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CYTOTOXIC XANTHONES FROM *HYPERICUM CHINENSE*

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Abstract – Two new xanthones, 1-hydroxy-4-(2-hydroxyethyl)-3,8-dimethoxy-9*H*-xanthen-9-one (**1**) and (*S*)-1-hydroxy-4-(1-hydroxy-3-oxobutyl)-3,8-dimethoxy-9*H*-xanthen-9-one (**2**), together with three known xanthones (**3**-**5**) were isolated from the leaves and stems of *Hypericum chinense*. Their structures were elucidated by spectroscopic methods, including extensive 1D- and 2D NMR techniques. Compounds **1-5** were tested for their cytotoxicity against five human tumor cell lines (NB4, A549, SHSY5Y, PC3, and MCF7). The results revealed that compound **1** showed high cytotoxicity against A549 and PC3 cell with IC_{50} values of 2.8 and 3.4 μM, and **2** showed high cytotoxicity against PC3 cell with IC_{50} valve of 2.8 μM, respectively.

The family Clusiaceae is a rich source of xanthones.[1,2] Xanthones are typically polysubstituted and occur as either fully aromatized, dihydro-, tetrahydro-, or, more rarely, hexahydro- derivatives.[2] This family of compounds appeals to medicinal chemists because of their pronounced biological activity within a notably broad spectrum of disease states, including anti-hepatitis B virus,[3] anti-tobacco mosaic virus,[4] antibacterial,[5,6] antioxidant,[7,8] anti-inflammatory,[9] tumor-promoting inhibition,[10] cytotoxicity,[11,12] and the like, as a result of their interaction with a correspondingly diverse range of target biomolecules.

The genus *Hypericum* belonging to Clusiaceae is distributed widely in temperate regions, and has been used for traditional medicines in various parts of the world. In China, *Hypericum. chinese* is used as a folk medicine for treatment of female disorders.[13] Previous phytochemical investigations on *H. chinese*

resulted in the isolation of xanthones,[12] acylphloroglucinols,[14] lactones,[15] and norlignans.[16] With the aim of multipurpose utilization of herb plants and identify bioactive natural products from this genus, the phytochemical investigation on *H. chinese* was carried out. As a result, two new xanthones (**1** and **2**), together with three known xanthones (**3**-**5**), were isolated. Compound **2** is the first naturally occurring xanthone possessing a 1-hydroxy-3-oxobutyl moiety. The structures of new compounds were elucidated by comprehensive analysis of their NMR data. In addition, the cytotoxicity of **1**-**5** were evaluated. The details of the isolation, structure elucidation, and cytotoxicity of the isolates are reported in this article.

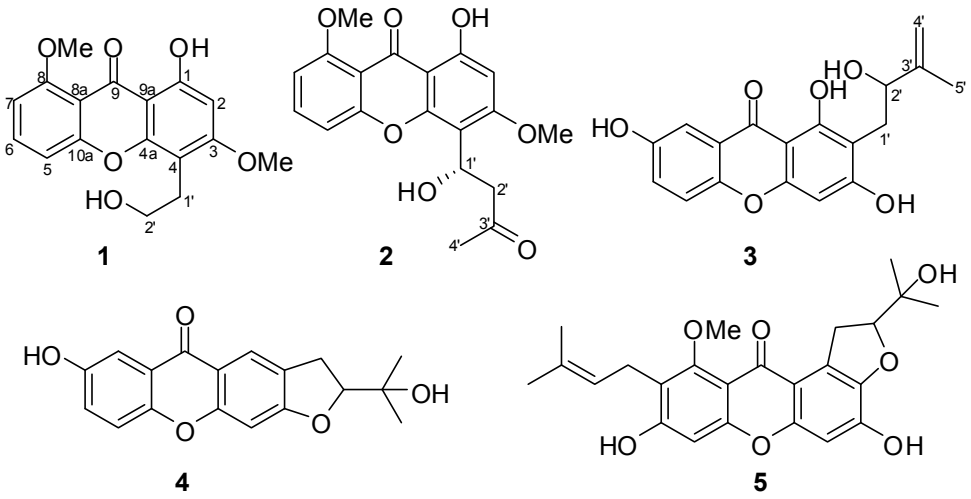

Figure 1. The structures of xanthones from *H. chinense*

A 70% aq. acetone extract prepared from the leaves and stems of *H. chinense* was subjected repeatedly to column chromatography on Silica gel, Sephadex LH-20, RP-18 and Preparative HPLC to afford compounds **1**-**5**, including two new xanthones, named 1-hydroxy-4-(2-hydroxyethyl)-3,8-dimethoxy-9*H*-xanthen-9-one (**1**) and (*S*)-1-hydroxy-4-(1-hydroxy-3-oxobutyl)-3,8-dimethoxy-9*H*-xanthen-9-one (**2**), together with three known xanthones, 1,3,7-trihydroxy-2-(2-hydroxy-3-methyl-3-butenyl)xanthone (**3**),[17] 1,7-dihydroxy-2,3-[2″-(1-hydroxy-1-methylethyl)dihydrofurano]xanthone (**4**),[17] cratoxylumxanthone D (**5**).[18] The structures of the compounds **1-5** were shown in Figure 1 and the ^{1}H and ^{13}C NMR data of **1** and **2** were listed in Table 1.

Compound **1** was isolated as a yellow gum. The HRESIMS of **1** gave the pseudomolecular $[M+Na]^{+}$ ion at *m*/*z* 339.0840, corresponding to a molecular formula of $C_{17}H_{16}O_6$. Its UV spectrum showed the maximum absorption at 302, 245, and 210 nm. Strong absorption bands accounting for hydroxy (3432 cm^{-1}), carbonyl (1658 cm^{-1}), and aromatic groups (1602, 1546, 1468 cm^{-1}) could also be observed in its IR spectrum. The ^{1}H- and ^{13}C NMR spectrum (Table 1) displayed signals for all 17 carbons and 16 protons, including a xanthones skeleton[19] (C-1 ~ C-9, C-4a, C-8a ~ C-10a; H-2, H-5 ~ H-7), two

methoxy groups (δ_C 56.0 q, 56.2 q; δ_H 3.85 s, 3.81 s), a hydroxyethyl unit [11] [δ_C 34.0 t, 63.8 t; δ_H 2.54 t (7.2), 3.67 t (7.2)], and a phenolic hydroxy group (δ_H 13.43 s). The typical proton signals of ring A [δ_H 6.85 d (8.3), 7.42 t (8.3), 6.70 d (8.3)] and ring B (δ_H 6.59 s) suggested that **1** should be a 1,3,4,8-tetrasubstituted xanthone.[19] The HMBC correlation (Figure 2) of one methoxy proton signal (δ_H 3.85) with C-3 (δ_C 161.0) showed this methoxy group was located at C-3. The correlation between the proton signal (δ_H 3.81) and C-8 (δ_C 165.4) indicated the other methoxy group located at C-8. The long-range correlations of H_2-1′ (δ_H 2.54) to C-3 (δ_C 161.0), C-4 (δ_C 108.8) and C-4a (δ_C 155.3), of H_2-2′ (δ_H 3.67) to C-4 (δ_C 108.8) were observed in **1**. This led us to conclude that the hydroxyethyl unit was located at C-4. Finally, the HMBC correlations between the phenolic hydroxy proton (δ_H 13.43) and C-1 (δ_C 162.8), C-2 (δ_C 98.1), and C-9a (δ_C 104.1) led to the assignment of the phenolic hydroxy group at C-1. Therefore, compound **1** was assigned as 1-hydroxy-4- (2-hydroxyethyl)-3,8-dimethoxy-9*H*-xanthen-9-one.

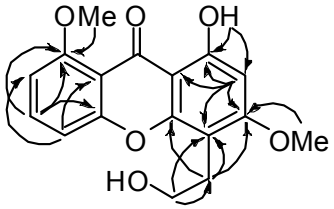

Figure 2. Key HMBC (↷) correlations of **1**

Table 1. ^{1}H and ^{13}C NMR Data of Compounds **1 and 2** (δ in ppm, 500 and 125 MHz)

No.	Compound **1**[a]		Compound **2**[b]	
	δ_C (m)	δ_H (m, *J*, Hz)	δ_C (m)	δ_H (m, *J*, Hz)
1	162.8 s		162.2 s	
2	98.1 d	6.59 s	97.1 d	6.40 s
3	161.0 s		161.4 s	
4	108.8 s		110.8 s	
5	110.8 d	6.85 d (8.3)	112.1 d	6.87 d (8.3)
6	135.9 d	7.42 t (8.3)	135.2 d	7.48 t (8.3)
7	106.5 d	6.70 d (8.3)	106.1 d	6.62 d (8.3)
8	165.4 s		165.5 s	
9	182.2 s		183.5 s	
4a	155.3 s		156.8 s	
8a	107.4 s		107.2 s	
9a	104.1 s		105.0 s	
10a	158.2 s		159.2 s	
1′	34.0 t	2.54 t (7.2)	63.1 d	5.13 dd (8.8, 3.2)
2′	63.8 t	3.67 t (7.2)	51.6 t	2.84 dd (15.4, 3.2) 2.43 dd (15.4, 8.8)
3′			205.0 s	
4′			31.5 q	2.14 s
3-OMe	56.0 q	3.85 s	55.8 q	3.83 s
8-OMe	56.2 q	3.81 s	56.1 q	3.80 s
Ar-OH		13.43 s		

[a] data obtained in C_5D_5N, [b] data obtained in CD_3OD

Compounds (**2**) was also isolated as a yellow gum, and its molecular formula was determined as $C_{19}H_{18}O_7$ through HRESI-MS analysis (pseudomolecular ion $[M+Na]^+$ at *m*/z 381.0956). The ^1H- and ^{13}C spectra data of **2** was very similar to those of **1** (see Table 1), except for the hydroxyethyl unit in **1** was replaced by a 1-hydroxy-3-oxobutyl moiety[20] [δ_C 63.1 d, 51.6 t, 205.0 s, 31.5 q; δ_H 5.13 dd (8.8, 3.2), 2.84 dd (15.4, 3.2), 2.43 dd (15.4, 8.8), 2.14 s] in compound **2**. Two methoxy groups located at C-3 and C-8, a phenolic hydroxy group located at C-1, and the 1-hydroxy-3-oxobutyl moiety at C-4 were also be concluded by the analysis of its HMBC spectrum. To determine the absolute configuration of **2**, the circular dichroism (CD) analysis was employed. The experimental CD spectrum of **2** exhibited a positive Cotton effect (CE) at 219 nm and a negative CE near 246 nm. The CEs, optical rotation, and coupling constant valves of **2** were in excellent agreement with these of known compound,[20] (1′*S*)-7-hydroxy-3-(1′-hydroxy-3′-butanoyl)chromone-5-carboxylic acid. Thus, compound **2** was determined as (*S*)-1-hydroxy-4-(1-hydroxy-3-oxobutyl)-3,8-dimethoxy-9*H*-xanthen-9-one.

Since xanthones are known to exhibit potent cytotoxicity,[2,11,12] the cytotoxicity of compounds **1**-**5** were tested using a previously reported procedure.[21] All treatments were performed in triplicate. In the MTT assay, the IC_{50} was defined as the concentration of the test compound resulting in a 50% reduction of absorbance compared with untreated cells. The cytotoxic abilities against NB4, A549, SHSY5Y, PC3, and MCF7 tumor cell lines by MTT-assay (with taxol as the positive control) were shown in Table **2**. The results revealed that compound **1** showed high cytotoxicity against A549 and PC3 cell with IC_{50} values of 2.8 and 3.4 μM, and **2** showed high cytotoxicity against PC3 cell with IC_{50} valves of 2.8 μM, respectively. The other compounds also showed moderate cytotoxicity for some tested cell lines with IC_{50} values below 10.

Table 2. Cytotoxicity data (IC_{50}, μM) for compounds **1** - **5**

Compounds	NB4	A549	SHSY5Y	PC3	MCF7
1	5.2	2.8	8.2	3.4	7.5
2	6.1	8.5	7.1	2.8	6.6
3	5.4	>10	7.8	>10	5.5
4	>10	9.6	>10	8.8	7.2
5	6. 7	>10	6.5	9.0	>10
taxol	0.03	0.02	0.2	0.2	0.1

NB4, human leukemia cell; A549, carcinomic human alveolar basal epithelial cell; SHSY5Y, human neuroblastoma cell; PC3, Human prostate cancer cell; MCF7, Human breast adenocarcinoma cell.

EXPERIMENTAL

General. Optical rotation was measured in Horiba SEPA-300 high sensitive polarimeter. UV spectra

were obtained using a Shimadzu UV-2401A spectrophotometer. IR spectra were obtained in KBr disc on a Bio-Rad Wininfmred spectrophotometer. ESI-MS were measured on a VG Auto Spec-3000 MS spectrometer. ^{1}H, ^{13}C, and 2D NMR spectra were recorded on Bruker DRX-500 instrument with TMS as internal standard. Column chromatography was performed on silica gel (200-300 mesh), or on silica gel H (10～40 μm, Qingdao Marine Chemical Inc., China). Second separation was performed by an Agilent 1100 HPLC equipped with ZORBAX-C_{18} (21.2 mm × 250 mm, 7.0 μm) column and DAD detector.

Plant material. The leaves and stems of *Hypericum chinense* L. were collected in Xishuangbanna Prefecture, Yunnan Province, People's Republic of China, in September 2010. The identification of the plant material was verified by Prof. Ren P. Y (Xishuangbanna Botanical Garden). A voucher specimen (YNNI-2010-9-22) has been deposited in our laboratory.

Extraction and Isolation. The air-dried and powdered leaves and stems of *H. chinense* (4.0 kg) were extracted four times with 70% acetone (4 ×6 L) at room temperature and filtered. The crude extract (256 g) was applied to silica gel (200–300 mesh) column chromatography, eluting with a $CHCl_3$-acetone gradient system (9:1, 8:2, 7:3, 6:4, 5:5), to give five fractions A–E. The further separation of fraction A (9:1, 18.5 g) by silica gel column chromatography, eluted with petroleum ether-EtOAc (9:1, 8:2, 7:3, 6:4, 1:1), yielded the mixtures A1–A5. The subfraction A2 (8:2, 4.2 g) was subjected to preparative HPLC (68% MeOH, flow rate 12 mL/min) to give **5** (14.8 mg). The further separation of subfraction A3 (7:3, 3.8 g) by silica gel column chromatography, and preparative HPLC (55~65% MeOH, flow rate 12 mL/min) to give **1** (8.5 mg), **2** (10.3 mg), **3** (15.7 mg), and **4** (16.0 mg).

Cytotoxicity Assay. The IC_{50} values of compounds were measured using the MTT assay. The MTT assay is a colorimetric assay for measuring the activity of cellular enzymes that reduce the tetrazolium dye, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a yellow tetrazole), to its insoluble formazan, giving a purple color. Firstly, 2500 cells suspended in 100 μL MEM medium were seeded, respectively, in a 96-well plate. After 24 h incubation, fresh medium containing various concentrations of each compound were added into the 96-well plate to replace the old medium. The concentrations were ranged from 100 μM to 1.5625 μM, which was achieved by doing twofold dilutions six times. The OD_{595} values of the control groups at 0 h and 72 h together with the compound treated groups at 72 h from the MTT assay were measured using a plate reader. IC_{50} is the concentration of a compound inhibiting 50% of the cell growth.

1-Hydroxy-4-(2-hydroxyethyl)-3,8-dimethoxy-9*H*-xanthen-9-one (**1**): Obtained as a yellow gum; UV (MeOH) λ_{max} (log ε) 210 (4.15), 245 (3.38), 302 (3.92) nm; IR (KBr) ν_{max} 3432, 3078, 2930, 2885, 1658, 1602, 1546, 1468, 1375, 1186, 1065, 885, 764 cm^{-1}; ESIMS *m/z* (positive ion mode) 339 $[M+Na]^+$; HRESIMS (positive ion mode) *m/z* 339.0840 $[M+Na]^+$ (calcd $C_{17}H_{16}O_6Na$ for 339.0845).

(*S*)-1-Hydroxy-4-(1-hydroxy-3-oxobutyl)-3,8-dimethoxy-9*H*-xanthen-9-one (**2**): Obtained as a yellow gum; $[\alpha]_D^{22.5}$ -45.6 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 210 (4.15), 246 (3.78), 305 (3.78) nm; CD (MeOH, *c* 0.25) $\Delta\varepsilon_{219}$ +0.94, $\Delta\varepsilon_{237}$ -5.56, $\Delta\varepsilon_{278}$ +0.28, $\Delta\varepsilon_{324}$ -0.92; IR (KBr) ν_{max} 3425, 3062, 2872, 2806, 1705, 1649, 1600, 1568, 1472, 1349, 1167, 1059, 875, 764 cm^{-1}; ESIMS *m/z* (positive ion mode) 381 $[M+Na]^+$; HRESIMS (positive ion mode) *m/z* 381.0956 $[M+Na]^+$ (calcd $C_{19}H_{18}NaO_7$ for 381.0950).

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