Golden Penda' because of its spectacular golden flowers. Some species of genus Xanthostemon had been used as traditional herbal medicines [2]. However, the phytochemical investigation on Xanthostemon plants is limited, only the chemical compositions of volatile oils of several plants were analyzed [3]. As part of our search for structurally unique and biologically active constituents from Myrtaceae plants [4–7], four new cinnamoyl-phloroglucinols, xanchryones A–D (**1–4**), were isolated from the leaves of *X. chrysanthus*. Compounds **1** and **2** represent the first example of natural phloroglucinols with an oxazole unit. Herein, we describe the isolation, structural elucidation, plausible biosynthetic pathway and cytotoxic activities of these new compounds.

2. Experimental

2.1. General methods

Melting points were obtained on an X-5 melting point instrument (Fukai, Beijing, China) without correction. UV spectra were recorded on a Jasco V-550 UV/VIS spectrophotometer (Jasco, Tokyo, Japan). IR spectra were determined on a Jasco FT/IR-4600 plus Fourier transform infrared spectrometer (Jasco, Tokyo, Japan) using KBr pellets. HR-ESI-MS was carried out on Agilent 6210 LC/MSD TOF mass spectrometer (Agilent Technologies, CA, USA). NMR spectra were measured on Bruker AV-400 and AV-500 spectrometers (Bruker, Switzerland) with TMS as internal standard, and chemical shifts were denoted in δ values (ppm). Single-crystal data were performed using Oxford-Diffraction SuperNova diffractometer and Cu Kα radiation. Column chromatography (CC) were performed on Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden), silica gel (200–300 mesh; Qingdao Marine Chemical Inc., Qingdao, P. R. China), and ODS (YMC, Kyoto, Japan). Preparative HPLC was performed on an Agilent 1260 Chromatograph equipped with a G1311C pump and a G1315D photodiode array detector (Agilent Technologies, CA, USA) with a semi-preparative Cosmosil C18 (10 × 250 mm) column. All solvents used in CC and HPLC were of analytical (Shanghai Chemical Plant, Shanghai, China) grade and chromatographic grade (Fisher Scientific, NewJersey, USA), respectively.

2.2. Plant material

The leaves of *Xanthostemon chrysanthus* were collected in Guangzhou city, Guangdong province of P. R. China, in October of 2016. A voucher specimen (No. 2016100501) identified by Prof. Guang-Xiong Zhou (Jinan University) was deposited in the Institute of Traditional Chinese Medicine & Natural Products, Jinan University, Guangzhou, P. R. China.

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2.3. Extraction and isolation

The air-dried leaves of *X. chrysanthus* (20 kg) were powdered and extracted with 95% EtOH for 4 times (each 12 h) at room temperature. The pooled solution was evaporated to yield a crude extract (3.0 kg), which was suspended in H_2O and extracted with petroleum ether (PE, b.p. 60–90 °C). The PE extract (930 g) was subjected to a silica gel column chromatography eluted with a gradient mixture of PE-EtOAc (100:0 → 0:100) to afford ten fractions (Fr.1–10). Fr.4 (26.7 g) was separated on Sephadex LH-20 (6 × 160 cm, CH_3OH) to yield four subfractions (Fr.4A-4D). Fr.4D (10.2 g) was subjected to ODS column chromatography using CH_3OH-H_2O (50:50 → 100:0) as eluent to yield Fr.4D-1-4D-20. Then, Fr.4D-3 (200 mg) was separated on Sephadex LH-20 (2 × 150 cm, CH_3OH) to afford compound **3** (63 mg). Fr.4D-5 (300 mg) was purified by reversed-phase semi-preparative HPLC (CH_3CN-H_2O, 70:30, 3 mL/min) to afford compound **4** (45.8 mg, t_R 15.5 min). Fr.4D-10 (70 mg) was further purified by HPLC (CH_3CN-H_2O, 70:30, 3 mL/min) to afford compounds **1** (4.5 mg, t_R 26.8 min) and **2** (7.2 mg, t_R 30.9 min).

Compound 1: colorless blocks (CH_3OH); mp 188–189 °C; HRESIMS *m/z* 312.1235 $[M + H]^+$ (calcd for $C_{18}H_{18}NO_4$: 312.1230); UV (MeOH) λ_{max} (log ε): 206 (3.61), 293 (3.32) nm; IR (KBr) ν_{max} 3294, 2925, 1629, 1543, 1494, 1400, 1293, 1213, 1171, 1098, 1061, 797, 739, 697 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 1.

Compound 2: amorphous powder; HRESIMS *m/z* 326.1387 $[M + H]^+$ (calcd for $C_{19}H_{20}NO_4$: 326.1387); UV (MeOH) λ_{max} (log ε): 206 (3.82), 294 (3.70) nm; IR (KBr) ν_{max} 3422, 2926, 1626, 1498, 1453, 1398, 1316, 1248, 1209, 1176, 1103, 829, 746, 699 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Table 1.

Compound 3: colorless prisms (CH_3OH); mp 119–120 °C; HRESIMS *m/z* 317.1385 $[M + H]^+$ (calcd for $C_{18}H_{21}O_5$: 317.1384); UV (MeOH) λ_{max} (log ε): 207 (4.28), 282 (4.09), 354 (3.35) nm; IR (KBr) ν_{max} 3369, 2946, 1623, 1598, 1456, 1419, 1370, 1309, 1283, 1234, 1127, 1089, 1040, 1025, 953, 895, 866, 737, 698 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 1.

Table 1

^1H and ^{13}C NMR data of **1–4** ($CDCl_3$)[a].

NO.	**1**[b]		**2**[c]		**3**[b]		**4**[b]	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		150.1		150.9		149.7		162.6
2		122.6		123.8		131.7		106.0
3		155.5		154.9		156.9		152.3
4		111.5		110.9		110.5		127.8
5		162.3		161.2		158.9		161.6
6		100.9		100.9		105.8		104.5
7		201.4		201.2		205.2		206.0
8	3.51 3.49	44.1	3.47 3.45	44.0	3.48 3.44	45.7	3.49 3.47	45.7
9	3.12 3.09	30.1	3.11 3.08	30.4	3.06 3.03	30.5	3.04 3.02	30.1
10		141.2		141.3		141.6		141.1
11,15	7.30	128.6	7.31	128.6	7.28	128.5	7.28	128.6
12,14	7.30	128.7	7.31	128.7	7.28	128.5	7.28	128.6
13	7.23 (m)	126.4	7.22 (m)	126.3	7.21 (m)	126.1	7.21 (m)	126.2
16	7.91 (s)	148.4		159.4	3.84 (s)	61.3	10.04 (s)	193.1
17	4.49 (s)	61.0	4.43 (s)	60.9	3.91 (s)	60.4	4.23 (s)	61.6
18	2.15 (s)	8.2	2.13 (s)	8.2	2.08 (s)	7.9		
19			2.60 (s)	14.5				
OH-1					6.79 (br s)		14.14 (br s)	
OH-5	13.43 (br s)		13.33 (br s)		13.35 (br s)		15.35 (br s)	

[a] Overlapped signals were reported without designating multiplicity.

[b] ^1H NMR measured at 400 MHz and ^{13}C NMR measured at 100 MHz.

[c] ^1H NMR measured at 500 MHz and ^{13}C NMR measured at 125 MHz.

Compound 4: yellow needles (CH_3OH); mp 175–176 °C; HRESIMS *m/z* 339.0840 $[M + Na]^+$ (calcd for $C_{17}H_{16}NaO_6$: 339.0839); UV (MeOH) λ_{max} (log ε): 205 (4.01), 272 (4.17), 368 (3.48) nm; IR (KBr) ν_{max} 3289, 1605, 1478.17, 1443, 1386, 1321, 1295, 1263, 1155, 955, 848, 797, 700, 610 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 1.

2.4. X-ray analysis

Crystal data for ***1***: $C_{18}H_{17}NO_4$, monoclinic, space group *I*2/*m*, $a = 8.49350(10)$ Å, $b = 6.68410(10)$ Å, $c = 26.1980(3)$ Å, $\beta = 95.3770(10)°$, $V = 1480.75(3)$ Å^3, $Z = 4$, $T = 100.00(10)$ K, μ(Cu Kα) = 0.815 mm^{-1}, $D_{calcd} = 1.396$ g/cm^3, 13,836 reflections measured ($6.77° \le \theta \le 147.69°$), 1634 unique ($R_{int} = 0.0329$, $R_{sigma} = 0.0135$) which were used in all calculations. The final R_1 was 0.0408 [$I > 2\sigma(I)$] and wR_2 was 0.1086 (all data). CCDC-1819352 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via http://www.ccdc.cam.ac.uk/data_request/cif.

Crystal data for ***3***: $C_{18}H_{20}O_5$, monoclinic, space group $P2_1/c$, $a = 13.6908(4)$ Å, $b = 14.6122(3)$ Å, $c = 8.4187(2)$ Å, $\alpha = \gamma = 90.0°$, $\beta = 107.731(3)°$, $V = 1604.18(8)$ Å^3, $Z = 4$, $T = 100.00(10)$ K, μ(Cu Kα) = 0.786 mm^{-1}, $D_{calcd} = 1.310$ g/cm^3, 6893 reflections measured ($9.09° \le \theta \le 148.05°$), 3140 unique ($R_{int} = 0.0258$, $R_{sigma} = 0.0266$) which were used in all calculations. The final R_1 was 0.0453 [$I > 2\sigma(I)$] and wR_2 was 0.1229 (all data). CCDC-1823235 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via http://www.ccdc.cam.ac.uk/data_request/cif.

2.5. Cytotoxic assay

Human breast cancer cell line (MDA-MB-231) and human gastric cancer carcinoma cell line (SGC-7901) were obtained from American Type Culture Collection (ATCC). All of the cell lines were cultured in RPMI 1640 medium, supplemented with 10% fetal bovine serum (FBS) at 37 °C in a humidified atmosphere of 5% CO_2. Cells were cultured in 96-well plates for 24 h. Then the cells were treated with compounds **1–4** at various concentrations for 72 h. After incubated for another 4 h with 30 μL aliquot of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) solution (5 mg/mL in PBS), the medium was discarded, and 100 μL of DMSO was added to dissolve the produced formazan. The absorbance was measured at 570 nm using a microplate Reader (Thermo scientific multiskan MK3, USA).

3. Results and discussion

The EtOH extract of the leaves of *X. chrysanthus* was separated by column chromatographies over silica gel, Sephadex LH-20, ODS and preparative HPLC to yield four new cinnamoyl-phloroglucinols (**1–4**) (Fig. 1).

Compound **1** was obtained as colorless blocks. The molecular formula of **1** was established as $C_{18}H_{17}NO_4$ by its HR-ESI-MS data (*m/z* 312.1235 $[M + H]^+$, calcd for $C_{18}H_{18}NO_4$: 312.1230). The UV spectrum showed absorption maxima at 206 and 293 nm, suggesting the presence of a conjugated system. The IR spectrum showed characteristic absorptions for hydroxyl group (3294 cm^{-1}), conjugated carbonyl group (1629 cm^{-1}), and aromatic ring (1590, 1494 cm^{-1}). The ^1H NMR spectrum displayed signals for one methyl [δ_H 2.15 (3H, s, H-18)], a methoxyl [δ_H 4.49 (3H, s, H-17)], one ethylene group [δ_H 3.51 (1H, overlapped, H-8a), 3.49 (1H, overlapped, H-8b), 3.12 (1H, overlapped, H-9a), 3.09 (1H, overlapped, H-9b)], five aromatic protons [δ_H 7.30 (4H, overlapped, H-11, H-12, H-14, H-15) and 7.23 (1H, m, H-13)], an olefinic proton [δ_H 7.91 (1H, s, H-16)] and a chelated hydroxyl group [δ_H 13.34 (1H, s, OH-5)]. The ^{13}C NMR and DEPT spectra of **1** exhibited eighteen carbon signals including a carbonyl, seven quaternary