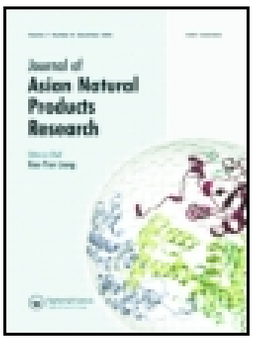

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Six new furostanol glycosides from *Smilax glauco-china* and their cytotoxic activity

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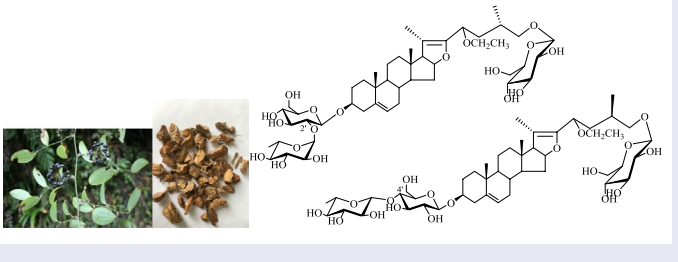
ABSTRACT

Six new steroidal saponins, namely glauco-chinaosides A–F, and one known compound were isolated from the tubers of *Smilax glauco-china*. Their structures were elucidated by a combination of spectroscopic analysis and hydrolysis followed by spectral and chromatographic analysis. Compounds **1**–**7** were tested *in vitro* for their cytotoxic activities against four human tumor cell lines (SH-SY5Y, SGC-7901, HCT-116, and Lovo). Compounds **1**, **2,** and **5** exhibited cytotoxic activity against SGC-7901, with IC_{50} values of 2.7, 11.5, and 6.8 μM, respectively.



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KEYWORDS
Smilax glauco-china; Smilacaceae; furostanol glycosides; cytotoxic activity

1. Introduction

Smilaceae comprises approximately 370 species of shrubs which grow in tropical and temperate areas around the world. *Smilax glauco-china,* an evergreen climbing shrub mainly distributed in the south of China (i.e. Zhejiang, Jiangxi, Hunan and Guizhou Province), is a member of the *Smilax* family [1]. The tubers of *S. glauco-china* are commonly used as herbal materials in traditional Chinese medicine (TCM). In TCM, the efficacy of *Smilax glauco-china* was regarded as dispelling wind-evil, cleaning heat, detoxicating. and eliminating damp. Based on the previous phytochemical investigation on the Smilaceae family, a conclusion can be safely arrived at that the genus *Smilax* is rich in steroidal saponins [2–4].

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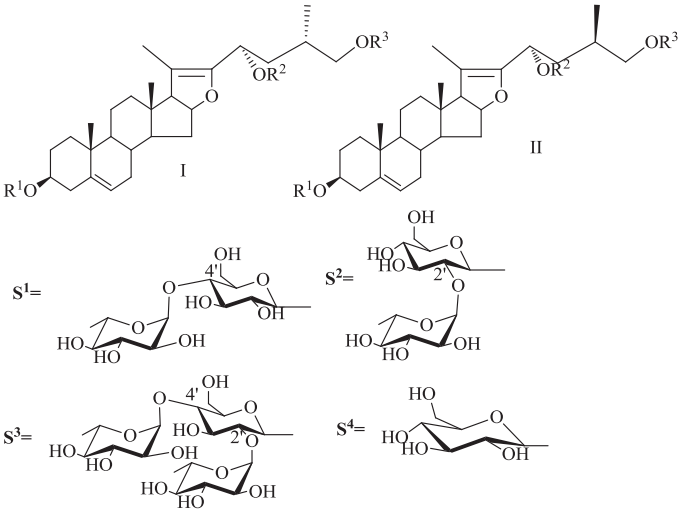

Aglycone moiety	compounds	R^1	R^2	R^3
I	**1**	S^1	CH_2CH_3	S^4
	3	S^3	CH_2CH_3	S^4
	5	S^2	CH_2CH_3	S^4
	7	S^3	CH_3	S^4
II	**2**	S^1	CH_2CH_3	S^4
	4	S^3	CH_2CH_3	S^4
	6	S^3	CH_3	S^4

Figure 1. The structures of compounds **1–7**.

Steroidal saponins exhibit a range of bioactivities, such as anti-inflammatory, cytotoxic, and anti-tumor effects [3–7]. However, to our best knowledge, there is almost no reference regarding the constituents of *S. glauco-china*. As part of our continuous interests in steroidal saponins in *Smilax* (Smilaceae) plants [8,9], a chemical investigation has been undertaken. The current article reports six new steroidal saponins and one known relative from the plant.

2. Results and discussion

Compound **1** (Figure 1) was obtained as a white amorphous powder with a molecular formula of $C_{47}H_{76}O_{18}$, determined by the deprotonated molecule at *m/z* 927.4989 $[M - H]^-$ in the HR-ESI-MS. The UV spectrum showed absorption maximum at 240 nm. In the IR spectrum, absorption bands for hydroxyl (3409 cm^{-1}), methyl (2934 cm^{-1}), olifin (1718 cm^{-1}), and ether (1038 cm^{-1}) groups were observed. Its structure was identified by comparison of its ^{1}H-NMR and ^{13}C-NMR (Table 1), HSQC, and HMBC data with the congener from *Dioscorea panthaica* [10,11].

Table 1. ^{13}H-NMR of compounds **1–7** at 400 MHz in pyridine (δ in ppm).

Aglycone moiety	1	2	3	4	5	6	7	Suger portion	1	2	3	4	5	6	7
1a	0.98 m	1.1m	1.0 m	1.0m	1.1m	1.0m	1.1m	*3-Glc*							
1b	1.7 m	1.74 m	1.7 m	1.8 m	1.68m	1.8m	1.68 m	1	4.98 d (7.6)	4.98 d (7.76)	4.98 d(6.04)	4.98m	5.08d(7.32)	4.98m	5.08d(7.32)
2a	1.7 m	1.71 m	1.86 m	1.89 m	1.27m	1.87m	1.27m	2	4.26 m	4.27 br d(2.28)	4.26 m	4.29 m	4.31m	4.3 m	4.31m
2b	2.08 m	2.10 m	2.09 m	2.09 m	2.15m	2.06m	2.15 m	3	4.23 m	4.24 m	3.66 m	3.66 m	4.33 m	3.67 m	4.33m
3	3.88 m	4.49 m	4.23 m	4.27 m	3.95 m	4.24m	3.95 m	4	4.5 m	4.51m	4.43 m	4.45 m	4.23 m	4.45 m	4.23m
4a	2.45 br d (11.32)	2.44 m	2.76 br d(11.90)	2.73 br d(10.7)	2.35m	2.74 br d	2.35m	5	3.75 m	3.72 m	4.23 m	4.27 m	4.33 m	4.28 m	4.33m
4b	2.72 dd(2.613.3)	2.71 m	2.82 dd(4.2013.4)	2.8 dd(3.314.0)	2.83 m	2.82 dd(3.413.8)	2.83 m	6	4.23m 4.26 d(1.96)	4.43 m 4.58 br d(3.32)	4.11 m 4.20 m	4.13 m 4.25 m	4.4 m 4.53m	4.12 m 4.21 m	4.53m
5	–	–	–	–	–	–	–	*2′-Rha*							
6	5.34 br d(4.36)	5.32m	5.33 br d(4.2)	5.31 br d(4.9)	5.33 br d(3.6)	5.31br d(3.64)	5.33 br d(13.2)	1			6.44 br s	6.45 br s	6.43 br s	6.44 br s	6.43 br s
7a	1.47 m	1.51m	1.85 m	1.83 m	1.50 m	1.83 m	1.50 m	2			4.85 m	4.86 m	4.84m	4.86 m	4.84m
7b	1.86 dd(2.167.04)	1.87 m	1.89 m	1.88 m	1.87 m	1.88 m	1.87 m	3			4.57 m	4.58 m	4.65dd(28.2)	4.57 dd(1.68.2)	4.65dd(9.16)
8	1.48m	1.49 m	1.50 m	1.54 m	1.49 m	1.54 m	1.49 m	4			4.39 m	4.36 m	4.39m	4.38 m	4.39m
9	0.9 m	0.87 m	0.91 m	0.92 m	0.87 m	0.94 m	0.87 m	5			4.98 m	4.99 m	5.06m	4.99m	5.06m
10	–	–	–	–	–	–	–	6			1.79 d(6.12)	1.79 d(6.24)	1.8 d(7.6)	1.79 d(7.64)	1.82s
11a	1.42 m	1.41m	1.48 m	1.49 m	1.41m	1.49 m	1.41 m	*4′-Rha*							
11b	1.48 m	1.47m	1.50 m	1.50 m	1.5m	1.50 m	1.5 m	1	5.94 br s	5.95 br s	5.89 br s	5.90br s		5.9 br s	
12a	1.17 m	1.18m	1.17 m	1.15m	1.15m	1.18 m	1.15 m	2	4.73 m	4.61m	4.71 m	4.71 m		4.71 m	
12b	1.75 m	1.75s	1.74 m	1.76 m	1.78 m	1.76 m	1.78 m	3	4.86 m	4.74m	4.65 m	4.66 m		4.66 dd(2.49.8)	
13	–	–	–	–	–	–	–	4	4.37 m	4.36m	4.39 m	4.36 m		4.38 m	
14	0.88 m	0.86 m	0.88 m	0.89 m	0.86 m	0.88 m	0.86 m	5	5.04 m	5.07m	4.96 m	4.96 m		4.99m	
15a	1.44 m	1.49 m	1.47 m	1.47 m	1.49 m	1.46 m	1.49 m	6	1.75 d (6.2)	1.75m	1.65 d(6.2)	1.66 d(6.16)		1.66 d(6.16)	
15b	2.11 m	2.11m	2.14 m	2.11 m	2.15 m	2.11m	2.15m	*26-Glc*							
16	4.74 br s	4.8 br s	4.88 m	4.83 m	4.84 dd(1.53.4)	4.87m	4.84dd(1.523.4)	1	4.88 d(7.7)	4.87 d(7.76)	4.86 m	4.88 d(7.7)	4.87d(7.76)	4.88 d(7.7)	4.87d(7.76)
17	2.52 d (10.2)	2.48 m	2.53 d(10.1)	2.50 d(10.3)	2.51d(10.1)	2.5d(10.6)	2.51d (10.1)	2	3.73 m	4.98s	4.08 m	4.02 m	3.72m	4.02 m	3.72m
18	0.72 s	0.78s	0.72 s	0.79 s	0.73s	0.79s	0.73 s	3	4.27 m	4.25m	3.98 m	3.98 m	4.23m	3.98 m	4.23m
19	0.95 s	0.95s	1.08 s	1.09 s	1.09s	1.08s	1.09 s	4	4.25 m	4.27br d(2.28)	4.25 m	4.29 m	4.37m	4.3 m	4.37m
20	–	–	–	–	–	–	–	5	3.97 m	3.97m	3.90 m	3.91 m	4.27m	3.91 m	4.27m

(*Continued*)

Table 1. (*Continued*)

Aglycone moiety	1	2	3	4	5	6	7	Suger portion	1	2	3	4	5	6	7
21	1.79 s	1.76s	1.80 s	1.76 s	1.8br s	1.75s	1.8 br s	6	4.44 m 4.60 m	4.19m 4.27 br d(2.28)	4.41 m 4.57 m	4.40 m 4.61 m	4.46 d(5.32) 4.58m	4.4 m 4.62 m	4.46 d(5.32) 4.58m
22	–	–	–	–	–	–	–								
23	4.35 m	4.34 m	4.35 m	4.33 m	4.53m	4.35 m	4.53 m								
24a	1.71 m	1.78 m	1.74 m	1.91 m	1.73 m	1.79 m	1.73 m								
24b	2.24 m	2.09 m	2.25 m	2.06 m	2.26 m	2.06 m	2.26 m								
25	2.33 dd(6.212.5)	2.19 m	2.33 m	2.18 m	2.34 m	2.18 m	2.34 m								
26a	3.69 m	3.99 m	3.73 m	3.70 m	4.03 m	3.7 m	4.03 m								
26b	4.0 m	4.0 m	4.05 m	4.07 m	4.04 m	4.05 m	–								
27	1.14 d (6.56)	1.15 d(6.64)	1.14 d(6.60)	1.11 d(6.68)	1.15 d(6.6)	1.1 d(6.64)	1.15 d(6.6)								
(23-OCH_2)a	3.4 m	2.46m	3.40 m	3.46 m	3.72m	–	3.72 m								
(23-OCH_2)b	3.7 m	3.75m	3.71 m	3.74 m	3.42m	–	3.42 m								
23-CH_3	1.21 t (6.96)	1.26t(6.96)	1.21t(7)	1.25 t(7)	1.22t(7)	3.39s	1.22t(7)								

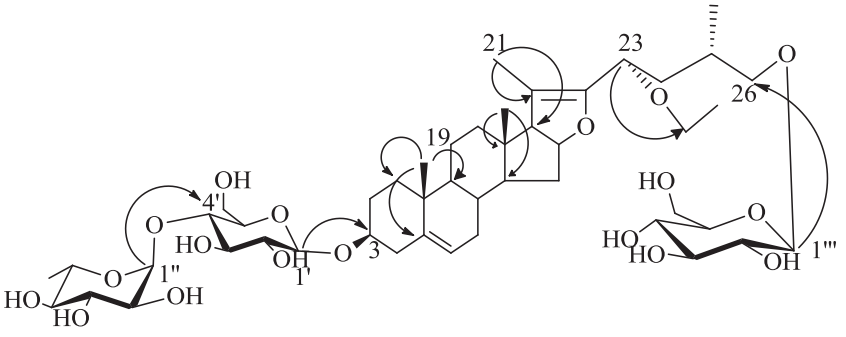

Figure 2. Key HMBC (H→C) correlations of compound **1**.

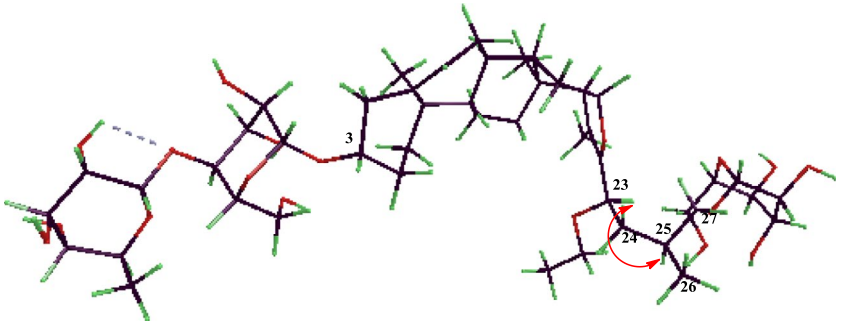

Figure 3. Important NOE (H–H) enhancement of compound **1**.

The ^{1}H-NMR spectrum of **1** showed signals belonging to an olefinic proton at δ_H 5.34 (br d, J = 4.4 Hz, H-6), and six methyl protons at δ_H 0.95(s, H-19), 0.72 (s, H-18), 1.79(s, H-21), 1.21(t, J = 7.0, H-23-CH_3), δ_H 1.14 (d, J = 6.6 Hz, H-27), δ_H 1.75(d, J = 6.2 Hz, H-6″). The ^{13}C-NMR spectrum displayed a total of 47 carbon signals, which were composed of 27 signals due to aglycone moiety, 2 carbon signals due to the ethoxy group at C-23, and 18 carbon signals due to the presence of three hexoses. The DEPT spectrum of **1** showed the presence of 6 primary carbons, 12 secondary carbons, 24 tertiary carbons, 3 quaternary carbons, and 2 SP^2 hybrid quaternary carbons. All of the spectral data showed compound **1** possessed the same partial structure in A, B, C, D, and E rings as dioscoreside C [10], except for the different substituent of C-23. Comparison of the ^{13}C-NMR data with those in the reference indicated the upfield shifts of C-22 and C-23 by 0.7 and 1.4 ppm, respectively, together with one more carbon signal at δ_C 15.7. Furthermore, in the HMBC spectrum, the correlation of H-23/C-23-OCH_2 was observed (Figure 2). These data indicated a typical ethoxy group at C-23.

The spatial configuration of compound **1** was confirmed according to the known compound of dioscoreside C. The ^{1}H-NMR spectrum indicated that **1** possessed one less sugar moiety than dioscoreside C. In the NOESY spectrum of **1**, the correlation of H-23/H-25 (Figure 3) indicated that the configuration of C-23 is similar to the dioscoreside C. Thus, the ethoxy group of C-23 had the α-configuration. Therefore, the absolute configuration of C-23 was assigned as *S*. The resonance of the protons and carbons (C-24, C-25, C-26, and C-27) around the C-25 center and the $^{3}J_{HH}$ values (12.5, 6.2 Hz) between

H-25 and H-26 provided the evidence for the C-25 *R* configuration of **1,** as described in the previous report [11,12]. Based on the inspection on the ^{1}H and ^{13}C NMR spectral data of compound **1**, the structure of the aglycone moiety was found to be identical to 3*β*,26-dihydroxy-23(*S*)-ethoxy-25(*R*)-furosta-5,20(22)-diene.

The ^{1}H-NMR spectrum of **1** displayed signals for three anomeric protons at δ_H 4.98 (d, J = 7.6 Hz), δ_H 5.94 (br s), and δ_H 4.88 (d, J = 7.7 Hz), which gave correlations in the HSQC spectrum with ^{13}C NMR signals at δ_C 102.5, δ_C 102.7, and δ_C 104.9, respectively. The identity of the monosaccharides and the sequence of the oligosaccharide chain were determined by the analysis of HMQC, HMBC, COSY, and NOESY spectra. All of the data showed that compound **1** possessed two glucoses and one rhamnose. Acid hydrolysis of **1** yielded D-glucose and L-rhamnose, as revealed by HPLC analysis and comparison with authentic standards. The *α*-anomeric configuration for the rhamnose was determined by its C-5 data (δ_C 70.4) [13], and the *β*-anomeric configurations for the two glucoses were determined from their large $^{3}J_{1,2}$ coupling constants (7.6, 7.7 Hz). The attachments of the rhamnose moiety to C-4′ of the glucose moiety were established by the correlation of H-1″/C-4′, and the glucose moiety to C-3 of the aglycone was based on a correlation of H-1′/C-3. In addition, the remaining glucose moiety to C-26 of the aglycone was based on a correlation of H-1‴/C-26. Based on all the data mentioned above, compound **1** was determined to be 26-*O*-*β*-D-glucopyranosyl-3*β*,26-dihydroxy-23(*S*)–ethoxy -25(*R*)-furosta-5,20(22)-diene-3-*O*-*α*-L-rhamnopyranosyl (1→4)-*β*-D-glucopyranoside, and given the trivial name glauco-chinaoside A.

Compound **2** (Figure 1) was isolated as a white amorphous powder with a molecular formula of $C_{47}H_{76}O_{18}$, determined by the deprotonated molecular ion at *m/z* 927.4965 $[M - H]^-$ in the HR-ESI-MS. The UV spectrum showed absorption maximum at 260 nm. In the IR spectrum, absorption bands of hydroxyl (3406 cm^{-1}), methyl (2934 cm^{-1}), olifine (1664 cm^{-1}), and ether (1074 cm^{-1}) groups can be found. ^{1}H-NMR and ^{13}C-NMR spectral data (Table 1) indicated that compound **2** had the same aglycone moiety and sugar arrangement as that of **1**. The HMQC, HMBC, and NOESY spectra indicated that the aglycone of **2** possessed the same partial structure of A, B, C, D, and E rings, together with the ethoxy group at C-23, of which absolute configuration of C-23 was assigned as *S***.** Indeed, the NMR spectroscopic data of compound **2** were very similar to that of **1**, with the main difference at the downfield shifts of C-27 (δ_C 18.1) and C-25 (δ_C 31.0) and the upfield shift of C-24 (δ_C 37.5). According to the reference, the C-25 *S* configuration of **2** could be deduced [10]. Based on all the data mentioned above, compound **2** was determined to be 26-*O*-*β*-D-glucopyranosyl-3*β*,26-dihydroxy-23(*S*)-ethoxy-25(*S*)-furosta- 5,20(22)-diene-3-*O*-*α*-L-rhamnopyranosyl(1→4)-*β*-D-glucopyranoside, and was named glauco-chinaoside B.

Compound **3** (Figure 1) was obtained as a white amorphous powder. The molecular formula of compound **3** was $C_{53}H_{86}O_{22}$, determined by the deprotonated molecule at *m/z* 1073.5599 $[M - H]^-$ in the HR-ESI-MS. The UV spectrum showed absorption maximum at 210 nm. In the IR spectrum, absorption bands of hydroxyl (3426 cm^{-1}), methyl (2924 cm^{-1}), olifine (1742 cm^{-1}), and ether (1041 cm^{-1}) groups can be found. According to the NMR data (Table 1), it had the same aglycone moiety as compound **1**, but as its molecular mass was 146 Da higher, an additional 6-deoxyhexose moiety could be presumed. The spatial configuration of compound **3** was confirmed according to the resonance data, which were very similar to that of compound **1**. The ^{1}H-NMR spectrum of **3** displayed signals for four anomeric protons of δ_H 4.98, 6.44, 5.89, and 4.86, which gave correlations in the HSQC spectrum with ^{13}C NMR signals at δ_C 100.3, 102.0, 102.9, and 104.9, respectively. According

to the HMBC data, the attachments of the rhamnoses moiety to C-2′ and C-4′ of the glucose moiety were established by the correlation of H-1″/C-2′, H-1‴/C-4′. In conclusion, compound **3** was elucidated as 26-*O*-*β*-D-glucopyranosyl-3*β*,26-dihydroxy-23(*S*)-ethoxy-25(*R*)-furosta-5,20(22)-diene-3-*O*-*α*-L-rhamnopyranosyl-(1→2)-[*α*-L-rhamnopyranosyl(1→4)]-*β*-D-glucopyranoside, and was named glauco-chinaoside C.

Compound **4** was isolated as a white amorphous powder with a molecular formula of $C_{53}H_{86}O_{22}$, determined by the pseudo-molecular ion at *m/z* 1097.546 $[M + Na]^+$ in the HR-ESI-MS. The UV spectrum showed absorption maximum at 265 nm. In the IR spectrum, absorption bands for hydroxyl (3431 cm^{-1}), methyl (2925 cm^{-1}), olifin (1737 cm^{-1}), and ether (1164 cm^{-1}) groups can be found. ^{1}H-NMR and ^{13}C-NMR spectral data (Table 1) indicated that compound **4** had the same aglycone moiety and spatial configuration as that of compound **2**. ^{1}H-NMR and ^{13}C-NMR spectral data indicated the configuration of C-25 in compounds **2** and **3** was different. So, compounds **4** and **3** were different in the configuration of C-25. Similar sugar part and molecular weight of compounds **4** and **3** all indicated that they are structural isomers. Thus, the structure of **4** was established as 26-*O*-*β*-D-glucopyranosyl-3*β*,26-dihydroxy-23(*S*)-ethoxy-25(*S*)-furosta-5,20(22) -diene-3-*O*-*α*-L-rhamnopyranosyl-(1→2)-[*α*-L-rhamnopyranosyl(1→4)]-*β*-D-glucopyranoside, and was named glauco-chinaoside D.

Compound **5** was obtained as a white amorphous powder with a molecular formula of $C_{47}H_{76}O_{18}$, determined by the deprotonated molecular ion at *m/z* 927.5032 $[M - H]^-$ in the HR-ESI-MS, similar molecular weight to compounds **1** and **2**. The UV spectrum showed absorption maximum at 260 nm. In the IR spectrum, absorption bands for hydroxyl (3432 cm^{-1}), methyl (2924 cm^{-1}), olifin (1736 cm^{-1}), and ether (1163 cm^{-1}) groups can be found. From the NMR data (Table 1), the same aglycone moiety and spatial configuration as that of compound **1** could be identified. The resonance of the protons and carbons provided the evidence for the C-23 *S* and C-25 *R* configurations of **5**. Compound **5** possessed two glucoses and one rhamnose based on the ^{1}H-NMR spectrum that displayed signals for three anomeric protons at δ_H 5.08, 6.43, and 4.87 and three anomeric carbons at δ_C 100.4, 102.7, and 105.0, respectively. Indeed, the NMR spectroscopic data of compound **5** were very similar to that of **1**, whereas the main difference was the attachment of rhamnose. The attachment of the rhamnose moiety to C-2′ of the glucose moiety was established by the HMBC correlation of H-1″/C-2′. These data allowed the structure of **5** to be assigned as 26-*O*-*β*-D-glucopyranosyl-3*β*,26-dihydroxy-23(*S*)-ethoxy-25(*R*)- furosta-5,20(22)-diene-3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranoside, and was named glauco-chinaoside E.

The molecular formula of compound **6** was determined as $C_{52}H_{84}O_{22}$ by the ion peak at *m/z* 1083.5369 $[M + Na]^+$ in HR-ESI-MS, which is 14 Da less than compound **4**. The UV (MeOH) spectrum showed absorption maximum at 210 nm. In the IR (KBr)spectrum, absorption bands for hydroxyl (3425 cm^{-1}), methyl (2922 cm^{-1}), olifin (1731 cm^{-1}), and ether (1040 cm^{-1}) groups can be found. From the NMR data (Table 1), the same sugar chain could be identified. Further interpretation of the NMR data revealed the difference in C-23, and the ethoxy group (δ_C 63.8 and δ_C 15.7) was displaced by the methoxy group (δ_C 56.3). In addition, the absolute configuration of C-23 was assigned as *S* according to the reference [10]. The resonance of the protons and carbons (C-24, C-25, C-26, and C-27) around the C-25 center provided the evidence for the C-25 *S* configuration of compound **6** as described in compound **2**. Thus, compound **6** was elucidated as

26-*O*-*β*-D-glucopyranosyl-3*β*,26-dihydroxy-23(*S*)-methoxy-25(*S*)-furosta-5,20(22)-diene-3-*O*-*α*-L-rhamnopyranosyl-(1→2)-[*α*-L-rhamnopyranosyl(1→4)]-*β*-D-glucopyranoside, and was named glauco-chinaoside F.

Additionally, one known compound **7** was isolated. Based on the NMR spectroscopic data and comparison with the literature [10], its structure was determined to be 26-*O*-*β*-D-glucopyranosyl-3*β*,26-dihydroxy-23(*S*)-methoxy-25(*R*)-furosta-5,20(22)-diene-3-*O*-*α*-L-rhamnopyranosyl-(1→2)-[*α*-L-rhamnopyranosyl(1→4)]-*β*-D-glucopyranoside, and was named dioscoreside C.

Compounds **1–7** were evaluated for cytotoxic activities against four human cell lines (SH-SY5Y, SGC-7901, HCT-116, and Lovo), with Vero as a positive control. The purity of compounds **1–7** was 94, 93.2, 92.1, 91.0, 91.7, 94.2, and 91.2%, respectively. Cisplatin (≥99.9%; Sigma–Aldrich, St. Louis, MO, USA), a well-known anticancer drug, was used in this experiment as a positive control. Compounds **1–7** were inactive (IC_{50} > 100 μM) to SH-SY5Y, Lovo, and HCT-116 cell lines. Compounds **1**, **2,** and **5** exhibited cytotoxic activity against SGC-7901, with IC_{50} values of 2.7, 11.5, and 6.8 μM, respectively.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured with a Perkin–Elmer 241 MC polarimeter (Co. Perkin Elmer, Liantrisant, UK). IR spectra were recorded on L1600401 Spectrum TWO DTGS (Co. Perkin Elmer, Liantrisant, UK). NMR spectra were obtained on a Bruker AM-400 spectrometer (400-MHz) in pyridine-d_5 at room temperature (25 °C) (Bruker, Fällanden, Switzerland); Electrospray ionization (ESI) mass spectra were employed in the positive ion mode on a LCQ DECAXP instrument (Thermo Finnigan, San Jose, CA, USA) equipped with an ion-trap mass analyzer. HR-ESI-MS were obtained in the positive ion mode using a Waters UPLC Premier Q-TOF system. Preparative HPLC was operated using a Prevail C_{18} column (5 μm, 10.0 mm I.D × 250 mm) at rate of 3.0 ml/min and detection wavelength of 210 nm. UV spectra and HPLC analysis was carried out on an Agilent 1200 system equipped with a quaternary solvent delivery system, an autosampler, and a DAD detector. The column was a Thermo C_{18} (5 μm, 4.6 mm I.D × 250 mm). Column chromatography was performed using MCI GEL (Mitsubishi Chemical Corporation, Japan), Sephadex LH-20 (Amersham Bio-sciences AB, Uppsala, Sweden) and C_{18} SPE by Bulk Sorbent (Grace Davison Discovery Sciences), and silica gel (SiO_2; 200–300 mesh; Qingdao Haiyang Chemical Co., Ltd., Qingdao, China). TLC plates were HSGF254 SiO2 from Yantai Jiangyou Silica Gel Development Co., Ltd. (Yantai, China).

3.2. Plant material

Rhizomes of *Smilax glauco-china* (Smilacaceae) were collected in Wuyunjie Nature Reserve, Taoyuan County, Hunan Province, China, in May 2013. All plants were identified by Daigui Zhang, a lecturer at Jishou University. A voucher specimen (No. 20130513) was deposited at the Key Laboratory of Modern Preparation of TCM, Jiangxi University of TCM, China.

Table 2. ^{13}C-NMR of compounds **1–7** at 100 MHz in pyridine (δ in ppm).

Aglycone moiety	1	2	3	4	5	6	7	Suger portion	1	2	3	4	5	6	7
1	37.5	37.5	37.6	37.5	37.6	37.4	37.6	*3-Glc*							
2	30.2	30.2	30.2	30.2	29.9	30.2	30.2	1	102.5	102.5	100.3	100.3	100.4	100.3	100.3
3	78.3	78.3	77.8	77.8	78.3	78.1	78	2	75.2	75.2	78.7	78.7	79.7	78.7	78.7
4	39.4	39.4	39	39	39	39	39	3	76.7	76.7	77	77	77.8	77	77
5	140.9	140.9	140.9	140.8	140.9	140.8	140.8	4	78.4	78.2	78.5	78.5	71.8	78.6	78.6
6	121.7	121.7	121.8	121.8	121.7	121.8	121.8	5	77.2	77.2	77.8	78	77.9	77.8	77.8
7	32.4	32.3	32.4	32.4	32.4	32.4	32.4	6	61.6	61.6	61.3	61.3	62.7	61.3	61.3
8	31.4	31.4	31.4	31.4	31.4	31.4	31.4	*2′-Rha*							
9	50.3	50.2	50.3	50.3	50.3	50.3	50.3	1			102	102	102.7	102	102.1
10	37.1	37.1	37.1	37.1	37.1	37.1	37.1	2			72.6	72.5	72.6	72.6	72.6
11	21.3	21.2	21.3	21.2	21.2	21.2	21.2	3			72.8	72.8	72.9	72.8	72.8
12	39.6	39.6	39.6	39.6	39.6	39.6	39.6	4			74.2	74.1	74.2	74.1	74.1
13	43.5	43.3	43.5	43.3	43.5	43.3	43.5	5			69.5	69.5	69.5	69.6	69.6
14	54.9	55.1	54.9	55.1	54.9	55.1	54.9	6			18.7	18.7	18.7	18.7	18.7
15	34.5	34.4	34.5	34.4	34.5	34.4	34.5	*4′-Rha*							
16	84.6	85.1	84.6	85.1	84.6	85.2	84.6	1	102.7	102.7	102.9	102.9		102.9	102.9
17	64.9	64.8	64.9	64.8	64.9	64.8	64.9	2	72.7	72.7	72.6	72.6		72.6	72.6
18	14.3	14.4	14.3	14.4	14.3	14.6	14.3	3	72.9	72.9	72.9	72.8		72.8	72.9
19	19.4	19.4	19.4	19.4	19.4	19.4	19.4	4	74	74	74	73.9		74	74
20	107.8	108.7	107.9	108.7	107.9	108.7	108.6	5	70.4	70.4	70.5	70.5		70.5	70.4
21	11.5	11.6	11.5	11.6	11.5	11.6	11.5	6	18.6	18.6	18.5	18.5		18.5	18.5
22	151.1	150.9	151.1	150.9	151	150.9	151.1	*26-Glc*							
23	71.7	71.7	71.7	71.7	71.7	71.8	73.4	1	104.9	104.8	104.9	104.8	105	104.8	105
24	37.9	37.5	37.9	37.5	37.8	37.6	37.7	2	75.3	75.3	75.2	75.1	75.3	75.2	75.2
25	30.7	31	30.7	31	30.7	31	30.7	3	78.7	78.7	78.7	78.7	78.7	78.6	78.6
26	75.6	75.6	75.3	75.3	75.2	75.3	75.3	4	71.8	71.8	71.8	71.8	71.8	71.8	71.8
27	17.6	18.1	17.6	18.1	17.6	18.1	17.6	5	78.5	78.6	78	78.1	78.1	78.1	78
23-OCH_2	63.8	63.9	63.8	63.9	63.8	23-OCH3(56.3)	23-OCH3(56.1)	6	62.9	62.9	62.9	62.9	62.9	62.9	62.9
23-CH_3	15.7	15.8	15.7	15.7	15.7										

3.3. Extraction and isolation

Air-dried and powdered rhizomes and roots of *S. glauco-china* (5 kg) were extracted twice with 70% EtOH and concentrated *in vacuum* to give crude extract, which was suspended in H_2O and partitioned successively with EtOAc and n-BuOH. The n-BuOH soluble portion (201 g) was first chromatographed on a HP-20 macroporous adsorption resin and eluted with 30, 50, 70, and 95% EtOH, successively, to obtain four fractions (Fr. A, B, C, and D). Fr. A (31 g) was subjected to a silica gel column using a stepwise gradient of $CHCl_3$: MeOH (from 7:1 to 0:1), then purified by a Sephadex LH-20 column, and eluted with MeOH. A further purification was made by a preparative HPLC with solvent of CH_3CN:H_2O (22:78) to obtain **3** (15 mg, t_R = 15.3 min), **4** (22.8 mg t_R = 15.2 min), **6** (8.6 mg, t_R = 12.2 min), and **7** (12.9 mg, t_R = 20.5 min). Fr. B (26 g) was subjected to a MCI column using a stepwise gradient of MeOH: H_2O (20:80, 40:60, 60:40, 80:20) to obtain four sub-fractions. Fr.B.3 (5.2 g) was subjected to a silica gel column using a stepwise gradient of $CHCl_3$: MeOH (from 8:1 to 0:1); it was further purified by preparative HPLC with solvent of CH_3CN:H_2O (18:82) to obtain compounds **1** (23 mg, t_R 17.3 min) and **2** (33.8 mg, t_R 19.1 min). Likewise, Fr.B.4 (3.9 g) was further purified by preparative HPLC with CH_3CN:H_2O (16:84) to obtain compound **5** (5.5 mg, t_R 22.5 min). All the detection was carried out using wavelength of 210 nm and a flow rate of 3.0 ml/min.

3.3.1. Glauco-chinaoside A (1)

White amorphous powder (MeOH); $[\alpha]_D^{25}$ – 53.19 (c 0.0188, MeOH); UV (MeOH) λ_{max} (log ε) 240 (0.59) nm; IR (KBr) ν_{max} cm^{-1}: 3409, 2934, 1718, 1038; ^1H NMR and ^{13}C NMR spactral data see Tables 1 and 2; HR-ESI-MS: *m/z* 927.4989 $[M - H]^-$ (calcd for $C_{47}H_{75}O_{18}$, 927.4953).

3.3.2. Glauco-chinaoside B (2)

White amorphous powder (MeOH); $[\alpha]_D^{25}$ – 35.02 (c 0.0256, MeOH); UV (MeOH) λ_{max} (log ε) 260 (0.27) nm; IR (KBr) ν_{max} cm^{-1}: 3406, 2934, 1664, 1074; ^1H NMR and ^{13}C NMR spactral data see Tables 1 and 2; HR-ESI-MS: *m/z* 927.4965 $[M - H]^-$ (calcd for $C_{47}H_{75}O_{18}$, 927.4953).

3.3.3. Glauco-chinaoside C (3)

White amorphous powder (MeOH); $[\alpha]_D^{25}$ – 44.94 (c 0.0178, MeOH); UV (MeOH) λ_{max} (log ε) 210 (0.66) nm; IR (KBr) ν_{max} cm^{-1}: 3426, 2924, 1742, 1041; ^1H NMR and ^{13}C NMR spactral data see Tables 1 and 2; HR-ESI-MS: *m/z* 1073.5599 $[M - H]^-$ (calcd for $C_{53}H_{85}O_{22}$, 1073.5532).

3.3.4. Glauco-chinaoside D (4)

White amorphous powder (MeOH); $[\alpha]_D^{25}$ – 35.9(c 0.0195, MeOH); UV (MeOH) λ_{max} (log ε) 265 (0.67) nm; IR (KBr) ν_{max} cm^{-1}: 3431, 2925, 1737, 1164; ^1H NMR and ^{13}C NMR spactral data see Tables 1 and 2; HR-ESI-MS: *m/z* 1097.5460 $[M + Na]^+$ (calcd for $C_{53}H_{86}O_{22}Na$, 1097.5508).

3.3.5. Glauco-chinaoside E (5)

White amorphous powder (MeOH); $[\alpha]_D^{25}$ – 28.57 (c 0.021, MeOH); UV (MeOH) λ_{max} (log ε) 260 (0.31) nm; IR (KBr) ν_{max} cm^{-1}: 3432, 2924, 1736, 1163; ^1H NMR and ^{13}C NMR spactral data see Tables 1 and 2; HR-ESI-MS: *m/z* 927.5032 $[M - H]^-$ (calcd for $C_{47}H_{75}O_{18}$, 927.4953).

3.3.6. Glauco-chinaoside F (6)

White amorphous powder (MeOH); $[\alpha]_D^{25}$ – 29.85 (c 0.0067, MeOH); UV (MeOH) λ_{max} (log ε) 210 (0.67) nm; IR (KBr) ν_{max} cm^{-1}: 3425, 2922, 1731, 1040; ^1H NMR and ^{13}C NMR spactral data see Tables 1 and 2; HR-ESI-MS: *m/z* 1083.5369 $[M + Na]^+$ (calcd for $C_{52}H_{84}O_{22}Na$, 1083.5352).

3.4. Cytotoxic activity

The cytotoxicity against four human tumor cell lines (SH-SY5Y, SGC-7901, HCT-116, and Lovo), with Vero as a positive control, was evaluated using the MTT method. Cells were seeded in 96-well microplates at a density of 150 per well and were cultured in cell culture medium (RPMI1640 medium supplemented with 10% FBS (fetal bovine serum), 100 U/ml penicillin, and 100 g/ml streptomycin) for 12 h, then treated with the stock solution of test compounds dissolved in DMSO. The final DMSO concentration never exceeded 0.2% (v/v). After 48 h of cultivation, cells were incubated with MTT (0.5 mg/ml, 4 h) and subsequently resolved in DMSO. The absorbance in the control and drug-treated wells was measured by an automated microplate reader at 570/630 nm. All experiments were carried out in triplicate and repeated twice. The cytotoxicity was expressed as IC_{50} values (50% inhibitory concentration).

3.5. Acid hydrolysis of 1–6

Compounds **1–6** (1.5 mg, each) were hydrolyzed with 2 M CF_3COOH (5 ml), and the hydrolyzed products were treated and detected through HPLC following the methods of Lin et al., 2012 and Guo et al., 2004 [7,8].

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Disclosure statement

No potential conflict of interest was reported by the authors

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