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Acylated iridoid glycosides and acylated rhamnopyranoses from *Gmelina arborea* flowers

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ABSTRACT

Nine acylated iridoid glycosides (**1**–**9**), five acylated rhamnopyranoses (**10**–**14**) and verbascoside (**15**) were isolated from *Gmelina arborea* flowers, including 5 new compounds (**1**, **2**, and **10**–**12**). The cytoprotective activity of 11 selected compounds (**1**–**8**, **10**, **11**, and **15**) against CCl_4-induced cytotoxicity on liver was determined. Compounds **1**, **2**, **4**, **7**, **8** and **15** displayed hepatoprotective activity. 6-*O*-α-L-(2″, 3″-di-*O*-*trans*-*p*-hydroxycinnamoyl)rhamnopyranosylcatalpol (**2**) exhibited the most potent cytoprotective effect with an EC_{50} value of 42.5 μM (SI = 19.3) compared with biphenyldimethylesterate (DDB, EC_{50} = 277.3 μM, SI = 9.8) and bicylo-ethanol (EC_{50} = 279.2 μM, SI = 12.2). Among the acylated iridoid glycosides, the compounds (**2** and **8**) containing phenolic hydroxy groups were more active than were those lacking them.

1. Introduction

The genus *Gmelina* (ca. 40 species), which belongs to the Verbenaceae family, is widely distributed in the tropical and subtropical regions of Australia, Asia and Africa (Greaves, 1981). In China, it is naturally distributed in the Xishuangbanna, Dehong, Pu'er and Lincang prefectures in Yunnan Province. The fruit is edible and the flower is used as coloring and flavoring ingredients for festival cakes in Water Splashing Festival by the Dai minority people in Xishuangbanna. The flower is also used for pigment extraction (Wang, 2004). In addition, *Gmelina arborea* is an important folk medicine for the Dai and Hani ethnic groups in Xishuangbanna. The folklore of India described the use of *G. arborea* bark and leaves to treat liver disorders, loosen phlegm, act as a diuretic or galactogogue and stimulate the appetite (Kawamura and Ohara, 2005). In the ethnobotanical regimes of the indigenous tribes in Tamil Nadu, India, the aerial part of the *G. arborea* is used to treat jaundice and other hepatic diseases (Merlin and Parthasarathy, 2011).

Previous phytochemical studies demonstrated the presence of flavonoids (Nair and Subramanian, 1975), lignans (Anjaneyulu et al., 1972, 1975, 1977; Satyanarayana et al., 1986; Kawamura et al., 2004), iridoid glycosides (Kawamura and Ohara, 2005; Hosny and Rosazza, 1998; Tiwari et al., 2008), and other chemical constituents (Olatunji, 1999; Barik et al., 1992; Satyanarayana et al., 1985; Rao et al., 1967; Falah et al., 2008) in the aerial parts of *G. arborea*; however, these chemical constituents from *G. arborea* have not been reported to possess cytoprotective activity. In this study, an investigation of the constituents of *G. arborea* flowers was performed, which led to the isolation of 15 glycosides, including 5 new ones. Selected compounds were then tested for activity against hepatic injury.

2. Results and discussion

2.1. Phytochemical investigation

The ethyl acetate and *n*-butanol fraction of *G. arborea* flowers extract was separated by a combination of column chromatography on silica gel, followed by further purification using reversed phase chromatography (RP-18) and Sephadex LH-20 column chromatography as well as high-pressure liquid chromatography

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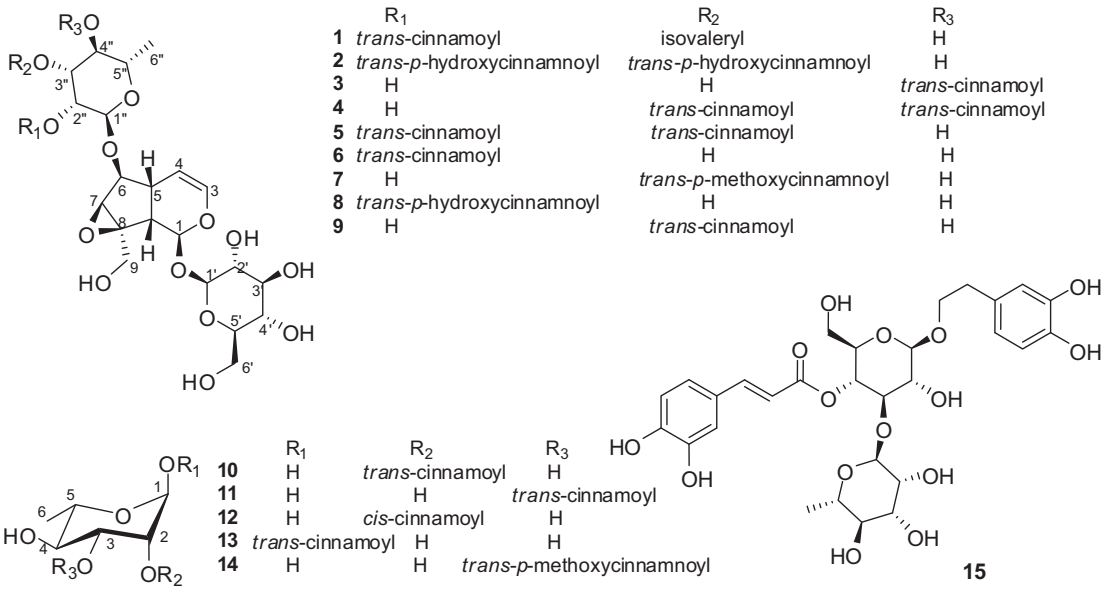

Fig. 1. The structures of compounds **1**–**15**.

(HPLC). Fifteen compounds (Fig. 1) were identified, including 2 new iridoid glycosides (**1** and **2**), 3 new acylated rhamnosides (**10**–**12**), and 10 known compounds.

Compound **1** was found to have the elemental composition $C_{35}H_{46}O_{16}$ by HR-ESI-MS (*m*/*z* 757.2476 $[M+Cl]^-$. Its IR spectrum indicated the presence of hydroxy (3412 cm^{-1}) and conjugated carbonyl (1723 and 1636 cm^{-1}) groups. The NMR data (Table 1) demonstrated the signals of two carbonyl groups (δ_C 174.1 and 167.4), a monosubstituted phenyl ring, a *trans* double bond [δ_H 7.71 (d, *J* = 16.0) and 6.58 (d, *J* = 16.0)], a β-glucosyl group [δ_H 4.75 (d, *J* = 7.9)], and an α-rhamnosyl group [δ_H 5.03 (br s) and 1.31 (d, *J* = 6.1)]. By comparing its NMR data with those of the known compounds, **3**–**9** (Taskova et al., 2006; Tatli et al., 2003; Otsuka et al., 1990, 1991b), compound **1** was deduced to be an acylated iridoid glycoside containing a characteristic fragment of 6-*O*-α-L-rhamnopyranosylcatalpol. The NMR data for compound **1** was very similar to those of 6-*O*-α-L-(2″-*O*-*trans*-cinnamoyl)rhamnopyranosylcatalpol (**6**) except for the presence of additional signals from an isovaleryl group in **1** (Taskova et al., 2006; Tatli et al., 2003). The *trans*-cinnamoyl and isovaleryl groups were located at C-2″ and C-3″ by the HMBC correlations (Fig. 2) from H-2″ to C-9‴ and H-3″ to C-1‴', respectively. These deductions were supported by the ^1H–^1H COSY spectra (Fig. 2 and Supporting Information) of compound **1**. Accordingly, compound **1** was identified as 6-*O*-α-L-(2″-*O*-*trans*-cinnamoyl-3″-*O*-isovaleryl)rhamnopyranosylcatalpol, for which we give the trivial name gmelinoside M.

The molecular formula of compound **2** was determined to be $C_{39}H_{44}O_{18}$ by HRESIMS, indicating 18 degrees of unsaturation. The NMR data (Table 1) for compound **2** was very similar to those of 6-*O*-α-L-(2″-*O*-*trans*-*p*-hydroxycinnamoyl)rhamnopyranosylcatalpol (**8**) except that there were signals for two *trans*-*p*-hydroxycinnamoyl groups in compound **2,** whereas there was only one *trans*-*p*-hydroxycinnamoyl group in compound **8** (Otsuka et al., 1990). The two *trans*-*p*-hydroxycinnamoyl groups in compound **2** were located at C-2″ and C-3″ by the HMBC correlations (Supporting Information) from H-2″ to C-9‴ and H-3″ to C-1‴', respectively. Therefore, compound **2** was identified as 6-*O*-α-L-(2″, 3″-di-*O*-*trans*-*p*-hydroxycinnamoyl)rhamnopyranosylcatalpol, and it was named gmelinoside N.

The MS, HRMS and NMR data (Table 2) for **10**–**12** indicated these compounds to be isomers containing two fragments, namely a *trans*- (compounds **10** and **11**) or a *cis*-cinnamoyl (compound **12**) moieties and a rhamnopyranosyl group in their molecular structures, like those known to be present in 1-*O*-*trans*-cinnamoyl-α-L-rhamnopyranose (**13**) (Salib et al., 2008). The downfield chemical shifts were observed for H-2 (δ_H 5.07, br s) in **10**, H-3 (δ_H 5.12, dd, *J* = 9.5, 2.5 Hz) in **11** and H-2 (δ_H 5.03, br s) in **12**, which implied that the cinnamoyl groups should be attached at C-2 of compounds **10** and **12**, and C-3 of compound **11**, respectively. This deduction was further confirmed by their HMBC spectra (Fig. 2 and Supporting Information). Thus, compounds **10**–**12** were identified as 2-*O*-*trans*-cinnamoyl-α-L-rhamnopyranose, 3-*O*-*trans*-cinnamoyl-α-L-rhamnopyranose and 2-*O*-*cis*-cinnamoyl-α-L-rhamnopyranose, respectively.

The known glycosides were determined to be 6-*O*-α-L-(4″-*O*-*trans*-cinnamoyl)rhamnopyranosylcatalpol (**3**) (Taskova et al., 2006), 6-*O*-α-L-(3″,4″-di-*O*-*trans*-cinnamoyl)rhamnopyranosylcatalpol (**4**) (Taskova et al., 2006), 6-*O*-α-L-(2″,3″-di-*O*-*trans*-cinnamoyl)rhamnopyranosylcatalpol (**5**) (Taskova et al., 2006), 6-*O*-α-L-(2″-*O*-*trans*-cinnamoyl)rhamnopyranosylcatalpol (verbaspinoside, **6**) (Taskova et al., 2006; Tatli et al., 2003), 6-*O*-α-L-(3″-*O*-*trans*-*p*-methoxycinnamoyl)rhamnopyranosylcatalpol (**7**) (Otsuka et al., 1991a), 6-*O*-α-L-(2″-*O*-*trans*-*p*-coumaroyl)rhamnopyranosylcatalpol, (**8**) (Otsuka et al., 1990), 6-*O*-α-L-(3″-*O*-*trans*-cinnamoyl)rhamnopyranosylcatalpol (**9**) (Tatli et al., 2003), 1-*O*-*trans*-cinnamoyl-α-L-rhamnopyranose (**13**) (Salib et al., 2008), 3-*O*-*trans*-*p*-methoxycinnamoyl-α-L-rhamnopyranose (**14**) (Otsuka et al., 1991a), and verbascoside (**15**) (Miyase et al., 1982), by comparing their spectroscopic data with those reported in the literature.

2.2. Hepatoprotective activity

The cytoprotective activity against CCl_4-induced cytotoxicity of 11 selected compounds (**1**–**9**, **10**, **11** and **15**) that were isolated from *G. arborea* flowers was investigated. The preliminary screening results revealed that compounds **1**, **2**, **4**, **7**, **8** and **15** displayed significant activity against CCl_4-induced cytotoxicity, whereas the other compounds did not. Therefore compounds **1**, **2**, **4**, **7**, **8** and **15** were selected for determinations of their EC_{50} and CC_{50} values (Table 3). Among the compounds tested, compounds **2** exhibited the most potent suppressing effects, with EC_{50} values of

Table 1
^{1}H (400 MHz) and ^{13}C NMR (100 MHz) data of **1** and **2** in CD_3OD, *J* in Hz.

Position	**1**		**2**	
	δ_C	δ_H	δ_C	δ_H
Aglycon				
1	95.1	5.06 (m)	95.3	5.06 (d, *J* = 9.5)
3	142.4	6.36 (d, *J* = 5.8)	142.4	6.35 (m)
4	103.4	5.09 (m)	103.5	5.09 (m)
5	37.2	2.46 (m)	37.3	2.45 (m)
6	84.3	4.04 (d, *J* = 8.2)	84.5	4.03 (d, *J* = 8.0)
7	59.3	3.64 (br s)	59.5	3.63 (br s)
8	66.5		66.6	
9	43.2	2.56 (dd, *J* = 9.3, 7.8)	43.4	2.54 (m)
10	61.4	4.13 (d, *J* = 13.1), 3.78 (d, *J* = 13.1)	61.5	4.12 (d, *J* = 13.1), 3.76 (d, *J* = 13.1)
Glucosyl				
1′	99.7	4.75 (d, *J* = 7.9)	99.8	4.74 (d, *J* = 7.9)
2′	74.8	3.22 (dd, *J* = 8.9, 7.9)	74.9	3.23 (m)
3′	77.6	3.37 (t, *J* = 8.9)	77.8	3.35 (t, *J* = 8.9)
4′	71.8	3.25 (overlapped)	71.8	3.22 (m)
5′	78.6	3.28 (overlapped)	78.7	3.28 (overlapped)
6′	63.0	3.89 (d, *J* = 11.8), 3.60 (m)	63.0	3.87 (br d, *J* = 11.8), 3.58 (dd, *J* = 11.8, 6.6)
Rhamnosyl				
1″	97.7	5.03 (br s)	98.0	5.04 (br s)
2″	71.6	5.34 (m)	71.6	5.39 (m)
3″	72.8	5.15 (dd, *J* = 10.1, 3.4)	73.1	5.22 (dd, *J* = 9.8, 3.4)
4″	71.5	3.61 (m)	70.4	3.67 (m)
5″	70.3	3.85 (m)	70.4	3.87 (m)
6″	18.0	1.31 (d, *J* = 6.1)	18.1	1.32 (d, *J* = 6.0)
Acyl moiety A (R^1)				
1′	135.5		127.1	
2′, 6′	129.4	7.61 (m)	131.5	7.42 (d, *J* = 8.5)
3′, 5′	130.1	7.40 (m)	117.0	6.76 (d, *J* = 8.5)
4′	131.8	7.40 (m)	161.5	
7′	147.4	7.71 (d, *J* = 16.0)	147.7	7.59 (d, *J* = 15.9)
8′	118.1	6.58 (d, *J* = 16.0)	114.5	6.36 (d, *J* = 15.9)
9′	167.4		168.1	
Acyl moiety B (R^2)				
1″			127.2	
2″, 6″			131.2	7.33 (d, *J* = 8.5)
3″, 5″			116.9	6.69 (d, *J* = 8.5)
4″			161.3	
7″			147.1	7.54 (d, *J* = 16.0)
8″			115.0	6.25 (d, *J* = 16.0)
9″			168.5	
3″-O-isovaleryl (R^2)				
1″	174.1			
2″	44.1	2.16 (d, *J* = 7.0)		
3″	26.7	2.03 (m)		
4″	22.7	0.89 (d, *J* = 6.5)		
5″	22.8	0.90 (d, *J* = 6.5)		

42.45 μM, it was more effective than the DDB (EC_{50} = 277.3 μM) and bicylo-ethanol (EC_{50} = 279.2 μM). These observations indicated that the acylated iridoid glycosides (**2** and **8**) containing phenolic hydroxy groups were more active than were the other one.

3. Materials and methods

3.1. General experimental procedues

The optical rotation values were determined using a Jasco P-1020 polarimeter. The UV spectra in methanol were obtained using a Shimadzu UV 2401PC spectrophotometer. The IR spectra were obtained using a BRUKER Tensor-27 spectrophotometer. The 1D and 2D NMR spectra were recorded using Bruker AM-400 and DRX-500 spectrometers with TMS as the internal standard. ESI-MS data were obtained using a Bruker HCT/Esquire system, whereas the HR-ESI-MS data were obtained using a Waters Autospec Premier P776 spectrometer at 70 eV. HR-EI-MS analyses were performed using a VG Auto Spec-3000 MS instrument. Column chromatography was performed using silica gel 80–100; 300–400 mesh; GF-254 (SiO_2; Qingdao Meigao Chemical Co.); C_{18} reversed-phase silica gel (SiO_2, 40–75 μm; Fuji SilysiaChemical Ltd.), and Sephadex LH-20 gel (GE Healthcare Bio-Sciences AB). HPLC analytical and purification separations were conducted using Agilent 1200 equipment with a diode array detector and an Agilent Zorbax SB-C_{18} reversed-phase column (9.4 mm × 250 mm).

3.2. Plant material

The *G. arborea* flowers were collected in Xishuangbanna, Yunnan Province, People's Republic of China, in February 2011. The plant was identified by Professor Chun-Lin Long at the Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (No. BN-0002) was deposited at the Key Laboratory of Economic Plants and Biotechnology, Kunming Institute of Botany.

3.3. Extraction and isolation

The air-dried *G. arborea* flowers (6.5 kg) were ground, and extracted three times with MeOH (4 h each time) at 70 °C. The MeOH-extracts were suspended in water and successively extracted with petroleum ether, EtOAc and *n*-butanol.

The butanol-soluble portion (135 g) was fractionated by CC on silica gel 60 (80–100 mesh) by elution a gradated series of $CHCl_3$–MeOH solutions (20:1, 10:1, 5:1, 2:1 and 1:1) and finally with MeOH to obtain 6 fractions (Fr. 1–Fr. 6). The components in Fr. 3 ($CHCl_3$–MeOH, 5:1) were separated using a C_{18} reversed-phase column with a H_2O–MeOH step-gradient (10% MeOH → 95% MeOH), yielding Fr. 3.1–3. Fr. 3.1 was fractionated on a Sephadex LH-20 column using methanol as the solvent and then successively by CC on 300–400 mesh SiO_2 and HPLC on RP-18 with MeOH-0.05% TFA as the solvent (35:65) to obtain compound **12** (9.2 mg; t_R = 39.3 min). Fr. 4 was fractionated on a C_{18} reversed-phase column with a H_2O–MeOH step-gradient (10% MeOH → 95% MeOH). A portion of the 40% MeOH fraction (23.4 g) was applied to a Sephadex LH-20 column and eluted with MeOH, yielding Fr. 4.1–4.5. Fr 4.1 was successively subjected to CC in $CHCl_3$–MeOH (15:1) and reversed-phase HPLC with MeCN–H_2O (20:80) as the eluent, yielding compound **9** (1.03 g; t_R = 12.2 min). Fr. 4.3 was subjected to CC in $CHCl_3$–MeOH (10:1), yielding compounds **3** (147.7 mg), **7** (71.5 mg) and **2** (20.3 mg). The 60% MeOH sample was fractionated on a Sephadex LH-20 column with MeOH as the eluent, and subsequently fractionated by column chromatography on a silica gel column with $CHCl_3$–MeOH (10:1) as the eluent, to obtain compound **1** (11.3 mg). The 70% MeOH fraction was subjected to CC on a Sephadex LH-20 column with MeOH as the eluent and subsequently to CC on a gel column and with $CHCl_3$–MeOH–H_2O (4:1:0.1) as the eluent, to obtain compounds **6** (55.2 mg), **4** (6.0 mg) and **5** (18.8 mg). Fr. 5 ($CHCl_3$–EtOH, 1:1) was subjected to a C_{18} reversed-phase column with a H_2O–MeOH step-gradient (10% MeOH → 95% MeOH). Compound **8** (60.2 mg) was obtained in the 20% EtOH fraction. A portion of the 30% MeOH fraction (8.8 g) was applied to a Sephadex LH-20 column and eluted with MeOH and then successively subjected to preparative TLC ($CHCl_3$–MeOH, 2:1) to yield compound **15** (80.7 mg).

The AcOEt-soluble portion (33.4 g) was subjected to column chromatography (SiO_2, $CHCl_3$–MeOH, 1:0 → 0:1) to obtain Fr. 1–7. Fr. 3 ($CHCl_3$–MeOH, 10:1) was fractionated on a C_{18} reversed-phase column using a H_2O–MeOH step-gradient (10% MeOH → 95% MeOH). A portion of the 40% MeOH fraction (1.2 g) was fractionated by successive column chromatography on a Sephadex

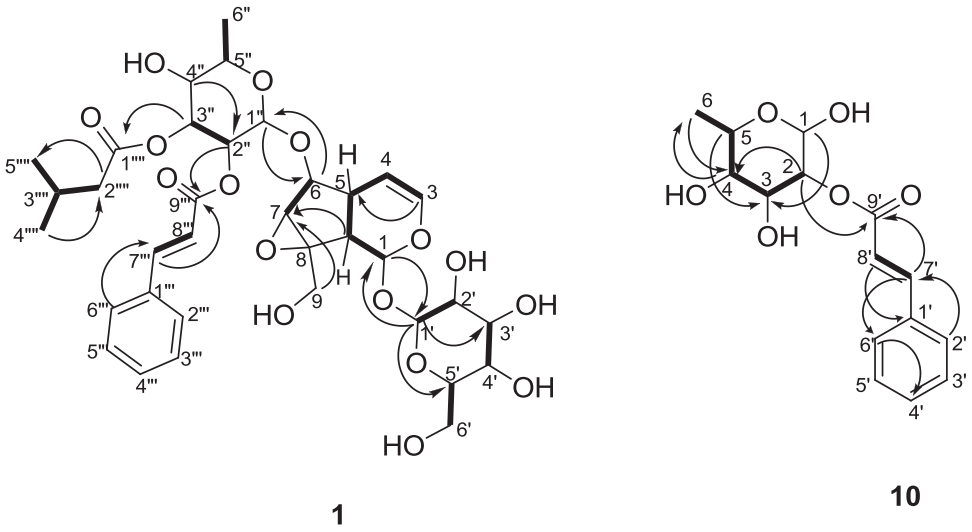

Fig. 2. Selected HMBC (arrow, H → C) and ^{1}H–^{1}H COSY (bold) correlations of compounds **1** and **10**.

Table 2
^{1}H NMR and ^{13}C NMR data of **10**–**12**, *J* in Hz.

Position	**10**[a]		**11**[a]		**12**[b]	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	5.03 (br s)	93.1	4.98 (s)	95.9	5.01 (br s)	95.1
2	5.07 (br s)	75.3	3.97 (m)	71.1	5.03 (br s)	75.2
3	3.93 (dd, *J* = 9.5, 2.8)	70.3	5.12 (dd, *J* = 9.5, 2.5)	75.8	3.90 (dd, *J* = 9.3 2.8)	70.4
4	3.42 (t, *J* = 9.5)	74.6	3.61 (t, *J* = 9.5)	71.7	3.26 (t, *J* = 9.3)	74.5
5	3.85 (m)	69.3	3.92 (m)	69.5	3.81 (m)	69.3
6	1.26 (d, *J* = 6.2)	18.2	1.25 (d, *J* = 6.2)	18.3	1.21 (d, *J* = 6.0)	18.1
1′		135.7		136.0		136.3
2′, 6′	7.61 (m)	129.3	7.56 (m)	129.4	7.60 (m)	130.9
3′, 5′	7.58 (m)	130.0	7.36 (m)	130.2	7.31 (m)	129.1
4′	7.53 (m)	131.6	7.35 (m)	131.7	7.39 (m)	130.1
7′	7.69 (d, *J* = 16.0)	146.7	7.73 (d, *J* = 16.0)	146.5	7.01 (d, *J* = 12.7)	144.6
8′	6.56 (d, *J* = 16.0)	118.8	6.57 (d, *J* = 16.0)	119.3	6.01 (d, *J* = 12.7)	120.3
9′		168.2		168.4		167.4

[a] Measured in CD_3OD at 400 and 100 MHz.
[b] Measured in CD_3OD at 500 and 125 MHz.

LH-20 column eluted with MeOH and a 300–400 mesh SiO_2 column eluted with $CHCl_3$–MeOH, 40:1, and then by HPLC on a RP-18 column eluted with MeOH-0.05% TFA (40:60), yielding compounds **10** (12.1 mg, t_R = 42.8 min), **11** (35.0 mg; t_R = 32.7 min), **13** (15.2 mg; t_R = 36.2 min), and **14** (18.3 mg; t_R = 45.3 min).

3.4. Characterization of the new compounds

3.4.1. 6-*O*-α-L-(2″-*O*-trans-cinnamoyl-3″-*O*-isovaleryl)rhamnopyranosylcatalpol (**1**)

Pale yellow amorphous solid; $\alpha_D^{18} = -131.2$ (*c* = 0.05, MeOH); UV (MeOH) λ_{max} (log ε): 279 (30.98), 203 (34.99) nm; IR (KBr)

Table 3
Cytoprotective effects of compounds **1**–**8**, **10**, **11**, and **15** against CCl_4-induced liver injury in HL-7702 cells in vitro (means ± SD, *n* = 3).[a]

Compound	EC_{50} (μM)	CC_{50} (μM)	SI (CC_{50}/EC_{50})
1	154.97 ± 0.019	1302.01 ± 0.084	8.4
2	42.45 ± 0.004	819.22 ± 0.096	19.3
4	148.24 ± 0.013	975.29 ± 0.107	6.6
7	142.00 ± 0.01	1645.74 ± 0.069	11.6
8	58.02 ± 0.008	922.14 ± 0.147	15.9
15	137.69 ± 0.014	1437.74 ± 0.134	10.4
DDB[b]	277.27 ± 0.026	2724.88 ± 0.014	9.8
Bicyclo-ethanol[b]	279.24 ± 0.033	3402.16 ± 0.028	12.2

[a] Because compounds **3**, **5**, **6**, **10**, and **11** did not show significant cytoprotective effects as compared with the control groups, the EC_{50} values of these compounds were not determined.
[b] Positive control.

ν_{max}: 3412, 1723, 1636, 1451, 1132, 1075, 1015, 768 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESI-MS (negative) *m/z* 757 $[M+Cl]^-$; HR-ESI-MS (negative) *m/z* 757.2476 $[M+Cl]^-$ (calculated for $C_{35}H_{46}O_{16}Cl$, 757.2474).

*3.4.2. 6-O-α-L-(2″,3″-di-O-trans-p-hydroxycinnamoyl)rhamnopyranosylcatalpol (**2**)*

Pale yellow amorphous solid; $\alpha_D^{18} = -3.7$ (*c* = 0.1, MeOH); UV (MeOH) λ_{max} (log ε): 313 (38.35), 229 (22.64), 204 (29.29) nm; IR (KBr) ν_{max}: 3432, 1705, 1690, 1630, 1514, 1075, 834, 578 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; ESI-MS (negative) *m/z* 799 $[M+H]^-$; HR-ESI-MS (negative) *m/z* 799.2465 $[M-H]^-$ (calculated for $C_{39}H_{43}O_{18}$, 799.2449).

*3.4.3. 2-O-trans-cinnamoyl-α-L-rhamnopyranose (**10**)*

White amorphous powder; $\alpha_D^{23} = +84.8$ (*c* = 0.06, MeOH); UV (MeOH) λ_{max} (log ε): 279 (14.64), 216 (9.87), 205 (9.23) nm; IR (KBr) ν_{max}: 3433, 1699, 1635, 1451, 1314, 1185, 1059, 982, 769 cm^{-1}; ^1H and ^{13}C NMR, see Table 3; ESI-MS (positive) *m/z* 317 $[M+Na]^+$; HR-ESI-MS (positive) *m/z* 317.1000 $[M+Na]^+$ (calculated for $C_{15}H_{18}O_6Na$, 317.1001).

*3.4.4. 3-O-trans-cinnamoyl-α-L-rhamnopyranose (**11**)*

White amorphous powder; $\alpha_D^{23} = +44.8$ (*c* = 0.06, MeOH); UV (MeOH) λ_{max} (log ε): 277 (18.67), 216 (13.06), 206 (11.95) nm; IR (KBr) ν_{max}: 3426, 1696, 1636, 1451, 1315, 1185, 1058, 979, 769 cm^{-1}; ^1H and ^{13}C NMR, see Table 3; ESI-MS (positive) *m/z* 317 $[M+Na]^+$; HR-EI-MS *m/z* $[M]^+$ 294.1103 (calculated for $C_{15}H_{18}O_6$, 294.1098).

*3.4.5. 2-O-cis-cinnamoyl-α-L-rhamnopyranose (**12**)*

White amorphous powder; $\alpha_D^{20} = +9.1$ (*c* = 0.34, MeOH); UV (MeOH) λ_{max} (log ε): 275 (15.76), 204 (16.79) nm; IR (KBr) ν_{max}: 3426, 1682, 1634, 1191, 1059, 981, 699 cm^{-1}; ^1H and ^{13}C NMR, see Table 3; ESI-MS (negative) *m/z* 329 $[M+Cl]^-$; HR-EI-MS *m/z* $[M]^+$ 294.1103 (calculated for $C_{15}H_{18}O_6$, 294.1100).

3.5. Evaluation of in vitro cytoprotective activities

The immortalized normal human hepatic cell line HL-7702, provided from Shanghai Bioleaf Biotech Co. Ltd, was used in these experiments. The HL-7702 cells were cultured in vitro (Guo et al., 2012). After the isolated human hepatocytes were plated, they were treated with each compound or with the positive control substances, biphenyldimethylesterate (DDB) or bicylo-ethanol, for 16 h. Then the hepatocytes were exposed to 3 μL CCl_4 to induce cytotoxocity. The MTT method was used to determine the number of cells, based on the absorbance at 490 nm in an enzyme-linked immunosorbent assay (ELISA) (Shimoda et al., 2008).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.phytol.2013.08.016.

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