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Asprecosides A–J, ten new pentacyclic triterpenoid glycosides with cytotoxic activity from the roots of *Ilex asprella*

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Abstract

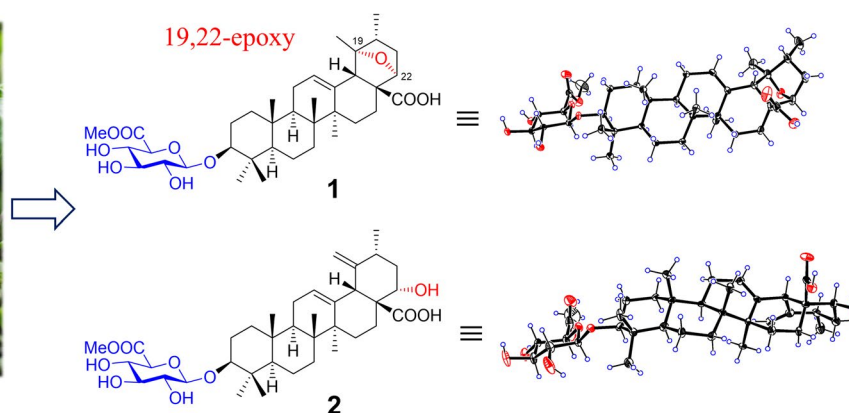
Phytochemical study of the *n*-BuOH extract of *Ilex asprella* resulted in the discovery of ten new pentacyclic triterpenoid glycosides, comprising nine ursane-type glycosides (**1–9**) and one oleanane-type glycoside (**10**), along with seven known compounds (**11–17**). Compound **1** is the first reported 19,22-epoxy ursane triterpenoid glycoside, whereas **4** and **5** are rare examples of ursane triterpenoid glycosides containing a 28,19-lactone group. The structural characterization of these compounds was achieved using spectroscopic and chemical techniques, as well as single-crystal X-ray analysis. Compounds **7**, **12**, **15**, and **17** exhibited moderate cytotoxic activities against H1975 and HCC827 cancer cells.

Keywords *Ilex asprella*, Pentacyclic triterpenoid glycoside, Ursane, Oleanane, Cytotoxic activity

Graphical abstract



Ilex asprella



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1 Introduction

Pentacyclic triterpenoid glycosides represent a large class of secondary metabolites predominantly found in higher plants, consisting of diverse triterpenoid aglycones and glycosyl moieties [1, 2]. Common pentacyclic triterpenoid aglycones mainly include oleanane-type, ursane-type, and friedelane-type (6/6/6/6/6 carbocyclic ring system), as well as lupane-type (6/6/6/6/5 carbocyclic ring system) [3]. The structural variability resulting from various functional modifications of triterpenoid aglycones endows these compounds with notable biological properties [4, 5]. In recent years, numerous pentacyclic triterpenoid glycosides exhibiting remarkable bioactivities have been identified, as exemplified by the PTP1B inhibitors gymlatinosides GL2 and GL3 [6], the neuroprotective medicaginside A [7], the β -glucuronidase inhibitor astraleanoside H [8], and the cytotoxic compound ilekudinoside B *n*-butyl ester [9].

Ilex asprella (Hook. & Arn.) Champ. ex Benth (Aquifoliaceae) is a deciduous shrub and predominantly distributed in southern regions of China, particularly in Guangdong, Hunan, and Guangxi provinces [10]. The

roots of *I. asprella* are extensively utilized in traditional Chinese medicine to treat of headache, cough, and pharyngitis, etc. [10]. Earlier phytochemical studies indicated that triterpenoids and their glycosides are characteristic components of this plant [11–17], with some exhibiting antiviral and cytotoxic activities. As part of ongoing research to identify structurally diverse terpenoids from medicinal plants [18, 19], nine new ursane triterpenoid glycosides (1–9), one new oleanane glycoside (10), and seven known analogues (11–17) were isolated from the roots of *I. asprella* (Fig. 1). Compound 1 represents the first 19,22-epoxy ursane triterpenoid glycoside, whereas 4 and 5 are rare ursane triterpenoid glycosides with a 28,19-lactone group. This study presents the isolation, structural characterization, and cytotoxic properties of these triterpenoid glycosides (1–17).

2 Results and discussion

Asprecoside A (1) was isolated as colorless needles, and its molecular formula $C_{37}H_{56}O_{10}$ was determined by the HRESIMS ion peak at m/z 659.3803 $[M-H]^-$ (calcd for $C_{37}H_{55}O_{10}^-$, 659.3801). The 1H NMR data of

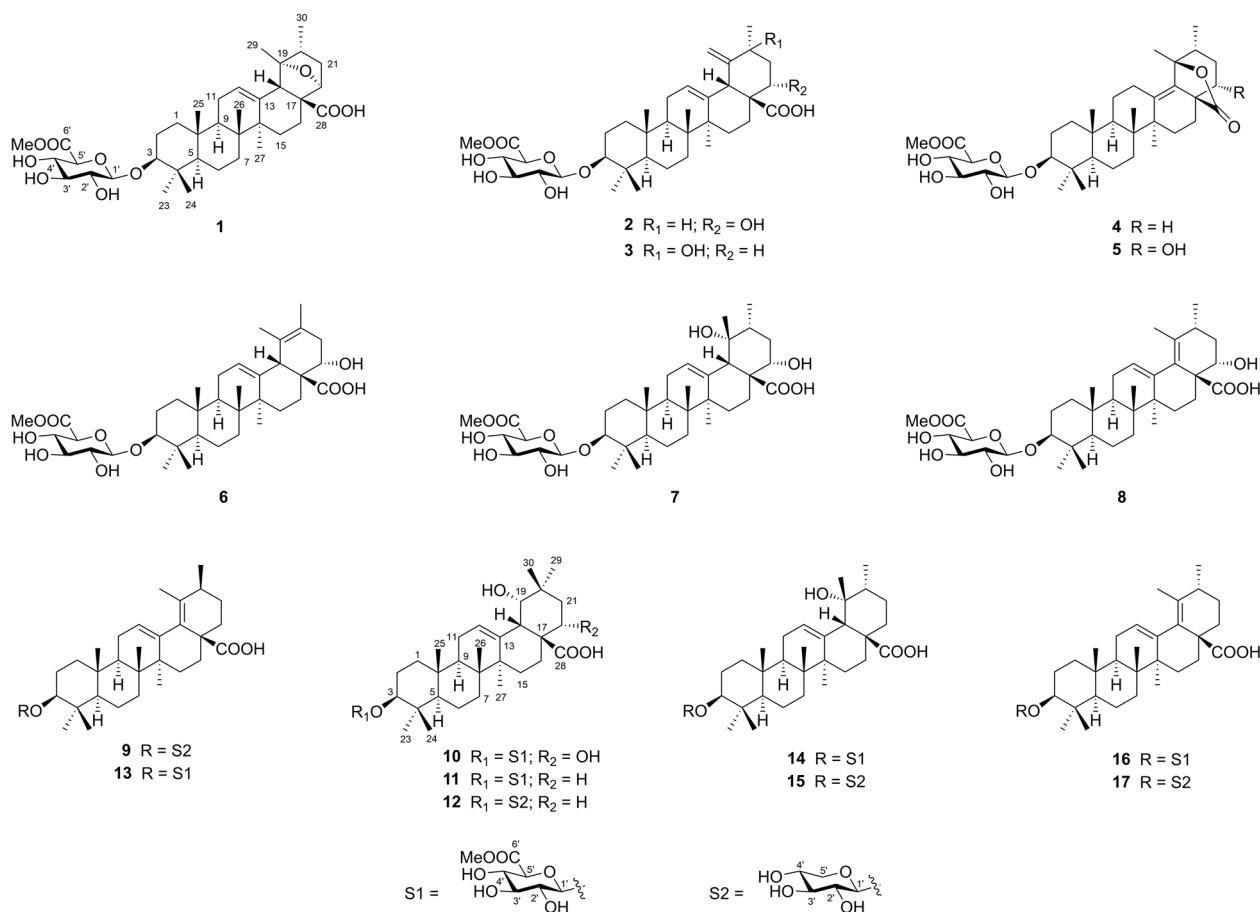


Fig. 1 Chemical structures of compounds 1–17

1 (Table 1) exhibited characteristic signals, including six methyl singlets (δ_{H} 0.86, 0.93, 0.99, 1.28, 1.30, and 1.32), a methyl doublet [δ_{H} 0.91 (d, $J=6.7$ Hz)], a methoxy group [δ_{H} 3.76 (s)], seven oxygenated methines [δ_{H} 3.42 (1H, dd, $J=11.7$, 4.3 Hz), 4.43 (1H, d, $J=5.5$ Hz), 5.03 (1H, d, $J=7.8$ Hz), 4.11 (1H, dd, $J=9.0$, 7.8 Hz), 4.28 (1H, t, $J=9.0$ Hz), 4.49 (1H, dd, $J=9.7$, 9.0 Hz),

and 4.62 (1H, d, $J=9.7$ Hz)], and an olefinic proton [δ_{H} 5.42 (1H, t, $J=3.3$ Hz)]. The ^{13}C NMR and DEPT data (Table 2) of **1** revealed the presence of 37 carbon resonances, including a carboxyl group (δ_{C} 178.7), a methoxycarbonyl group (δ_{C} 171.2 and 52.4), a trisubstituted double bond (δ_{C} 137.3 and 126.6), an anomeric carbon (δ_{C} 107.7), an oxygenated sp^3 tertiary carbon (δ_{C} 90.4),

Table 1 ^1H NMR data of compounds **1–5** (500 MHz, J in Hz, δ in ppm)

No	1 ^a	2 ^a	3 ^b	4 ^a	5 ^a
1 α	0.79, m	0.88, m	1.01, m	0.81, m	0.84, m
1 β	1.45, m	1.42, m	1.64, m	1.57, m	1.59, m
2 α	2.17, m	2.17, m	1.80, m	2.20, m	2.19, m
2 β	1.88, m	1.86, m	1.69, m	1.90, m	1.91, m
3	3.42, dd (11.7, 4.3)	3.42, dd (11.7, 4.3)	3.17, dd (11.6, 4.5)	3.40, dd (11.8, 4.5)	3.39, dd (11.7, 4.3)
5	0.81, m	0.83, br d (10.7)	0.81, br d (10.9)	0.76, m	0.77, br d (11.8)
6 α	1.49, m	1.54, m	1.57, m	1.51, m	1.51, m
6 β	1.25, m	1.29, m	1.45, m	1.29, m	1.24, m
7 α	1.39, m	1.35, m	1.51, m	1.35, m	1.23, m
7 β	1.49, m	1.53, m	1.34, m	1.44, m	1.45, m
9	1.58, dd (9.1, 8.3)	1.74, m	1.72, m	1.38, m	1.49, m
11	1.90, m	α 1.97, m β 1.90, m	1.99, m	α 1.41, m β 1.18, m	α 1.42, m β 1.32, m
12	5.42, t (3.3)	5.58, t (3.3)	5.34, t (3.4)	α 1.87, m β 2.58, m	α 2.09, td (14.7, 5.2) β 2.78, br d (14.7)
15 α	1.19, m	1.27, m	1.05, m	1.20, m	1.60, m
15 β	1.68, m	2.53, td (13.2, 3.0)	1.83, m	1.72, m	1.66, m
16 α	2.24, m	1.75, m	1.61, m	1.59, m	2.24, m
16 β	2.48, m	2.73, br d (13.2)	1.73, m	2.03, m	2.28, m
18	3.31, s	3.98, br s	3.85, br s		
20	1.91, m	2.28, m		2.05, m	2.20, m
21 α	1.37, m	1.80, m	1.61, m	1.34, m	1.78, m
21 β	2.30, dd (12.4, 8.1)	2.10, dt (12.3, 3.9)	1.66, m	2.02, m	2.32, m
22	4.43, d (5.5)	4.51, dd (11.7, 3.9)	α 1.55, m β 2.21, td (13.0, 5.3)	α 1.54, m β 1.71, m	4.18, br dd (4.6, 3.1)
23	1.32, s	1.32, s	1.05, s	1.33, s	1.32, s
24	0.99, s	0.98, s	0.86, s	0.98, s	0.99, s
25	0.86, s	0.82, s	0.98, s	0.78, s	0.83, s
26	0.93, s	1.05, s	0.86, s	0.77, s	0.87, s
27	1.28, s	1.37, s	1.13, s	1.17, s	1.49, s
29	1.30, s	a 5.27, s b 5.13, s	a 5.28, s b 5.09, s	1.67, s	1.79, s
30	0.91, d (6.7)	1.18, d (6.4)	1.40, s	0.85, d (7.1)	1.33, d (7.1)
1'	5.03, d (7.8)	5.02, d (7.8)	4.39, d (7.8)	5.04, d (7.8)	5.04, d (7.8)
2'	4.11, dd (9.0, 7.8)	4.10, dd (9.0, 7.8)	3.23, dd (9.2, 7.8)	4.12, dd (9.0, 7.8)	4.12, dd (9.0, 7.8)
3'	4.28, t (9.0)	4.28, t (9.0)	3.35, t (9.2)	4.29, t (9.0)	4.30, t (9.0)
4'	4.49, dd (9.7, 9.0)	4.49, dd (9.7, 9.0)	3.51, dd (9.8, 9.2)	4.51, dd (9.7, 9.0)	4.51, dd (9.7, 9.0)
5'	4.62, d (9.7)	4.62, d (9.7)	3.83, d (9.8)	4.63, d (9.7)	4.64, d (9.7)
OMe-6'	3.76, s	3.76, s	3.77, s	3.76, s	3.76, s
OH-22					6.91, d (4.6)

^a In pyridine- d_5 , ^b in CD_3OD

Table 2 ^{13}C NMR data (125 MHz) of compounds **1–10**

No	1 ^a	2 ^a	3 ^b	4 ^a	5 ^a	6 ^a	7 ^a	8 ^a	9 ^b	10 ^a
1	39.3	39.1	39.8	39.5	39.5	39.4	39.1	39.6	40.3	38.8
2	27.0	27.0	27.0	27.2	27.1	27.1	27.0	27.1	27.2	26.9
3	89.5	89.5	91.1	89.4	89.4	89.5	89.6	89.5	90.6	89.5
4	39.9	39.9	40.2	40.0	40.0	39.9	39.9	39.9	40.2	39.9
5	56.3	56.3	57.1	56.2	56.1	56.3	56.2	56.3	57.2	56.2
6	18.8	18.8	19.3	18.8	18.8	18.7	19.0	18.8	19.2	19.0
7	34.5	33.7	34.2	35.4	35.9	34.6	33.8	35.7	35.8	33.5
8	39.6	40.1	40.5	42.7	42.4	40.1	40.8	39.9	40.2	40.5
9	48.5	48.3	48.9	52.3	51.8	48.5	48.0	48.6	49.2	48.5
10	37.2	37.3	38.0	37.7	37.7	37.2	37.3	37.1	37.8	37.4
11	24.0	24.2	24.6	22.4	22.1	23.9	24.3	23.6	24.2	24.5
12	126.6	128.8	129.3	26.6	27.7	127.6	128.5	125.4	127.2	124.1
13	137.3	138.2	138.3	137.1	141.3	138.9	140.2	141.1	139.9	144.8
14	42.2	43.8	43.6	43.5	43.5	44.7	43.0	46.4	45.7	42.7
15	27.0	29.1	29.6	28.3	28.6	28.5	29.3	29.5	29.8	29.2
16	28.9	19.7	26.3	27.0	23.6	18.7	19.7	27.5	35.9	21.1
17	59.8	55.5	49.6	48.9	55.2	53.4	54.9	57.4	49.5	53.1
18	55.5	53.4	48.7	133.5	130.5	54.1	55.8	135.0	134.5	46.4
19	90.4	153.5	154.1	91.4	92.2	129.5	72.9	133.1	137.0	81.5
20	44.1	35.8	71.0	40.2	39.6	123.4	41.0	35.1	35.7	36.8
21	37.1	39.6	35.6	27.4	36.8	39.0	36.3	38.0	27.5	38.3
22	82.6	74.7	32.7	32.5	72.8	72.5	75.4	72.3	32.4	72.0
23	28.5	28.5	28.5	28.4	28.4	28.6	28.5	28.6	28.6	28.5
24	17.3	17.3	17.0	17.2	17.2	17.4	17.2	17.4	17.1	17.2
25	16.4	15.9	16.1	17.3	17.2	16.3	15.8	16.6	16.7	15.7
26	17.7	17.5	17.6	19.2	19.0	18.5	17.4	18.8	18.6	17.7
27	21.9	27.1	26.5	25.0	21.9	22.7	25.4	22.6	22.3	25.6
28	178.7	179.3	181.3	178.5	178.2	178.9	180.1	178.7	180.5	180.9
29	16.6	110.9	113.3	23.3	23.4	17.5	27.3	17.5	19.8	26.3
30	19.9	19.7	29.2	15.8	18.5	20.8	17.0	21.4	19.1	29.5
1'	107.7	107.7	107.1	107.8	107.7	107.7	107.7	107.7	107.4	107.7
2'	75.8	75.8	75.3	75.8	75.8	75.8	75.8	75.8	75.4	75.8
3'	78.2	78.3	77.5	78.3	78.3	78.3	78.3	78.3	78.0	78.2
4'	73.5	73.5	73.2	73.6	73.6	73.5	73.6	73.5	71.2	73.6
5'	77.5	77.6	76.6	77.7	77.6	77.6	77.6	77.6	66.7	77.6
6'	171.2	171.2	171.4	171.3	171.3	171.2	171.2	171.2		171.3
OMe	52.4	52.4	52.8	52.4	52.4	52.4	52.4	52.4		52.4

^a In pyridine-*d*₅, ^bin CD₃OD

five sp^3 quaternary carbon, ten sp^3 methines (six oxygenated), eight sp^3 methylenes, and seven methyls. The above NMR data indicated that the compound exhibited the primary structural characteristics of ursane triterpenoid glycosides and closely resembled ilexasoside A (**14**) [13], a previously reported triterpenoid glycoside, except that the sp^3 methylene (CH₂-22: δ_{H} 2.17 and 2.07; δ_{C} 38.5) in **14** was replaced by an oxygenated methine group (δ_{H} 4.43; δ_{C} 82.6) in **1**, as confirmed by the ^1H – ^1H COSY correlation between H₂-21 and H-22.

Additionally, two heavily deshielded O-bearing carbons (C-19: δ_{C} 90.4; C-22: δ_{C} 82.6) implied the existence of an epoxy bridge connecting C-19 and C-22, which was further substantiated by the HMBC correlation of H-22/C-19. Comprehensive analysis of 2D NMR data enabled the establishment of the gross structure of **1**, as illustrated in Fig. 2.

The relative configuration of **1** was partially elucidated through the analysis of NOESY spectra and coupling constant data. The β -configuration of the glycosyl group

was confirmed based on the coupling constant of its anomeric proton (d, $J=7.8$ Hz, H-1'). The observed NOESY correlations (Fig. 3) of H-3/H-5, H-5/H-9, H-9/H₃-27 revealed that these protons, together with Me-27, were cofacial and were arbitrarily assigned α -orientations. Consequently, the correlations of H-11 β /H₃-25 and H₃-26 indicated that Me-25 and Me-26 were β -oriented. Furthermore, the β -orientations of H-18 and H-20 were supported by the NOE correlations of H-18/H-12 and H-20. Nevertheless, due to the absence of useful NOESY signals, the configurations of the other stereocenters could not be determined. Ultimately, the complete structure of **1**, including its absolute configuration, was confirmed through single-crystal X-ray diffraction analysis (Fig. 4), with a Flack parameter of $-0.02(3)$.

Asprecoside B (**2**) was identified with a molecular formula of C₃₇H₅₆O₁₀, based on the HRESIMS data. A comparison of the NMR data of **2** (Tables 1 and 2) with those of **14** revealed key distinctions: the presence of an additional terminal double bond [δ_{H} 5.27 and 5.13 (each

1H, s); δ_{C} 153.5 and 110.9] in place of the oxygenated sp^3 tertiary carbon and a methyl group in **14**, along with an oxymethine [δ_{H} 4.51 (1H, dd, $J=11.7, 3.9$ Hz); δ_{C} 74.7] in **2** instead of the methylene in **14**. The location of $\Delta^{19(29)}$ double bond was confirmed by HMBC, which showed correlations (Fig. 2) from the olefinic protons (δ_{H} 5.27 and 5.13, H₂-29) to C-18, C-19, and C-20; as well as from H₃-30 to C-19. Furthermore, the position of the hydroxy group at C-22 was verified through the HMBC correlations of H-22/C-17 and C-28 and H-18/C-22, alongside the $^1\text{H}-^1\text{H}$ COSY correlation of H-22/H₂-21. The relative configuration of **2** was determined based on analysis of its NOE data. In particular, the NOESY correlations (Fig. 3) of H-18/H-22 and H-22/H-20 indicated the β -configurations of H-20 and H-22. The structure of **2** was further confirmed by single-crystal X-ray diffraction (Fig. 4).

Asprecoside C (**3**) shared the same molecular formula (C₃₇H₅₆O₁₀) as that of **2**, suggesting that they were structural isomers. The 1D NMR spectra of **3** revealed

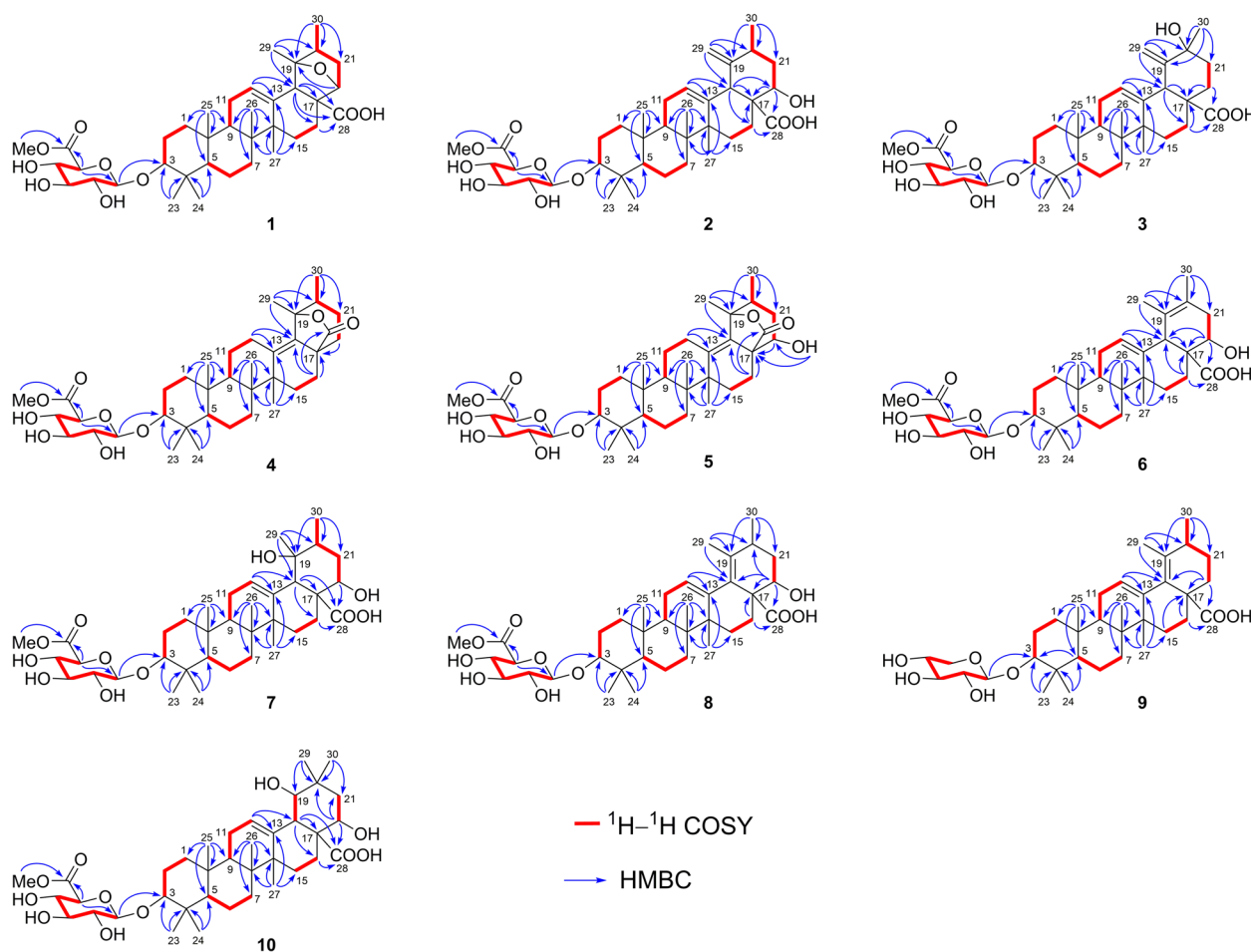
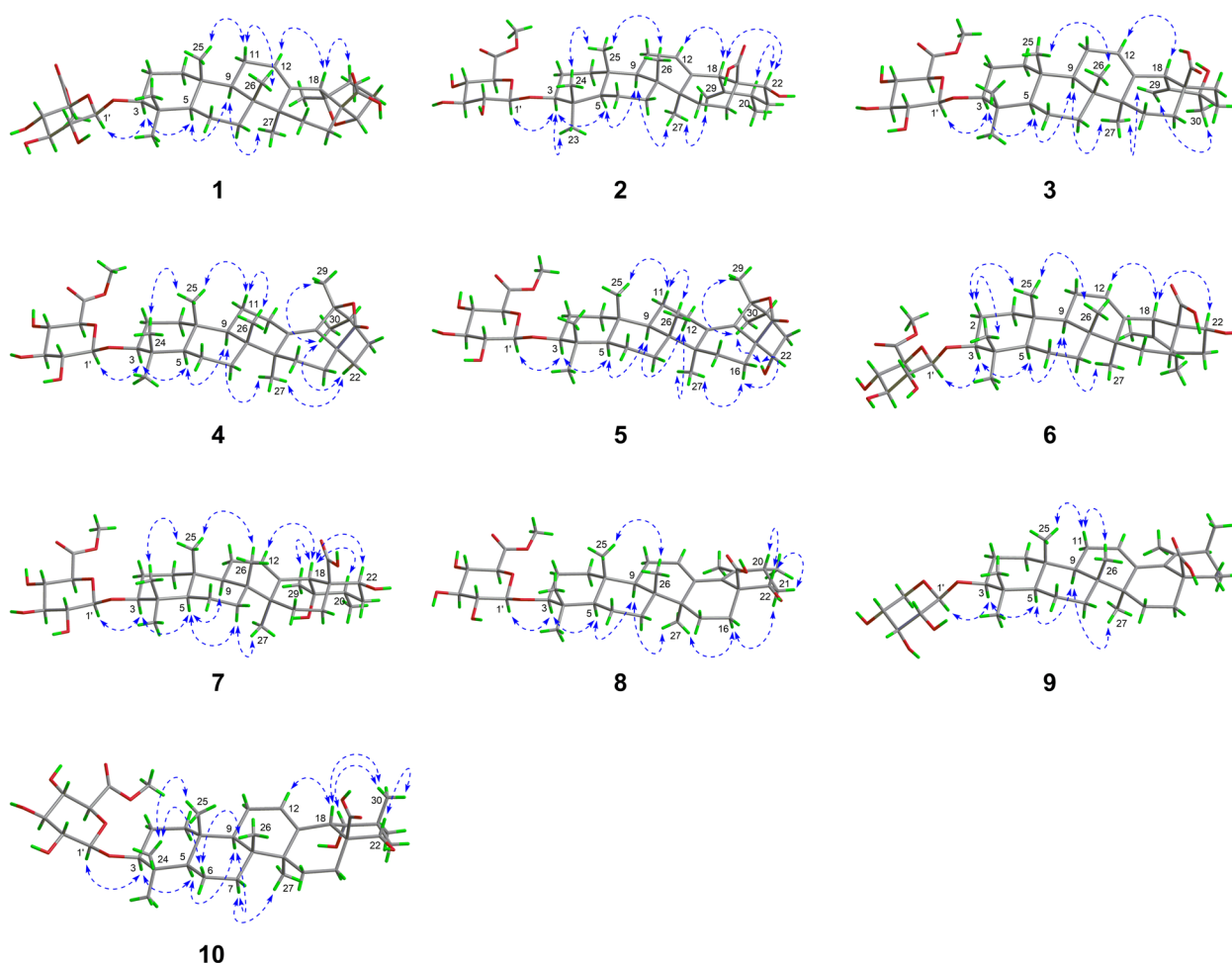
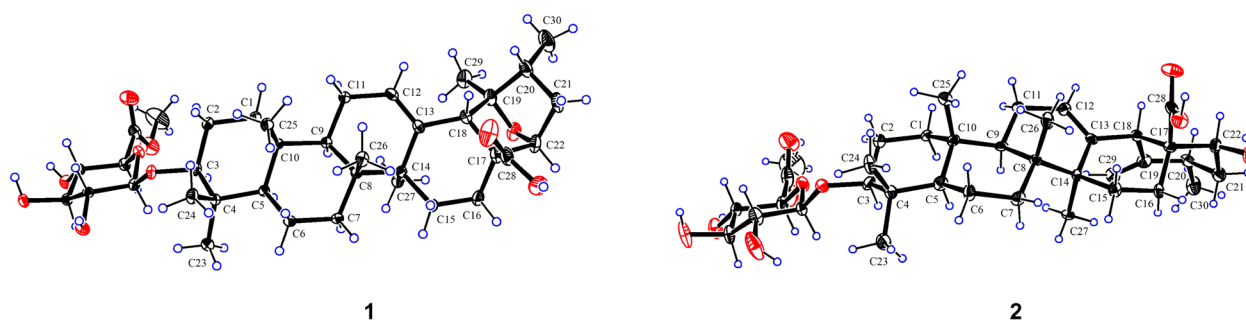


Fig. 2 Key $^1\text{H}-^1\text{H}$ COSY and HMBC correlations of **1**–**10**

**Fig. 3** Key NOESY correlations of **1–10****Fig. 4** X-ray crystal structures of compounds **1** and **2**

similar structural characteristics found in **2**, with the key difference being the hydroxy group relocating from C-22 in **2** to C-20 in **3**. This was supported by the HMBC correlations (Fig. 2) from a methyl singlet (δ_{H} 1.40, H₃-30) to an oxygenated sp^3 tertiary carbon (δ_{C} 71.0, C-20), as well as the ^1H – ^1H COSY correlation of

H₂-21/H₂-22. The stereochemistry of **3** was assigned to be the same as that of **2** by comparison of their NOE data (Fig. 3).

The molecular formula of asprecoside D (**4**) was determined to be C₃₇H₅₆O₉ based on its HRESIMS data. The ^1H and ^{13}C NMR data (Tables 1 and 2) of **4** exhibited a

close resemblance to those of **14**, except for the presence of a tetrasubstituted double bond instead of the trisubstituted double bond in **14**. This suggested that $\Delta^{12(13)}$ in **14** shifted to $\Delta^{13(18)}$ in **4**, which was validated by the HMBC correlations (Fig. 2) from H₂-12 and H₃-27 to a hydrogen-free sp^2 carbon (δ_C 137.1, C-13) and from H₂-12 and H₃-29 to the other hydrogen-free sp^2 carbon (δ_C 133.5, C-18). Additionally, the significant downfield shift of C-19 (δ_C 91.4 in **4**; δ_C 72.6 in **14**) and slight upfield shift of C-28 (δ_C 178.5 in **4**; δ_C 180.8 in **14**) suggested that C-19 and C-28 were linked via an O atom, leading to the formation of a five-membered lactone ring. The relative stereochemistry of **4** was established by analysis of its NOESY correlations (Fig. 3).

Asprecoside E (**5**) exhibited a molecular formula of C₃₇H₅₆O₁₀, containing one additional O atom compared to **4**. The 1D NMR spectra of **5** resembled those of **4**, except that the sp^3 methylene in **4** was substituted by an oxymethine [δ_H 4.18 (1H, br dd, $J=4.6, 3.1$ Hz); δ_C 72.8], indicating that **5** was a hydroxylated analogue of **4**. HMBC correlations (Fig. 2) revealed that the hydroxy group was attached at C-22, as evidenced by signals from OH-22 [δ_H 6.91, 1H, d ($J=4.6$ Hz)] to C-17 and C-22. Especially, the α -orientation of OH-22 was assigned by the NOE correlations (Fig. 3) of OH-22/H₃-30 and H-16 α as well as H-16 α /H₃-27.

The molecular formula of asprecoside F (**6**), C₃₇H₅₆O₁₀, was determined to be identical to that of **2** through HRESIMS analysis ($[M-H]^-$ m/z 659.3805). When comparing its 1D NMR data (Tables 2 and 3) of with those of **2**, the only structural difference between these two compounds was that $\Delta^{19(29)}$ double bond in **2** migrated to $\Delta^{19(20)}$ in **6**. This was confirmed by the HMBC correlations (Fig. 2) from a vinyl methyl (δ_H 1.73, H₃-29) to C-18, C-19, and C-20 and from the other vinyl methyl (δ_H 1.70, H₃-30) to C-19, C-20, and C-21. Comparison of their NOE data demonstrated that the relative stereochemistry of **6** aligned with that of **2**.

Asprecoside G (**7**) was assigned a molecular formula of C₃₇H₅₈O₁₁, as determined based on HRESIMS data at m/z 677.3905 $[M-H]^-$ (calcd for C₃₇H₅₇O₁₁⁻, 677.3906). Comparing the NMR data (Tables 2 and 3) of **7** with those of **14** demonstrated their structural resemblance. The primary distinction was the substitution of the sp^3 methylene (CH₂-22) in **14** with an oxymethine [δ_H 4.44 (1H, dd, $J=11.6, 4.1$ Hz); δ_C 75.4] in **7**, indicating that **7** was a 22-hydroxylated derivative of **14**. This was corroborated by the analysis of 2D NMR data (Fig. 2), especially the ¹H–¹H COSY correlation of the H₂-21/H-22. The β -configuration of H-22 was established based on the NOE correlation between H-18 and H-22 (Fig. 3).

Asprecoside H (**8**) showed a molecular formula of C₃₇H₅₆O₁₀, as proved by the HRESIMS data. Its 1D NMR

data resembled those of **7**, except for the presence of a tetrasubstituted double bond (δ_C 135.0 and 133.1) in **8** replacing the sp^3 methine at C-18 (δ_C 55.8) and the oxygenated sp^3 tertiary carbon at C-19 (δ_C 72.9) in **7**. This suggested that **8** was a C-18–C-19 dehydrated derivative of **7**, and was further confirmed by the HMBC correlations (Fig. 2) from a vinyl methyl (δ_H 1.96, H₃-29) to C-18 and C-19. The similar NOE correlations observed in **8** and **7** (Fig. 3) indicated that **8** shared the same relative stereochemistry as **7**.

The molecular formula of asprecoside I (**9**) was determined as C₃₅H₅₄O₇ by its HRESIMS data at m/z 585.3799 $[M-H]^-$ (C₃₅H₅₃O₇⁻, 585.3797). Comparison of its NMR data (Tables 2 and 3) with those of known compound (**13**) [20] revealed that they shared the same aglycone moiety but a different sugar unit. In the ¹H and ¹³C NMR spectra of **9**, the chemical shifts [δ_H 4.27 (1H, d, $J=7.5$ Hz), 3.82 (1H, dd, $J=11.4, 5.3$ Hz), 3.46 (1H, ddd, $J=10.1, 8.9, 5.3$ Hz), 3.28 (1H, t, $J=8.9$ Hz), 3.19 (1H, dd, $J=11.4, 10.1$ Hz), and 3.18 (1H, dd, $J=8.9, 7.5$ Hz); δ_C 107.4, 78.0, 75.4, 71.2, and 66.7] indicated the presence of a β -xylosyl group, which was attached to C-3 of the aglycone by the HMBC correlation (Fig. 2) from H-1' (δ_H 4.27) to C-3 (δ_C 90.6). Compound **9** shared the same relative stereochemistry with that of **13** regarding the aglycone moiety, by comparing their 1D NMR and NOESY data, especially the NOE correlations of H-1/H-3, H-3/H-5, H-5/H-9, H-9/H₃-27, H₃-25/H-11 β , H-11 β /H₃-26. The relative configuration of the chiral center C-20 was specifically determined by quantum chemical calculations of the 1D NMR chemical shifts of aglycone moiety in **9**, for both the 20S*-isomer and 20R*-isomer. The improved probability DP4+ analysis of the experimental and calculated 1D NMR data yielded the final score, with 20S*-isomer (100.00%) showing an absolute advantage over 20R*-isomer (0.00%), suggesting a 20S*-configuration for compound **9**.

Asprecoside J (**10**) exhibited an $[M-H]^-$ ion peak at m/z 677.3898 (C₃₇H₅₇O₁₁⁻, 677.3906), indicating a molecular formula of C₃₇H₅₈O₁₁. The NMR data (Tables 2 and 3) of **10** bore a resemblance to those of known oleanane glycoside (**11**) [21]. The key difference was the substitution of the sp^3 methylene in **11** by an oxymethine [δ_H 4.62 (1H, dd, $J=12.0, 3.8$ Hz); δ_C 72.0] in **10**, identifying **10** as a hydroxylated derivative of **11**. The HMBC correlations (Fig. 2) of H-22/C-20, C-21, and C-28, along with ¹H–¹H COSY correlation of H-22/H₂-21 confirmed the position of the hydroxyl group at C-22. The relative stereochemistry of **10** was elucidated through comparison of its 1D NMR data and NOE correlations with those of **11**. Notably, the β -orientation of H-22 was verified by the observed NOE correlations of H-18/H₃-30 and H₃-30/H-22.

Table 3 ^1H NMR data of compounds **6–10** (500 MHz, J in Hz, δ in ppm)

No	6 ^a	7 ^a	8 ^a	9 ^b	10 ^a
1 α	0.79, m	0.85, m	0.82, m	1.03, m	0.86, m
1 β	1.46, m	1.43, m	1.50, m	1.73, m	1.39, m
2 α	2.15, m	2.14, m	2.16, m	1.84, m	2.13, br d (11.4)
2 β	1.87, m	1.86, m	1.85, m	1.68, m	1.86, m
3	3.40, dd (11.7, 4.3)	3.37, dd (11.7, 4.2)	3.42, dd (11.7, 4.3)	3.15, dd (11.8, 4.1)	3.36, dd (10.8, 2.8)
5	0.80, m	0.84, m	0.83, m	0.81, br d (11.2)	0.81, m
6 α	1.49, m	1.50, m	1.49, m	1.55, m	1.50, m
6 β	1.27, m	1.34, m	1.30, m	1.39, m	1.33, m
7 α	1.58, m	1.63, m	1.62, m	1.58, m	1.53, m
7 β	1.44, m	1.42, m	1.51, m	1.50, m	1.37, m
9	1.48, m	1.78, m	1.47, m	1.44, m	1.81, m
11	1.84, m	2.00, m	1.91, m	1.96, m	1.98, m
12	5.66, t (3.3)	5.59, t (3.2)	5.71, t (3.3)	5.38, t (3.8)	5.57, t (3.7)
15 α	1.35, m	1.39, m	1.45, m	1.17, dt (13.7, 3.8)	1.37, m
15 β	2.71, m	2.51, m	2.69, td (13.2, 4.6)	1.90, td (13.7, 3.8)	2.29, br t (12.1)
16 α	2.04, m	3.02, td (13.1, 6.4)	2.18, td (13.0, 4.3)	1.38, m	2.75, m
16 β	2.73, m	2.78, br d (13.1)	2.91, br d (13.0)	2.17, m	2.83, m
18	3.74, s	3.08, s			3.70, br s
19					3.63, br s
20		1.72, m	2.58, m	2.19, m	
21 α	2.67, dd (17.0, 10.7)	1.90, m	1.99, m	1.36, m	2.49, t (12.0)
21 β	2.46, dd (17.0, 5.4)	2.49, m	2.29, m	1.83, m	1.73, m
22	4.62, dd (10.7, 5.4)	4.44, dd (11.6, 4.1)	4.83, dd (9.9, 3.2)	α 1.63, m β 1.81, m	4.62, dd (12.0, 3.8)
23	1.31, s	1.31, s	1.33, s	1.06, s	1.31, s
24	0.97, s	0.98, s	0.98, s	0.86, s	0.98, s
25	0.83, s	0.83, s	0.84, s	1.01, s	0.83, s
26	1.06, s	1.12, s	1.12, s	0.94, s	1.06, s
27	1.25, s	1.80, s	1.26, s	1.00, s	1.72, s
29	1.73, s	1.46, s	1.96, s	1.74, s	1.22, s
30	1.70, s	1.19, d (6.6)	1.28, d (7.1)	1.10, d (7.0)	1.26, s
1'	5.02, d (7.8)	5.01, d (7.8)	5.03, d (7.8)	4.27, d (7.5)	5.02, d (7.7)
2'	4.10, dd (9.0, 7.8)	4.10, dd (8.7, 7.8)	4.11, dd (9.0, 7.8)	3.18, dd (8.9, 7.5)	4.11, dd (9.0, 7.7)
3'	4.28, t (9.0)	4.28, t (8.7)	4.28, t (9.0)	3.28, t (8.9)	4.29, t (9.0)
4'	4.49, dd (9.7, 9.0)	4.50, dd (9.7, 8.7)	4.50, dd (9.7, 9.0)	3.46, ddd (10.1, 8.9, 5.3)	4.49, t (9.6, 9.0)
5'	4.62, d (9.7)	4.62, d (9.7)	4.63, d (9.7)	α 3.19, dd (11.4, 10.1) β 3.82, dd (11.4, 5.3)	4.64, d (9.6)
OMe-6'	3.76, s	3.76, s	3.77, s		3.74, s

^a In pyridine- d_5 , ^b in CD₃OD

To ascertain the absolute configurations of the glycosyl moiety in **1–10**, HPLC sugar analysis following acid hydrolysis and thiazolidine thiocarbamoyl derivatization was conducted [22]. The results demonstrated the occurrence of a D-xylosyl group in **9** and a D-glucuronosyl group in **1–8** and **10**.

The known compounds were identified as 19 α -hydroxy oleanolic acid 3-*O*- β -D-glucuronopyranoside-6'-*O*-methyl ester (**11**) [21], ilexoside A (**12**) [23],

3 β -hydroxy-20-*epi*-ursa-12,18-dien-28-oic acid 3-*O*- β -D-glucuronopyranoside-6'-*O*-methyl ester (**13**) [20], ilexasoside A (**14**) [13], ilexoside B (**15**) [24], 3 β -hydroxyursa-12,18-dien-28-oic acid 3-*O*- β -D-glucuronopyranoside-6'-*O*-methyl ester (**16**) [25], and ilexasprellanoside A (**17**) [14], through comparison of their NMR data with reported values.

Compounds **1–17** exhibited similar structures, especially the triterpenoid aglycone moiety with diverse

functional groups. Based on the well-known triterpenoids, oleanolic acid and ursolic acid, the proposed biosynthetic pathways and structural relationships of **1–17** are shown in Additional file 1: Fig S1.

The cytotoxic activities of **1–17** against H1975 and HCC827 cells were evaluated using the CCK-8 method, with gefitinib serving as the positive control. Apart from four compounds (**7**, **12**, **15**, and **17**) showing moderate cytotoxicity with IC_{50} values ranging from 10.85 to 48.41 μM , the other compounds did not display obvious activity ($IC_{50} > 50 \mu M$). Among these, compound **12** was identified as the most potent, exhibiting cytotoxicity against H1975 cells ($IC_{50} = 10.85 \pm 0.34 \mu M$).

3 Experimental section

3.1 General experimental procedures

Optical rotations were determined using a Rudolph Autopol I automatic polarimeter, and X-ray crystallographic data were collected on an Agilent Xcalibur Nova X-ray diffractometer. Melting points were measured on an X-4 melting instrument. UV and IR spectra were recorded using a Shimadzu UV-2600i spectrophotometer and a Bruker Tensor 37 infrared spectrophotometer, respectively. 1D and 2D NMR spectra acquired on a Bruker AM-500 spectrometer at 25 °C. HR-ESI-MS was performed using a Waters Micromass Q-TOF spectrometer. Semipreparative HPLC was performed on a Shimadzu LC-40B XR liquid chromatograph equipped with a YMC-Pack ODS-A column (250 mm $\times$ 10 mm, 5 μm). Silica gel (200–300 mesh, Qingdao Marine Chemical, Inc, Qingdao, China), Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Sweden), and reversed-phase C_{18} (RP- C_{18}) silica gel (50 μm , Quebec City Canada) were employed for column chromatography (CC).

3.2 Plant material

The roots of *I. asprella* were collected in April 2023 from Hezhou City, Guangxi Zhuang Autonomous Region, China. The plants were authenticated by one of the authors (W. Li) and a voucher specimen (No. GM202304) has been deposited in the Zhongshan Institute for Drug Discovery.

3.3 Extraction and isolation

The dried roots of *I. asprella* (20 kg) were crushed and subjected to reflux extraction using 65% EtOH for three times (3 $\times$ 100 L, 2 h each), producing a crude extract (1.6 kg). The extract was suspended in hot water (2.0 L) and successively partitioned with EtOAc (3 $\times$ 3.0 L) and *n*-BuOH (3 $\times$ 3.0 L), yielding EtOAc (105 g) and *n*-BuOH (303 g) fractions.

The *n*-BuOH extract was chromatographed on a silica gel column using a gradient of petroleum ether (PE)/

EtOAc/MeOH solvent system (5:1:0 $\rightarrow$ 0:0:1), yielding six fractions (Frs. A–F). Fr. C (24.8 g) was processed on an ODS gel column (MeOH/H₂O, 3:7 $\rightarrow$ 1:0) to afford four sub-fractions (Frs. C1–C4). Fr. C2 (2.8 g) was separated on a Sephadex LH-20 column (MeOH) and further purified by semipreparative RP-HPLC with a YMC-park ODS-A column (CH₃CN/H₂O, 45:55, 3 mL/min) to obtain compounds **15** (17.5 mg, t_R 38 min), **9** (16.6 mg, t_R 41 min), **13** (2.7 mg, t_R 43 min), and **17** (7.5 mg, t_R 33 min). Fr. D (33.5 g) was subjected to repeated silica gel column chromatography with CH₂Cl₂/MeOH (100:1 $\rightarrow$ 0:1), affording five fractions (Frs. D1–D5). Fr. D4 (778.2 mg) was further separated using a Sephadex LH-20 column (MeOH), resulting in five subfractions (Frs. D4a–Fr. D4e). Fr. D4b (94.1 mg) was purified via HPLC (MeOH/H₂O, 77:23, 2 mL/min) to yield **5** (1.9 mg, t_R 28 min), **11** (7.2 mg, t_R 34 min), and **12** (10.7 mg, t_R 35 min). Similarly, Fr. D4c (39.1 mg) was processed using HPLC (CH₃CN/H₂O, 43:57, 2 mL/min) to yield **1** (19.7 mg, t_R 50 min). Fr. D4e (88.5 mg) was also purified using HPLC (CH₃CN/H₂O, 52:48, 2 mL/min) to afford **3** (4.1 mg, t_R 12 min), **14** (15.5 mg, t_R 15 min), and **16** (15.7 mg, t_R 17 min). Chromatography of Fr. D5 on an ODS gel column (MeOH/H₂O, 1:1 $\rightarrow$ 1:0) produced six fractions (Fr. D5a–D5f). Fr. D5b (17.5 mg) was further refined using HPLC (CH₃CN/H₂O, 30:70, 2 mL/min) to isolate **7** (5.7 mg, t_R 64 min) and **10** (9.6 mg, t_R 74 min). Compounds **8** (8.0 mg, t_R 18 min), **4** (5.6 mg, t_R 21 min), and **6** (12.7 mg, t_R 22 min) were purified from Fr. D5c (50.5 mg) by HPLC (CH₃CN/H₂O, 45:55, 2 mL/min), and **2** (11.7 mg, t_R 55 min) was obtained from Fr. D5f (24.2 mg) by HPLC (CH₃CN/H₂O, 38:62, 2 mL/min).

3.4 Spectroscopic data of compounds

3.4.1 Asprecoside A (1)

Colorless needles; $[\alpha]_D^{20} -16$ (*c* 0.1, CH₃CN); IR (KBr) ν_{max} 3417, 2950, 1738, 1444, 1374, 1238, 1092, 1045, 1027, 982 cm^{-1} ; ¹H and ¹³C NMR data (Tables 1 and 2); HRESIMS m/z 659.3803 $[M-H]^-$ (calcd for C₃₇H₅₅O₁₀[−], 659.3801).

3.4.2 Asprecoside B (2)

Colorless needles; $[\alpha]_D^{20} -12$ (*c* 0.1, CH₃CN); IR (KBr) ν_{max} 3424, 2925, 1738, 1458, 1376, 1168, 1051, 1025, 910 cm^{-1} ; ¹H and ¹³C NMR data (Tables 1 and 2); HRESIMS m/z 659.3799 $[M-H]^-$ (calcd for C₃₇H₅₅O₁₀[−], 659.3801).

3.4.3 Asprecoside C (3)

White amorphous powder; $[\alpha]_D^{20} -21$ (*c* 0.1, MeOH); IR (KBr) ν_{max} 3434, 2948, 1737, 1452, 1387, 1227, 1171, 1046 cm^{-1} ; ¹H and ¹³C NMR data (Tables 1 and 2); HRESIMS m/z 659.3816 $[M-H]^-$ (calcd for C₃₇H₅₅O₁₀[−], 659.3801).

3.4.4 Asprecoside D (4)

White amorphous powder; $[\alpha]_D^{20} -11$ (*c* 0.1, MeOH); IR (KBr) $\nu_{\max}$ 3424, 2943, 1747, 1456, 1373, 1167, 1053 cm^{-1} ; ^1H and ^{13}C NMR data (Tables 1 and 2); HRESIMS m/z 689.3907 $[\text{M} + \text{HCOO}]^-$ (calcd for $\text{C}_{38}\text{H}_{57}\text{O}_{11}^-$, 689.3906).

3.4.5 Asprecoside E (5)

White amorphous powder; $[\alpha]_D^{20} -14$ (*c* 0.1, MeOH); IR (KBr) $\nu_{\max}$ 3441, 2925, 1743, 1456, 1376, 1049 cm^{-1} ; ^1H and ^{13}C NMR data (Tables 1 and 2); HRESIMS m/z 705.3857 $[\text{M} + \text{HCOO}]^-$ (calcd for $\text{C}_{38}\text{H}_{57}\text{O}_{12}^-$, 705.3856).

3.4.6 Asprecoside F (6)

White amorphous powder; $[\alpha]_D^{20} -25$ (*c* 0.1, CH_3CN); IR (KBr) $\nu_{\max}$ 3423, 2925, 1739, 1441, 1374, 1244, 1164, 1046, 1025, 914 cm^{-1} ; ^1H and ^{13}C NMR data (Tables 2 and 3); HRESIMS m/z 659.3805 $[\text{M} - \text{H}]^-$ (calcd for $\text{C}_{37}\text{H}_{55}\text{O}_{10}^-$, 659.3801).

3.4.7 Asprecoside G (7)

White amorphous powder; $[\alpha]_D^{20} -69$ (*c* 0.1, CH_3CN); IR (KBr) $\nu_{\max}$ 3423, 2925, 1738, 1443, 1375, 1249, 1166, 1048, 1026, 982 cm^{-1} ; ^1H and ^{13}C NMR data (Tables 2 and 3); HRESIMS m/z 677.3905 $[\text{M} - \text{H}]^-$ (calcd for $\text{C}_{37}\text{H}_{57}\text{O}_{11}^-$, 677.3906).

3.4.8 Asprecoside H (8)

White amorphous powder; $[\alpha]_D^{20} +18$ (*c* 0.1, CH_3CN); UV (CH_3CN) $\lambda_{\max}$ (log ϵ) 225 (3.97) nm; IR (KBr) $\nu_{\max}$ 3417, 2924, 1738, 1442, 1372, 1238, 1166, 1084, 1044 cm^{-1} ; ^1H and ^{13}C NMR data (Tables 2 and 3); HRESIMS m/z 659.3817 $[\text{M} - \text{H}]^-$ (calcd for $\text{C}_{37}\text{H}_{55}\text{O}_{10}^-$, 659.3801).

3.4.9 Asprecoside I (9)

White amorphous powder; $[\alpha]_D^{20} +14$ (*c* 0.1, CH_3CN); UV (CH_3CN) $\lambda_{\max}$ (log ϵ) 226 (3.99) nm; IR (KBr) $\nu_{\max}$ 3414, 2937, 2873, 1696, 1460, 1372, 1239, 1163, 1043 cm^{-1} ; ^1H and ^{13}C NMR data (Tables 2 and 3); HRESIMS m/z 585.3799 $[\text{M} - \text{H}]^-$ (calcd for $\text{C}_{35}\text{H}_{53}\text{O}_7^-$, 585.3797).

3.4.10 Asprecoside J (10)

White amorphous powder; $[\alpha]_D^{20} -13$ (*c* 0.1, CH_3CN); IR (KBr) $\nu_{\max}$ 3418, 2924, 1729, 1443, 1375, 1251, 1168, 1045, 1023, 983 cm^{-1} ; ^1H and ^{13}C NMR data (Tables 2 and 3); HRESIMS m/z 677.3898 $[\text{M} - \text{H}]^-$ (calcd for $\text{C}_{37}\text{H}_{57}\text{O}_{11}^-$, 677.3906).

3.5 X-ray crystallographic data of 1 and 2

The X-ray crystallographic data of asprecosides A (1) and B (2) have been deposited at the Cambridge Crystallographic Data Centre, with the following CCDC deposition numbers: 2413613 (1) and 2,413,615 (2).

3.5.1 Crystallographic data of asprecoside A (1)

$\text{C}_{37}\text{H}_{56}\text{O}_{10} \cdot 4(\text{H}_2\text{O})$ ($M = 732.88$ g/mol): monoclinic, space group $\text{P}2_1$ (no. 4), $a = 13.2729(3)$ Å, $b = 7.0751(2)$ Å, $c = 20.4063(5)$ Å, $\beta = 96.8560(10)^\circ$, $V = 1902.59(8)$ Å³, $Z = 2$, $T = 170.00$ K, $\mu(\text{CuK}\alpha) = 0.801$ mm⁻¹, $D_{\text{calc}} = 1.279$ g/cm³, 22,369 reflections measured ($6.708^\circ \leq 2\theta \leq 133.79^\circ$), 6424 unique ($R_{\text{int}} = 0.0391$, $R_{\text{sigma}} = 0.0367$) which were used in all calculations. The final R_1 was 0.0387 ($I > 2\sigma(I)$) and wR_2 was 0.1039 (all data). Flack parameter = $-0.02(3)$.

3.5.2 Crystallographic data of asprecoside B (2)

$\text{C}_{37}\text{H}_{56}\text{O}_{10}$ ($M = 660.81$ g/mol): monoclinic, space group $\text{P}2_1$ (no. 4), $a = 7.0183(2)$ Å, $b = 26.3891(6)$ Å, $c = 23.1192(5)$ Å, $\beta = 98.73^\circ$, $V = 4232.22(18)$ Å³, $Z = 4$, $T = 170.15$ K, $\mu(\text{CuK}\alpha) = 0.605$ mm⁻¹, $D_{\text{calc}} = 1.037$ g/cm³, 12,902 reflections measured ($5.116^\circ \leq 2\theta \leq 133.192^\circ$), 12,902 unique ($R_{\text{sigma}} = 0.0806$) which were used in all calculations. The final R_1 was 0.0631 ($I > 2\sigma(I)$) and wR_2 was 0.1789 (all data). Flack parameter = 0.09(4).

3.6 Acid hydrolysis of asprecosides A – J (1–10)

Each compound (1 mg) was heated under refluxed in 1.5 mL of 2 M HCl (dioxane/ H_2O , 1:1) at 90 °C for 4 h. Following hydrolysis, 5 mL of water was added, followed by extraction with EtOAc. The aqueous layer was evaporated under vacuum and the residue was dissolved in anhydrous pyridine (400 μL), followed by the addition of 2 mg of L-cysteine methyl ester hydrochloride. After stirring the mixture at 60 °C for 1 h, 50 μL of *o*-tolyl isothiocyanate was added, and the reaction continued at the same temperature for an additional hour. The authentic samples D-xylose and D-glucuronic acid underwent identical treatment and were analyzed by reversed-phase HPLC. D-xylose (2 mL/min, MeCN/ H_2O , 25:75, $t_R = 51.7$ min) was identified in 9, whereas D-glucuronic acid (2 mL/min, MeCN/ H_2O , 50:50, $t_R = 25.2$ min) was observed in 1–8 and 10.

3.7 Cell culture

H1975 and HCC827 cells, sourced from the American Type Culture Collection, were cultured in RPMI 1640 medium (Gibco BRL, USA) containing 10% fetal bovine serum (FBS). These cells were grown under conditions of 5% CO_2 and 37 °C.

3.8 Cell viability assay

Cell viability was evaluated through the Cell Counting Kit-8 (CCK-8) assay. In brief, Briefly, H1975 and HCC827 cells were seeded into 96-well plates (1×10^3 cells/well)

and incubated for 24 h. Then, the attached cells were treated with different concentrations of compounds. Following a 3-days incubation, CCK-8 reagent (Dojindo) was added, and luminescence was measured according to the manufacturer's protocol.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1007/s13659-025-00499-7>.

Additional file 1.

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Author contributions

Yuwei Wu: investigation, methodology, and writing of original draft; Baihui Zhang and Wenxian Li: methodology; Lihua Peng and Welin Qiao: resources; Wei Li: data curation, revised the manuscript, and supervision; De-an Guo: supervision and project administration. All authors read and approved the final manuscript.

Availability of data and materials

All data generated and analyzed during this study are included in this published article and its supplementary information file.

Declarations

Competing interests

The authors declare that there are no conflicts of interest associated with this work.

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