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Six new compounds from *Atractylodes lancea* and their hepatoprotective activities

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ABSTRACT

Two new phenolic glycosides with a rare *β*-D-glucopyranosyl-(1→3)-*α*-L-rhamnopyranosyl moiety (**1**, **2**), one new dihydrobenzofuran derivative (**3**), one new pyrazine derivative (**4**), two new furofuran lignan glycosides (**5**, **6**), and six known compounds (**7**−**12**) were isolated from the rhizomes of *Atractylodes lancea*. The structures of these compounds were elucidated by extensive spectroscopic analyses combined with the experimental and calculated electronic circular dichroism and the $Rh_2(OCOCF_3)_4$-induced circular dichroism for configurational assignments. Notably, compounds **1**−**3** showed significant hepatoprotective activities against *N*-acetyl-*p*-aminophenol-induced HepG2 cell injury. This study is also the first report on the isolation of furofuran lignans and pyrazine derivatives (**4**−**7**) from the genus *Atractylodes*.

key words:

Atractylodes lancea

phenolic glycosides

furofuran lignan

pyrazine

dihydrobenzofuran

Genus *Atractylodes* are distributed widely in eastern Asia and has a long medical history. The rhizomes of *Atractylodes lancea*, known as 'Cangzhu' in traditional Chinese medicine, were reputed to treat rheumatic diseases, digestive disorders, influenza, and other diseases.[1−3] Previous phytochemical investigations revealed a series of sesquiterpenoids, monoterpenes, polyacetylenes, phenolic acids, and steroids from *A. lancea*.[4−9] A survey of the literature revealed that the extract and chemical constituents of this plant possess potent hepatoprotective effects.[10] In our search for bioactive constituents from *A. lancea*,[11] six new compounds including two phenolic glycosides (**1**, **2**),[12,13] one prenylated dihydrobenzofuran derivative (**3**),[14] one pyrazine derivative (**4**),[15] two furofuran-type lignan glycosides (**5**, **6**),[16,17] and six known compounds (**7**−**12**) were isolated from the *n*-butanol fraction. The structures of these compounds were determined by extensive spectroscopic analyses. The stereochemistry of **3**−**6** was defined using the experimental and calculated electronic circular dichroism (ECD) and $Rh_2(OCOCF_3)_4$-induced CD. All of the compounds were assessed for the hepatoprotective activities against *N*-acetyl-*p*-aminophenol (APAP)-induced HepG2 cell injury.[18]

Compound **1** has the molecular formula of $C_{19}H_{26}O_{12}$ with seven degrees of unsaturation, as deduced by the HRESIMS adduct ion at *m*/*z* 445.1353 $[M - H]^-$ and ^{13}C NMR data. Its IR spectrum showed absorption signals of hydroxy (3389 cm^{-1}) and phenyl (1694, 1606, and 1510 cm^{-1}) groups. The four aromatic proton resonances (Table 1) at δ_H 7.14 (2H, d, 8.5 Hz) and 7.89 (2H, d, 8.5 Hz) in the ^1H NMR spectrum, in association with the aromatic carbon resonances (Table 1) at δ_C 116.2, 124.3, 131.3, 159.4, and 166.9 in the ^{13}C NMR spectrum suggested the *p*-hydroxybenzoic acid skeleton. The remaining 12 carbon signals

were assigned to a rhamnopyranosyl moiety (δ_C 98.1, 69.2, 80.9, 70.6, 69.3, and 17.9) and a glucopyranosyl moiety (δ_C 104.7, 74.0, 76.8, 69.8, 76.2, and 61.0). In the HMBC experiment, a key long-range correlation (Figure 2) from the anomeric proton at δ_H 5.51 (Rha-C-1′) to the aromatic carbon at δ_C 159.4 (C-4) defined the location of the rhamnosyl moiety. In accordance with the ^{1}H-^{1}H spin system [C(1′)H-C(2′)H-C(3′)H] (Figure 2), an HMBC correlation from the anomeric proton (Glc-H-1′) at δ_H 4.47 to the carbon (Rha-C-3′) at δ_C 80.9 confirmed that the glucosyl moiety was substituted at Rha-C-3′, which was also evidenced by the $^3J_{H\text{-}2',3'}$ (3.0 Hz) and $^3J_{H\text{-}3',4'}$ (9.0 Hz) values. The L-rhamnose and D-glucose were identified by GC analyses following hydrolysis of **1** and chiral derivatization.[19–21] Their relative absolutions were deduced based on the $^3J_{1',2'}$ value (1.5 Hz) of the L-rhamnopyranosyl moiety and the $^3J_{1'',2''}$ value (7.5 Hz) of the D-glucopyranosyl moiety. Consequently, the structure of compound **1** was elucidated as *p*-hydroxybenzoic acid-4-O-*β*-D-glucopyranosyl-(1→3)-*α*-L-rhamnopyranoside.

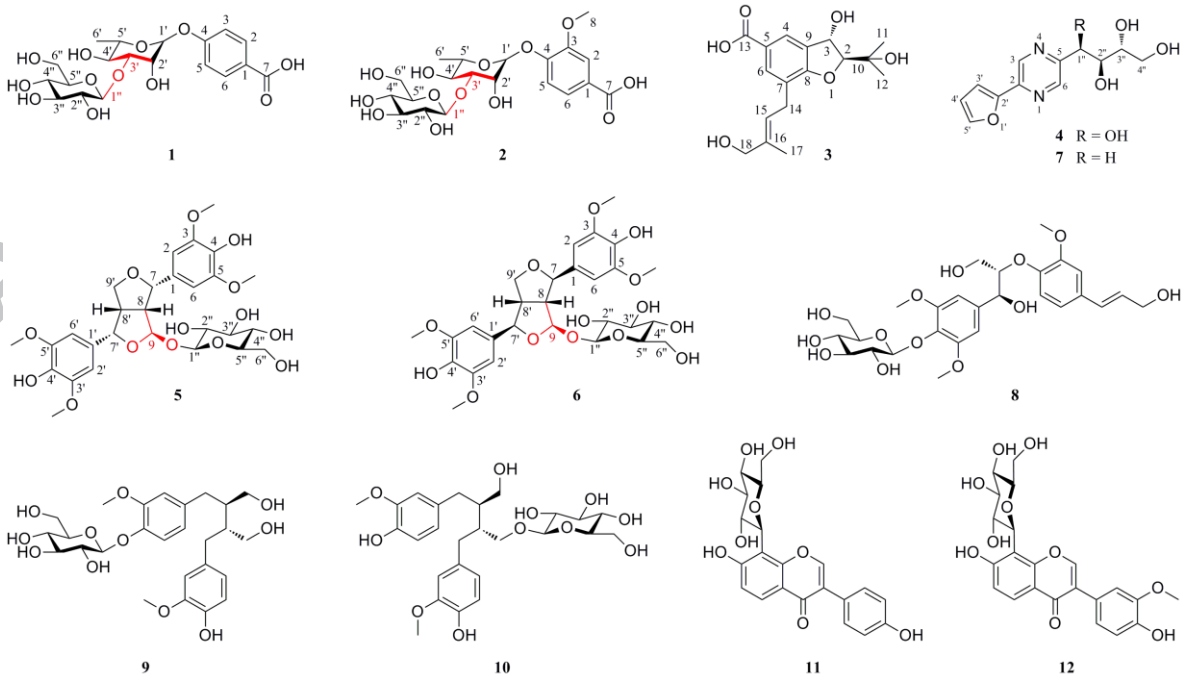

Figure 1. The chemical structures of compounds **1**–**12**.

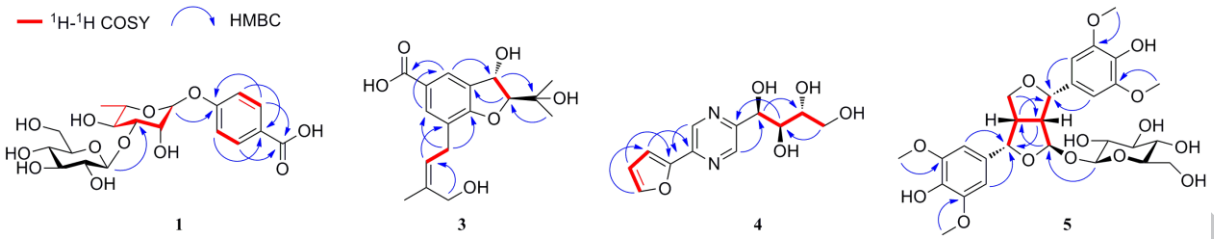

Figure 2. The key ^{1}H-^{1}HCOSY and HMBC correlations of compounds **1** and **3**–**5**.

The molecular formula of compound **2** was defined as $C_{20}H_{28}O_{13}$ by the HRESIMS adduct ion at *m/z* 475.1461 $[M - H]^-$. Interpretation of the ^{1}H and ^{13}C NMR data (Table 1) revealed an ABX benzoic acid system at δ_H 7.19 (1H, d, 8.5 Hz), 7.50 (1H, d, 2.0 Hz), and 7.52 (1H, dd, 2.0, 8.5 Hz), coupled with δ_C 113.0, 117.1, 122.8, 125.8, 148.5, 149.6, and 167.2. A methoxyl group resonated at δ_H 3.82 and δ_C 55.8 was determined to be attached to C-3 on the basis of their HMBC cross-peaks (Figure 2). The consistent ^{13}C NMR data (Table 1) of the sugar moieties of **1** and **2** are indicative of the same carbohydrate chain present in both compounds. A long-range correlation from the anomeric proton at δ_H 5.42 (Rha-C-1′) to the aromatic carbon at δ_C 148.5 (C-4) confirmed the location of the rhamnosyl moiety. The absolute configurations of the sugar moieties were determined by GC analyses as above. Thus, compound **2** was identified as vanillic acid-4-O-*β*-D-glucopyranosyl-(1→3)-*α*-L-rhamnopyranoside.

Table 1

^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) data (δ in ppm, *J* in Hz) for compounds **1**−**3** in DMSO-d_6

NO.	1		2		NO.	3	
	δ_H	δ_C	δ_H	δ_C		δ_H	δ_C
1		124.3		125.8	2	4.21, d (4.0)	97.3
2	7.89, d (8.5)	131.3	7.50, d (2.0)	113.0	3	5.21, d (4.0)	71.1
3	7.14, d (8.5)	116.2		149.6	4	7.75, d (1.5)	125.0
4		159.4		148.5	5		130.4
5	7.14, d (8.5)	116.2	7.19, d (8.5)	117.1	6	7.65, d (1.5)	131.0
6	7.89, d (8.5)	131.3	7.52, dd (2.0, 8.5)	122.8	7		120.3
7		166.9		167.2	8		161.6

8			3.82, s	55.8	9		122.5
Rha-1'	5.51, d (1.5)	98.1	5.42, d (1.5)	99.3	10		69.8
2'	4.10, brs	69.2	4.12, brs	69.2	11	1.17, s	25.9
3'	3.77, dd (3.0, 9.0)	80.9	3.77, dd (3.0, 9.0)	81.0	12	1.08, s	25.0
4'	3.50, overlap	70.6	3.50, t (9.0)	70.6	13		167.4
5'	3.50, overlap	69.3	3.61, m	69.3	14	3.31, d (7.5)	27.0
6'	1.11, d (6.0)	17.9	1.10, d (6.0)	17.8	15	5.50, td (1.5, 7.5)	120.3
Glc-1"	4.47, d (7.5)	104.7	4.46, d (7.5)	104.6	16		136.7
2"	3.08, overlap	74.0	3.08, overlap	74.0	17	1.66, s	13.6
3"	3.17, overlap	76.8	3.17, overlap	76.8	18	3.81, brs	66.1
4"	3.08, overlap	69.8	3.10, overlap	69.8			
5"	3.19, overlap	76.2	3.19, overlap	76.2			
6"a	3.67, brd (11.5)	61.0	3.66, brd (11.0)	60.9			
6"b	3.47, overlap		3.47, dd (5.0, 11.0)				

Compound **3** showed the molecular formula of $C_{17}H_{22}O_6$, as established by the negative HRESIMS ion at *m/z* 321.1349 $[M - H]^-$. The IR absorption spectrum indicated the presence of hydroxyl (3357 cm^{-1}), carbonyl (1687 cm^{-1}), and aromatic (1610 and 1480 cm^{-1}) groups. In combination with the HSQC data, two aromatic protons at δ_H 7.75 (d, 1.5 Hz) and 7.65 (d, 1.5 Hz), an olefinic proton at δ_H 5.50 (td, 1.5, 7.5 Hz), three methyl groups at δ_H 1.08, 1.17, and 1.66, two methylene groups at δ_H 3.31 and 3.81 were observed in the ^1H NMR spectrum. The ^{13}C NMR data (Table 1) revealed eight olefinic carbons (δ_C 120.3, 120.3, 122.5, 125.0, 130.4, 131.0, 136.7, and 161.6), a carbonyl carbon (δ_C 167.4), three methyl carbons (13.6, 25.0, and 25.9), two methylene carbons (δ_C 27.0 and 66.1), two methine carbons (δ_C 71.1 and 97.3), and an oxygenated tertiary carbon (δ_C 69.8). With the exception of the chemical shifts of an isopentene group, the aforementioned data were similar to those of a known dihydrobenzofuran derivative isolated from *A. lancea*.[22] The major difference detected by the HMBC experiment was that H_2-18 resonant at δ_H 3.81 was correlated with C-15 resonant at δ_C 120.3. Accordingly, the structure of compound **3** was

determined as shown in Figure 1. The coupling constant (4.0 Hz) of H-2 and H-3 suggested a *trans* configuration.[23] To designate the absolute configuration, ECD calculation was performed using the MMFF94 force field and TDDFT method at the B3LYP/6-31+G(d,p) level (supplementary data, S32). The calculated data obtained for the (2*S*,3*S*)-conformer and the experimental data of **3** were well-matched (Figure 3).

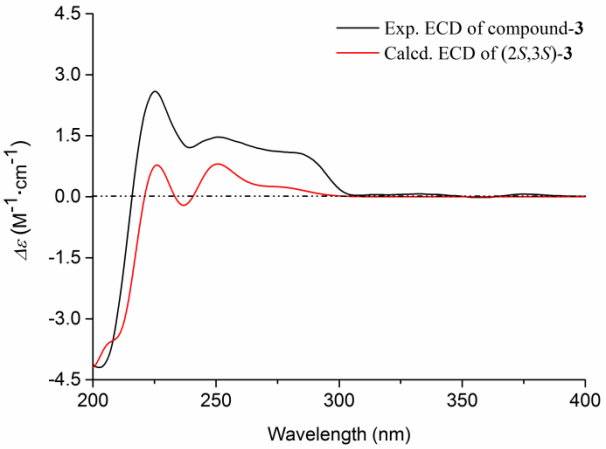

Figure 3. The experimental and calculated spectra of compound **3**.

Compound **4** exhibited a positive HRESIMS quasi-molecular ion at *m/z* 267.0975 $[M + H]^+$ that was correlated to the molecular formula of $C_{12}H_{14}N_2O_5$. Its IR absorption signals were attributed to hydroxyl (3289 cm^{-1}) and aromatic (1605, 1503 cm^{-1}) groups. The ^1H NMR data (Table 2) revealed three methines at δ_H 4.98 (1H, d, 5.0 Hz) and 3.60 (2H, overlap), one methylene at δ_H 3.43 (1H, m), 3.60 (1H, overlap), and five olefinic protons at δ_H 6.70 (dd, 1.5, 3.0 Hz), 7.23 (d, 3.0 Hz), 7.91 (brs), 8.71 (s), and 8.89 (d, 1.0 Hz). Correspondingly, four olefinic carbons δ_H 110.1, 112.5, 138.0, 142.1, 142.9, 144.9, 151.0, and 157.3, one oxygenated primary carbon at δ_C 63.6, and three oxygenated carbons at δ_C 71.2, 71.7, and 73.8 were observed in the ^{13}C NMR spectrum. The obtained spectral data were highly similar to those reported for crotonine (**7**).[24] Comparison of the HRESIMS data

of **4** and **7** disclosed an additional hydroxy in **4**. The deshielded resonance at δ_C 71.7 indicated that the hydroxy was substituted at C-1". This was evidenced by further analyses of the 2D NMR data. Thus, compound **4** was elucidated as 1"-hydroxylcrotonine. Based on biogenetic considerations, C-5, C-6, and the side chain ($C_{1"}$-$C_{4"}$) may be derived from glucose. This plausible hypothesis was further supported by the ECD calculation showing that the experimental ECD spectrum (Figure 4) of **4** was in good agreement with the theoretical spectra of a simplified structure (**4a**). Therefore, the absolute configuration of compound **4** was defined as (1"*R*,2"*S*,3"*R*).

Table 2

^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) data (δ in ppm, *J* in Hz) for compounds **4**−**6** in DMSO-d_6

NO.	4		NO.	5		6	
	δ_H	δ_C		δ_H	δ_C	δ_H	δ_C
2		142.1	1		131.8		132.9
3	8.89, d (1.0)	138.0	2, 6	6.63, s	103.5	6.60, s	102.9
5		157.3	3, 5		148.0		148.0
6	8.71, s	142.9	4		135.0		134.7
2'		151.0	7	4.77, d (8.0)	82.5	4.78, d (5.5)	82.8
3'	7.23, d (3.0)	110.1	8	2.85, t (8.0)	61.0	2.99, overlap	60.7
4'	6.70, dd (1.5, 3.0)	112.5	9	5.66, s	101.4	5.57, s	107.9
5'	7.91, brs	144.9	10, 11	3.76, s	56.0	3.76, s	56.0
1"	4.98, d (5.0)	71.7	1'		132.0		132.2
2"	3.60, overlap	73.8	2', 6'	6.77, s	104.2	6.83, s	103.8
3"	3.60, overlap	71.2	3', 5'		148.0		147.8
4"a	3.60, overlap	63.6	4'		135.0		134.5
4"b	3.43, m		7'	4.88, d (8.0)	88.2	4.87, d (5.5)	87.7
			8'	3.09, overlap	53.0	3.01, overlap	53.2
			9'a	4.16, dd (5.5, 8.5)	70.8	4.14, dd (5.5, 8.5)	71.7
			9'b	3.96, d (8.5)		3.99, d (8.5)	
			10', 11'	3.77, s	56.2	3.76, s	56.0
			Glc-1"	4.52, d (7.5)	97.9	4.38, d (7.5)	101.9
			2"	3.00, m	73.3	2.98,overlap	73.8
			3"	3.08, overlap	77.2	3.08, overlap	77.2
			4"	3.02, m	70.1	3.07, overlap	69.9
			5"	3.15, t (8.5)	76.8	3.13, overlap	76.7

	6"a	3.62, brd (11.0)	61.2	3.64, dd (5.5,11.0)	61.0
	6"b	3.37, overlap		3.44, overlap	

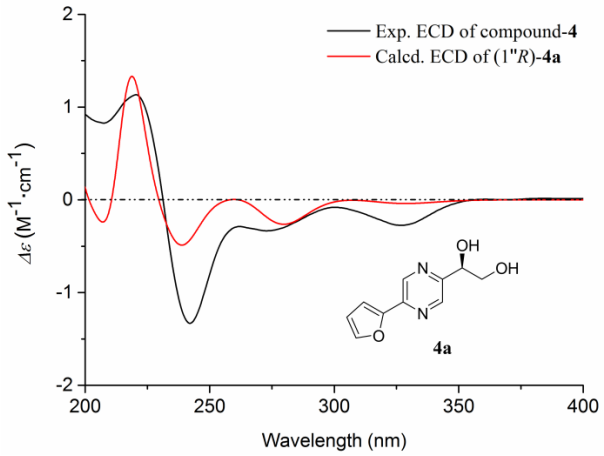

Figure 4. The experimental and calculated spectra of compound **4**.

Compound **5** exhibited a positive HRESIMS quasi-molecular ion at *m/z* 619.1996 [M + Na]$^+$, compatible with the molecular formula of $C_{28}H_{36}O_{14}$. The IR spectrum exhibited absorption signals of hydroxy (3429 cm^{-1}) and aromatic (1613, 1518 cm^{-1}) groups. The ^{1}H and ^{13}C NMR data (Table 2) showing the four aromatic protons at δ_H 6.63 (2H, brs, H-2, 6) and 6.77 (2H, brs, H-2', 6'), coupled with the twelve aromatic carbons at δ_C 131.8 (C-1), 103.5 (C-2, 6), 148.0 (C-3, 5, 3', 5'), 135.0 (C-4, 4'), 132.0 (C-1'), and 104.2 (C-2', 6') implied the presence of two 3,4,5-*O*-trisubstituted aromatic rings. Additionally, based on HMBC analyses, four methoxy groups at δ_H 3.76 (6H) and 3.77 (6H) were determined to be attached to C-3, 3', 5, and 5'. The ^{13}C NMR data exhibited a total of 18 carbons except for the methoxy groups and glucosyl moiety, which indicated two C6-C3 systems. Moreover, the carbons resonant at δ_C 82.5 (C-7), 61.0 (C-8), 101.4 (C-9), 88.2 (C-7'), 53.0 (C-8'), and 70.8 (C-9') further suggested of a 9-hydroxylfurofuran lignan.[25] Based on the HMBC data (Figure 2), the glucosyl moiety (δ_C 97.9, 73.3, 77.2, 70.1, 76.8, and 61.2) was located at C-9. Thus, compound **5** was defined as hydroxysyringaresinol-9-O-*β*-D-glucopyranoside. In the

NOESY experiment, the correlations (Figure 5) of H-7 with H-8', H-7' with H-8 revealed that these were on the same face of the furofuran-ring, which was supported by the ^{1}H NMR coupling constant (8.0 Hz).[26] H-9 was determined on the opposite side of the the furofuran-ring by the chemical shift (δ_C 101.4) of C-9.[27,28] The absolute configuration was established as (7*S*,7'*S*,8*R*,8'*S*,9*R*) by comparing the experimental and theoretical ECD spectra of **5** (Figure 6). The above configurational assignment of C-9 was in agreement with the $Rh_2(OCOCF_3)_4$-induced CD (supplementary data, S25) of **5a** that was hydrolyzed from **5** with snailase.[29–31] The *β*-D-form of the glucopyranosyl moiety was deduced based on the $^{3}J_{1'',2''}$ value (7.5 Hz) and GC analyses after chiral derivatization.[19–21]

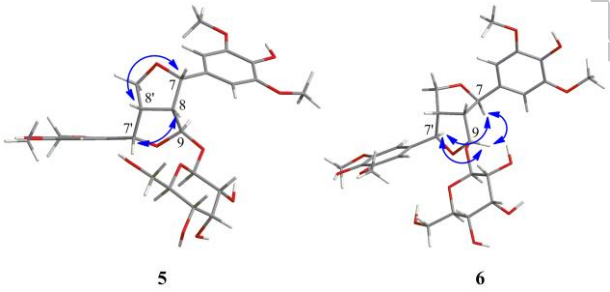

Figure 5. The key NOESY correlations of compounds **5** and **6**.

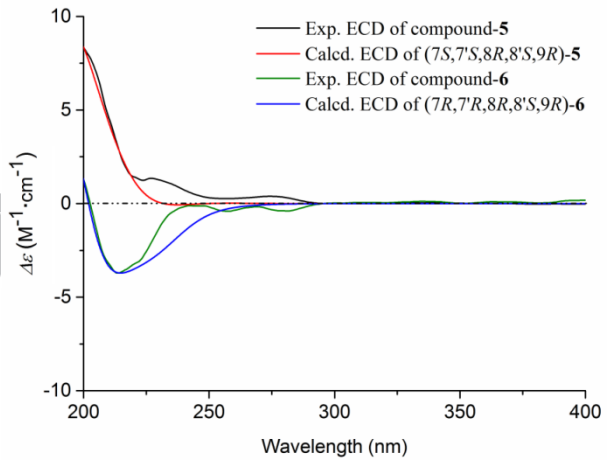

Figure 6. The experimental and calculated spectra of compounds **5** and **6**.

Compound **6** showed a planar structure identical to that of **5** as determined by detailed analyses of the 1D and 2D NMR spectroscopic data and the HRESIMS adduct ion at *m/z*

595.2053 [M − H]$^-$. The NOESY correlations (Figure 5) of H-7 with H-7'/H-9, and H-7' with H-9 indicated that H-7, H-7', and H-9 were on the same face of the furofuran-ring, whereas H-8 and H-8' were on the opposite side. This assignment was unambiguously supported by the ^{1}H NMR coupling constant (5.0 Hz) of H-7/H-8, H-7'/8' and the chemical shift (δ_C 107.9) of C-9.[26–28] The absolute configuration was established by ECD calculation. The experimental ECD data of **6** were in good agreement with its calculated data (Figure 6). Thus, the structure of compound **6** was elucidated as (7*R*,7'*R*,8*R*,8'*S*,9*R*)-hydroxysyringaresinol-9-O-*β*-D-glucopyranoside .

In addition to the above new compounds, six compounds (**7**−**12**) were identified as the known compounds crotonine (**7**),[24] longifloroside B (**8**),[32] secoisolariciresinol-4-O-*β*-D-glucopyranoside (**9**),[33] secoisolariciresinol-9-O-*β*-D-glucopyranoside (**10**),[34] puerarin (**11**),[35] and 3'-methoxy puerarin (**12**)[36]. Their structures were determined by comparison of the 1D NMR spectroscopic data with the reported literature data.

All compounds were evaluated for hepatoprotective activities against APAP-induced HepG2 cell injury.[18] Compared with the model group (cell survival rate of 39.87%), compounds **1**, **2**, and **3** showed significant hepatoprotective activities (Figure 7) at the concentration of 10 μM with the cell survival rates of 54.69% ($p < 0.001$), 54.57% ($p < 0.001$), and 53.58% ($p < 0.001$), respectively (bicyclol with 52.87%, $p < 0.01$).

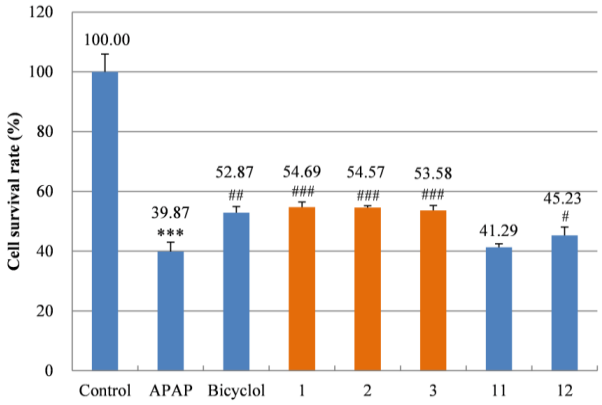

Figure 7. Hepatoprotective effects of compounds **1**–**3**, **11**, and **12** (10 μM) against APAP (8 mM)-induced HepG2 cell injury. Results are expressed as the mean ± SD (n = 3). ***p < 0.001 (*vs* control group), $^{###}p$ < 0.001, $^{##}p$ < 0.01, $^{#}p$ < 0.05 (*vs* model group).

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Supplementary data

Supplementary data associated with this article can be found online at///////.

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11. The dried rhizomes of *A. lancea* (100 kg) were pulverized and extracted with 80% EtOH-H_2O (*v*/*v*) at 85 °C for 2 h. After evaporating the solvent, the resultant residue (25.6 kg) was partitioned with 30 L H_2O and then extracted with petroleum ether, EtOAc, and *n*-BuOH. The *n*-BuOH extract (1.2 kg) was chromatographed with an HP-20 column, with a step gradient of EtOH-H_2O (*v*/*v*), to give Fr. A (824 g, H_2O-soluble), Fr. B (88.6 g, 15% EtOH-soluble), Fr. C (106.4 g, 30% EtOH-soluble), Fr. D (53.3 g, 50% EtOH-soluble) and Fr. E (19.5 g, 95% EtOH-soluble). Fr. C was chromatographed on an RP-18 column, with a gradient of MeOH-H_2O (0−100%), to yield Fr. C1−Fr. C7 via HPLC analyses. Fraction C1 (30.5 g) was chromatographed on an LH-20 column using H_2O to obtain 123 subfractions (Fr. C1.1−Fr. C1.123). Fr. C1.49−Fr. C1.95 were further separated using reversed-phase preparative liquid chromatography (P-HPLC), with 25% MeOH (*v*/*v*) to yield **1** (35.0 mg), **2** (8.9 mg), and **4** (4.5 mg). Subfraction Fr. C1.116 afforded **8** (29.2 mg). Fraction C2 (8.2 g) was eluted using a LH-20 column with H_2O to obtain 30 subfractions (Fr. C2.1−Fr. C2.30). Subtractions Fr. C2.18−Fr. C2.20 was further purified by P-HPLC, with 30% MeOH-H_2O (*v*/*v*), to afford **7** (86.9 mg). Fraction C3 (10.0 g) was separated by LH-20 using H_2O to obtain 42 subfractions (Fr. C3.1−Fr. C3.42). Then, subfraction Fr. C3.25−Fr. C3.30 produced **11** (63.3 mg) and **12** (23.2 mg). Fraction C4 (10.2 g) was separated by an LH-20 column using H_2O to yield 32 subfractions (Fr. C4.1−Fr. C4.32). These subfractions were further separated by P-HPLC using a MeOH-H_2O ratio of 30:70 (*v*/*v*). Fr. C4.17−Fr. C4.22 produced **9** (24.1 mg). Fraction C5 (12.9 g) was chromatographed on an LH-20 column using H_2O to yield 38 subfractions (Fr. C5.1−Fr. C5.38). These subfractions were separated by P-HPLC with 35% MeOH-H_2O (*v*/*v*). Subfractions Fr. C5.25−Fr. C5.28 afforded **6** (5.7 mg). Fraction C7 (9.7 g) was separated using LH-20 with H_2O (Fr. C7.1−Fr. C7.35) and further purified using P-HPLC with MeOH:H_2O (40:60, *v*/*v*). Fr. C7.29−Fr. C7.32 produced **3** (13.4 mg), **5** (19.4 mg), and **10** (38.5 mg).

12. Compound **1**: white amorphous powder; $[\alpha]_D^{20}$ −108.6 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.21), 246 (4.10) nm; IR (KBr) ν_{max} 3389, 2977, 2934, 1694, 1606, 1510, 1076, 1020 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 445.1353 $[M - H]^-$ (calcd for $C_{19}H_{25}O_{12}$, 445.1346).

13. Compound **2**: white amorphous powder; $[\alpha]_D^{20}$ −72.6 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 209 (4.41), 249 (3.95), 291 (3.48) nm; IR (KBr) ν_{max} 3392, 2975, 2936, 1693, 1601, 1512, 1078, 1034 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 475.1461 $[M - H]^-$ (calcd for $C_{20}H_{27}O_{13}$, 475.1452).

14. Compound **3**: white amorphous powder; $[\alpha]_D^{20}$ +69.0 (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ε) 206 (4.58), 258 (4.07) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 225 (+2.59), 251 (+1.47), 283 (+1.08) nm; IR (KBr) ν_{max} 3357, 2978, 2936, 1687, 1610, 1480, 1008 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 321.1349 $[M - H]^-$ (calcd for $C_{17}H_{21}O_6$, 321.1338).

15. Compound **4**: grey amorphous powder; $[\alpha]_D^{20}$ +138.5 (*c* 0.01, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.26), 272 (4.11), 330 (4.02) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 221 (+2.44), 241 (−3.42), 276 (−0.81), 325 (−0.72) nm; IR (KBr) ν_{max} 3289, 2929, 1605, 1503 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; HRESIMS *m/z* 267.0975 $[M + H]^+$ (calcd for $C_{12}H_{15}N_2O_5$, 267.0981).

16. Compound **5**: white amorphous powder; $[\alpha]_D^{20}$ −11.3 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 209 (4.81), 242 (4.03), 272 (3.17) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 225 (+1.25), 275 (+0.39) nm; IR (KBr) ν_{max} 3429, 2939, 2885, 1613, 1518, 1114, 1076 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; HRESIMS *m/z* 619.1996 $[M + Na]^+$ (calcd for $C_{28}H_{36}O_{14}Na$, 619.2003).

17. Compound **6**: white amorphous powder; $[\alpha]_D^{20}$ −88.2 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 208 (4.51), 240 (3.87), 279 (3.48) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 214 (−3.71) nm; IR (KBr) ν_{max} 3406, 2940, 1614, 1518, 1116, 1049 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; HRESIMS *m/z* 595.2053 $[M - H]^-$ (calcd for $C_{28}H_{35}O_{14}$, 595.2027).

18. The hepatoprotective activity was evaluated by an MTT assay. Human hepatoma cells (HepG2) were cultured in the DMEM medium supplemented with 10% fetal calf serum and penicillin (100 U/mL)-streptomycin (100 μg/mL) solution, at 37 °C in a humidified atmosphere with 5% CO_2. The growing cells were seeded in 96-well plates and incubated for 12 h. These cells were treated with APAP (8 mM) and various test samples (10 μM) and were further incubated for 48 h. Then, 100 μL

of MTT solution (0.5 mg/mL) was added to each well following the removal of the medium, and the solutions were incubated for an additional 4 h. The residuum was dissolved in 150 μL DMSO after emptying the culture medium, and the absorbance was quantified at 570 nm with a microplate reader. Bicyclol was used as the positive contrast.

19. Compounds **1** and **2** (each 5 mg) were separately hydrolyzed with 1 mol/L HCl-dioxane (*v*/*v* = 1:1, 5 mL) at 60 °C for 6 h. Compound **5** (5 mg) was hydrolyzed with snailase (*w*/*w* = 1:2, 3 mL H_2O as the solvent) at 37 °C for 24 h. The obtained hydrolysates were extracted with EtOAc three times (each 3 mL) to yield EtOAc extracts and monosaccharide residues after the evaporation of the solvents. The EtOAc extracts of **5** was further purified by P-HPLC and eluted with 20% CH_3CN-H_2O to yield the corresponding aglycones (**5a**). Its ^{1}H NMR spectrum is presented in the 'supplementary data' (S25). These dried monosaccharide residues were processed using the reported method and then analyzed by GC. The retention times of the trimethylsilyl-L-cysteine derivatives were as follows: L-rhamnose, 16.61 min and D-glucose, 20.56 min.
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GRAPHIC ABSTRACT