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Xanthones from the stems of *Cudrania tricuspidata* and their inhibitory effects on pancreatic lipase and fat accumulation

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ABSTRACT

Nine new xanthones, cudracuspixanthones I - Q (**12–14**, **25**, **32–36**), and 30 known xanthones (**1–11**, **15–24**, **26–31**, **37–39**) were isolated from the stems of *Cudrania tricuspidata* (Moraceae). The structures of isolated compounds were established by using 1D and 2D NMR in combination with HR-TOF-MS. Xanthones from the stems of *C. tricuspidata* exerted pancreatic lipase inhibitory activity. In addition, cudracuspixanthone P (**35**), a new xanthone, reduced the fat accumulation in liver cells stimulated with fatty acids. Therefore, these compounds might be beneficial in the treatment of metabolic diseases.

1. Introduction

Fat is one of the three main macronutrients, along with carbohydrate and protein. It is an essential part of our diet and provides energy for living. It is also a source of essential fatty acids and contributes to the regulation of fat-soluble nutrients such as vitamins A, D and E. However, excess fat causes obesity and various other diseases. Fat is absorbed in the small intestine after being hydrolyzed by pancreatic lipase. Therefore, pancreatic lipase inhibition is one of the important therapeutic strategies. Pancreatic lipase inhibitors are used clinically for the prevention/treatment of obesity [1–3].

Fat is distributed in various parts of our body. The liver is one of the organs that is vulnerable to the accumulation of excessive fat. The accumulation of fat in the liver is considered to be a cause of chronic hepatic diseases [4–5]. Nonalcoholic fatty liver disease (NAFLD) is one of the most common liver diseases and the retention of triglyceride (TG) is the prerequisite for its onset. NAFLD accompanies not only various hepatic diseases including fibrosis, cirrhosis, and hepatocarcinoma, but also insulin resistance, hypertriglyceridemia and metabolic syndrome [6–7]. The development of an agent that can alleviate obesity and hepatic lipid accumulation may be one of the therapeutic approaches to treat NAFLD and associated hepatic disorders.

Cudrania tricuspidata (Moraceae) is a deciduous tree that belongs to the Moraceae family. It is indigenous to East Asia, mainly South Korea. The roots have been traditionally used for the treatment of inflammatory diseases such as hepatitis, rheumatism, and inflammation. A wide array of therapeutic effects of *C. tricuspidata* such as hepatoprotective, antitumor, and antioxidant effects [8–10] have been reported. Plants contain different constituents depending on the part, which exhibit different activities. Our previous investigations of *C. tricuspidata* have led to the isolation of anti-proliferative and anti-inflammatory xanthones from the roots [11,12] and anti-obesity isoflavonoids from the fruits [13]. In a continuation of our research on various parts of *C. tricuspidata*, the stems were investigated for their constituent and biological activity. As a result, nine new xanthones, named cudracuspixanthones I - Q (**12–14**, **25**, **32–36**) together with 30 known xanthones (**1–11**, **15–24**, **26–31**, **37–39**) were isolated. The effect of isolated compounds on lipid metabolism was also evaluated by measuring pancreatic lipase activity and fat accumulation.

2. Experimental

2.1. General experimental procedures

Optical rotations were measured with a Jasco DIP-1000 polarimeter. UV and IR spectra were obtained on a Jasco UV-550 and Jasco FT-IR 4100. ESI-MS data was obtained on VG Autospec Ultima mass spectrometers using H_2 as carrier gas with a Hypersil Gold column (flow rate 0.2 mL/min). HR-ESI-MS data was obtained using a maXis 4G (Bruker, Germany). NMR spectra were acquired using a Bruker Avance 400, 500, 700, or 900 MHz spectrometer using acetone-d_6, $CDCl_3$, and CD_3OD as solvents. Semi-preparative HPLC was performed using a

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Waters HPLC system equipped with Waters 600 Q-pumps, a 996-photodiode array detector, and Waters Empower software using a Gemini-NX ODS-column (150 × 10.0 mm and 150 × 21.2 mm). Column chromatography (CC) procedures were performed using silica gel (200–400 mesh, Fisher Scientific) and Sephadex LH-20 (25–100 μm, Pharmacia Fine Chemical Industries Co.). Thin-layer chromatography (TLC) was performed on precoated silica gel 60 F_{254} (0.25 mm, Merck, Germany) plates. After spraying with a color reagent (10% vanillin-H_2SO_4 and 10% H_2SO_4 in EtOH), heating revealed the spots.

2.2. Plant material

The dried stems of *C. tricuspidata* were purchased from a local herbal market in Chungbuk, Korea in March 2014. They were identified by the herbarium of the College of Pharmacy at Chungbuk National University, where a voucher specimen was deposited (CBNU201403-CTS).

2.3. Extraction and isolation

The dried stems of *C. tricuspidata* (2.5 kg) were extracted two times with 100% MeOH, which yielded a methanolic extract (532.2 g). The methanolic extract was then suspended in H_2O and partitioned successively with *n*-hexane, CH_2Cl_2, EtOAc and *n*-BuOH.

The EtOAc fraction (93.3 g) was subjected to silica gel column chromatography with a mixture of *n*-hexane-CH_2Cl_2 of increasing polarity to give eight fractions (E1 - E8). Fraction E1 was subjected to MPLC with a mixture of *n*-hexane-EtOAc to give five subfractions (E1A - E1E). Subfraction E1C was subjected to column chromatography over Sephadex LH-20 eluted with *n*-hexane-CH_2Cl_2-MeOH (10:10:1) to yield three subfractions (E1C1 - E1C3). Subfraction E1C1 was purified by semi-preparative HPLC eluting with AcCN-H_2O (100:0) to give compounds **9** (0.5 mg) and **33** (2.8 mg). Fraction E4 was subjected to RP-MPLC with a mixture of MeOH-H_2O to give six subfractions (E4A - E4F). Subfraction E4D was purified by semi-preparative HPLC eluting with AcCN-H_2O (80:20) to give compounds **15** (0.6 mg), **28** (1.1 mg), **34** (6.8 mg), and **37** (2.6 mg). Fraction E4F was subjected to column chromatography over Sephadex LH-20 eluted with CH_2Cl_2-MeOH (1:1) to yield four subfractions (E4F1 - E4F4). Subfraction E4F4 was purified by semi-preparative HPLC eluting with AcCN-H_2O (80:20) to give compounds **4** (0.4 mg), **8** (0.4 mg), and **36** (2.7 mg). Fraction E5 was subjected to RP-MPLC with a mixture of MeOH-H_2O to give five subfractions (E5A - E5E). Subfraction E5C was subjected to column chromatography over Sephadex LH-20 eluted with MeOH to yield four subfractions (E5C1 - E5C4). Subfraction E5C3 was purified by semi-preparative HPLC eluting with AcCN-MeOH-H_2O (35:40:25) to give compound **16** (0.4 mg). Fraction E5D was subjected to column chromatography over Sephadex LH-20 eluted with MeOH to yield four subfractions (E5D1 - E5D4). Fraction E5D4 was purified by semi-preparative HPLC eluting with AcCN-H_2O (80:20) to give compounds **1** (1.4 mg) and **2** (1.1 mg). Fraction E5E was subjected to column chromatography over Sephadex LH-20 eluted with MeOH to yield four subfractions (E5E1 - E5E4). Subfraction E5E3 was purified by semi-preparative HPLC eluting with AcCN-H_2O (85:15) to give compounds **29** (2.4 mg) and **30** (0.4 mg). Subfraction E5E4 was purified by semi-preparative HPLC eluting with AcCN-H_2O (90:10) to give compound **35** (0.5 mg). Fraction E6 was subjected to silica gel column chromatography with a mixture of CH_2Cl_2-MeOH of increasing polarity to give eight subfractions (E6A – E6H). Subfraction E6B was purified by semi-preparative HPLC eluting with AcCN-H_2O (80:20) to give compounds **23** (0.3 mg) and **32** (0.2 mg). Subfraction E6C was subjected to column chromatography over Sephadex LH-20 eluted with MeOH to yield three subfractions (E6C1 - E6C3). Subfraction E6C3 was purified by semi-preparative HPLC eluting with AcCN-H_2O (75:25) to give compounds **5** (0.2 mg) and **22** (0.1 mg). Fraction E6D was subjected to column chromatography over Sephadex LH-20 eluted with MeOH to yield three subfractions (E6D1 - E6D3). Subfraction E6D3 was purified by semi-preparative HPLC eluting with AcCN-H_2O (90:10) to yield four subfractions (E6D3A - E6D3D). Subfraction E6D3D was purified by semi-preparative HPLC eluting with AcCN-H_2O (70:30) to give compound **39** (1.7 mg). Fraction E6E was subjected to column chromatography over Sephadex LH-20 eluted with MeOH to yield three subfractions (E6E1 – E6E3). Subfraction E6E3 was purified by semi-preparative HPLC eluting with AcCN-H_2O (80:20) to give subfraction E6E3C and compounds **10** (4.5 mg), **13** (1.0 mg), **17** (3.0 mg), **25** (4.0 mg), and **38** (3.8 mg). Subfraction E6E3C was purified by semi-preparative HPLC eluting with AcCN-H_2O (60:40) to give compound **31** (0.5 mg). Fraction E6F was subjected to MPLC with a mixture of *n*-hexane-EtOAc to give four subfractions (E6F1 – E6F4). Subfraction E6F2 was subjected to column chromatography over Sephadex LH-20 eluted with *n*-hexane-CH_2Cl_2-MeOH (5:5:1) to yield seven subfractions (E6F2A – E6F2G). Subfraction E6F2E was purified by semi-preparative HPLC eluting with AcCN-H_2O (70:30) to give compound **27** (0.7 mg). Compound **26** (0.5 mg) was obtained from E6F2F by semi-preparative HPLC eluting with AcCN-H_2O (80:20). Compound **11** (1.1 mg) was obtained from E6F2G by semi-preparative HPLC eluting with AcCN-H_2O (80:20). Subfraction E6F3 was subjected to column chromatography over Sephadex LH-20 eluted with *n*-hexane-CH_2Cl_2-MeOH (5:5:1) to yield 10 subfractions (E6F3A - E6F3J). Subfraction E6F3E was purified by semi-preparative HPLC eluting with AcCN-H_2O (60:40) with 0.1% acetic acid to give compounds **3** (3.6 mg), **12** (5.5 mg), **14** (0.5 mg), and **20** (0.3 mg). Subfraction E6F3I was purified by semi-preparative HPLC eluting with AcCN-H_2O (65:35) with 0.1% acetic acid to give compounds **7** (3.8 mg), **21** (5.7 mg), and **24** (4.9 mg). Compound **6** (17.6 mg) was obtained from E6F3J by semi-preparative HPLC eluting with AcCN-H_2O (80:20). Subfraction E6F4 was subjected to column chromatography over Sephadex LH-20 eluted with *n*-hexane-CH_2Cl_2-MeOH (5:5:1) to yield five subfractions (E6F4A - E6F4E). Subfraction E6F4A was purified by semi-preparative HPLC eluting with AcCN-H_2O (80:20) to give compound **19** (3.6 mg). Fraction E6H was subjected to RP-MPLC with a mixture of MeOH-H_2O to give 12 subfractions (E6H1 - E6H12). Subfraction E6H5 was subjected to column chromatography over Sephadex LH-20 eluted with MeOH to yield four subfractions (E6H5A - E6H5D). Subfraction E6H5B was purified by semi-preparative HPLC eluting with AcCN-H_2O (80:20) to give compound **18** (0.4 mg).

2.3.1. Cudracuspixanthone I (12)

Light brown amorphous powder; $[\alpha]_D^{25}$ −2.3 (*c* 0.08, MeOH); UV (MeOH) λ_{max}: 213, 246, 284, 323 nm; IR ν_{max} 3390, 1658, 1436 cm^{-1}; HRESI-TOF-MS *m/z* 363.0839 $[M+Na]^+$ (calcd. for $C_{19}H_{16}NaO_6$, 363.0845); ESI-MS *m/z* 341 $[M+H]^+$; ^1H NMR (acetone-d_6, 700 MHz), see Table 1; ^{13}C NMR (acetone-d_6, 176 MHz), see Table 2.

Table 1
^1H NMR spectroscopic data for compounds **12–14**.

H	12[a]	13[a]	14[b]
4	6.54, s	6.53, s	6.46, s
7	7.03, d (9.1)	7.25, d (9.1)	7.03, d (8.8)
8	7.85, d (9.1)	7.96, d (9.1)	7.85, d (8.8)
11	3.42, dd (15.4, 9.8) 3.00, dd (15.4, 7.7)	3.42, dd (15.4, 9.8) 3.01, dd (15.4, 7.7)	–
12	5.48, dd (9.8, 7.7)	5.49, dd (9.8, 7.7)	4.58, q (6.8)
13	–	–	1.42, d (6.8)
14	5.14, s 4.98, s	5.15, s 4.98, s	1.51, s
15	1.81, s	1.81, s	1.27, s
5-OCH_3	4.02, s	3.98, s	4.02, s
6-OCH_3	–	4.06, s	–
1-OH	13.34, s	13.28, s	13.43, s
6-OH	9.55, s	–	–

[a] Recorded at 700 MHz in acetone-d_6.
[b] Recorded at 400 MHz in acetone-d_6.

Table 2
^{13}C NMR spectroscopic data for compounds **12–14**.

C	**12**[a]	**13**[a]	**14**[b]
1	158.0	158.2	158.8
2	107.6	107.6	116.9
3	167.2	167.4	166.1
4	89.0	89.1	89.4
4a	158.0	158.2	ND[c]
5	134.7	136.4	134.7
6	156.2	158.1	157.8
7	113.6	109.4	113.7
8	121.0	120.9	121.0
8a	114.1	114.9	114.1
9	180.2	180.3	180.4
9a	103.0	103.0	103.3
10a	150.7	150.3	150.8
11	30.2	30.1	43.3
12	88.1	88.1	90.9
13	143.8	143.7	13.7
14	111.8	111.8	24.6
15	16.2	16.2	20.0
5-OCH_3	60.9	60.6	60.9
6-OCH_3	–	56.1	–

[a] Recorded at 176 MHz in acetone-d_6.
[b] Recorded at 125 MHz in acetone-d_6.
[c] Not detected.

2.3.2. Cudracuspixanthone J (13)

Yellow amorphous powder; $[\alpha]_D^{25}$ −9.2 (*c* 0.01, MeOH); UV (MeOH) λ_{max}: 211, 248, 323 nm; IR ν_{max} 3355, 1646, 1455 cm^{-1}; HRESI-TOF-MS *m/z* 377.0996 $[M+Na]^+$ (calcd. for $C_{20}H_{18}NaO_6$, 377.1001); ESI-MS *m/z* 355 $[M+H]^+$; ^{1}H NMR (acetone-d_6, 700 MHz), see Table 1; ^{13}C NMR (acetone-d_6, 176 MHz), see Table 2.

2.3.3. Cudracuspixanthone K (14)

Brown amorphous powder; $[\alpha]_D^{25}$ −25.1 (*c* 0.02, MeOH); UV (MeOH) λ_{max}: 213, 246, 285, 323 nm; IR ν_{max} 3396, 1657, 1436 cm^{-1}; HRESI-TOF-MS *m/z* 365.0996 $[M+Na]^+$ (calcd. for $C_{19}H_{18}NaO_6$, 365.1001); ESI-MS *m/z* 343 $[M+H]^+$; ^{1}H NMR (acetone-d_6, 400 MHz), see Table 1; ^{13}C NMR (acetone-d_6, 125 MHz), see Table 2.

2.3.4. Cudracuspixanthone L (25)

Brown amorphous powder; $[\alpha]_D^{25}$ +5.9 (*c* 0.06, MeOH); UV (MeOH) λ_{max}: 211, 261, 317, 364 nm; IR ν_{max} 3326, 1651, 1449 cm^{-1}; HRESI-TOF-MS *m/z* 395.1500 $[M-H]^-$ (calcd. for $C_{23}H_{23}O_6$, 395.1495); ESI-MS *m/z* 397 $[M+H]^+$; ^{1}H NMR (acetone-d_6, 400 MHz), see Table 3; ^{13}C NMR (acetone-d_6, 100 MHz), see Table 4.

Table 4
^{13}C NMR data of compounds **25** and **33–36**.

C	**25**[a]	**33**[b]	**34**[b]	**35**[c]	**36**[d]
1	161.5	160.9	160.9	161.0	163.3
2	98.7	99.0	98.8	98.5	98.2
3	162.8	161.0	160.4	160.7	159.6
4	110.2	98.8	98.9	99.0	100.2
4a	155.2	154.1	154.0	154.1	151.1
5	100.2	125.2	100.4	102.7	100.8
6	152.8	116.6	151.5	153.2	153.7
7	139.1	150.1	138.0	138.1	139.3
8	121.1	121.3	121.4	119.9	121.5
8a	110.0	118.5	111.4	107.6	110.4
9	182.7	183.6	182.7	182.4	182.5
9a	103.6	104.2	103.8	103.3	103.5
10a	153.3	151.7	153.1	152.8	153.1
11	40.8	16.1	16.1	15.8	114.7
12	–	31.9	31.9	31.4	127.0
13	–	76.0	75.8	75.9	77.9
14	150.6	26.7	26.5	26.0	27.5
15	107.2	26.7	26.5	26.0	27.5
16	22.2	22.5	22.3	120.6	22.3
17	32.4	32.6	32.9	132.7	32.3
18	74.5	73.9	75.6	76.0	74.5
19	25.6	26.5	26.7	26.2	25.6
20	25.6	26.5	26.7	26.2	25.6

[a] Recorded at 100 MHz in acetone-d_6.
[b] Recorded at 100 MHz in $CDCl_3$.
[c] Recorded at 226 MHz in acetone-d_6.
[d] Recorded at 176 MHz in acetone-d_6.

2.3.5. Cudracuspixanthone m (32)

Light brown amorphous powder; $[\alpha]_D^{25}$ +20.0 (*c* 0.03, MeOH); UV (MeOH) λ_{max} : 244, 262, 333, 375 nm; IR ν_{max} 3372, 1647 cm^{-1}; HRESI-TOF-MS *m/z* 433.1258 $[M+Na]^+$ (calcd. for $C_{23}H_{22}NaO_7$, 433.1263); ESI-MS *m/z* 409 $[M-H]^-$; ^{1}H NMR (acetone-d_6, 700 MHz), see Table 3.

2.3.6. Cudracuspixanthone n (33)

Light yellow powder; $[\alpha]_D^{25}$ +22.4 (*c* 0.01, MeOH); UV (MeOH) λ_{max} : 238, 267, 311 nm; IR ν_{max} 3316, 1448 cm^{-1}; HRESI-TOF-MS *m/z* 403.1516 $[M+Na]^+$ (calcd. for $C_{23}H_{24}NaO_5$, 403.1521); ESI-MS *m/z* 381 $[M+H]^+$; ^{1}H NMR ($CDCl_3$, 400 MHz), see Table 3; ^{13}C NMR

Table 3
^{1}H NMR data of compounds **25** and **32–36**.

H	**25**[a]	**32**[b]	**33**[c]	**34**[d]	**35**[e]	**36**[b]
2	6.25, s	–	6.22, s	6.21, s	6.09, s	6.12, s
4	–	6.30, s	–	–	–	–
5	–	–	7.18, d (9.2)	6.87, s	6.93, s	6.91, s
6	–	–	7.27, d (9.2)	–	–	–
11	–	–	2.85, t (6.8)	2.84, t (7.0)	2.86, t (7.2)	6.85, d (10.0)
12	1.69, s	1.58, s	1.89, t (6.8)	1.88, t (7.0)	1.93, t (7.2)	5.74, d (10.0)
13	1.69, s	1.37, s	–	–	–	–
14	6.38, dd (17.6, 10.4)	4.47, t (6.3)	1.38, s	1.42, s	1.39, s	1.48, s
15	5.04, dd (17.6, 1.2) 4.93, dd (10.4, 1.2)	3.92, d (6.3)	1.38, s	1.42, s	1.39, s	1.48, s
16	3.47, t (6.8)	8.05, d (9.8)	3.54, t (6.8)	3.53, t (6.5)	8.06, d (9.9)	3.48, t (7.0)
17	1.90, t (6.8)	5.96, d (9.8)	1.87, t (6.8)	1.92, t (6.5)	5.96, d (9.9)	1.91, t (7.0)
19	1.37, s	1.48, s	1.40, s	1.40, s	1.48, s	1.38, s
20	1.37, s	1.48, s	1.40, s	1.40, s	1.48, s	1.38, s
1-OH	13.70, s	13.74, s	13.04, s	13.18, s	13.12, s	13.64, s

[a] Recorded at 400 MHz in acetone-d_6.
[b] Recorded at 700 MHz in acetone-d_6.
[c] Recorded at 400 MHz in $CDCl_3$.
[d] Recorded at 500 MHz in $CDCl_3$.
[e] Recorded at 900 MHz in acetone-d_6.

($CDCl_3$, 100 MHz), see Table 4.

*2.3.7. Cudracuspixanthone O (**34**)*

Light yellow powder; $[\alpha]_D^{25}$ +8.1 (*c* 0.04, MeOH); UV (MeOH) λ_{max} : 260, 316, 361 nm; IR ν_{max} 3373, 1647 cm^{-1}; HRESI-TOF-MS *m/z* 397.1646 $[M+H]^+$ (calcd. for $C_{23}H_{25}O_6$, 397.1651); ESI-MS *m/z* 397 $[M+H]^+$; ^1H NMR ($CDCl_3$, 500 MHz), see Table 3; ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 4.

*2.3.8. Cudracuspixanthone p (**35**)*

Light brown amorphous powder; $[\alpha]_D^{25}$ +23.8 (*c* 0.01, MeOH); UV (MeOH) λ_{max} : 245, 265, 331, 380 nm; IR ν_{max} 3355, 1647, 1473 cm^{-1}; HRESI-TOF-MS *m/z* 417.1309 $[M+Na]^+$ (calcd. for $C_{23}H_{22}NaO_6$, 417.1314); ESI-MS *m/z* 395 $[M+H]^+$; ^1H NMR (acetone-d_6, 900 MHz), see Table 3; ^{13}C NMR (acetone-d_6, 226 MHz), see Table 4.

*2.3.9. Cudracuspixanthone Q (**36**)*

Yellow powder; $[\alpha]_D^{25}$ +4.7 (*c* 0.1, MeOH); UV (MeOH) λ_{max} : 263, 285, 322, 380 nm; IR ν_{max} 3374, 1647, 1461 cm^{-1}; HRESI-TOF-MS *m/z* 417.1309 $[M+Na]^+$ (calcd. for $C_{23}H_{22}NaO_6$, 417.1314); ESI-MS *m/z* 395 $[M+H]^+$; ^1H NMR (acetone-d_6, 700 MHz), see Table 3; ^{13}C NMR (acetone-d_6, 176 MHz), see Table 4.

2.4. Assessment of biological activity

2.4.1. Measurement of pancreatic lipase inhibitory activity

Pancreatic lipase inhibitory activity was determined using the procedure reported with minor modifications. Briefly, an enzyme solution was prepared by the reconstitution of porcine pancreatic lipase (Sigma, St. Louis, MO) in 0.1 M Tris-HCl buffer (pH 8). Then, all isolated compounds were mixed with enzyme buffer and incubated for 15 min at 37 °C. Then, 10 mM *p*-nitrophenylbutyrate (*p*-NPB) was added after the incubation of the test compounds with pancreatic lipase solution at 37 °C for 15 min, and absorbance at 405 nm was recorded using an ELISA plate reader. Inhibition of lipase activity was expressed as the percentage decrease in the OD when incubated with the test compounds. Orlistat (Sigma, St. Louis, MO) was used as a positive control.

2.4.2. Measurement of accumulation of neural lipid and triglyceride in HepG2 cells

HepG2 cells were obtained from American Type Culture Collection (ATCC, USA). Cells were cultured in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin in an incubator under an atmosphere of 5% CO_2 at 37 °C. Prior to exposing a 1 mM free fatty acid (FFA) mixture (oleate/palmitate, 2:1 ratio), 50 μM samples were pretreated in HepG2 cells (5×10^5 cells/ml) for 1 h. After incubation, oil red O staining was performed to observe quantitative changes in intracellular neutral lipids. Cells were fixed with 4% paraformaldehyde and stained with working solution of oil red O for 10 min at room temperature. To quantify the oil red O content, 100% isopropanol was added to each sample and read using a microplate reader at 500 nm. Intracellular triglyceride content was measured by enzyme-colorimetric method according to the manufacturer's instructions using a triglyceride colorimetric assay kit (Cayman, USA) after dissolving the cells with 5% NP-40.

3. Results and discussion

Nine new xanthones together with 30 known xanthones were isolated from the stems of *C. tricuspidata*. The structures of the isolated compounds were established on the basis of combined spectroscopic analysis (Fig. 1).

3.1. Structure determination of new compounds

Compound **12** was isolated as a light brown amorphous powder. The molecular formula of **12** was determined as $C_{19}H_{16}O_6$ by HRESIMS (*m/z* 363.0839 $[M + Na]^+$, calcd. 363.0845). The UV spectrum showed characteristic xanthone absorption maxima at 213, 246, 284, 323 nm [14]. In accordance with the UV spectrum, 12 aromatic signals at [δ_C 158.0 (C-1), 107.6 (C-2), 167.2 (C-3), 89.0 (C-4), 158.0 (C-4a), 134.7 (C-5), 156.2 (C-6), 113.6 (C-7), 121.0 (C-8), 114.1 (C-8a), 103.0 (C-9a), 150.7 (C-10a)] together with a carbonyl carbon at δ_C 180.2 (C-9) were observed in the ^{13}C NMR spectrum. The ^1H NMR spectrum showed the signals for a tetrasubstituted benzene ring at [δ_H 7.03 (1H, d, J = 9.1 Hz, H-7), 7.85 (1H, d, J = 9.1 Hz, H-8)], a pentasubstituted benzene ring at δ_H 6.54 (1H, s, H-4) and a hydrogen-boned hydroxyl group at [δ_H 13.34 (1H, s, 1-OH)], which corresponded to a xanthone skeleton in the HSQC spectrum. Additionally, the presence of a 2-hydroxy-3-methyl-3-butenyl group was deduced by the signals at [δ_H 3.42 (1H, dd, J = 15.4, 9.8 Hz, H-11a), 3.00 (1H, dd, J = 15.4, 7.7 Hz, H-11b), 5.48 (1H, dd, J = 9.8, 7.7 Hz, H-12), 5.14 (1H, s, H-14a), 4.98 (1H, s, H-14b), 1.81 (3H, s, CH_3-15); δ_C 30.2 (C-11), 88.1 (C-12), 143.8 (C-13), 111.8 (C-14), 16.2 (C-15)], which was supported by HMBC correlations (Fig. 2). A signal for a methoxyl group was also observed at [δ_H 4.02 (3H, s); δ_C 60.9]. These data revealed **12** as a xanthone derivative with a 2-hydroxy-3-methyl-3-butenyl group and a methoxyl group. In the HMBC spectrum, the correlations from H-11 to C-2 and C-3 allowed the deduction that the position of the 2-hydroxy-3-methyl-3-butenyl group was at C-2 and C-3. The position of the methoxyl group was determined to be located at C-5 on the basis of HMBC correlation between OCH_3 and C-5. Therefore, compound **12** was formulated as illustrated, and named cudracuspixanthone I.

The molecular formula of **13** was determined to be $C_{20}H_{18}O_6$ by HRESIMS analysis. The ^1H and ^{13}C NMR spectra of **13** were almost identical to those of **12** except for the presence of an additional methoxyl group at [δ_H 4.06 (3H, s); δ_C 56.1]. The position of the additional methoxyl group was deduced to be C-6 by the correlation between OCH_3 and C-6 in the HMBC spectrum. On the basis of the obtained data, compound **13** was determined as shown and named cudracuspixanthone J.

Compound **14** was isolated as a brown amorphous powder and gave a pseudomolecular ion $[M + Na]^+$ at *m/z* 365.0996 (calcd. for 365.1001) in the HRESIMS analysis, consistent with the molecular formula of $C_{19}H_{18}O_6$. The ^1H and ^{13}C NMR spectra of **14** were similar to those of **12**. Detailed examination suggested the replacement of the 2-hydroxy-3-methyl-3-butenyl group in **12** by the 2,3,3-trimethyldihydrofuran group in **14** from the signals at [δ_H 4.58 (1H, q, J = 6.8 Hz, H-12), 1.42 (3H, d, J = 6.8 Hz, CH_3-13), 1.51 (3H, s, CH_3-14), 1.27 (3H, s, CH_3-15); δ_C 43.3 (C-11), 90.9 (C-12), 13.7 (C-13), 24.6 (C-14), 20.0 (C-15)]. The positions of the 2,3,3-trimethyldihydrofuran group were determined based on the HMBC correlations between H-14, H-15 and C-2 and between OCH_3 and C-5. Therefore, compound **14** was determined as shown and named cudracuspixanthone K.

Compound **25** was isolated as a brown amorphous powder. HRESIMS analysis gave a pseudomolecular ion at *m/z* 395.1500 (calcd. for 395.1495), consistent with a molecular formula of $C_{23}H_{24}O_6$. In combination with characteristic UV absorption and ^{13}C NMR spectrum, **25** was also thought to be a xanthone derivative. Besides signals for a xanthone skeleton, 10 additional carbons were observed in the ^{13}C NMR spectrum, which suggested the presence of two isoprenyl derivatives. In the ^1H and ^{13}C NMR spectra together with the HSQC and HMBC spectra, the presence of a 1,1-dimethylallyl group was deduced from the signals at [δ_H 1.69 (6H, s, CH_3-12, 13), 6.38 (1H, dd, J = 17.6, 10.4 Hz, H-14), 5.04 (1H, dd, J = 17.6, 1.2 Hz, H-15a), 4.93 (1H, dd, J = 10.4, 1.2 Hz, H-15b); δ_C 40.8 (C-11), 150.6 (C-14), 107.2 (C-15)] and a 2,2-dimethyldihydropyran group from the signals at [δ_H 3.47 (2H, t, J = 6.8 Hz, H-16), 1.90 (2H, t, J = 6.8 Hz, H-17), 1.37 (6H, s, CH_3-19, 20); δ_C 22.2 (C-16), 32.4 (C-17), 74.5 (C-18), 25.6 (C-19, 20)]. The positions of the 1,1-dimethylallyl group and 2,2-dimethyldihydropyran group were determined to be located at C-4 and C-7, C-8, respectively, on the basis of HMBC correlations between H-12 and C-4 and between H-16 and C-7, C-8, C-8a. Therefore, compound **25** was

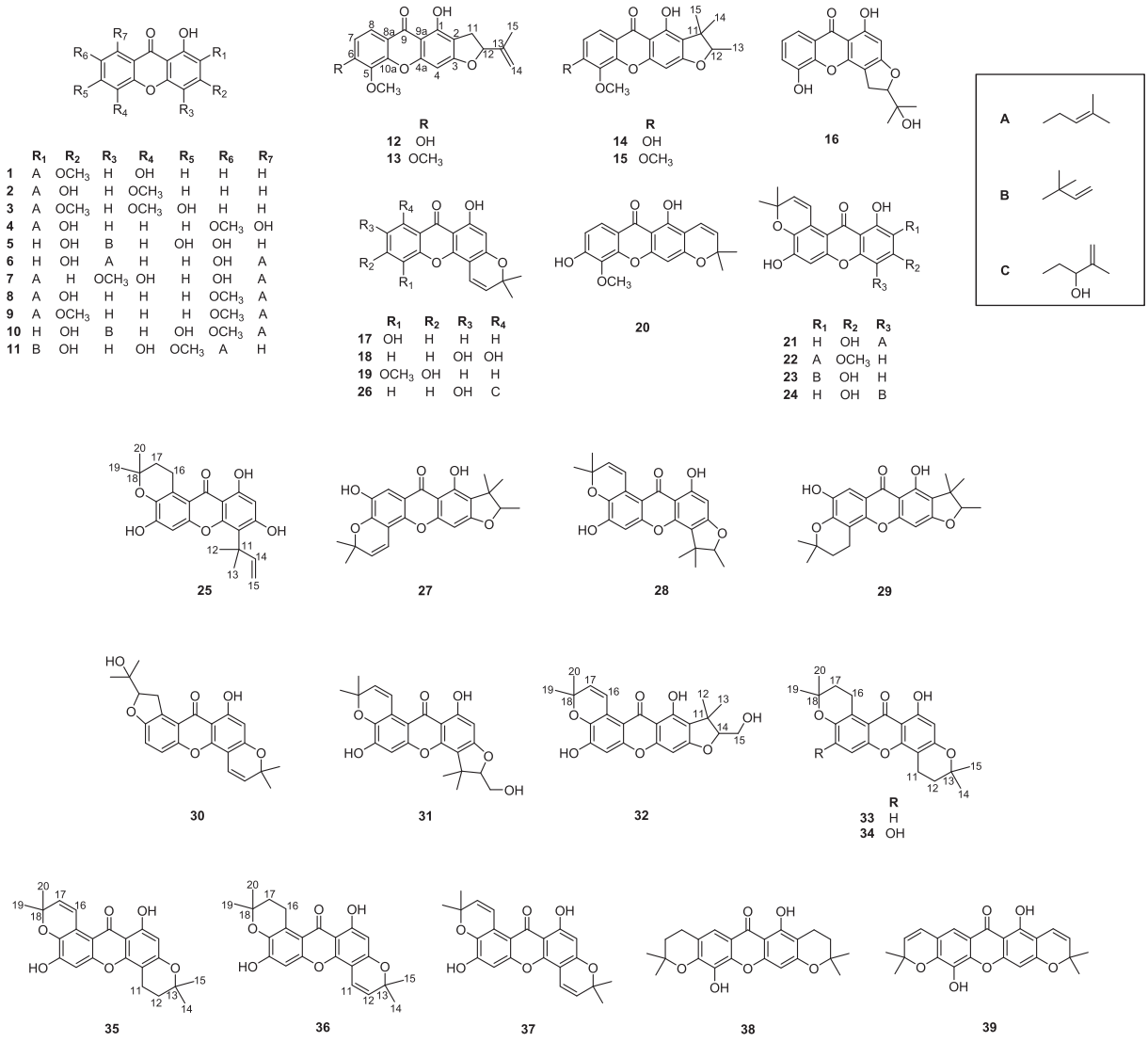

Fig. 1. Chemical structures of compounds **1–39** from *C. tricuspidata* stems.

determined as shown, and named cudracuspixanthone L.

The molecular formula of **32** was determined to be $C_{23}H_{22}O_7$ from a pseudomolecular ion at *m/z* 433.1258 $[M + Na]^+$ (calcd. for 433.1263) in the HRESIMS analysis. The ^1H NMR data of **32** was quite similar to those of **25**, revealing a xanthone derivative with two prenyl groups. Detailed examination showed the presence of a 2-hydroxymethyl-3,3-dimethyldihydrofuran group and 2,2-dimethylpyran group, which were very similar to those of cudraxanthone K (**31**). The position of the 2-hydroxymethyl-3,3-dimethyldihydrofuran group in the NOESY spectrum of **31** showed correlations between H-5 and H-12, H-13, whereas correlations between 1-OH and H-12, H-13 were observed in the NOESY spectrum of **32**. These differences allowed the deduction that the 2-hydroxymethyl-3,3-dimethyldihydrofuran group was attached at C-2 and C-3 in **32** Therefore, the structure of **32** was determined as shown, and named cudracuspixanthone M.

Compound **33**, isolated as a light yellow powder, gave a pseudomolecular ion $[M + Na]^+$ at *m/z* 403.1516 in HRESIMS, consistent with the molecular formula of $C_{23}H_{24}O_5$ (calcd. for $C_{23}H_{24}O_5Na$ *m/z* 403.1521). The ^{13}C NMR spectrum showed characteristic signals for a xanthone skeleton with 12 aromatic carbons and carbonyl carbons, similar to the aforementioned compounds. In the ^1H NMR spectrum, signals at [δ_H 7.18 (1H, d, J = 9.2 Hz, H-5), 7.27 (1H, d, J = 9.2 Hz, H-6)] and at δ_H 6.22 (1H, s, H-2) suggested the presence of a tetrasubstituted benzene ring and a pentasubstituted benzene ring, respectively, in a xanthone skeleton. Additionally, two 2,2-dimethyldihydropyran groups were deduced from the signals at [δ_H 2.85 (2H, t, J = 6.8 Hz, H-11), 1.89 (2H, t, J = 6.8 Hz, H-12), 1.38 (6H, s, CH_3-14, 15); δ_C 16.1 (C-11), 31.9 (C-12), 76.0 (C-13), 26.7 (C-14, 15)] and [δ_H 3.54 (2H, t, J = 6.8 Hz, H-16), 1.87 (2H, t, J = 6.8 Hz, H-17), 1.40 (6H, s, CH_3-19, 20); δ_C 22.5 (C-16), 32.6 (C-17), 73.9 (C-18), 26.5 (C-19, 20)] in the ^1H and ^{13}C NMR spectra, which was supported by the HSQC and HMBC analysis. The linkages between the xanthone skeleton and two 2,2-dimethyldihydropyran groups were determined as C-3/4 and C-7/8, respectively, on the basis of HMBC correlations between H-12 and C-4 and between H-17 and C-8. Therefore, compound **33** was determined as shown, and named cudracuspixanthone N.

The molecular formula of **34** was determined to be $C_{23}H_{24}O_6$ by HRESIMS analysis. The ^1H and ^{13}C NMR spectra of **34** were very close to those of **33**, except for the replacement of *ortho*-coupled aromatic signals at [δ_H 7.18 (1H, d, J = 9.2 Hz, H-5), 7.27 (1H, d, J = 9.2 Hz, H-6)] by a singlet proton at [δ_H 6.87 (1H, s, H-5)], which suggested the addition of a hydroxyl group. Therefore, compound **34** was determined as shown and named cudracuspixanthone O.

Compounds **35** and **36** were isolated as a light brown and yellow amorphous powder, respectively. The molecular formulas of both **35** and **36** were determined as $C_{23}H_{22}O_6$ from their HRESIMS data (*m/z* 417.1309 $[M + Na]^+$, calcd. 417.1314). Comparison of the ^1H and ^{13}C NMR data of **35** and **36** with those of compound **34** indicated that they

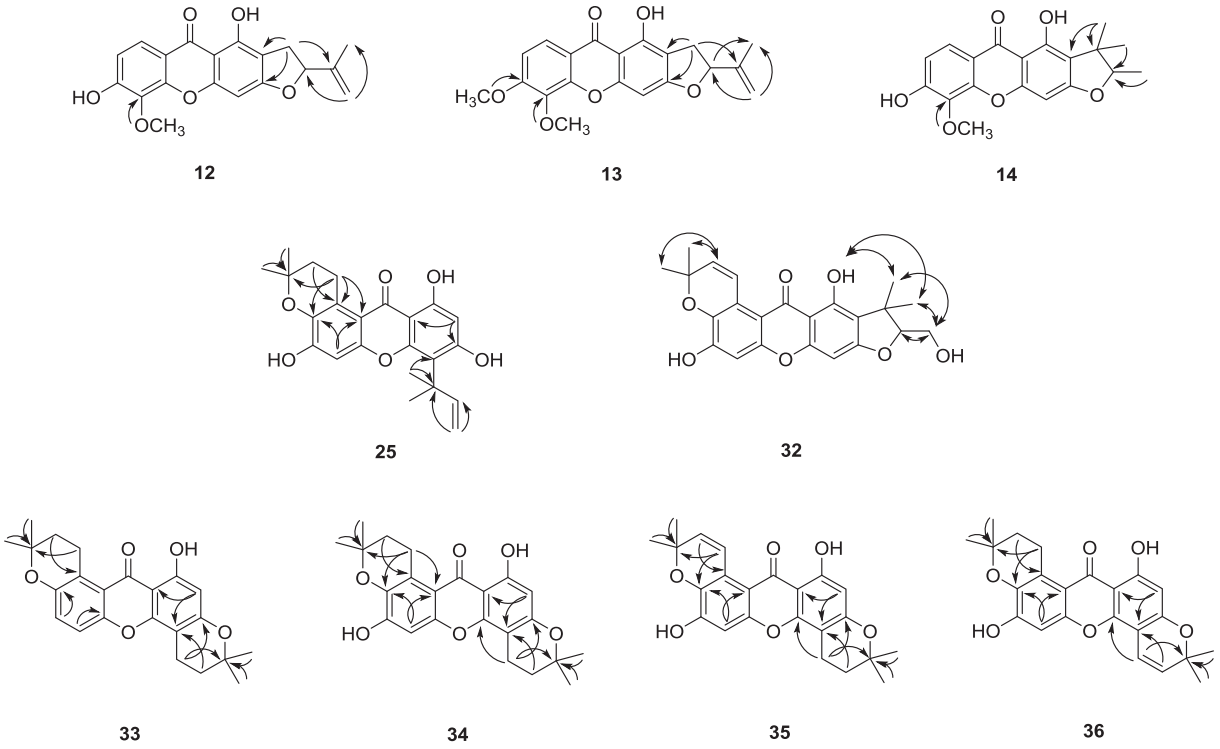

Fig. 2. Key HMBC (→) and NOESY (↔) correlations of new compounds.

share the same xanthone skeletons with the 2,2-dimethyldihydropyran group. The only differences were the observation of signals for a 2,2-dimethylpyran group in **35** and **36** instead of one 2,2-dimethyldihydropyran group in **34**. Therefore, compounds **35** and **36** were xanthone derivatives with one 2,2-dimethyldihydropyran and one 2,2-dimethylpyran group. The positions of the 2,2-dimethyldihydropyran and 2,2-dimethylpyran groups in **35** and **36** were determined by the HMBC correlations as shown, and named cudracuspixanthones P and Q, respectively.

3.2. Structure identification of known compounds

The known xanthones were identified as gudraxanthone (**1**), 1,3-dihydroxy-5-methoxy-2-(3-methyl-2-buten-1-yl)-9*H*-xanthen-9-one (**2**), cowaxanthone A (**3**), 1,3,8-trihydroxy-7-methoxy-2-(3-methyl-2-butenyl)-9*H*-xanthen-9-one (**4**), cudratricusxanthone L (**5**), cudraxanthone H (**6**), umbilicaxanthone B (**7**), calocalabaxanthone (**8**), 1-hydroxy-3,7-dimethoxy-2,8-bis(3-methyl-2-butenyl)-9*H*-xanthen-9-one (**9**), cudraxanthone C (**10**), cudrafrutixanthone A (**11**), 2,3-dihydro-4-hydroxy-8,9-dimethoxy-2,3,3-trimethyl-5*H*-Furo[3,2-b]xanthen-5-one (**15**), morusignin H (**16**), 6-deoxyisojacareubin (**17**), globulixanthone C (**18**), 5-*O*-methylisojacareubin (**19**), 1,6-dihydroxy-5-methoxy-6,6-dimethylpyrano[2′,3′:2,3]-xanthone (**20**), paxanthone B (**21**), dulcisxanthone F (**22**), cudraxanthone K (**23**), cudraxanthone B (**24**), cudratrixanthone A (**26**), cudracuspixanthone B (**27**), cudratrixanthone N (**28**), 2,3,9,10-tetrahydro-5,8-dihydroxy-3,3,9,9,10-pentamethyl-1*H*,7*H*-Furo[3,2]pyrano[3,2]xanthen-7-one (**29**), cudraxanthone O (**30**), cudratrixanthone K (**31**), brasilixanthone A (**37**), 3,4,9,10-tetrahydro-5,12-dihydroxy-2,2,10,10-tetramethyl-2*H*,6*H*,8*H*-dipyrano[3,2:2′,3′]xanthen-6-one (**38**), and pyranojacareubin (**39**) by comparison with reported data [11,15–39] (Fig. 1).

3.3. Effect of isolated compounds on pancreatic lipase activity and fat accumulation

All isolates were first evaluated for their inhibitory effects on pancreatic lipase activity using porcine pancreatic lipase *in vitro*. Overall, the xanthones isolated from *C. tricuspidata* in this study showed quite good inhibition of pancreatic lipase activity. These compounds inhibited 40–60% of pancreatic lipase activity at a concentration of 100 μM (Table 5).

We further measured the effects of these xanthones on intracellular neutral lipid and triglyceride accumulation in HepG2 cells. Compounds **34**, **35** and **37** significantly decreased FFA-induced lipid accumulation in HepG2 liver cells (Fig. 3). Compound **35**, a new xanthone, inhibited intracellular lipid and triglyceride accumulation by 54% and 75%, respectively, at 50 μM.

Taken together, xanthones from the stems of *C. tricuspidata* exerted pancreatic lipase inhibitory activity and reduced fat accumulation in liver cells stimulated with fatty acids. Therefore, these compounds

Table 5
Pancreatic lipase inhibitory activity of compounds **1–39.**

Compounds	Inhibition (%)	Compounds	Inhibition (%)
1	56.4 ± 8.8	**21**	38.4 ± 9.7
2	54.0 ± 2.0	**22**	68.6 ± 4.3
3	66.7 ± 1.9	**23**	50.5 ± 7.5
4	54.6 ± 9.3	**24**	65.9 ± 5.7
5	57.8 ± 7.6	**25**	48.1 ± 3.9
6	76.9 ± 2.3	**26**	48.5 ± 1.6
7	56.2 ± 3.8	**27**	43.6 ± 7.1
8	65.9 ± 6.3	**28**	53.3 ± 2.8
9	54.6 ± 7.4	**29**	41.9 ± 8.2
10	73.9 ± 1.7	**30**	54.6 ± 8.1
11	66.1 ± 4.8	**31**	59.4 ± 1.8
12	44.6 ± 8.8	**32**	56.2 ± 7.3
13	47.7 ± 5.4	**33**	42.9 ± 4.1
14	67.6 ± 3.5	**34**	49.3 ± 6.2
15	48.9 ± 8.3	**35**	59.2 ± 9.1
16	62.7 ± 5.0	**36**	50.2 ± 6.6
17	57.2 ± 1.5	**37**	46.5 ± 9.0
18	53.0 ± 5.5	**38**	52.0 ± 6.2
19	58.1 ± 9.8	**39**	18.5 ± 3.2
20	59.3 ± 8.3	**orlistat**	76.2 ± 2.1

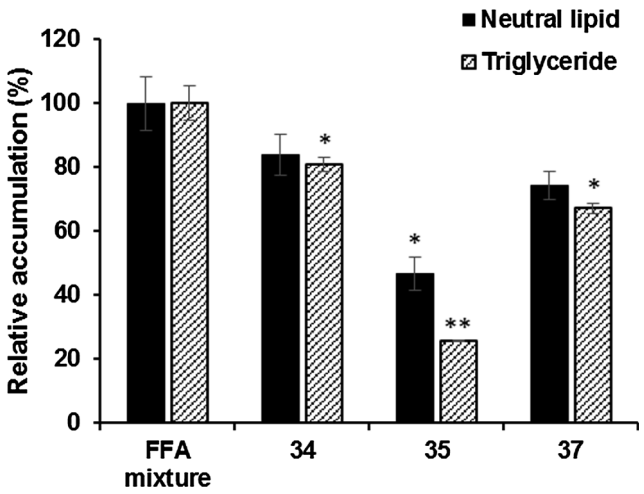

Fig. 3. Effect of compounds **34**, **35** and **37** on the accumulation of neutral lipid and triglyceride in HepG2 cells. Cells were pretreated with 50 μM samples for 1 h, followed by exposure to 1 mM FFA for 24 h. An oil red O staining assay was performed to measure the neutral lipid accumulation. Quantification of triglyceride content was determined using a commercial kit. The bar graphs show the mean ± S.D. (*$p < 0.05$ and **$p < 0.01$ compared with the FFA-treated control).

might be effective in the treatment of metabolic diseases such as obesity and nonalcoholic fatty liver disease.

4. Conclusions

The phytochemical study of the stems of *C. tricuspidata* resulted in the isolation of 39 compounds. Structural elucidation demonstrated that nine xanthones named cudracuspixanthones I - Q were newly reported. Xanthones from the stems of *C. tricuspidata* exerted pancreatic lipase inhibitory activity and reduced fat accumulation in liver cells stimulated with fatty acids in liver cells. Therefore, these compounds might be beneficial in the treatment of metabolic diseases.

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