

Article

Prenylated Xanthenes from the Bark of *Garcinia xanthochymus* and Their 1,1-Diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Activities

Yu Chen^{1,2,3}, Hua Fan⁴, Guang-zhong Yang^{1,2,4,*}, Yan Jiang⁴, Fang-fang Zhong⁴ and Hong-wu He^{1,2,*}

¹ Key Laboratory of Pesticide and Chemical Biology, Ministry of Education, Central China Normal University, Wuhan 430079, China

² State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China

³ College of Chemistry and Material Sciences, South Central University for Nationalities, Wuhan 430074, China

⁴ Laboratory for Natural Product Chemistry, College of Pharmacy, South Central University for Nationalities, Wuhan 430074, China

* Authors to whom correspondence should be addressed; E-Mail: yanggz888@126.com (G.-z.Y.); he1208@mail.ccnu.edu.cn (H.-w.H.); Tel.: 86-27- 67841196; Fax: 86-27-67841196.

Received: 25 September 2010; in revised form: 7 October 2010 / Accepted: 11 October 2010 /

Published: 22 October 2010

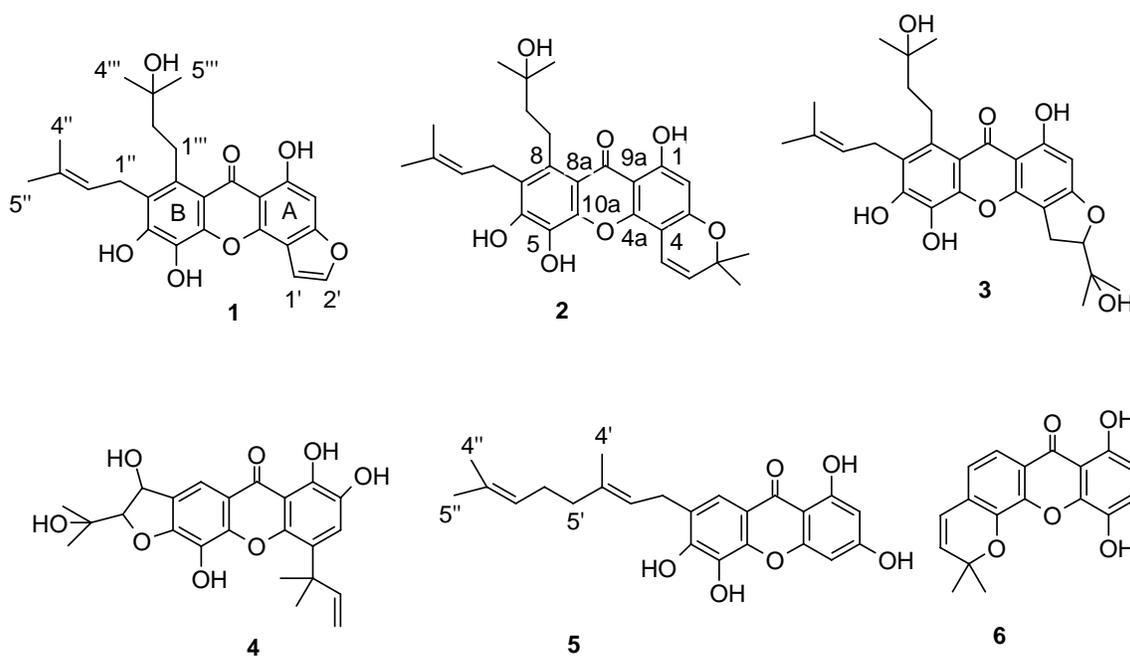
Abstract: *Garcinia xanthochymus* has been widely used in traditional Chinese medicine for expelling worms and removing food toxins. Bioassay-guided fractionation of an EtOAc-soluble extract of *G. xanthochymus* stem bark led to the isolation of six new xanthenes. Their structures were elucidated by spectroscopic methods, especially 2D-NMR techniques. Free-radical-scavenging activities of the isolated compounds were elucidated through DPPH method. Most of the isolated compounds showed considerable free radical scavenging activity on DPPH assay. Compound **1** exhibited effective antioxidant scavenging activity against DPPH radical with an IC₅₀ value of 19.64 μM, and compound **6** showed the lowest activity among all the tested molecules, with an IC₅₀ value of 66.88 μM. These findings support the notion that the plant genus *Garcinia* is a good source of bioactive compounds.

Keywords: Xanthenes; *Garcinia xanthochymus*; DPPH radical scavenging activity

1. Introduction

Reactive oxygen species (ROS) such as superoxide anion radical ($O_2^{\cdot-}$) and hydroxyl radical ($\cdot OH$) play an important role in the human body, and they are linked to the pathology of cirrhosis, cancer, and neurodegenerative diseases [1,2]. Oxidation could damage DNA, proteins, lipids and other small molecules. In order to prevent oxidative reactions in biological tissues against molecular targets, various synthetic or natural antioxidants can be used. However, synthetic antioxidants are not used extensively due to their toxicity and unwanted side effects. It has been suggested that natural antioxidants are safer and healthier than synthetic antioxidants. Therefore, more and more attention has been paid to the use of naturally occurring antioxidants for treatment or prophylaxis of various oxidative stress-related diseases [3]. Phenolic compounds play an important role in the antioxidative properties of many plant-derived antioxidants and they were also reported to possess a wide range of biological effects, such as antioxidant, antimicrobial, anti-inflammatory and vasodilatory actions [4].

Figure 1. Structures of compounds 1-6.



The genus *Garcinia* belongs to the Guttiferae family, which comprises 200 species confined to the tropics as trees or shrubs, and rarely subshrubs. It is well known to be a rich source of oxygenated and prenylated xanthenes [5]. Xanthenes are a class of polyphenolics that exhibit well-documented pharmacological properties, such as antioxidative, antileukaemic, antitumour, antiulcer, antimicrobial, antihepatotoxic, and CNS depressant activities [6], mainly due to their oxygenated heterocyclic nature and diversity of functional groups [7]. *Garcinia xanthochymus* is a traditional *Dai* medicine native to

the south and southwest of Yunnan Province, P. R. China which can grow up to 10-20 m. It has been widely used as a traditional medicine for expelling worms and removing food toxins [8]. Previous phytochemical studies of the leaves, seeds, fruits, twig bark, and wood have demonstrated the presence of benzophenones [9-15], flavonoids [16,17], triterpenes [18] and xanthenes [19-21]. In order to clarify the bioactive components, bioassay-guided fractionation has led to the isolation of six novel xanthenes **1-6** (Figure 1). Herein we report the isolation and structural elucidation of these new xanthenes and DPPH-radical scavenging activities of the isolated compounds.

2. Results and Discussion

2.1. Structural elucidations of xanthenes

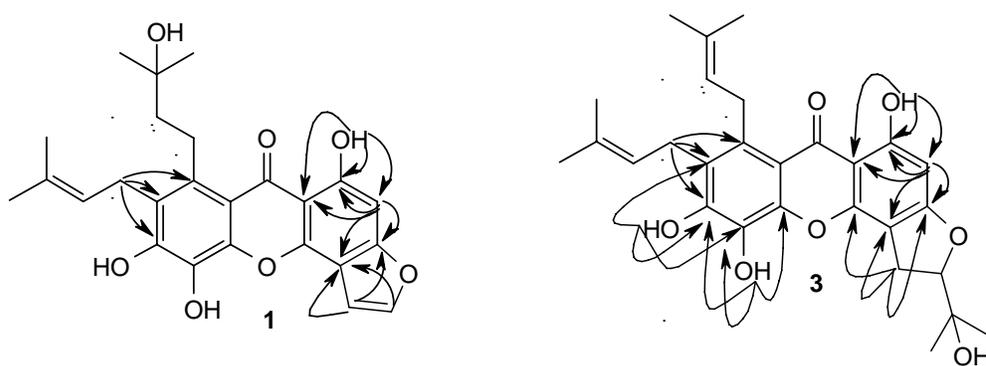
Compound **1** was obtained as a yellow powder. Its molecular formula $C_{25}H_{26}O_7$ was determined by the molecular ion peak at m/z 438.1698 in the HREIMS (calcd 438.1679). The UV spectrum of **1** had characteristic xanthone absorptions at 231, 265, 350 nm. In the 1H -NMR spectrum of **1**, three hydroxyl groups [δ_H 8.90, 9.12(1H each, s) and 13.61(1H, s, chelated)] and an aromatic proton [δ_H 6.85 (1H, s)] appeared, in addition to a 3-methyl-2-butenyl group, a 3-hydroxy-3-methylbutyl group and a fused furan ring [δ_H 7.39, 7.80 (1H each, br s)]. The presence of the fused furan ring was substantiated by the methine carbons (δ_C 104.9 and 144.8) in the ^{13}C -NMR spectrum. The HMBC correlations of the hydrogen-bonded proton (1-OH) with an oxygenated aromatic carbon at δ_C 160.9, a quaternary aromatic carbon at δ_C 105.8 and a methine aromatic carbon at δ_C 94.1 corresponding to an aromatic proton [δ_H 6.85 (1H, s)] in HSQC spectrum. It suggested that this proton may be attributed to H-2. The position of the furan ring was determined as follow. In the HMBC spectrum, one proton signal at δ_H 7.39 (1H, br s) of the furan ring showed correlations with a quaternary aromatic carbon at δ_C 108.5 (C-4) and an oxygenated aromatic carbon at δ_C 160.6 (C-3). The signals at δ_C 108.5 and 160.6 also correlated with the aromatic proton signal at δ_H 6.85 (1H, s, H-2). Therefore, the furan ring was fused at C-4 through an oxygen at C-3. The locations of other substituents were determined as follows. In the ^{13}C -NMR spectrum, the aromatic carbons with an oxygen function were observed at δ_C 149.4, 130.3 and 150.5, which suggested the presence of a 1, 2, 3-trioxygenated benzene ring in partial structure B. In HMBC spectrum (Figure 2), the correlations of $H_2-1''/C-6$ (δ_C 150.5), C-7 (δ_C 126.1) and C-8 (δ_C 136.4) indicated that one 3-methyl-2-butenyl group was located at C-7. Thus, the remaining 3-hydroxy-3-methylbutyl group should be located at C-8. Compound **1** was thus identified to be 1,5,6-trihydroxy-7-(3-methyl-2-butenyl)-8-(3-hydroxy-3-methylbutyl)furano(2',3':3,4) xanthone.

Compound **2** was obtained as a yellow powder. The molecular formula was determined as $C_{28}H_{32}O_7$ (m/z 480.2118) by HREIMS. Comparing its ^{13}C -NMR and DEPT data with those of **1**, it was found that compound **2** had almost the same chemical shifts as those of **1**, except for the dimethylpyran ring carbon signals at δ_C 115.9 (d), 127.2 (d), 78.3 (s), 28.0 (q) and 28.0 (q) in **2** instead of furan ring carbon signals at δ_C 104.9 (d) and 144.8 (d) in **1**. These facts suggested that dimethylpyran ring in the structure of **2** replaced furan ring found in **1**. Thus, **2** was identified to 1,5,6-trihydroxy-7-(3-methyl-2-butenyl)-8-(3-hydroxy-3-methylbutyl)-6', 6'-dimethylpyrano (2',3':3,4) xanthone.

Compound **3** was obtained as a yellow powder, whose molecular formula was determined as $C_{28}H_{34}O_8$ by the HREIMS (m/z 498.2256, calcd. 498.2254). Comparison of NMR data of **3** with those

of **1** indicated that the two compounds were closely related. The obvious spectroscopic differences between them resulted from the presence of a 2-(1-hydroxy-1-methylethyl)-2,3-dihydrofuran ring in **3**, instead of furan ring in **1**. The location of 2-(1-hydroxy-1-methylethyl)-2,3-dihydrofuran ring was fused at C-3 and C-4 of the xanthone nucleus with an ether linkage at C-3 by the HMBC correlations (Figure 2) between signals at δ_H 6.19 (H-2) to δ_C 163.7(C-1), 166.7(C-3) and δ_H 3.27(H₂-1') to δ_C 166.7 (C-3), 102.5 (C-4). Therefore, the structure of **3** was determined as 1,5,6-trihydroxy-7-(3-methyl-2-butenyl)-8-(3-hydroxy-3-methylbutyl)-5'-(1-hydroxy-1-methylethyl)-4', 5'-dihydrofurano (2',3':3,4) xanthone.

Figure 2. Significant HMBC correlations of compound **1** and **3**.



Compound **4** was obtained as a yellow amorphous powder. The $[M^+]$ at m/z 428.1464 in the HREIMS corresponds to $C_{23}H_{24}O_8$ (calcd 428.1472). The 1H -NMR spectrum of **4** exhibited one chelated hydroxy group [δ_H 13.0 (1H, s)], two aromatic proton as a singlet at δ_H 7.34 (1H, s) and δ_H 7.79 (1H, s), a typical signal of a 1,1-dimethylallyl group at δ_H 6.36 (1H, dd, $J = 17.7, 10.8$ Hz); 5.16 (1H, d, $J = 17.7$ Hz); 5.02 (1H, d, $J = 10.8$ Hz) and 1.65 (6H, s), as well as a 2-(1-hydroxy-1-methylethyl)-2, 3-dihydrofuran-3-ol moiety from resonances at δ_H 5.53 (1H, d, $J = 3.9$ Hz); 4.49 (1H, d, $J = 3.9$ Hz); 1.32 (3H, s) and 1.30 (3H, s). The ^{13}C -NMR and DEPT experiments displayed the presence of four methyl, five methine, one methylene, 12 quaternary carbons and one carbonyl. A partial structure A of **4**, 1, 2-dihydroxy-4-(1, 1-dimethylallyl)xanthone, was deduced by comparison of the 1H -NMR and ^{13}C -NMR data of **4** with those of subelliptenone H [22], and supported by a HMBC experiment (Table 3). The position of 2, 3-dihydrofuran ring was determined as follow. In HMBC spectrum, a singlet aromatic proton at δ_H 7.79 caused cross-peaks with δ_C 183.0 (C-9), which suggested that this proton was assigned to C-8. The aromatic carbons with an oxygen function were observed at δ_C 146.6, 129.6 and 154.0 in ^{13}C NMR spectrum, which indicated the presence of a 1, 2, 3-trioxygenated benzene ring in partial structure B. Therefore, the 2, 3-dihydrofuran ring was fuse at C-7 through an oxygen at C-6. Thus, the structure of **4** was determined as 1, 2, 5, 4'-tetrahydroxy-4-(1,1-dimethylallyl)-5'-(2-hydroxypropan-2-yl)-4', 5'-dihydrofurano-(2', 3' : 6, 7)xanthone.

Compound **5** was obtained as a yellow powder, whose molecular formula was determined as $C_{23}H_{24}O_6$ by the HREIMS (m/z 396.1574, calcd. 396.1573). By comparing the 1H -NMR spectrum of **5** with that of the previously isolated compounds from the same plant, **5** was identified to be an isomer of 1, 2, 5, 6-tetrahydroxy-7-geranyl xanthone [21]. The geranyl group was located at C-7 based on the HMBC correlations (Table 3) between δ_H 7.51 (H-8) with δ_C 180.7(C-9), 150.4(C-6), 29.1(C-1') and

δ_{H} 3.43 (H₂-1') with δ_{C} 126.5 (C-7). The ¹H NMR spectrum of **7** exhibited one chelated hydroxy group [δ_{H} 13.22 (1H, s)], two *meta*-aromatic proton at δ_{H} 6.20 (1H, br s) and δ_{H} 6.40 (1H, br s). Therefore, a coupling of *meta*-aromatic protons were assigned to C-2 and C-4 respectively, which was further supported by the HMBC correlations of H-2 with δ_{C} 165.5(C-3), 94.3(C-4) and 102.8(C-9a). Based on the above observation, the structure of **5** was established as 1, 3, 5, 6-tetrahydroxy-7-geranyl-xanthone.

Table 1. ¹H-NMR data of compounds **1-3** and **6**.

Position	1	2	3	6
1-OH	13.61 s		14.03 s	12.02 s
5-OH	8.90 s		9.43 s	
6-OH	9.12 s		9.82 s	
2	6.85 s	6.14 s	6.19 s	6.64 d (8.9)
3				7.32 d (8.9)
7				7.18 d (8.1)
8				7.72 d (8.1)
1'	7.39 br s	7.11 d (9.3)	3.27 m	6.59 d (9.8)
2'	7.80 br s	5.72 d (9.3)	4.77 m	6.02 d (9.8)
4'		1.42 s	1.17 s	1.54 s
5'		1.42 s	1.17 s	1.54 s
1''	3.56 d (5.6)	3.35 d (6.0)	3.39 d (5.4)	
2''	5.14 br s	5.15 br s	5.02 br s	
4''	1.83 s	1.84 s	1.77 s	
5''	1.68 s	1.70 s	1.65 s	
1'''	3.45 m	3.44 m	3.34 m	
2'''	1.76 m	1.74 m	1.55 m	
4'''	1.32 s	1.32 s	1.20 s	
5'''	1.32 s	1.32 s	1.20 s	

Compound **6** was obtained as a yellow powder. The molecular formula was determined as C₁₈H₁₄O₅ (*m/z* 310.0848) by HREIMS. The ¹³C NMR spectrum showed 18 carbon signals, which were classified into 2 methyl, 6 methine and 10 quaternary carbons by analysis of the DEPT spectra. The ¹H NMR data showed two sets of *ortho*-aromatic protons at δ_{H} 6.64 (1H, d, *J* = 8.9 Hz) and 7.32 (1H, d, *J* = 8.9 Hz); 7.18 (1H, d, *J* = 8.1 Hz) and 7.72 (1H, d, *J* = 8.1 Hz), one chelated hydroxyl group at δ_{H} 12.02 (1H, s), one *cis* olefinic group [δ_{H} 6.59 (1H, d, *J* = 9.8 Hz), 6.02 (1H, d, *J* = 9.8 Hz)] as well as two tertiary methyls attached to an oxygenated carbon [δ_{H} 1.54 (6 H, s)] indicating the presence of a dimethylpyran ring system. The positions of the substituents were deduced by analysis of HMBC. In the HMBC spectrum, there were correlations between the chelated hydroxyl group at δ_{H} 12.02 and the carbon signal at δ_{C} 154.2 (C-1) and 109.6 (C-2) corresponding to one of the *ortho*-coupled proton δ_{H} 6.64 (1H, d, *J* = 8.9 Hz) in the HSQC spectrum. These results indicated that **6** was a 1, 4-dihydroxyxanthone derivative. The other coupling of *ortho*-aromatic protons at δ_{H} 7.18 (1H, d, *J* = 8.1 Hz) and 7.72 (1H, d, *J* = 8.1 Hz) were assigned as H-7 and H-8, respectively, by the HMBC correlation of δ_{H} 7.72 with carbonyl carbon C-9 (182.4). The dimethylpyran ring was fused with the xanthone in an angular form which was further supported by HMBC correlation of δ_{H} 6.59 (1H, d, *J* = 9.8 Hz) with δ_{C} 141.7 (s, C-5), 127.6 (s, C-6) and 122.3 (d, C-7) and δ_{H} 6.02 (1H, d, *J* = 9.8 Hz) with δ_{C} 127.6 (s, C-6). Thus, the structure of compound **6** was established as 1, 4-dihydroxy-6', 6'-dimethylpyrano (2', 3': 5, 6) xanthone.

Table 2. ^{13}C -NMR data of compounds **1-3** and **6**.

Position	1	2	3	6
1	160.9 (qC)	163.8 (qC)	163.7 (qC)	154.2 (qC)
2	94.1 (CH)	99.0 (CH)	92.4 (CH)	109.6 (CH)
3	160.6 (qC)	160.3 (qC)	166.7 (qC)	124.5 (CH)
4	108.5 (qC)	104.0 (qC)	102.5 (qC)	138.2 (qC)
4a	146.1 (qC)	150.9 (qC)	150.4 (qC)	144.6 (qC)
5	130.3 (qC)	130.0 (qC)	129.7 (qC)	141.7 (qC)
10a	149.4 (qC)	146.8 (qC)	145.8 (qC)	146.1 (qC)
6	150.5 (qC)	151.3 (qC)	150.6 (qC)	127.6 (qC)
7	126.1 (qC)	125.6 (qC)	124.8 (qC)	122.3 (CH)
8	136.4 (qC)	136.7 (qC)	134.9 (qC)	117.3 (CH)
8a	112.0 (qC)	111.6 (qC)	110.1 (qC)	121.5 (qC)
9	183.8 (qC)	183.1 (qC)	181.9 (qC)	182.4 (qC)
9a	105.8 (qC)	101.1 (qC)	103.3 (qC)	109.4 (qC)
1'	104.9 (CH)	115.9 (CH)	26.7 (CH ₂)	122.1 (CH)
2'	144.8 (CH)	127.2 (CH)	91.6 (CH)	134.9 (CH)
3'		78.3 (qC)	70.0 (qC)	78.6 (qC)
4'		28.0 (CH ₃)	26.0 (CH ₃)	27.6 (CH ₃)
5'		28.0 (CH ₃)	24.9 (CH ₃)	17.6 (CH ₃)
1''	25.2 (CH ₂)	25.1 (CH ₂)	24.1 (CH ₂)	
2''	123.7 (CH)	123.7 (CH)	123.3 (CH)	
3''	131.5 (qC)	131.4 (qC)	130.6 (qC)	
4''	18.0 (CH ₃)	17.9 (CH ₃)	18.0 (CH ₃)	
5''	25.6 (CH ₃)	25.6 (CH ₃)	25.6 (CH ₃)	
1'''	24.8 (CH ₂)	24.8 (CH ₂)	24.5 (CH ₂)	
2'''	45.3 (CH ₂)	45.3 (CH ₂)	44.9 (CH ₂)	
3'''	70.0 (qC)	69.9 (qC)	69.0 (qC)	
4'''	28.9 (CH ₃)	28.9 (CH ₃)	29.1 (CH ₃)	
5'''	28.9 (CH ₃)	28.9 (CH ₃)	29.1 (CH ₃)	

Table 3. ^1H - and ^{13}C -NMR, HMBC data of compounds **4-5** in acetone- d_6 .

	4			5		
	δ_{H}	δ_{C}	HMBC	δ_{H}	δ_{C}	HMBC
1		148.0			164.4	
2		139.6		6.20 br s	98.4	C-3, 9a, 4
3	7.34 s	122.4	C-2, 4a, 1'		165.5	
4		126.1		6.40 br s	94.3	
4a		147.4			158.3	
5		129.6			132.1	
10a		146.6			145.3	
6		154.0			150.4	
7		126.1			126.5	
8	7.79 s	112.8	C-9	7.51 s	116.1	C-9, 6, 10a, 1'
8a		115.2			113.4	
9		183.0			180.7	
9a		109.0			102.8	
1'		40.5		3.43 d (6.6)	29.1	C-7, 2', 3'
2'	6.36dd (17.7,10.8)	147.8		5.41 t (6.6)	122.4	C-7, 1', 4', 5'

Table 3. Cont.

3'	5.16 d (17.7) 5.02 d (10.8)	110.9	C-1'		136.8	
4'	1.65 s	27.3	C-2', 3', 4	1.74 s	15.9	C-2', 3', 5'
5'	1.65 s	27.2	C-2', 3', 4	2.08 m	40.2	C-2', 3', 2''
1''	5.53 d (3.9)	72.4		2.12 m	28.3	C-2'', 3''
2''	4.49 d (3.9)	99.8		5.13 t (6.0)	124.7	C-5', 1'', 4'', 5''
3''		70.9			131.5	
4''	1.32 s	25.2	C-2'', 3''	1.63 s	25.5	C-2'', 3'', 5''
5''	1.30 s	25.5	C-2'', 3''	1.58 s	17.4	C-2'', 3'', 5''
1-OH	13.0 s		C-2	13.22 s		

2.2. DPPH radical-scavenging activities of the purified compounds

The six xanthenes were evaluated for their antioxidant activities by DPPH free radical scavenging method (Table 4). Most of the isolated compounds showed considerable free radical scavenging activity on DPPH assay. The potency of DPPH radical-scavenging activity was in a decreasing order: **1** > **3** > **2** > **5** > **4** > **6**.

Table 4. *In vitro* DPPH radical scavenging activities of prenylated xanthenes isolated from the bark of *G. xanthochymus*.

Compound	DPPH radical-scavenging activity (IC ₅₀ , μM)
1	19.64 ± 0.39
2	31.82 ± 0.08
3	22.07 ± 0.25
4	40.70 ± 0.10
5	34.27 ± 0.25
6	66.88 ± 0.19
ascorbic acid	13.16 ± 0.03
gallic acid	5.86 ± 0.03

Compound **1** exhibited effective antioxidant scavenging activity against DPPH radical, with an IC₅₀ value of 19.64 μM, and compound **6** showed the lowest activity with an IC₅₀ value of 66.88 μM among all the tested molecules. The DPPH radical scavenging activities of these compounds seemed to be related to the number of phenol-like OH groups at the xanthone skeleton. It was reported previously that the radical scavenging activity was increased in the presence of an increasing number of phenol like OH groups in a molecule [23]. However, compound **5**, having four phenol-like OH groups, showed a lower radical scavenging activity compared to that of compound **1** having three phenol-like OH groups. This was because the presence of furan ring in compound **1** extended the conjugation system to participate in stabilizing the phenoxy radical by resonances, therefore increasing the radical-scavenging activity of compound **1** [24]. From above the data, it can be deduced that the main components responsible for the antioxidant activities of *Garcinia xanthochymus* were the phenolic compounds, such as xanthone derivatives.

3. Experimental

3.1. General

UV spectra were measured on an SP-2102UVPC spectrometer using MeOH as the solvent. NMR spectra were run in DMSO- d_6 or Me₂CO- d_6 on a Bruker AM-400 (1D) or Varian Inova-600 (2D) spectrometer with TMS as an internal standard. EIMS and HREIMS measurements were conducted with a Finnigan MAT 95 instrument. Thin-layer chromatography (TLC) was performed on silica gel 60 GF₂₅₄, while column chromatography was carried out using silica gel (200-300 mesh) from Qingdao Haiyang Chemical Group Co., P. R. China and C₁₈ reversed-phase silica gel from YMC CO., LTD., Japan.

3.2. Plant material

The bark of *Garcinia xanthochymus* was collected from Xishuangbanna Prefecture, Yunnan Province, P.R. China and identified by Xishuangbanna Prefecture National Medicine Research Institute. The voucher specimen (06061201) was deposited with the Herbarium of College of Pharmacy, South Central University for Nationalities.

3.3. Extraction and isolation procedures

The powdered bark of *G. xanthochymus* (6.5 kg) was extracted with 95% EtOH (25 L × 3) and then successively partitioned with petroleum ether (P.E.) (3.0 L × 3), EtOAc (3.0 L × 3) and *n*-BuOH (3.0 L × 3). The combined EtOAc extract (590 g) was chromatographed on silica gel with P.E-Me₂CO (9:1, 8:2, 7:3, 1:1, 3:7, 0:1, v/v) to give thirteen fractions (fr.1–fr.13). Fr.6 (17.0 g) was separated on a silica column (toluene/Me₂CO 95:5→3:7 gradient system), and then purified by chromatography on a silica gel (CHCl₃-MeOH, 1:0→1:1 gradient system) and RP-18 (MeOH-H₂O, 8:2) to afford compound **6** (2.8 mg). Fr.7 (33.8 g) was extensively separated over a silica column (toluene/Me₂CO 95:5→3:7 gradient system) and RP-18 (MeOH-H₂O, 3:7→7:3 gradient system) to afford **1** (3.2 mg), **2** (9.8 mg) and **5** (4.8 mg). Fr. 9 (10.8 g) was also subjected to silica gel with a gradient elution (toluene-Me₂CO, 9:1→3:7 gradient system) and RP-18 (MeOH-H₂O, 3:7→7:3 gradient system) to afford compounds **3** (7.4 mg) and **4** (8.8 mg).

3.4. Physical data of new compounds

1,5,6-Trihydroxy-7-(3-methyl-2-butenyl)-8-(3-hydroxy-3-methylbutyl)-furano(2',3':3,4) xanthone (1). Yellow amorphous powder; UV λ_{\max} (MeOH) nm (log ϵ): 231 (3.50), 265 (3.49), 350 (3.58); For ¹H-NMR and ¹³C-NMR spectroscopic data (in Me₂CO- d_6), see Tables 1 and 2; EIMS (70 eV) *m/z* (%): 438 (M⁺, 28), 420 (36), 377 (76), 364 (48), 349 (100), 323 (80); HREIMS *m/z* 438.1698 (calcd. for C₂₅H₂₆O₇, 438.1679).

1,5,6-Trihydroxy-7-(3-methyl-2-butenyl)-8-(3-hydroxy-3-methylbutyl)-6',6'-dimethylpyrano (2',3' 3,4) xanthone (2). Yellow amorphous powder; UV λ_{\max} (MeOH) nm (log ϵ): 229 (3.52), 263 (3.52), 350 (3.63); For ¹H-NMR and ¹³C-NMR spectroscopic data (in Me₂CO- d_6), see Tables 1 and 2; EIMS

(70 eV) m/z (%): 480 (M^+ , 36), 463 (76), 447 (100), 419 (72), 391(84), 365 (64), 349 (56); HREIMS m/z 480.2118 (calcd. for $C_{28}H_{32}O_7$, 480.2148).

1,5,6-Trihydroxy-7-(3-methyl-2-butenyl)-8-(3-hydroxy-3-methylbutyl)-5'-(1-hydroxy-1-methyl-ethyl)-4',5'-dihydrofurano(2',3':3,4) xanthone (3). Yellow amorphous powder; UV λ_{max} (MeOH) nm (log ϵ): 250 (4.21), 285 (4.00), 334 (4.11); For 1H -NMR and ^{13}C -NMR spectroscopic data (in DMSO- d_6), see Tables 1 and 2; EIMS (70 eV) m/z (%): 498 (M^+ , 8), 480 (40), 437 (100), 424 (44), 409 (52), 383 (100), 365 (32); HREIMS m/z 498.2256 (calcd. for $C_{28}H_{34}O_8$, 498.2254).

1,2,5,4'-Tetrahydroxy-4-(1,1-dimethylallyl)-5'-(2-hydroxypropan-2-yl)-4',5'-dihydro furano-(2',3':6,7) xanthone (4). Yellow amorphous powder; UV λ_{max} (MeOH) nm (log ϵ): 230 (3.46), 264 (3.47), 337 (3.46), 389(sh)(3.09); For 1H -NMR and ^{13}C -NMR spectroscopic data (in Me_2CO-d_6), see Table 3; EIMS (70 eV) m/z (%): 428 (M^+ , 8), 410 (6), 392 (16), 352 (56), 319 (56), 319 (100); HREIMS m/z 428.1464 (calcd. for $C_{23}H_{24}O_8$, 428.1472).

1,3,5,6-Tetrahydroxy-7-geranyl xanthone (5). Yellow amorphous powder; UV λ_{max} (MeOH) nm (log ϵ): 260 (4.00), 341(4.01); For 1H -NMR and ^{13}C -NMR spectroscopic data (in Me_2CO-d_6), see Table 3; EIMS (70 eV) m/z (%): 396 (M^+ , 28), 327 (32), 311 (100), 274 (56), 123 (181), 69 (24); HREIMS m/z 396.1574 (calcd. for $C_{23}H_{24}O_6$, 396.1573).

1,4-Dihydroxy-6',6'-dimethylpyrano (2',3':5,6) xanthone (6). Yellow amorphous powder; UV λ_{max} (MeOH) nm (log ϵ): 230 (3.33), 264 (3.33), 354 (3.45), 400 (sh) (3.26); For 1H -NMR and ^{13}C -NMR spectroscopic data (in Me_2CO-d_6), see Table 1 and 2; EIMS (70 eV) m/z (%): 310 (M^+ , 36), 295 (100), 148 (15); HREIMS m/z 310.0848 (calcd. for $C_{18}H_{14}O_5$, 310.0841).

3.5. DPPH radical scavenging activity

Scavenging activities of the purified compounds from *G. xanthochymus* towards DPPH radical were assessed by using the method described by Scherer and Godoy with a slight modification [25,26]. Briefly, a 0.08 mM solution of DPPH radical solution in methanol was prepared and then, the solvent extracts and purified compounds at different concentrations (0.1 mL) were added to the prepared DPPH radical solution (3.9 mL); the mixture was shaken vigorously, after a 30 min incubation period at 37 °C in the dark, the absorbance was measured at 517 nm by using a UV-visible spectrophotometer. Obviously, decreasing of the DPPH solution absorbance indicates an increase of the DPPH radical-scavenging activity. The radical scavenging activity is given as DPPH radical scavenging effect that is calculated using equation (1):

$$\text{DPPH radical scavenging effect (\%)} = [(A_0 - A_1)/A_0] \times 100 \quad (1)$$

where A_0 was the absorbance of control and A_1 was the absorbance in the presence of the standard, solvent extracts or purified compounds at different concentrations. Ascorbic acid (V_C) and gallic acid were used as positive controls, respectively. All the tests were performed in triplicate. The scavenging activities of the purified compounds towards DPPH radical were expressed as IC_{50} , which was determined to be the effective concentration at which DPPH radical was scavenged by 50%. The IC_{50} value was obtained by interpolation from linear regression analysis.

3.6. Statistical analyses of results of activity studies

The results were performed as mean \pm standard deviation (SD) of three determinations. Analysis of significance differences among means were tested by one-way analysis of variance. The IC₅₀ values were calculated by linear regression analysis.

4. Conclusions

In the course of our ongoing research project on bioactive natural products from *G. xanthochymus*, an EtOAc-soluble partition of the EtOH extract of the bark of *G. xanthochymus* displayed significant antioxidant activity in the 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging bioassay. This prompted us to perform a detailed bioassay-guided isolation from this plant, which led to the isolation of six new xanthenes. Notably, most of the isolated compounds showed considerable free radical scavenging activity in the DPPH assay. Compound **1** exhibited effective antioxidant scavenging activity against DPPH radicals with an IC₅₀ value of 19.64 μ M, and compound **6** showed the lowest activity among all the tested molecules, with an IC₅₀ value of 66.88 μ M. These findings support that plant genus *Garcinia* is a good source of bioactive compounds.

Acknowledgements

This work was supported by the State key Laboratory of Drug Research (SIMM0901KF-02), National Basic Research Program of China (No. 2010CB126100), the National Natural Science Foundation of China (No.30670215; 20772042) and in part by the PCSIRT (No. IRT0953).

References and Notes

1. Hail, N., Jr.; Cortes, M.; Drake, E.N.; Spallholz, J.E. Cancer chemoprevention: A radical perspective. *Free Radic. Biol. Med.* **2008**, *45*, 97-110.
2. Aruoma, O.I.; Spencer, J.P.E.; Warren, D.; Jenner, P.; Butler, J.; Halliwell, B. Characterization of food antioxidants, illustrated using commercial garlic and ginger preparations. *Food Chem.* **1997**, *60*, 149-156.
3. Ningappa, M.B.; Dinesha, R.; Srinivas, L. Antioxidant and free radical scavenging activities of polyphenol-enriched curry leaf (*Murraya koenigii* L.) extract. *Food Chem.* **2008**, *106*, 720-728.
4. Wang, B.G.; Zhang, W.W.; Duan, X.J.; Li, X.M. *In vitro* antioxidative activities of extract and semi-purified fractions of the marine red alga, *Rhodomela confervoides* (*Rhodomelaceae*). *Food Chem.* **2009**, *113*, 1101-1105.
5. Mbwambo, Z.H.; Kapingu, M.C.; Moshi, M.J.; Machumi, F.; Apers, S.; Cos, P., Ferreira, D.; Marais, J.P.; Vanden Berghe, D.; Maes, L.; Vietinck, A.; Pieters, L. Antiparasitic activity of some xanthenes and biflavonoids from the root bark of *Garcinia livingstonei*. *J. Nat. Prod.* **2006**, *69*, 369-372.
6. Peres, V.; Nagem, T.J.; Oliveira, D.F.F. Tetraoxygenated naturally occurring xanthenes. *Phytochemistry* **2000**, *55*, 683-710.

7. Franklin, G.; Conceicao, L.F.R.; Kombrink, E.; Dias, A.C.P. Xanthone biosynthesis in *Hypericum perforatum* cells provides antioxidant and antimicrobial protection upon biotic stress. *Phytochemistry* **2009**, *70*, 60-68.
8. Lin, Y.F.; Zhuan, Y.; Zhao, Y.H. *Chinese Dai Medicine Colorful Illustrations* (in Chinese); Yunnan National Publishing House: Kunming, China, 2003; p. 6.
9. Karanjgoakar, C.G.; Rao, A.V.R.; Venkataraman, K.; Yemul, S.S.; Palmer, K.J. The constitution of xanthochymol and isoxanthochymol. *Tetrahedron Lett.* **1973**, *50*, 4977-4980.
10. Blount, J.F.; Williams, T.H. Revised structure of xanthochymol. *Tetrahedron Lett.* **1976**, *34*, 2921-2924.
11. Basa, S.C.; Mahanty, P.; Das, D.P. Isoxanthochymol: a revised structure. *Chem. Ind.* **1978**, *4*, 166-167.
12. Tandon, R.N.; Srivastava, O.P.; Baslas, R.K.; Kumar, P. Preliminary investigation of the antimicrobial activity of a phytochemical, xanthochymol from the fruits of *Garcinia xanthochymus* Hook. F. *Curr. Sci.* **1980**, *49*, 472-473.
13. Rao, A.V.R.; Venkatswamy, G.; Yemul, S.S. Xanthochymol & isoxanthochymol, two novel polyisoprenylated benzophenones from *Garcinia xanthochymus*. *Indian J. Chem., Sect. B.* **1980**, *19*, 627-633.
14. Baslas, R.K.; Kumar, P. Isolation and characterisation of biflavone and xanthenes in the fruits of *Garcinia xanthochymus*. *Acta Cienc. Indica* **1981**, *7*, 31-34.
15. Baggett, S.; Protiva, P.; Mazzola, E.P.; Yang, H.; Ressler, E.T.; Basile, M.J.; Bernard, W.; Edward, J.K. Bioactive benzophenones from *Garcinia xanthochymus* Fruits. *J. Nat. Prod.* **2005**, *68*, 354-360.
16. Konoshima, M.; Ikeshiro, Y.; Miyahara, S.; Yen, K.Y. The constitution of biflavonoids from *Garcinia plants*. *Tetrahedron Lett.* **1970**, *48*, 4203-4206.
17. Baslas, R.K.; Kumar, P. Chemical examination of the fruits of *Garcinia xanthochymus*. *Curr. Sci.* **1979**, *48*, 814-815.
18. Singh, M.P.; Parveen, N.; Khan, N.; Achari, B.; Dutta, P. Constituents of *Garcinia xanthochymus*. *Fitoterapia* **1991**, *62*, 286-289.
19. Chanmahasathien, W.; Li, Y.S.; Satake, M.; Oshima, Y.; Ruangrunsi, N.; Ohizumi, Y. Prenylated xanthenes with NGF-potentiating activity from *Garcinia xanthochymus*. *Phytochemistry* **2003**, *64*, 981-986.
20. Chanmahasathien, W.; Li, Y.S.; Satake, M.; Oshima, Y.; Ishibashi, M.; Ruangrunsi, N.; Ohizumi, Y. Prenylated xanthenes from *Garcinia xanthochymus*. *Chem. Pharm. Bull.* **2003**, *51*, 1332-1334.
21. Han, Q.B.; Qiao, C.F.; Song, J.Z.; Yang, N.Y.; Cao, X.W.; Peng, Y.; Yang, D.J.; Chen, S.L.; Xu, H.X. Cytotoxic prenylated phenolic compounds from the twig bark of *Garcinia xanthochymus*. *Chem. Biodivers.* **2007**, *4*, 940-946.
22. Inuma, M.; Tosa, H.; Tanaka, T.; Asai, F.; Shimano, R. Two new xanthone from the root bark of *Garcinia subelliptica*. *Heterocycles* **1995**, *40*, 279-284.
23. Hay, A.E.; Aumond, M.C.; Mallet, S.; Dumontet, V.; Litaudon, M.; Rondeau, D.; Richomme, P. Antioxidant xanthenes from *Garcinia vieillardii*. *J. Nat. Prod.* **2004**, *67*, 707-709.
24. Pinedo, A.T.D.; Penalver, P.; Morales, J.C. Synthesis and evaluation of new phenolic-based antioxidants: Structure-activity relationship. *Food Chem.* **2007**, *103*, 55-61.

25. Scherer, R.; Godoy, H.T. Antioxidant activity index (AAI) by the 2,2-diphenyl-1-picrylhydrazyl method. *Food Chem.* **2009**, *112*, 654-658.
26. Alma, M.H.; Mavi, A.; Yilderim, A.; Digrak, M.; Hirata, T. Screening chemical composition and in vitro antioxidant and antimicrobial activities of the essential oils from *Origanum syriacum* L. growing in Turkey. *Biol. Pharm. Bull.* **2003**, *26*, 1725-1729.

Sample Availability: Samples of the compounds are available from the authors.

© 2010 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution license (<http://creativecommons.org/licenses/by/3.0/>).