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Aromatic Glucosides from the Seeds of *Prunus davidiana*

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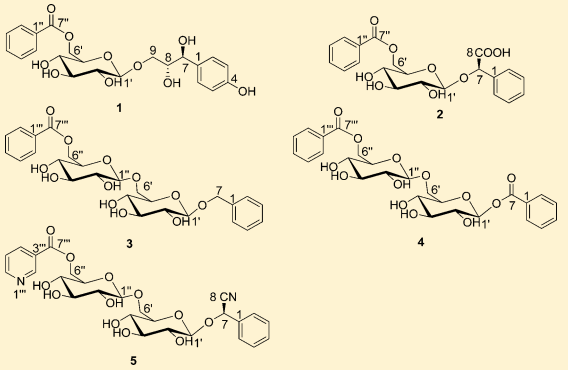
Supporting Information

ABSTRACT: Chemical investigation of the seeds of *Prunus davidiana* afforded seven new aromatic glucosides, i.e., the prupersins A–E (**1**–**5**) and compounds **6** and **7**, as well as 11 known compounds. The structures of **1**–**7** were elucidated by spectroscopic data analysis and chemical evidence, and configurations were determined by hydrolysis experiments (**1**, **2**, and **5**) or electronic circular dichroism (**6**). Compounds **1**–**6** exhibited antioxidant activity aganist Fe^{2+}-cysteine-induced rat liver microsomal lipid peroxidation, with malondialdehyde inhibitory rates of 50–67% and 53–57% at concentrations of 10^{-5} and 10^{-6} mol/L, respectively.



The seeds of *Prunus davidiana* (Carr.) Franch or *Prunus persica* (L.) Batsch, persicae semen, have been used in traditional Chinese medicine to treat cardiovascular, cerebrovascular, and gynecological diseases.[1] Previous phytochemical studies on persicae semen have led to the isolation and identification of cyanogenic glycosides, glycerides, sterols, and emulsins.[2] As part of our program to study traditional Chinese medicine, an ethanolic extract of the seeds of *P. davidiana* (Carr.) Franch was investigated. This led to the identification of 18 aromatic glucosides: the new prupersins A–E (**1**–**5**); two synthetic compounds, **6** and **7**, that have not yet been found in nature; and 11 known analogues. Herein, we report the isolation and structural elucidation of these new glucosides. In addition, we report the results of antioxidant activity assays against Fe^{2+}-cysteine-induced rat liver microsomal lipid peroxidation.

RESULTS AND DISCUSSION

Prupersin A (**1**) was obtained as an amorphous powder, $[\alpha]^{20}_{D}$ −25.3 (*c* 2.35, MeOH). The IR spectrum of **1** showed absorption bands at 3339, 1717, 1614, 1600, and 1516 cm^{-1}, indicating the presence of hydroxy, ester carbonyl, and aromatic functional groups. The molecular formula, $C_{22}H_{26}O_{10}$, was determined from positive-ion HRESIMS (473.1428 $[M + Na]^+$, calcd 473.1418) and also supported by the NMR spectroscopic data. The ^1H NMR spectrum (Table 1) revealed the presence of two oxymethine hydrogens at δ_H 4.35 (d, J = 4.5 Hz, H-7) and 3.65 (m, H-8), one oxymethylene at δ_H 3.82 (dd, J = 10.0, 2.5 Hz, H-9a) and 3.37 (overlapped, H-9b), nine aromatic protons at δ_H 7.97 (dd, J = 8.0, 1.0 Hz, H-2″, 6″), 7.68 (br dd, J = 8.0, 8.0 Hz, H-4″), 7.55 (dd, J = 8.0, 8.0 Hz, H-3″, 5″), 7.07 (d, J = 8.0 Hz, H-2, 6), and 6.62 (d, J = 8.0 Hz, H-3, 5), and a glucopyranosyl unit. The ^{13}C NMR spectrum (Table 2) displayed characteristic signals for benzoyl, 1-(4-hydroxyphenyl)glycerol, and β-glucopyranosyl moieties. In the HMBC spectrum, the correlations of H-2″, H-6″, H-3″ (weak), and H-5″ (weak)/C-7″ confirmed the presence of a benzoyl moiety, and the correlations of H-2, H-6/C-7; H-7/C-1, C-2, C-8, and C-9; H-8/C-9 and C-7 (weak); and H-9/C-7; H-9b/C-8 confirmed the presence of a phenylglycerol moiety. In addition, the correlations of H-6′/C-7″; H-9/C-1′; and H-1′/C-9 indicated the benzoyl group was attached to C-6′ of the glucopyranosyl moiety, and C-1′ of the glucopyranosyl unit was connected to C-9 of the phenylglycerol moiety.

Alkaline hydrolysis of **1** (Scheme 1) gave benzoic acid, glucose, and 1-(4-hydroxyphenyl)glycerol (**1c**). The presence of benzoic acid and glucose was confirmed by comparison with authentic samples. The D-configuration of glucose was determined by optical rotation (Experimental Section). A β-anomeric configuration for the glucosyl unit was assigned via its large $^3J_{1,2}$ coupling constant (7.5 Hz). The ^1H NMR spectrum of 1-(4-hydroxyphenyl)glycerol (**1c**) was in good agreement with that of *threo*-1-(4-hydroxyphenyl)glycerol,[3] and the positive $[\alpha]_D$ value {$[\alpha]^{20}_{D}$ +18.0 (*c* 0.06, EtOH)} indicated that the absolute configuration was 7*S*, 8*S*.[4] On the basis of the above data, prupersin A was characterized as **1** (Figure 1).

Prupersin B (**2**) was obtained as a colorless gum, $[\alpha]^{20}_{D}$ −82.0 (*c* 0.37, MeOH). The molecular formula was determined to be $C_{21}H_{22}O_9$ on the basis of positive-ion HRESIMS (441.1158 $[M + Na]^+$, calcd 441.1156), corresponding to 11 indices of hydrogen deficiency. Similar to **1**, the 1D NMR data (Tables 1 and 2) indicated the presence of a benzoyl group and a β-glucopyranosyl moiety. HMBC correlations of H-6′a and H-6′b with C-7″ confirmed that the connection between the benzoyl

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Table 1. ^{1}H NMR Spectroscopic Data of Compounds 1−5[a]

position	1	2	3	4	5
2	7.07, d (8.0)	7.36, d (6.0)	7.36, d (7.5)	7.98, dd (7.5, 2.0)	7.57,[b] m
3	6.62, d (8.0)	7.20−7.27,[b] m	7.31, dd (7.5, 7.5)	7.52, dd (7.5, 7.5)	7.45,[b] m
4		7.20−7.27,[b] m	7.26, dd (7.5,7.5)	7.67, dd (7.5, 7.5)	7.45,[b] m
5	6.62, d (8.0)	7.20−7.27,[b] m	7.31, dd (7.5, 7.5)	7.52, dd (7.5, 7.5)	7.45,[b] m
6	7.07, d (8.0)	7.36, d (6.0)	7.36, d (7.5)	7.98, dd (7.5, 2.0)	7.57,[b] m
7	4.35, d (4.5)	5.21, s	a 4.78, d (12.5)		5.98, s
			b 4.53, d (12.5)		
8	3.65, m				
9a	3.82, dd (10.0, 2.5)				
9b	3.37[b]				
1′	4.22, d (7.5)	3.99, d (7.5)	4.19, d (7.8)	5.57, dd (6, 2.0)	4.21, d (7.5)
2′	3.03, dd (7.5, 7.5)	3.20−3.44,[b] m	2.97−3.08,[b] m	3.30,[b] m	3.08,[b] m
3′	3.22[b]	3.20−3.44,[b] m	3.11, ddd (8.5, 8.5, 5.0)	3.30,[b] m	3.08,[b] m
4′	3.21[b]	3.20−3.44,[b] m	2.97−308,[b] m	3.18,[b] m	3.08,[b] m
5′	3.48[b]	3.20−3.44,[b] m	3.31,[b] m	3.52,[b] m	3.2−3.4,[b] m
6′a	4.55, dd (11.5, 1.5)	4.64, d (11.0)	4.00, d (11.4)	3.97, br d (11.5)	4.00, br d (12.0)
6′b	4.27, dd (11.5, 6)	4.41, dd (11.0, 6.5)	3.6, dd (11.4, 7.5)	3.63, dd (11.5, 6.0)	3.62, dd (12.0, 7.5)
2′-OH			5.14, d (5.0)		
3′-OH			5.02, d (5.0)		
4′-OH			5.02, d (5.0)		
1″			4.38, d (7.5)	4.29, d (8)	4.50, d (7.5)
2″	7.97, dd (8.0, 1.0)	8.08, d (7.5)	2.97−3.08,[b] m	3.01, dd (6.0, 6.0)	3.08,[b] m
3″	7.55, dd (8.0, 8.0)	7.52, dd (7.5, 7.5)	3.20, m	3.20,[b] m	3.2−3.4,[b] m
4″	7.68, br dd (8.0, 8.0)	7.64, dd (7.5, 7.5)	3.23, m	3.18,[b] m	3.2−3.4,[b] m
5″	7.55, dd (8.0, 8.0)	7.52, dd (7.5, 7.5)	3.46, dd (6.0, 6.0)	3.42,[b] m	3.53, m
6″a	7.97, dd (8.0, 1.0)	8.08, d, (7.5)	a 4.56, d (11.5)	4.51, dd (12.0, 2.0)	4.60, dd (11.5, 2.0)
6″b			b 4.29, dd (11.5, 6)	4.23, dd (12.0, 6.5)	4.35, dd (11.5, 6.0)
2″-OH			5.09, d (5.5)		
3″-OH			5.10, d (5.5)		
4″-OH			5.27, d (5.5)		
2‴			7.98, dd (7.5, 1.0)	7.95, dd (7.5, 2.0)	9.11, d (2.0)
3‴			7.53, dd (7.5, 7.5)	7.52, dd (7.5, 7.5)	
4‴			7.66, dd (7.5, 7.5)	7.65, dd (7.5, 7.5)	8.30, ddd (8.0, 2.0, 2.0)
5‴			7.53, dd (7.5, 7.5)	7.52, dd (7.5, 7.5)	7.59,[b] m
6‴			7.98, dd (7.5, 1.0)	7.95, dd (7.5, 2.0)	8.83, dd (8.0, 2.0)

[a] ^{1}H NMR data (δ) were measured at 500 MHz in DMSO-d_6 for **1**, **3**, **4**, **5** and in methanol-d_4 for **2**. Coupling constants (J) in Hz are given in parentheses. The assignments were based on HSQC and HMBC experiments. [b] Signal overlapped.

and β-glucopyranosyl moieties was identical to that of **1**. In addition, the ^{1}H NMR data revealed the presence of one oxymethine proton at δ_H 5.21 (s, H-7) and the protons of a monosubstituted aromatic ring at δ_H 7.36 (d, J = 6.0 Hz, 2H) and 7.20−7.27 (m, 3H), which in combination with the ^{13}C NMR data suggests the presence of a mandelic acid moiety. The position of the 6-benzoylglucosyl group was determined to be at C-7 of the mandelic acid moiety through the connectivity of C-1′−*O*−C-7, based on the HMBC correlations of H-1′/C-7. Hydrolysis of **2** yielded benzoic acid, β-D-glucopyranose with $[\alpha]^{20}_D$ +27.4 (*c* 0.06, H_2O), and (−)-mandelic acid with $[\alpha]^{20}_D$ −95.3 (*c* 0.19, H_2O). The(2*R* absolute configuration was confirmed by the negative $[\alpha]_D$ value of mandelic acid.[5] Therefore, prupersin B was determined to have structure **2** (Figure 1).

Prupersin C (**3**) was obtained as an amorphous powder, $[\alpha]^{20}_D$ −29.8 (*c* 0.24, MeOH). The molecular formula was determined to be $C_{26}H_{32}O_{12}$ from analysis of HRESIMS data (*m*/*z* 559.1796 $[M + Na]^+$, calcd 559.1786), corresponding to 11 indices of hydrogen deficiency. The IR spectrum indicated the presence of hydroxy (3362 cm^{-1}), ester carbonyl (1716 cm^{-1}), and aromatic (1601, 1496 cm^{-1}) functionalities. Analysis of 1D NMR data (Tables 1 and 2) indicated that, as with compounds **1** and **2**, there was a 6-benzoylglucosyl moiety present in **3**, which was verified by correlations from H-2‴, H-6‴, H-6″a, and H-6″b to C-7‴ in the HMBC spectrum. The ^{1}H NMR spectrum showed signals attributable to a monosubstituted aromatic ring at δ_H 7.36 (d, J = 7.5 Hz, H-2, 6), 7.31 (dd, J = 7.5, 7.5 Hz, H-3, 5), and 7.26 (dd, J = 7.5, 7.5 Hz, H-4) and an oxymethylene group at δ_H 4.78 (d, J = 12.5, H-7a) and 4.53 (d, J = 12.5, H-7b), revealing the presence of a benzyl group. Two doublets attributed to anomeric protons (δ_H 4.19 and 4.38), together with the coupling patterns of oxymethylene and oxymethine protons resonating between δ_H 3.00 and 4.56, as well as six hydroxy signals, indicated the presence of two β-glucopyranosyl units. This was confirmed by acidic hydrolysis of **3**, which produced β-D-glucopyranose as the sole sugar, as identified by TLC comparison and the optical rotation value. In the HMBC spectrum, correlations of H-6′a, H-6′b/C-1″; H-1″/C-6′; H-2, H-6, and H-1′/C-7; and H-7a and H-7b/C-1′ showed the connections of C-1″−*O*−C-6′ and C-1′−*O*−C-7. Therefore, prupersin C was deduced to have structure **3** (Figure 1).

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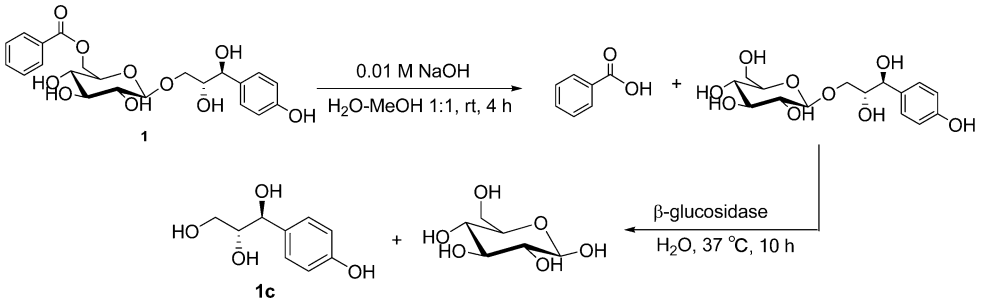
Table 2. ^{13}C NMR Spectroscopic Data of Compounds 1−5[a]

position	1	2	3	4	5
1	133.3 C	137.7, C	138.0, C	129.7, C	133.8, C
2	128.0 CH	129.7, CH	127.7, CH	129.5, CH	127.3, CH
3	114.3 CH	129.6, CH	128.1, CH	128.7, CH	129.0, CH
4	156.1 C	129.8, C	127.3, CH	133.7, CH	129.6, CH
5	114.3 CH	129.6, CH	128.1, CH	128.7, CH	129.0, CH
6	128.0 CH	129.7, CH	127.7, CH	129.5,CH	127.3, CH
7	73.4 CH	79.4, CH	69.6, CH2	164.6, C	66.7, CH
8	73.8 CH				118.8, C
9	71.4 CH_2				
1′	103.7 CH	99.7, CH	102.0, CH	94.9, CH	101.5, CH
2′	73.6 CH	74.9, CH	73.4, CH	72.4, CH	73.1, CH
3′	76.1 CH	77.3, CH	76.6, CH	76.1, CH	76.5, CH
4′	70.1 CH	72.2, CH	70.1, CH	69.4, CH	70.0, CH
5′	73.6 CH	75.9, CH	75.8, CH	76.5, CH	76.5, CH
6′	64.3 CH	65.4, CH	68.6, CH2	68.0, CH_2	68.5, CH_2
1″	129.8 CH	131.5, C	103.5, CH	102.9, CH	103.8, CH
2″	129.2 CH	130.8, CH	73.5, CH	73.4, CH	73.5, CH
3″	128.8 CH	129.8, CH	76.5, CH	76.4. CH	76.1, CH
4″	133.4 CH	134.6, CH	70.0, CH	70.0, CH	70.0, CH
5″	128.8 CH	129.8, CH	73.6, CH	73.7, CH	73.7, CH
6″	129.2 CH_2	130.8, CH	64.2, CH2	64.2, CH_2	64.6, CH_2
7″	165.7 C	167.9, C			
1‴			129.7, C	129.1, C	
2‴			129.2, CH	129.2, CH	150.0, CH
3‴			128.7, CH	128.7, CH	125.7, C
4‴			133.3, CH	133.2, CH	136.9, CH
5‴			128.7, CH	128.7, CH	124.0, CH
6‴			129.2, CH	129.2, CH	153.7, CH
7‴			165.7, C	165.6, C	164.6, C

[a]^{13}C NMR data (δ) were measured in DMSO-d_6 at 100 MHz for **1** and **5** and at 125 MHz for **3** and **4** and in methanol-d_4 at 100 MHz for **2**. The assignments were based on HSQC and HMBC experiments.

Scheme 1. Hydrolysis of 1



Prupersin D (**4**) was obtained as an amorphous powder, $[\alpha]^{20}_{D}$ −24.3 (*c* 0.21, MeOH). The molecular formula was determined to be $C_{26}H_{30}O_{13}$ from analysis of HRESIMS data (*m*/*z* 573.1594 [M + Na]$^+$, calcd 573.1579), corresponding to 12 indices of hydrogen deficiency.

The UV, IR, and NMR spectra of **4** were similar to those of **3**. Comparison of the ^{1}H and ^{13}C NMR data of **4** (Tables 1 and 2) with those of **3** shows a carbonyl signal (δ_C 164.6) instead of the oxymethylene signal (δ_C 69.6) in **3**, and the signal of anomeric carbon 1′ shifts from δ_C 102.0 to 94.9, indicating the presence of a benzoyl group in **4** rather than the benzyl group in **3**.[6] This assignment was in agreement with HMBC correlations between H-1′, H-2, and H-6 and C-7. Acidic hydrolysis of **4** produced β-D-glucopyranose as the sole sugar. Therefore, prupersin D was elucidated to have structure **4** (Figure 1).

Prupersin E (**5**) was obtained as an amorphous powder that crystallized from MeOH, $[\alpha]^{20}_{D}$ −53.6 (*c* 0.02, MeOH). The molecular formula was determined to be $C_{26}H_{30}N_2O_{12}$ from analysis of HRESIMS data (*m*/*z* 585.1709 [M + Na]$^+$, calcd 585.1731), corresponding to 12 indices of hydrogen deficiency. The IR spectrum indicated the presence of ester carbonyl (1726 cm^{-1}) and aromatic (1592, 1494 cm^{-1}) functionalities. The NMR spectrum resembled that of the known compound amygdalin except for signals attributed to a 3-substituted pyridine moiety at δ_H 9.11 (d, *J* = 2.0 Hz, H-2″), 8.83 (dd, *J* = 8.0, 2.0 Hz, H-6‴), 8.30 (ddd, *J* = 8.0, 2.0, 2.0 Hz, H-4‴), and 7.59 (m, H-5‴) and one carbonyl carbon at δ_c 164.6. These data indicated **5** as an amygdalin derivative, which was further

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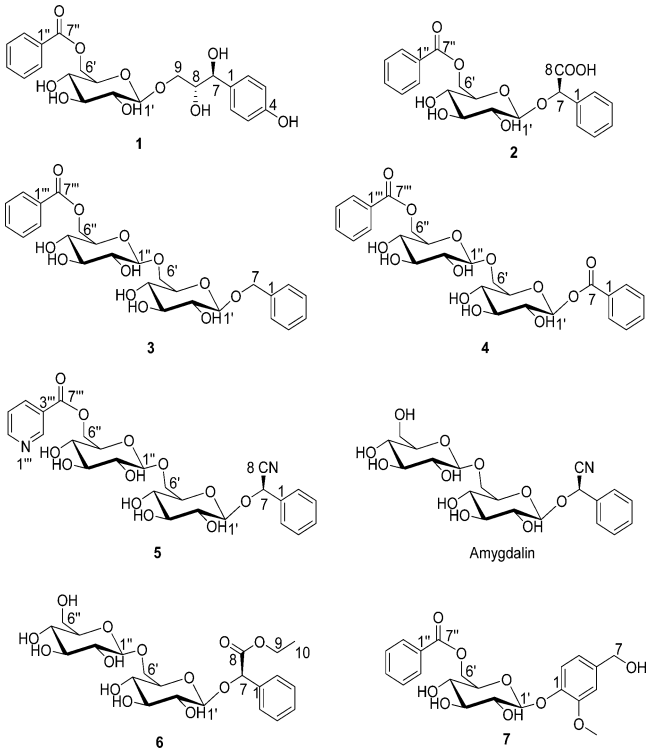

Figure 1. Structures of compounds **1**−**7** and amygdalin.

confirmed by hydrolysis (Experimental Section). HMBC correlations between H-6″a, H-6″b, H-2‴, and H-4‴ and the carbonyl carbon indicated the connectivity of C-3‴−C-7‴ and C-7‴−*O*−C-6″. Therefore, the structure of prupersin E was determined as **5** (Figure 1).

In addition to the five new compounds (**1**−**5**), two compounds that have not previously been found in nature, ethyl amygdalinate (**6**)[7,8] and 4-hydroxymethyl-2-methoxyphenyl 6-*O*-benzoyl-*β*-D-glucopyranoside (**7**),[9] were isolated from the seeds of *P. davidiana*. Full NMR data are presented here for the first time (Table 3). Compound **6** was obtained as an amorphous, white powder, $[\alpha]^{20}_{D}$ −100.2 (*c* 0.09, MeOH). The molecular formula was determined to be $C_{22}H_{32}O_{13}$ from HRESIMS data (*m*/*z* 527.1706 [M + Na]$^+$), corresponding to seven indices of hydrogen deficiency. The ^{1}H and ^{13}C NMR spectra for **6** corresponded to those for amygdalin, except for the presence of an ester in place of the cyano moiety. The NMR signals of the ester moiety and the HMBC correlations of H-7 and H-9/C-8 (Table 3) indicated the presence of an ethyl mandelate group. A 7*R* absolute configuration can be unambiguously assigned on the basis of the positive ^1L_b CEs in the ECD spectrum.[10] Acid hydrolysis of **6** yielded D-glucose. A *β*-anomeric configuration for the glucosyl unit was assigned from the large $^3J_{1,2}$ coupling constant (7.6 Hz). Thus, **6** was determined to be ethyl amygdalinate, which has been previously synthesized by Campbell and Haworth.[7]

Compound **7** was obtained as an amorphous powder. The molecular formula was determined to be $C_{21}H_{24}O_9$, based on HRESIMS data (*m*/*z* 443.1323 [M + Na]$^+$, calcd 443.1313),

Table 3. NMR Spectroscopic Data of Compounds 6 and 7[a]

	6			7		
position	δ_C	δ_H (*J* in Hz)	HMBC	δ_C	δ_H (*J* in Hz)	HMBC
1	136.5, C			147.0, C		
2	129.0, CH	7.48−7.51, m	4, 7	150.9, C		
3	129.7, CH	7.32−7.38, m	1	112.6, CH	6.89, d (1.8)	1, 2, 5, 7
4	130.0, CH	7.32−7.38, m	2, 6	137.8, C		
5	129.7, CH	7.32−7.38, m	1	120.5, CH	6.57, dd (8.1, 1.8)	1, 3, 7
6	129.0, CH	7.48−7.51, m	4, 7	118.1, CH	6.97, d (8.1)	1, 2, 4
7	78.9, CH	5.42, s	1, 2, 6, 8, 1′	65.0, CH_2	4.4, s	3, 5
8	172.9, C					
9	62.7, CH_2	4.07−4.21, m[b]	8, 10			
10	14.3, CH_3	1.05, t (6.9)	9			
1′	101.3, CH	4.07−4.21, m[b]		102.8, CH	4.82, d (7.0)	1
2′	74.8, CH	3.20−3.40		74.9, CH	3.32−3.48, m[b]	
3′	78.0, CH	3.20−3.40		77.8, CH	3.32−3.48, m[b]	
4′	71.4, CH	3.20−3.40		72.0, CH	3.32−3.48, m[b]	
5′	77.2, CH	3.20−3.40		75.6, CH	3.67, ddd (7.5, 7.5, 2.1)	
6′a	69.6, CH_2	4.07−4.21, m[b]	5′, 1″	65.3, CH_2	4.60, dd (12, 2.1)	7″, 5′
6′b		3.76, dd (11.6, 5.6)			4.32, dd (12, 7.5)	
1″	104.8, CH	4.39 d, (7.6)	6′, 2″	131.3, C		
2″	75.2, CH	3.20−3.40, m[b]		130.7, CH	7.92, d (7.2)	4″, 6″, 7″
3″	78.0, CH	3.20−3.40, m[b]		129.6, CH	7.40, dd (7.2, 7.2)	1″, 5″, 4″
4″	71.6, CH	3.20−3.40, m[b]		134.3, CH	7.53, dd (7.2, 7.2)	2″, 6″
5″	77.5, CH	3.20−3.40, m[b]		129.6, CH	7.40, dd (7.2, 7.2)	1″, 3″, 4″
6″a	62.7, CH_2	3.85, dd, (12.0, 2.0)	4″	130.7, CH	7.92, d (7.2)	2″, 4″, 7″
6″b		3.65, dd, (12.0,3.9)				
7″				167.8, C		
OMe				56.7	3.75, s	2

[a] ^{1}H NMR data (*δ*) were measured in methanol-d_4 at 400 MHz for **6** and in methanol-d_4 at 500 MHz for **7**. Coupling constants (*J*) in Hz are given in parentheses. The assignments were based on HSQC and HMBC experiments. [b] Signal overlapped.

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Table 4. Antioxidant Activity of Compounds 1−7

compound	concentration (M/L)	inhibitiory rates of MDA (%)	compound	concentration (M/L)	inhibitiory rates of MDA (%)
1	10^{-4}	88	**5**	10^{-4}	88
	10^{-5}	64		10^{-5}	67
	10^{-6}	57		10^{-6}	56
2	10^{-4}	67	**6**	10^{-4}	51
	10^{-5}	60		10^{-5}	51
	10^{-6}	53		10^{-6}	56
3	10^{-4}	55	**7**	10^{-4}	51
	10^{-5}	50		10^{-5}	42
	10^{-6}	59		10^{-6}	41
4	10^{-4}	56			
	10^{-5}	66			
	10^{-6}	56			

corresponding to 10 indices of hydrogen deficiency. Analysis of 1D NMR data indicated the presence of a 6-benzoylglucosyl moiety in **7**, as for compounds **1** and **2**, which was verified by correlations from H-2″, H-6″, H-6′a, and H-6′b to C-7″ in the HMBC spectrum of **7**. The presence of a 1,2,4-trisubstituted aromatic ring, a methoxy group, and an oxymethylene group was also determined from the ^{1}H NMR spectrum. According to the analysis of 2D NMR data (Table 3), the methoxy group is located at C-2 and the oxymethylene group at C-4. Thus, **7** was determined to be 4-hydroxymethyl-2-methoxyphenyl 6-*O*-benzoyl-*β*-D-glucopyranoside.

The known compounds were identified as amygdalin,[11] 1-*O*-vanilloyl-*β*-D-glucose,[12] vanilloloside,[13] androsin,[14] 2-*β*-D-glucopyranosyloxy-2-phenylacetic acid amide,[15] benzyl-*β*-D-glucopyranoside,[16] benzyl-*β*-D-glucopyranosyl(1−6)-*β*-D-glucopyranoside,[17] amygdalinic acid,[18] mandelic acid *β*-D-glucopyranoside,[19] and a mixture of prunasin and sambunigrin,[19] by NMR analysis and comparison with literature data.

The antioxidant activity of compounds **1**−**7** was assessed from the amount of malondialdehyde (MDA) that was produced during microsomal lipid peroxidation induced by Fe^{2+}-cysteine. As shown in Table 4, compounds **1**−**6** showed antioxidant activities with inhibitory rates of >50% at concentrations of 10 *μ*M. The compounds were also bioassayed for cytotoxicity against five human tumor cell lines, HCT-8 (human ileocecal adenocarcinoma cell line), Bel-7402 (human hepatoma cell line), BGC-823 (human gastric cancer cell line), A549 (human lung epithelial cell line), and A2780 (human ovarian cancer cell line). However, all the compounds were inactive against these cell lines (showing IC_{50} value of >10 *μ*M).

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured with a Jasco P-2000 polarimeter, and UV spectra with a Jasco V-650 spectrophotometer. ECD spectra were measured on a Jasco J-815 spectrometer. IR spectra were recorded on a Nicolet 5700 spectrometer by an FT-IR microscope transmission method. NMR measurements were performed using VNS-600, Inova-500, Bruker AV500-III, Mp-400, and Mercury-300 spectrometers. HRESIMS was performed using an Agilent 1100 series LC/MSD ion trap mass spectrometer. HPLC was performed on a Lumtech instrument equipped with an Alltech 500 ELSD detector, using a YMC-Pack ODS-A column (250 × 20 mm, 5 *μ*m). Silica gel (200−300 mesh, Qingdao Marine Chemical Factory, Qingdao, China), Sephadex LH-20 (GE), and ODS (50 *μ*m, YMC, Japan) were used for column chromatography. TLC was carried out with GF254 plates (Qingdao Marine Chemical Factory). Spots were visualized by spraying with 10% H_2SO_4 in EtOH followed by heating.

Plant Material. The seeds of *Prunus davidiana* (Carr.) Franch. were collected at Yuncheng, Shanxi Province, in China, in July 2010 and identified by Professor Lin Ma from the Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College. A voucher specimen (Annonaceae No. S-2443) was deposited in the Herbarium of the Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College.

Extraction and Isolation. Air-dried and powdered seeds of *P. davidiana* (17 kg) were exhaustively extracted with 95% aqueous EtOH (3 × 75 L) at reflux. The combined extracts were concentrated under reduced pressure to dryness. The residue was suspended in H_2O and partitioned with petroleum ether, EtOAc, and *n*-BuOH, successively. The *n*-BuOH-soluble residue (500 g) was crystallized in 95% aqueous EtOH to remove the major constituent amygdalin. The filtrate was concentrated under reduced pressure to dryness. The residue (350 g) was subjected to column chromatography on silica gel and eluted with a gradient of $CHCl_3$−MeOH (10:1; 5:1; 3:1; 1:1; 100% MeOH). Fraction B (19 g) was subjected to silica gel column chromatography ($CHCl_3$−MeOH, 7:1; 5:1; 1:1; 100% MeOH) to afford two subfractions. Fraction B-a (15.5 g) was further separated by MPLC (ODS, 50 *μ*m, YMC), eluted with 15, 20, 25, 35, and 100% MeOH−H_2O, to afford 40 subfractions. Fraction B-a-25 (42 mg) was purified by preparative HPLC using 14% MeCN−H_2O (8 mL/min) as the mobile phase to yield compound **1** (14 mg, t_R 60.3 min). Fraction B-a-37 (155 mg) was subjected to preparative HPLC using 30% MeCN−H_2O (8 mL/min) to give compound **3** (29 mg, t_R 56.3 min). Fraction B-a-34 (185 mg) was purified by preparative HPLC using 28% MeCN−H_2O (8 mL/min) to give compound **4** (34 mg, t_R 42.3 min). Fraction B-a-27 (121 mg) was crystallized in MeOH to afford compound **5** (19 mg). Fraction B-a-2 was separated on Sephadex LH-20 eluted with H_2O to afford three subfractions. Fraction B-b (4 g) was separated on Sephadex LH-20, eluted with MeOH−H_2O, 1:1, to afford **7** (5 mg). Fraction C (60 g) was subjected to silica gel column chromatography ($CHCl_3$−MeOH, 5:1; 2:1; 1:1; 100% MeOH) to give two subfractions. Fraction C-b (10 g) was further separated by MPLC (ODS, 50 *μ*m, YMC), eluted with 20, 50, 70, and 100% MeOH−H_2O, to afford 25 subfractions. Fraction C-b-20 (50 mg) was subjected to preparative HPLC using 60% MeOH−H_2O (8 mL/min) to give compound **2** (10 mg, t_R 45.3 min). Fraction B-a-34 (300 mg) was purified by preparative HPLC using 12% MeCN−H_2O (8 mL/min) to give compound **6** (200 mg, t_R 40.2 min).

*Prupersin A (**1**):* amorphous, white powder; $[\alpha]^{20}_D$ −25.3 (*c* 2.35, MeOH); UV(MeOH): λ_{max} (log *ε*) 205 (3.77) nm; IR ν_{max} 3339, 2890, 2257, 2128, 1717, 1614, 1600, 1516, 1452, 1317, 1278, 1172, 1051, 1025, 827, 764, 717, 834, 577 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) and ^{13}C NMR (DMSO-d_6, 100 MHz) see Tables 1 and 2; positive-ion ESIMS *m*/*z* 473.0 $[M + Na]^+$; positive-ion HRESIMS *m*/*z* 473.1428 $[M + Na]^+$ (calcd for $C_{22}H_{26}O_{10}Na$, 473.1418).

*Prupersin B (**2**):* colorless gum; $[\alpha]^{20}_D$ −82.0 (*c* 0.37, MeOH); UV (MeOH): λ_{max} (log *ε*) 194 (4.80) nm; IR ν_{max} 3372, 3066, 2894, 1719, 1603, 1492, 1452, 1411, 1317, 1278, 1196, 1071, 1025, 968, 801, 748,

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714, 629, 579, 517 cm^{-1}; ^1H NMR (methanol-d_4, 500 MHz) and ^{13}C NMR (methanol-d_4, 125 MHz) see Tables 1 and 2; negative-ion ESIMS m/z 417 $[M - H]^-$; positive-ion HRESIMS m/z 441.1158 $[M + Na]^+$ (calcd for $C_{21}H_{22}O_9Na$, 441.1156).

*Prupersin C (**3**):* amorphous, white powder; $[\alpha]^{20}_D$ −29.8 (*c* 0.24, MeOH); UV (MeOH) λ_{max} (log ε) 193 (4.99) nm; IR ν_{max} 3362, 2883, 2256, 2128, 1716, 1601, 1496, 1453, 1363, 1316, 1277, 1165, 1048, 1025, 825, 715 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) and ^{13}C NMR (DMSO-d_6, 125 MHz) see Tables 1 and 2; positive-ion ESIMS m/z 559 $[M + Na]^+$; positive-ion HRESIMS m/z 559.1796 $[M + Na]^+$ (calcd for $C_{26}H_{32}O_{12}Na$, 559.1786).

*Prupersin D (**4**):* amorphous, white powder; $[\alpha]^{20}_D$ −24.3 (*c* 0.21, MeOH); UV (MeOH) λ_{max} (logε) 193 (5.06) nm; IR ν_{max} 3445, 3073, 2920, 2879, 1727, 1602, 1585, 1493, 1453, 1421, 1352, 1318, 1274, 1180, 1079, 1026, 995, 909, 853, 808,713, 686, 565 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) and ^{13}C NMR (DMSO-d_6, 125 MHz) see Tables 1 and 2; positive-ion ESIMS m/z 573 $[M + Na]^+$; positive-ion HRESIMS m/z 573.1594 $[M + Na]^+$ (calcd for $C_{26}H_{30}O_{13}Na$, 573.1579).

*Prupersin E (**5**):* colorless needles (MeOH); mp 148−151 °C; $[\alpha]^{20}_D$ −53.6 (*c* 0.02, MeOH); IR ν_{max} 3371, 2964, 2935, 2887, 1726, 1661, 1592, 1495, 1457, 1422, 1371, 1336, 1295, 1266, 1231, 1197, 1161, 1145, 1124, 1079, 1046, 1018, 1000, 970, 895, 880, 851, 836, 761, 743, 703, 679, 610, 533 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) and ^{13}C NMR (DMSO-d_6, 100 MHz) see Tables 1 and 2; positive-ion ESIMS m/z 585 $[M + Na]^+$; positive-ion HRESIMS m/z 585.1709 $[M + Na]^+$ (calcd for $C_{26}H_{30}N_2O_{12}Na$, 585.1691).

*Ethyl amygdalinate (**6**):* amorphous, white powder; $[\alpha]^{20}_D$ −100.2 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε) 192 (5.04) nm; ECD (MeOH) 261 ($\Delta\varepsilon$ +8.56 × 10^{-3}) nm; IR ν_{max} 3564, 3536, 3292, 2955, 2917, 2871, 1740, 1611, 1499, 1452, 1418, 1370, 1322, 1301, 1270, 1238, 1211, 1165, 1134, 1109, 1080, 1035, 949, 894, 731,614 cm^{-1}; ^1H NMR (methanol-d_4, 300 MHz) and ^{13}C NMR (methanol-d_4, 100 MHz) see Table 3; positive-ion ESIMS m/z 527 $[M + Na]^+$; positive-ion HRESIMS m/z 527.1706 $[M + Na]^+$.

*4-Hydroxymethyl-2-methoxyphenyl 6-O-benzoyl-β-D-glucopyranoside (**7**):* amorphous, white powder; ^1H NMR (D_2O, 500 MHz) and ^{13}C NMR (D_2O, 125 MHz) see Table 3; positive-ion ESIMS m/z 443 $[M + Na]^+$; positive-ion HRESIMS m/z 443.1323 $[M + Na]^+$ (calcd for $C_{21}H_{24}O_9Na$, 443.1313).

*Alkaline and Enzymatic Hydrolysis of Compounds **1**, **2**, and **5**.* Compound **1** (5.5 mg) was dissolved in a 0.01 M solution of NaOH in H_2O−MeOH (2 mL, v/v, 1:1) at rt for 4 h. The reaction mixture was neutralized with dilute HCl, and the MeOH was removed under vacuum. The remaining aqueous solution was extracted with *n*-BuOH. The *n*-BuOH layer was evaporated *in vacuo* and purified by preparative HPLC using 14% MeCN−H_2O (5 mL/min) as the mobile phase to yield compound 1-(4-hydroxyphenyl)glycerol 3-*O*-*β*-D-glucopyranoside (2.5 mg, t_R 20 min) and benzoic acid (1.2 mg, t_R 35 min). Benzoic acid was identified by comparison with an authentic sample using analytical HPLC (14% MeCN−H_2O, t_R 6.5 min). 1-(4-Hydroxyphenyl)glycerol 3-*O*-*β*-D-glucopyranoside was hydrolyzed with 15 mg of *β*-glucosidase (BCBF2889 V, RS-Sigma) in 1.5 mL of H_2O at 37 °C for 10 h. The reaction mixture was extracted with *n*-BuOH. The *n*-BuOH layer was evaporated *in vacuo* and subjected to preparative HPLC using 3% MeOH−H_2O (5 mL/min) to give the aglycone (0.5 mg, t_R 25 min) as a colorless gum: $[\alpha]^{20}_D$ +18.0 (*c* 0.06, EtOH). The ^1H NMR spectrum was in agreement with that of *threo*-1-(4-hydroxyphenyl)propane-1,2,3-triol.[3] The aqueous layer was evaporated and subjected to column chromatography over silica gel with EtOAc−MeOH−H_2O (7:5:1) as eluent to yield (+)-D-glucose (0.91 mg), $[\alpha]^{20}_D$ +30.3 (*c* 0.09, H_2O), as confirmed by comparison with an authentic sample (EtOAc−MeOH−H_2O (7:5:1), R_f 0.59).

Hydrolysis of **2** (5 mg) as for **1** gave the aglycone (0.6 mg, $[\alpha]^{20}_D$ −95.3 (*c* 0.06, H_2O)) and (+)-D-glucose (0.7 mg, $[\alpha]^{20}_D$ +27.4 (*c* 0.06, H_2O)). The ^1H NMR spectrum of the aglycone of **2** was in agreement with that of mandelic acid.[19]

Compound **5** (10 mg) was subjected to alkaline hydrolysis in a 0.01 M solution of NaOH in H_2O−MeOH (2 mL, v/v, 1:1) at rt for 4 h as for **1**. The reaction mixture was neutralized with dilute HCl and was evaporated *in vacuo*. The residue was extracted with MeOH and purified by preparative HPLC using 20% MeOH−H_2O (5 mL/min) as the mobile phase to yield amygdalin (1 mg, t_R 40 min), as determined by comparison with an authentic sample with analaytical HPLC (20% MeOH−H_2O, t_R 10.5 min).

*Acid Hydrolysis of Compounds **3**−**7**.* Compounds **3** (5 mg), **4** (5 mg), **5** (5 mg), **6** (5 mg), and **7** (5 mg) were individually refluxed in 6% HCl (5.0 mL) at 80 °C for 2 h. Each reaction mixture was extracted with $CHCl_3$ (3 × 6 mL), and the H_2O phase was dried using a N_2 stream. The residues were separately subjected to column chromatography over silica gel with EtOAc−MeOH−H_2O (7:5:1) as eluent to yield (+)-D-glucose (2.10 mg) from **3**, $[\alpha]^{20}_D$ +44.3 (*c* 0.14, H_2O); (+)-D-glucose (2.60 mg) from **4**, $[\alpha]^{20}_D$ +52.5 (*c* 0.17, H_2O); (+)-D-glucose (2.00 mg) from **5**, $[\alpha]^{20}_D$ +25.5 (*c* 0.01, H_2O); (+)-D-glucose (3.20 mg) from **6**, $[\alpha]^{20}_D$ +51.5 (*c* 0.16, H_2O); and (+)-D-glucose (1.60 mg) from **7**, $[\alpha]^{20}_D$ +22.5 (*c* 0.01, H_2O). The solvent system EtOAc−MeOH−H_2O (7:5:1) was used for TLC identification.

Antioxidant Assay. The antioxidant activity of compounds **1**−**7** was assayed *in vitro* by measuring the inhibiton of MDA production using Fe^{2+}/cysteine-induced rat liver microsomal lipid peroxidation. MDA was detected using the thiobarbituric acid (TBA) method. Briefly, different concentrations of compound or vehicle (10 μL), 1 mg/mL of microsomal protein (100 μL), and 0.2 mM cysteine (10 μL) in 0.1 M PBS (0.82 mL) were incubated for 15 min at 37 °C, 0.5 mM $FeSO_4$ (50 μL) was added, and the solution was mixed and incubated for a further 15 min at 37 °C again. An equal volume of 20% trichloroacetic acid was added to terminate the reaction, and the mixture was centrifuged for 10 min at 3000 rpm. The supernatant (1 mL) was reacted with 0.67% TBA (1 mL) for 10 min at 100 °C. After cooling, the amount of MDA was quantified by determining the absorbance at 532 nm, and then the inhibition rate was calculated, from which the inhibition rate (*IR*) was calculated as $IR\ (\%) = 100\% - A_t/(A_p - A_c) \times 100$, where A_p, A_t, and A_c refer to the absorbance of Fe^{2+}-cysteine, the test compound, and control, respectively.[20]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

MS, NMR, and ECD spectra for compounds **1**−**7**, *threo*-1-(4-hydroxyphenyl)glycerol, and mandelic acid. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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