昆明杯冠藤中C21甾体成分及其抗肝癌活性研究

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摘要: 综合运用硅胶柱色谱、葡聚糖凝胶Sephadex LH-20柱色谱、制备薄层色谱和高效液相色谱等多种技术, 从昆明杯冠藤 (*Cynanchum wallichii* Wight) 氯仿提取物中分离得到14个C21甾体类化合物。通过质谱、核磁共振波谱、紫外、红外等波谱技术对化合物进行了结构鉴定。分别鉴定为cynanotonin E (**1**)、qingyangshengenin (**2**)、qinyangshengenin-3-*O*-*β*-*D*-cymaropyranosyl-(1→4)-*β*-*D*-cymaropyranoside (**3**)、qinyangshengenin-3-*O*-*β*-*D*-oleandropyranosyl-(1→4)-*β*-*D*-cymaropyranosyl-(1→4)-*β*-*D*-digitoxopyranoside (**4**)、qinyangshengenin-3-*O*-*β*-*D*-oleandropyranosyl-(1→4)-*β*-*D*-cymaropyranosyl-(1→4)-*β*-*D*-cymaropyranosyl-(1→4)-*β*-*D*-digitoxopyranoside (**5**)、12-*O*-benzoylsarcostin-3-*O*-*β*-*D*-oleandropyranosyl-(1→4)-*β*-*D*-cymaropyranosyl-(1→4)-*β*-*D*-cymaropyranosyl (**6**)、12-*O*-benzoylsarcostin-3-*O*-*β*-*D*-oleandropyranosyl-(1→4)-*β*-*D*-cymaropyranosyl-(1→4)-*β*-*D*-digitoxopyranoside (**7**)、caudatin-3-*O*-*β*-*D*-digitoxopyranoside (**8**)、caudatin-3-*O*-*β*-*D*-oleandropyranosyl-(1→4)-*β*-*D*-cymaropyranoside (**9**)、caudatin-3-*O*-*β*-*D*-cymaropyranosyl-(1→4)-*β*-*D*-cymaropyranoside (**10**)、caudatin-3-*O*-*β*-*D*-cymaropyranosyl-(1→4)-*β*-*D*-digitoxopyranoside (**11**)、otophylloside B (**12**)、otophylloside E (**13**) 和otophylloside F (**14**), 其中化合物**1**为新化合物。采用MTS法测试化合物**1**～**14**对人肝癌细胞HepG2的细胞毒活性, 结果表明, 所有化合物均表现出较好的抑制HepG2细胞增殖活性, 其IC_{50}值范围为4.3 ± 1.25～26.3 ± 1.65 μmol·L^{-1}, 其中化合物**1**、**3**和**6**～**14**的抑制活性强于阳性对照顺铂 (DDP, IC_{50}: 15.9 ± 1.25 μmol·L^{-1})。

关键词: 鹅绒藤属; 昆明杯冠藤; C21甾体; 结构鉴定; 细胞毒活性

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C21 steroidal components from *Cynanchum wallichii* Wight and their anti-hepatocellular carcinoma activity

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Abstract: By comprehensively applying various separation techniques such as silica gel, ODS, and Sephadex LH-20 column chromatography, as well as preparative HPLC, fourteen C21 steroidal compounds were isolated from the chloroform extract of *Cynanchum wallichii* Wight. The structures of these compounds were identified through spectroscopic techniques including mass spectrometry (MS), nuclear magnetic resonance spectroscopy (NMR), ultraviolet spectroscopy (UV), and infrared spectroscopy (IR) methods as cynanotonin E (**1**), qingyangshengenin (**2**), qinyangshengenin-3-*O*-*β*-*D*-cymaropyranoside-(1→4)-*β*-*D*-cymaropyranoside (**3**), qinyangshengenin-3-*O*-*β*-*D*-oleandropyranosyl-(1→4)-*β*-*D*-cymaropyranosy-(1→4)-*β*-*D*-digitoxopyranoside (**4**), qinyangshengenin-3-*O*-*β*-*D*-oleandropyranosyl-(1→4)-*β*-*D*-cymaropyranosyl-(1→4)-*β*-*D*-cymaropyranosyl-(1→4)-*β*-*D*-digitoxopyranoside (**5**), 12-*O*-benzoylsarcostin-3-*O*-*β*-*D*-oleandropyranosy-(1→4)-*β*-*D*-cymaropyranosyl-(1→4)-*β*-*D*-cymaropyranosyl (**6**), 12-*O*-benzoylsarcostin-3-*O*-*β*-*D*-oleandropyranosyl-(1→4)-*β*-*D*-cymaropyranosyl-(1→4)-*β*-*D*-digitoxopyranosyl (**7**), caudatin-3-*O*-*β*-*D*-digitoxopyranoside (**8**), caudatin-3-*O*-*β*-*D*-oleandropyranosyl-(1→

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4)-*β*-*D*-cymaropyranoside (**9**), caudatin-3-*O*-*β*-*D*-cymaropyranosyl-(1→4)-*β*-*D*-cymaropyranoside (**10**), caudatin-3-*O*-*β*-*D*-cymaropyranosyl-(1→4)-*β*-*D*-digitoxopyranoside (**11**), otophylloside B (**12**), otophylloside E (**13**) and otophylloside F (**14**). Among them, compound **1** is a new compound. The cytotoxic activities of compounds **1**−**14** against human hepatocellular carcinoma HepG2 cells were tested using the MTS method. The results indicated that all compounds exhibited good inhibitory activities against the proliferation of HepG2 cells, with IC_{50} values ranging from 4.3 ± 1.25 − 26.3 ± 1.65 μmol·L^{-1}. Among them, the inhibitory activities of compounds **1**, **3** and **6**−**14** were all stronger than that of the positive control cisplatin (DDP, IC_{50}: 15.9 ± 1.25 μmol·L^{-1}).

Key words: *Cynanchum*; *Cynanchum wallichii* Wight; C21 steroidal compound; structural identification; cytotoxic activity

昆明杯冠藤 (*Cynanchum wallichii* Wight.) 为萝藦科 (Asclepiadaceae) 鹅绒藤属 (*Cynanchum* Linn.) 多年生草质藤本中药。别名昆明白前、青洋参、断节参等。生于山坡草地、村边和路旁、灌木丛或山谷等处, 产于云南、贵州、广西和四川等省区, 并分布于印度[1]。是我国独具特色的民族中药材, 药用历史悠久, 民间常用其根作滋补强壮药, 并用于治疗跌打损伤、风湿关节炎、病后体虚以及肾虚腰痛等[2,3]。被纳入云南特色中成药“虎力散”的核心组方[4]。目前关于昆明杯冠藤化学成分研究报道较少, 主要为苯乙酮、C21 甾体类化学成分, 其中 C21 甾体类化合物是其主要活性成分, 具有抗炎、抗肿瘤、抗抑郁等多种药理活性[5-11]。C21 甾体类化合物的结构多样性主要体现在苷元类型差异, 目前已鉴定出断节参苷元 (wallicosid)、加加明 (gagamine)、青阳参苷元 (qingyangshengenin)、去乙酰萝摩苷元 (deacylmetaplexigenin) 及告达亭 (caudatin) 等主要苷元类型[12,13]。为了深入研究昆明杯冠藤主要的 C21 甾体类成分及其生物活性, 本实验对其甲醇提取物的氯仿萃取部位进行系统的提取分离, 从中分离得到 14 个 C21 甾体皂苷类化合物 (图 1), cynanotonin E (**1**)、qingyangshengenin (**2**)、qinyangshengenin-3-*O*-*β*-*D*-cymaropyranosyl-(1→4)-*β*-*D*-cymaropyranoside (**3**)、qinyangshengenin-3-*O*-*β*-*D*-oleandropyranosyl-(1→4)-*β*-*D*-cymaropyranosyl-(1→4)-*β*-*D*-digitoxopyranoside (**4**)、qinyangshengenin-3-*O*-*β*-*D*-oleandropyranosyl-(1→4)-*β*-*D*-cymaropyranosyl-(1→4)-*β*-*D*-cymaropyranosyl-(1→4)-*β*-*D*-digitoxopyranoside (**5**)、12-*O*-benzoylsarcostin-3-*O*-*β*-*D*-oleandropyranosyl-(1→4)-*β*-*D*-cymaropyranosyl-(1→4)-*β*-*D*-cymaropyranosyl (**6**)、12-*O*-benzoylsarcostin-3-*O*-*β*-*D*-oleandropyranosyl-(1→4)-*β*-*D*-cymaropyranosyl-

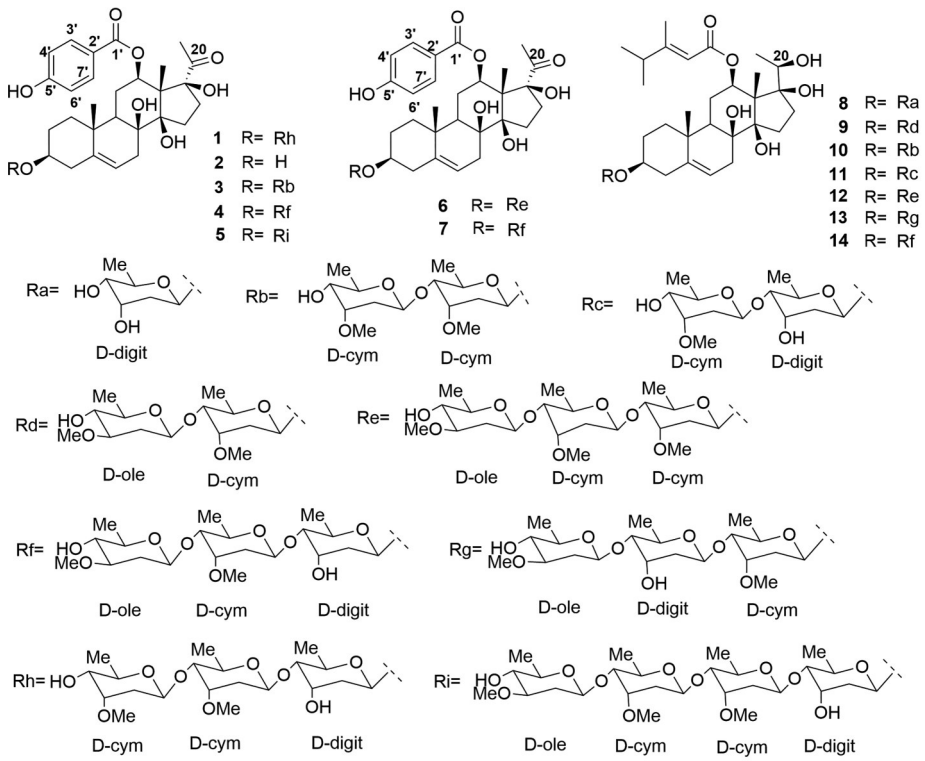


Figure 1 Structures of compounds **1**−**14**

(1→4)-β-*D*-digitoxopyranoside (**7**)、caudatin-3-*O*-β-*D*-digitoxopyranoside (**8**)、caudatin-3-*O*-β-*D*-oleandropyranosyl-(1→4)-β-*D*-cymaropyranoside (**9**)、caudatin-3-*O*-β-*D*-cymaropyranosyl-(1→4)-β-*D*-cymaropyranoside (**10**)、caudatin-3-*O*-β-*D*-cymaropyranosyl-(1→4)-β-*D*-digitoxopyranoside (**11**)、otophylloside B (**12**)、otophylloside E (**13**) 和 otophylloside F (**14**), 其中化合物 **1** 为新的C21甾体皂苷类化合物。采用MTS法测试了14个化合物对人肝癌细胞HepG2的细胞毒活性, 结果表明, 所有化合物均表现出较好的抑制HepG2细胞增殖活性, 其 IC_{50} 值为 4.3 ± 1.25～26.3 ± 1.65 μmol·L^{-1}, 其中化合物 **1**, **3** 和 **6**～**14** 的抑制活性都强于阳性对照顺铂 (DDP, IC_{50}: 15.9 ± 1.25 μmol·L^{-1})。

结果与讨论

1 化合物1结构鉴定

白色无定形粉末, $[\alpha]_D^{26.2}$ +4.0 (0.16, MeOH)。Liberman-Buchard 和 Keller-Kiliani 反应呈阳性, 提示化合物 **1** 可能为甾体2-去氧吡喃糖苷类化合物。高分辨电喷雾质谱HRESIMS显示准分子离子峰 $[M+K]^+$ 的 *m/z* 为957.424 5, 故化合物 **1** 的分子式为 $C_{48}H_{70}O_{17}$。在红外光谱中显示有羟基 (3 440 cm^{-1}), 羰基 (1 708 cm^{-1}) 和双键 (1 663 cm^{-1}) 的振动吸收。^{13}C NMR 和 DEPT 谱 (表1) 显示化合物 **1** 共有48个碳, 分别为6个甲基、2个甲氧基、10个亚甲基、20个次甲基 (包括1个 sp^3 次甲基, 5个 sp^2 次甲基, 14个氧化的次甲基) 以及10个季碳 (包括3个 sp^2 季碳、1个酯羰基、1个酮羰基、2个 sp^3 季碳、3个氧化的季碳)。综合以上的数据可以推测化合物 **1** 为典型的带3个去氧糖的C21甾体苷。通过比较 ^{1}H NMR 及 ^{13}C NMR 谱图, 发现化合物 **1** 的苷元部分与文献[14]中的青阳参苷元的数据基本一致, 也为青阳参苷元。在90～110 ppm处, ^{13}C NMR显示化合物 **1** 含有3个糖端基碳的共振信号: δ_C 97.0, δ_C 100.6, δ_C 101.3。HSQC实验指明 ^{1}H NMR 中相应的3个端基质子的共振信号: H-1″ (δ_H 4.97, dd, J = 2.0, 9.6 Hz), H-1‴ (δ_H 4.79, m), H-1⁗ (δ_H 4.77, m), 因此推断化合物 **1** 为三糖苷。通过将化合物 **1** 的 ^{1}H NMR 及 ^{13}C NMR 数据与文献[15,16]进行比对, 结合TLC检测结果, 对其在弱酸性条件下的水解产物与加拿大麻吡喃糖 (cymarose) 和毛地黄毒吡喃糖 (digitoxose) 对照品进行对照分析,

Table 1 ^{1}H NMR (400 MHz) and ^{13}C NMR (150 MHz) data of compound **1** in CD_3OD

No.	δ_H (J in Hz)	δ_C	No.	δ_H (J in Hz)	δ_C
1	1.10 m; 1.85 m	39.8 (CH_2)	*D*-Digit		
2	1.58 m; 1.84 m	30.2 (CH_2)	1″	4.97 dd (9.6, 2.0)	97.0 (CH)
3	3.57 m	79.3 (CH)	2″	1.69 m; 1.96 m	38.9 (CH_2)
4	2.22 m; 2.35 m	39.8 (CH_2)	3″	4.24 d (3.2)	74.1 (CH)
5		140.2 (C)	4″	3.32 m	83.6 (CH)
6	5.35 d (4.8)	119.7 (CH)	5″	3.96 m	69.5 (CH)
7	1.97 m; 2.14 m	35.5 (CH_2)	6″	1.3 d (6.1)	18.5 (CH_3)
8		75.0 (C)	*D*-Cym		
9	1.58 m	45.1 (CH)	1‴	4.79 m	100.6 (CH)
10		38.1 (C)	2‴	1.61 m; 2.14 m	36.2 (CH_2)
11	1.76 m; 1.96 m	25.5 (CH_2)	3‴	3.84 m	79.2 (CH)
12	4.75 dd (11.7, 4.3)	74.4 (CH)	4‴	3.29 m	83.8 (CH)
13		59.1 (C)	5‴	3.00 m	68.4 (CH)
14		90.0 (C)	6‴	1.27 s	18.5 (CH_3)
15	1.74 m; 1.93 m	34.3 (CH_2)	OMe	3.45 d (8.5)	58.5 (OCH_3)
16	1.72 m; 2.85 m	33.5 (CH_2)	*D*-Cym		
17		93.0 (C)	1⁗	4.77 m	101.3 (CH)
18	1.65 s	10.6 (CH_3)	2⁗	1.90 m; 2.24 m	37.4 (CH_2)
19	1.15 s	18.7 (CH_3)	3⁗	3.92 m	74.4 (CH)
20		212.1 (C)	4⁗	3.51 dd (9.6, 2.9)	78.5 (CH)
21	2.04 s	27.8 (CH_3)	5⁗	4.20 m	70.0 (CH)
p-OH-Benzoyl			6⁗	1.42 d (6.2)	18.4 (CH_3)
1′		166.8 (C)	OMe	3.46 s	58.1 (OCH_3)
2′		122.1 (C)			
3′	7.79 (d, 8.8)	132.8 (CH)			
4′	6.80 (dd, 8.9, 3.0)	116.3 (CH)			
5′		164.0 (C)			
6′	6.80 dd (8.9, 3.0)	116.3 (CH)			
7′	7.79 d (8.8)	132.8 (CH)			

结果表明, 该化合物中的3个糖基由2个加拿大麻吡喃糖和1个毛地黄毒吡喃糖组成, 且糖链中的糖均以*β*-苷键连接。进一步将糖的旋光OR值与文献[16]比较 (digitoxose: $[\alpha]_D^{20}$ +43.5, 文献为 *D*-digitoxose $[\alpha]_D^{20}$ +40.5; cymarose: $[\alpha]_D^{20}$ +56.0, 文献为 *D*-cymarose $[\alpha]_D^{20}$ +46.2), 确定三个糖的绝对构型为*D*型。图2给出了化合物**1**主要的^{1}H-^{1}H COSY和HMBC相关信号。尤其在HMBC谱中, 质子H-1″ (δ_H 4.97, dd, J = 9.6, 2.0 Hz)与苷元的C-3位 (δ_C 79.3) 远程相关, 说明化合物**1**的内侧糖基与苷元的C-3位上的羟基相连。进一步通过HMBC谱确定了化合物**1**的三个糖基之间的连接顺序: H-1″与C-3, H-1‴与C-4″ (δ_C 83.6), H-1⁗与C-4‴ (δ_C 83.8) 有较强的相关性, 说明糖链通过内侧的毛地黄毒吡喃糖基与苷元的C-3位连接, 糖链内的糖基通过1,4-糖苷键 (1→4) 进行连接。综上所述, 化合物**1**的结构推断为青阳参苷元-3-*O*-*β*-*D*-加拿大麻吡喃糖-(1→4)-*β*-*D*-加拿大麻吡喃糖-(1→4)-*β*-*D*-毛地黄毒吡喃糖苷, 命名为cynanotonin E (**1**)。

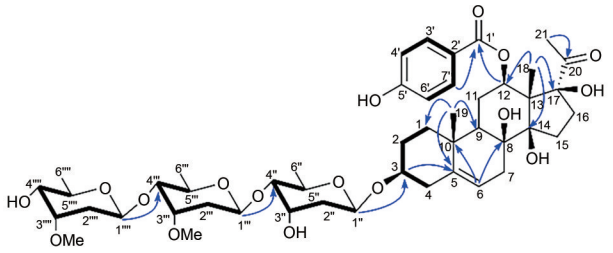


Figure 2 Key ^{1}H-^{1}H COSY and HMBC correlations of compounds **1**

2 化合物的细胞毒作用

采用MTS法测定化合物**1**～**14**对人肝癌细胞HepG2的细胞毒活性, 结果见表2。发现所有化合物均表现出较好的细胞毒活性, 其IC_{50}值从4.3 ± 1.25～26.3 ± 1.65 μmol·L^{-1}不等, 除了化合物**2**、**4**和**5**, 化合物**1**、**3**和**6**～**14**表现出显著的抑制HepG2细胞增殖活性, 它们的抑制效果比阳性对照顺铂 (DDP, IC_{50}: 15.9 ± 1.25 μmol·L^{-1}) 更强。通过比较化合物之间的活性差异, 发现化合物C-3位连的糖基数量对化合物的细胞毒活性有较大的影响, 含3个糖基的化合物活性明显增强。

Table 2 The cytotoxicity of compounds **1**−**14** against HepG2 cells. n = 3, $\bar{x} \pm s$. DDP: Cisplatin

Compd.	IC_{50}/μmol·L^{-1}	Compd.	IC_{50}/μmol·L^{-1}
1	9.2 ± 1.43	**9**	11.8 ± 1.33
2	26.3 ± 1.65	**10**	12.5 ± 2.15
3	12.7 ± 1.02	**11**	11.3 ± 1.67
4	19.2 ± 0.95	**12**	6.5 ± 1.53
5	23.6 ± 2.11	**13**	4.3 ± 1.25
6	14.6 ± 1.42	**14**	7.8 ± 1.27
7	12.1 ± 2.01	DDP	15.9 ± 1.25
8	14.5 ± 1.03		

实验部分

超导核磁共振仪AVANCEIII-400 MHz和AV-600 MHz (*δ*为ppm, J为Hz, 四甲基硅烷TMS作为内标)、傅里叶变换中红外光谱仪Tensor-27、液相离子阱色谱质谱联用仪 (HCT/Esquire), 德国Bruker公司; 全自动数字旋光仪 (日本Jasco P-1020); 液相四极杆飞行时间质谱仪 (APIQSTAR Pulsa)、多功能酶标仪 (MULTISKAN FC), 美国赛默飞公司; 紫外可见分光光度仪 (ShimadzuUV2401PC, 日本岛津公司); 高效液相色谱 (1100, 美国安捷伦公司); 反相材料RP-18 (日本Fuji公司); LH-20葡聚糖凝胶为进口分装; 柱色谱硅胶、薄层色谱硅胶 (青岛海洋化工); 显色剂: 10%硫酸−无水乙醇溶液, 喷洒后适当加热; 细胞株 (人肝癌细胞HepG2, 美国ATCC公司); 胎牛血清 (美国Hyclone公司, SH30406.05); DMEM培养基 (美国Gibco公司)。

昆明杯冠藤药材购买于云南省昆明市新螺蛳湾中药材市场, 并由昆明植物研究所裴盛基研究员鉴定为昆明杯冠藤 (*Cynanchum wallichii* Wight) 的干燥块根。凭证样品 (KUN No. 0776921) 存放于西南林业大学生物与食品工程学院生物化学实验室。

1 提取与分离

取昆明杯冠藤干燥根茎20公斤粉碎, 使用95%甲醇在80 ℃水浴条件下回流提取 (每次10倍量溶剂, 提取3次, 每次2 h)。合并提取液并减压回收溶剂, 得到浸膏共2 kg。加适量水使浸膏分散, 依次用石油醚、氯仿进行萃取 (10 L×3次), 减压回收得氯仿萃取物400 g。取氯仿萃取部位用硅胶柱色谱进行分离, 依次用氯仿−甲醇 (80∶1、50∶1、20∶1和5∶1) 洗脱, 根据薄层色谱结果, 合并洗脱液得到4个组分 (Fr.1～Fr.4)。将Fr.2 (60 g) 上正相硅胶柱分离, 经氯仿−甲醇 (60∶1) 洗脱, 得到5个组分 (Fr2.1～Fr2.5)。将Fr2.1组分 (45 g) 上反相ODS柱色谱分离, 甲醇−水 (40∶60～100∶0) 系统梯度洗脱, 得到11个组分 (Fr2.1.1～Fr2.1.11)。Fr2.1.9 (10 g) 上正相硅胶柱色谱, 经氯仿−丙酮 (15∶1) 梯度洗脱, 得到6个组分 (Fr2.1.9.1～Fr2.1.9.6)。将Fr2.1.9.4 (4 g) 上反相ODS柱色谱分离, 甲醇−水 (20∶80～100∶0) 系统梯度洗脱, 经点板确认, 得到3个组分 (Fr2.1.9.4.1～Fr2.1.9.4.3), 选取Fr2.1.9.4.2经半制备-HPLC (乙腈−水, 60∶40, 流速3 mL·min^{-1}) 后得到化合物**6** (t_R = 15.4 min; 8 mg), **7** (t_R = 22.5 min; 7 mg), **8** (t_R = 11.0 min; 10 mg), **9** (t_R = 34.5 min; 50 mg)。再选取Fr2.1.10经半制备-

HPLC (乙腈−水, 55∶45, 流速3 mL·min^{-1}) 富集纯化, 得到化合物**10** (t_R = 18.3 min; 50 mg)、**11** (t_R = 24.5 min; 15 mg) 和**12** (t_R = 36.4 min; 1 g)。将Fr2.3段 (30 g) 上反相ODS柱色谱分离, 经甲醇−水 (40∶60～100∶0) 系统梯度洗脱, 共得到15个组分 (Fr2.3.1～Fr2.3.15)。Fr2.3.7组分 (3 g) 上薄层层析硅胶柱进一步分离, 得到化合物**2** (200 mg)。将Fr2.3.12和Fr2.3.13组分分别上硅胶柱色谱分离, 经氯仿−甲醇 (35∶1) 洗脱, 分别得到6个组分 (Fr2.3.12.1～Fr2.3.12.6) 及 (Fr2.3.13.1～Fr2.3.13.6)。Fr2.3.12.2经半制备-HPLC (乙腈−水, 50∶50, 流速3 mL·min^{-1}) 富集纯化得到化合物**3** (t_R = 26.2 min; 6 mg) 和**4** (t_R = 37.5 min; 500 mg)。对Fr2.3.13.4进行薄层层析硅胶柱进一步分离, 得到5个组分 (Fr2.3.13.4.1～Fr2.3.13.4.5), 其中Fr2.3.13.4.3段经半制备-HPLC纯化 (乙腈−水, 40∶60, 流速3 mL·min^{-1}) 后得到化合物**1** (t_R = 16.0 min; 20 mg) 和**5** (t_R = 25.7 min; 5 mg)。将Fr2.3.15进行薄层层析硅胶柱进一步分离, 得到5个组分 (Fr2.3.15.1～Fr2.3.15.5)。其中Fr2.3.15.1 (250 mg) 经半制备-HPLC纯化 (乙腈−水, 45∶55, 流速3 mL·min^{-1}), 得到化合物**13** (t_R = 17.8 min; 10 mg) 和**14** (t_R = 27.5 min; 20 mg)。

2 化合物的理化常数

化合物**1** 白色无定形粉末; $[\alpha]_D^{26.2}$ +4.0 (0.16, MeOH); UV (MeOH) λ_{max} (log ε): 203(4.26), 259 (4.23), 368 (2.08); IR (KBr) ν_{max}: 3 440, 2 930, 1 708, 1 663, 1 399, 1 276, 1 194, 1 058和719 cm^{-1}; ESIMS *m*/*z* 957 $[M+K]^+$, HRESIMS *m*/*z* 957.424 5 $[M+K]^+$ (calcd. for $C_{48}H_{70}KO_{17}$, 957.424 7), ^{1}H NMR (400 MHz, CD_3OD) 和^{13}C NMR (150 MHz, CD_3OD) 数据, 见表1。

化合物**2** 白色无定形粉末, 分子式为$C_{28}H_{36}O_8$; ^{1}H NMR (CD_3OD, 400 MHz, *J* in Hz) δ_H: 7.81 (1H, d, *J* = 8.8 Hz, H-3′/7′), 6.82 (1H, d, *J* = 8.7 Hz, H-4′/6′), 5.33 (1H, s, H-6), 4.76 (1H, dd, *J* = 5.3, 10.2 Hz, H-12), 3.30 (1H, m, H-3), 2.07 (3H, s, H-21), 1.64 (3H, s, H-18), 1.15 (3H, s, H-19); ^{13}C NMR (CD_3OD, 150 MHz) δ_C: 39.8 (C-1), 31.7 (C-2), 72.6 (C-3), 42.9 (C-4), 140.7 (C-5), 119.3 (C-6), 35.2 (C-7), 75.0 (C-8), 45.1 (C-9), 38.0 (C-10), 25.5 (C-11), 74.1 (C-12), 59.1 (C-13), 90.0 (C-14), 34.3 (C-15), 33.5 (C-16), 93.1 (C-17), 10.6 (C-18), 18.6 (C-19), 212.1 (C-20), 27.8 (C-21), 166.8 (C-1′), 122.4 (C-2′), 132.8 (C-3′), 116.1 (C-4′), 163.6 (C-5′), 116.1 (C-6′), 132.8 (C-7′)。以上波谱数据与文献[17]报道对比, 确定化合物**2**为qingyangshengenin。

化合物**3** 白色无定形粉末, 分子式为$C_{42}H_{60}O_{14}$; ^{1}H NMR (CD_3OD, 400 MHz, *J* in Hz) δ_H: 7.81 (1H, d, *J* = 8.8 Hz, H-3′/7′), 6.82 (1H, d, *J* = 8.7 Hz, H-4′/6′), 5.36 (1H, s, H-6), 4.78 (1H, dd, *J* = 4.3, 11.8 Hz, H-1″), 4.77 (1H, dd, *J* = 5.3, 10.2 Hz, H-12), 4.76 (1H, dd, *J* = 4.3, 11.8 Hz, H-1‴), 3.84 (1H, m, H-3″), 3.84 (1H, m, H-3‴), 3.59 (1H, m, H-3), 3.59 (1H, m, H-5″), 3.45 (3H, s, OCH_3), 3.43 (1H, m, H-5‴), 3.42 (3H, s, OCH_3), 3.21 (1H, dd, *J* = 3.2, 9.6 Hz, H-4″), 3.18 (1H, dd, *J* = 3.2, 9.6 Hz, H-4‴), 2.10 (3H, s, H-21), 1.64 (3H, s, H-18), 1.23 (3H, d, *J* = 6.2 Hz, H-6″), 1.22 (3H, d, *J* = 6.2 Hz, H-6‴), 1.14 (3H, s, H-19); ^{13}C NMR (CD_3OD, 150 MHz) δ_C: 39.8 (C-1), 30.2 (C-2), 79.3 (C-3), 39.8 (C-4), 140.2 (C-5), 119.7 (C-6), 35.2 (C-7), 75.0 (C-8), 45.1 (C-9), 38.2 (C-10), 25.5 (C-11), 74.1 (C-12), 59.1 (C-13), 90.0 (C-14), 34.3 (C-15), 33.5 (C-16), 93.1 (C-17), 10.6 (C-18), 18.6 (C-19), 212.1 (C-20), 27.8 (C-21), 166.8 (C-1′), 122.3 (C-2′), 132.8 (C-3′), 116.2 (C-4′), 163.7 (C-5′), 116.2 (C-6′), 132.8 (C-7′), 97.2 (C-1″), 36.6 (C-2″), 79.2 (C-3″), 74.5 (C-4″), 71.3 (C-5″), 18.7 (C-6″), 58.4 (OCH_3), 101.2 (C-1‴), 35.6 (C-2‴), 78.6 (C-3‴), 74.1 (C-4‴), 70.0 (C-5‴), 18.5 (C-6‴), 58.1 (OCH_3)。以上波谱数据与文献[18]报道对比, 确定化合物**3**为qinyangshengenin-3-*O*-*β*-*D*-cymaropyranosyl-(1→4)-*β*-*D*-cymaropyranoside。

化合物**4** 白色无定形粉末, 分子式为$C_{48}H_{70}O_{17}$; ^{1}H NMR (CD_3OD, 400 MHz, *J* in Hz) δ_H: 7.72 (1H, d, *J* = 8.8 Hz, H-3′/7′), 6.74 (1H, d, *J* = 8.7 Hz, H-4′/6′), 5.35 (1H, s, H-6), 4.96 (1H, d, *J* = 9.6 Hz, H-1″), 4.85 (1H, dd, *J* = 1.8, 9.6 Hz, H-1‴), 4.80 (1H, dd, *J* = 5.3, 10.2 Hz, H-12), 4.60 (1H, d, *J* = 9.2 Hz, H-1⁗), 4.23 (1H, d, *J* = 2.8 Hz, H-3″), 3.85 (1H, m, H-3‴), 3.83 (1H, m, H-5‴), 3.82 (1H, m, H-5″), 3.56 (1H, m, H-3), 3.54 (3H, s, OCH_3), 3.51 (1H, m, H-3⁗), 3.43 (3H, s, OCH_3), 3.34 (1H, m, H-5⁗), 3.28 (1H, m, H-4‴), 3.20 (1H, m, H-4″), 3.20 (1H, m, H-4⁗), 2.10 (3H, s, H-21), 1.63 (3H, s, H-18), 1.30 (3H, d, *J* = 6.0 Hz, H-6⁗), 1.21 (3H, d, *J* = 6.0 Hz, H-6‴), 1.19 (3H, d, *J* = 6.3 Hz, H-6″), 1.15 (3H, s, H-19); ^{13}C NMR (CD_3OD, 150 MHz) δ_C: 39.8 (C-1), 30.2 (C-2), 79.3 (C-3), 39.8 (C-4), 140.1 (C-5), 119.7 (C-6), 35.2 (C-7), 75.0 (C-8), 45.1 (C-9), 38.2 (C-10), 25.5 (C-11), 74.1 (C-12), 59.1 (C-13), 90.0 (C-14), 34.3 (C-15), 33.5 (C-16), 93.1 (C-17), 10.6 (C-18), 18.6 (C-19), 212.1 (C-20), 27.8 (C-21), 166.8 (C-1′), 122.1 (C-2′), 132.8 (C-3′), 116.2 (C-4′), 163.6 (C-5′), 116.3 (C-6′), 132.8 (C-7′), 97.0 (C-1″), 38.8 (C-2″), 68.3 (C-3″), 83.6 (C-4″), 69.5 (C-5″), 18.6 (C-6″), 100.6 (C-1‴), 36.3 (C-2‴), 78.4 (C-3‴), 83.8 (C-4‴), 70.0 (C-5‴), 18.5 (C-6‴),

58.6 (OCH_3), 102.8 (C-1‴″), 37.4 (C-2‴″), 81.6 (C-3‴″), 76.9 (C-4‴″), 73.2 (C-5‴″), 18.5 (C-6‴″), 57.4 (OCH_3)。以上波谱数据与文献[18]报道对比, 确定化合物**4**为qinyangshengenin-3-*O*-*β*-*D*-oleandropyranosyl-(1→4)-*β*-*D*-cymaropyranosyl-(1→4)-*β*-*D*-digitoxopyranoside。

化合物**5** 白色无定形粉末, 分子式为$C_{55}H_{82}O_{20}$; ^{1}H NMR (CD_3OD, 400 MHz, *J* in Hz) δ_H: 7.81 (1H, d, *J* = 8.8 Hz, H-3′/7′), 6.81 (1H, d, *J* = 8.7 Hz, H-4′/6′), 5.35 (1H, s, H-6), 4.96 (1H, d, *J* = 9.6 Hz, H-1″), 4.85 (1H, dd, *J* = 1.8, 9.6 Hz, H-1‴), 4.85 (1H, dd, *J* = 1.8, 9.6 Hz, H-1‴″), 4.60 (1H, d, *J* = 9.2 Hz, H-1‴″), 4.58 (1H, dd, *J* = 5.3, 10.2 Hz, H-12), 4.23 (1H, d, *J* = 2.8 Hz, H-3″), 3.85 (1H, m, H-3‴), 3.85 (1H, m, H-3‴″), 3.83 (1H, m, H-5‴), 3.83 (1H, m, H-5‴″), 3.82 (1H, m, H-5″), 3.56 (1H, m, H-3), 3.54 (3H, s, OCH_3), 3.51 (1H, m, H-3‴″), 3.43 (3H, s, OCH_3), 3.34 (1H, m, H-5‴″), 3.28 (1H, m, H-4‴), 3.28 (1H, m, H-4‴″), 3.20 (1H, m, H-4″), 3.20 (1H, m, H-4‴″), 2.10 (3H, s, H-21), 1.63 (3H, s, H-18), 1.30 (3H, d, *J* = 6.0 Hz, H-6‴″), 1.21 (3H, d, *J* = 6.0 Hz, H-6‴), 1.21 (3H, d, *J* = 6.0 Hz, H-6‴″), 1.19 (3H, d, *J* = 6.3 Hz, H-6″), 1.15 (3H, s, H-19); ^{13}C NMR (CD_3OD, 150 MHz) δ_C: 39.8 (C-1), 30.2 (C-2), 79.3 (C-3), 39.8 (C-4), 140.1 (C-5), 119.7 (C-6), 35.2 (C-7), 75.0 (C-8), 45.1 (C-9), 38.2 (C-10), 25.5 (C-11), 74.1 (C-12), 59.1 (C-13), 90.0 (C-14), 34.3 (C-15), 33.5 (C-16), 93.1 (C-17), 10.6 (C-18), 18.6 (C-19), 212.1 (C-20), 27.8 (C-21), 166.8 (C-1′), 122.2 (C-2′), 132.8 (C-3′), 116.2 (C-4′), 163.8 (C-5′), 116.2 (C-6′), 132.8 (C-7′), 97.0 (C-1″), 38.8 (C-2″), 68.3 (C-3″), 83.6 (C-4″), 69.5 (C-5″), 18.6 (C-6″), 99.7 (C-1‴), 36.3 (C-2‴), 78.4 (C-3‴), 83.8 (C-4‴), 70.0 (C-5‴), 18.5 (C-6‴), 58.6 (OCH_3), 100.6 (C-1‴″), 36.3 (C-2‴), 78.4 (C-3‴″), 83.8 (C-4‴″), 70.0 (C-5‴″), 18.5 (C-6‴″), 58.0 (OCH_3), 102.7 (C-1‴″), 37.4 (C-2‴″), 81.6 (C-3‴″), 76.9 (C-4‴″), 73.2 (C-5‴″), 18.5 (C-6‴″), 57.6 (OCH_3)。以上波谱数据与文献[19]报道对比, 确定化合物**5**为qinyangshengenin-3-*O*-*β*-*D*-oleandropyranosyl-(1→4)-*β*-*D*-cymaropyranosyl-(1→4)-*β*-*D*-cymaropyranosyl-(1→4)-*β*-*D*-digitoxopyranoside。

化合物**6** 白色无定形粉末, 分子式为$C_{49}H_{72}O_{16}$; ^{1}H NMR (CD_3OD, 400 MHz, *J* in Hz) δ_H: 7.85 (1H, d, *J* = 7.2 Hz, H-3′/7′), 7.51 (1H, m, H-5′), 7.39 (1H, d, *J* = 8.0 Hz, H-4′/6′), 5.35 (1H, s, H-6), 4.73 (1H, dd, *J* = 1.6, 9.6 Hz, H-1″), 4.69 (1H, dd, *J* = 1.8, 9.6 Hz, H-1‴), 4.59 (1H, d, *J* = 9.2 Hz, H-1‴″), 4.50 (1H, dd, *J* = 5.3, 10.2 Hz, H-12), 3.89 (1H, m, H-5‴), 3.86 (1H, m, H-3″), 3.85 (1H, m, H-3‴), 3.82 (1H, m, H-5″), 3.54 (3H, s, OCH_3), 3.54 (3H, s, OCH_3), 3.50 (1H, m, H-3), 3.50 (1H, m, H-3‴″), 3.42 (3H, s, OCH_3), 3.42 (3H, s, OCH_3), 3.30 (1H, m, H-5‴″), 3.26 (1H, m, H-4″), 3.23 (1H, m, H-4‴), 3.18 (1H, m, H-4‴″), 2.10 (3H, s, H-21), 1.63 (3H, s, H-18), 1.30 (3H, d, *J* = 6.0 Hz, H-6‴″), 1.29 (3H, d, *J* = 6.4 Hz, H-6‴), 1.18 (3H, d, *J* = 6.3 Hz, H-6″), 1.15 (3H, s, H-19); ^{13}C NMR (CD_3OD, 150 MHz) δ_C: 39.8 (C-1), 30.2 (C-2), 79.3 (C-3), 39.8 (C-4), 140.1 (C-5), 119.8 (C-6), 35.2 (C-7), 75.0 (C-8), 45.1 (C-9), 38.2 (C-10), 25.5 (C-11), 74.1 (C-12), 59.1 (C-13), 90.0 (C-14), 34.3 (C-15), 33.5 (C-16), 93.1 (C-17), 10.6 (C-18), 18.6 (C-19), 212.3 (C-20), 27.8 (C-21), 166.7 (C-1′), 131.5 (C-2′), 129.6 (C-3′), 130.5 (C-4′), 134.3 (C-5′), 130.5 (C-6′), 129.6 (C-7′), 97.2 (C-1″), 36.6 (C-2″), 78.5 (C-3″), 83.9 (C-4″), 69.8 (C-5″), 18.6 (C-6″), 58.5 (OCH_3), 101.2 (C-1‴), 36.4 (C-2‴), 78.6 (C-3‴), 83.9 (C-4‴), 70.0 (C-5‴), 18.6 (C-6‴), 58.4 (OCH_3), 102.8 (C-1‴″), 37.4 (C-2‴″), 76.9 (C-3‴″), 81.7 (C-4‴″), 73.2 (C-5‴″), 18.5 (C-6‴″), 57.4 (OCH_3)。以上波谱数据与文献[20]报道对比, 确定化合物**6**为12-*O*-benzoylsarcostin-3-*O*-*β*-*D*-oleandropyranosyl-(1→4)-*β*-*D*-cymaropyranosyl-(1→4)-*β*-*D*-cymaropyranoside。

化合物**7** 白色无定形粉末, 分子式为$C_{48}H_{72}O_{16}$; ^{1}H NMR (CD_3OD, 400 MHz, *J* in Hz) δ_H: 7.60 (1H, d, *J* = 7.2 Hz, H-3′/7′), 7.58 (1H, m, H-5′), 7.37 (1H, d, *J* = 8.0 Hz, H-4′/6′), 5.35 (1H, s, H-6), 4.96 (1H, d, *J* = 9.6 Hz, H-1″), 4.85 (1H, dd, *J* = 1.8, 9.6 Hz, H-1‴), 4.60 (1H, d, *J* = 9.2 Hz, H-1‴″), 4.52 (1H, dd, *J* = 5.3, 10.2 Hz, H-12), 4.23 (1H, d, *J* = 2.8 Hz, H-3″), 3.85 (1H, m, H-3‴), 3.83 (1H, m, H-5‴), 3.82 (1H, m, H-5″), 3.54 (3H, s, OCH_3), 3.51 (1H, m, H-3‴″), 3.43 (3H, s, OCH_3), 3.34 (1H, m, H-5‴″), 3.28 (1H, m, H-4‴), 3.20 (1H, m, H-4″), 3.20 (1H, m, H-4‴″), 2.10 (3H, s, H-21), 1.63 (3H, s, H-18), 1.30 (3H, d, *J* = 6.0 Hz, H-6‴″), 1.21 (3H, d, *J* = 6.0 Hz, H-6‴), 1.19 (3H, d, *J* = 6.3 Hz, H-6″), 1.15 (3H, s, H-19); ^{13}C NMR (CD_3OD, 150 MHz) δ_C: 39.8 (C-1), 30.2 (C-2), 79.3 (C-3), 39.8 (C-4), 140.1 (C-5), 119.8 (C-6), 35.2 (C-7), 75.0 (C-8), 45.1 (C-9), 38.2 (C-10), 25.5 (C-11), 74.1 (C-12), 59.1 (C-13), 90.0 (C-14), 34.3 (C-15), 33.5 (C-16), 93.1 (C-17), 10.6 (C-18), 18.6 (C-19), 212.3 (C-20), 27.8 (C-21), 166.7 (C-1′), 131.5 (C-2′), 129.6 (C-3′), 130.5 (C-4′), 134.3 (C-5′), 130.5 (C-6′), 129.6 (C-7′), 97.0 (C-1″), 36.6 (C-2″), 78.5 (C-3″), 83.9 (C-4″), 69.8 (C-5″), 18.6 (C-6″), 100.6 (C-1‴), 36.4 (C-2‴), 78.6 (C-3‴), 83.8 (C-4‴), 70.0 (C-5‴), 18.5 (C-6‴),

58.7 (OCH_3), 102.8 (C-1‴), 37.4 (C-2‴), 76.9 (C-3‴), 81.7 (C-4‴), 73.2 (C-5‴), 18.4 (C-6‴), 58.5 (OCH_3)。以上波谱数据与文献[21]报道对比, 确定化合物**7**为12-*O*-benzoylsarcostin-3-*O*-*β*-*D*-oleandropyranosyl-(1→4)-*β*-*D*-cymaropyranosyl-(1→4)-*β*-*D*-digitoxopyranoside。

化合物**8** 白色无定形粉末, 分子式为$C_{34}H_{52}O_{10}$; ^{1}H NMR (CD_3OD, 400 MHz, *J* in Hz) δ_H: 5.84 (1H, s, H-2′), 5.35 (1H, d, *J* = 2.7 Hz, H-6), 4.98 (1H, d, *J* = 11.3 Hz, H-1″), 4.85 (1H, dd, *J* = 5.4, 10.1 Hz, H-12), 4.69 (1H, dd, *J* = 4.2, 11.6 Hz, H-3″), 3.84 (1H, m, H-3), 3.75 (1H, m, H-4″), 3.13 (1H, m, H-5″), 2.26 (3H, s, H-7′), 2.24 (1H, m, H-4′), 2.10 (3H, s, H-21), 1.63 (3H, s, H-18), 1.17 (3H, d, *J* = 5.8 Hz, H-6″), 1.14 (3H, s, H-19), 0.95 (3H, dd, *J* = 6.8, 8.7 Hz, H-5′/H-6′); ^{13}C NMR (CD_3OD, 150 MHz) δ_C: 39.8 (C-1), 30.2 (C-2), 79.2 (C-3), 39.8 (C-4), 140.2 (C-5), 119.7 (C-6), 35.2 (C-7), 74.9 (C-8), 45.2 (C-9), 38.1 (C-10), 25.4 (C-11), 73.2 (C-12), 58.7 (C-13), 89.9 (C-14), 34.2 (C-15), 33.2 (C-16), 93.0 (C-17), 10.5 (C-18), 18.7 (C-19), 211.6 (C-20), 27.6 (C-21), 167.5 (C-1′), 114.2 (C-2′), 167.5(C-3′), 39.3 (C-4′), 21.3 (C-5′), 21.3 (C-6′), 16.3 (C-7′), 97.1 (C-1″), 39.8 (C-2″), 75.6 (C-3″), 70.8(C-4″), 74.4 (C-5″), 18.6 (C-6″)。以上波谱数据与文献[22]报道对比, 确定化合物**8**为caudatin-3-*O*-*β*-*D*-digitoxopyranoside。

化合物**9** 白色无定形粉末, 分子式为$C_{42}H_{66}O_{13}$; ^{1}H NMR (CD_3OD, 400 MHz, *J* in Hz) δ_H: 5.86 (1H, s, H-2′), 5.34 (1H, d, *J* = 2.7 Hz, H-6), 4.89 (1H, m, H-1″), 4.84 (1H, dd, *J* = 5.4, 10.1 Hz, H-12), 4.59 (1H, dd, *J* = 2.0, 9.9 Hz, H-1‴), 3.86 (1H, m, H-3), 3.82 (1H, m, H-3″), 3.80 (1H, m, H-5″), 3.42 (3H, s, OCH_3), 3.42 (3H, s, OCH_3), 3.27 (1H, m, H-5‴), 3.25 (1H, m, H-4″), 3.19 (1H, m, H-3‴), 2.97 (1H, d, *J* = 9.0 Hz, H-4‴), 2.26 (3H, s, H-7′), 2.24 (1H, m, H-4′), 2.12 (3H, s, H-21), 1.62 (3H, s, H-18), 1.27 (3H, d, *J* = 6.3 Hz, H-6″), 1.21 (3H, d, *J* = 6.2 Hz, H-6‴), 1.13 (3H, s, H-19), 0.93 (3H, dd, *J* = 6.8, 8.7 Hz, H-5′/H-6′); ^{13}C NMR (CD_3OD, 150 MHz) δ_C: 39.8 (C-1), 30.1 (C-2), 79.3 (C-3), 39.8 (C-4), 140.2 (C-5), 119.7 (C-6), 35.2 (C-7), 75.0 (C-8), 45.2 (C-9), 38.1 (C-10), 25.4 (C-11), 73.3 (C-12), 58.7 (C-13), 89.9 (C-14), 34.2 (C-15), 33.2 (C-16), 93.0 (C-17), 10.4 (C-18), 18.4 (C-19), 211.6 (C-20), 27.6 (C-21), 167.5 (C-1′), 114.3 (C-2′), 167.4 (C-3′), 39.3 (C-4′), 21.3 (C-5′), 21.3 (C-6′), 16.7 (C-7′), 97.2 (C-1″), 36.6 (C-2″), 78.5 (C-3″), 83.9 (C-4″), 69.8 (C-5″), 18.6 (C-6″), 58.5 (OCH_3), 102.8 (C-1‴), 37.4 (C-2‴), 76.9 (C-3‴), 81.7 (C-4‴), 73.2 (C-5‴), 18.5 (C-6‴), 57.4 (OCH_3)。以上波谱数据与文献[23]报道对比, 确定化合物**9**为caudatin-3-*O*-*β*-*D*-oleandropyranosyl-(1→4)-*β*-*D*-cymaropyranoside。

化合物**10** 白色无定形粉末, 分子式为$C_{42}H_{66}O_{13}$; ^{1}H NMR (CD_3OD, 400 MHz, *J* in Hz) δ_H: 5.54 (1H, s, H-2′), 5.34 (1H, d, *J* = 2.6 Hz, H-6), 4.96 (1H, m, H-1″), 4.87 (1H, dd, *J* = 5.2, 10.2 Hz, H-12), 4.50 (1H, dd, *J* = 2.0, 9.9 Hz, H-1‴), 3.85 (1H, m, H-3), 3.82 (1H, m, H-3″), 3.80 (1H, m, H-5″), 3.42 (3H, s, OCH_3), 3.42 (3H, s, OCH_3), 3.27 (1H, m, H-5‴), 3.25 (1H, m, H-4″), 3.19 (1H, m, H-3‴), 2.97 (1H, d, *J* = 9.0 Hz, H-4‴), 2.26 (3H, s, H-7′), 2.24 (1H, m, H-4′), 2.12 (3H, s, H-21), 1.55 (3H, s, H-18), 1.27 (3H, d, *J* = 6.3 Hz, H-6″), 1.21 (3H, d, *J* = 6.2 Hz, H-6‴), 1.14 (3H, s, H-19), 1.10 (3H, dd, *J* = 6.8, 8.7 Hz, H-5′/H-6′); ^{13}C NMR (CD_3OD, 150 MHz) δ_C: 39.8 (C-1), 30.1 (C-2), 79.3 (C-3), 39.8 (C-4), 140.2 (C-5), 119.7 (C-6), 35.2 (C-7), 75.0 (C-8), 45.2 (C-9), 38.1 (C-10), 25.4 (C-11), 73.3 (C-12), 58.7 (C-13), 89.9 (C-14), 34.2 (C-15), 33.2 (C-16), 93.0 (C-17), 10.4 (C-18), 18.4 (C-19), 211.6 (C-20), 27.6 (C-21), 167.5 (C-1′), 114.3 (C-2′), 167.5 (C-3′), 39.3 (C-4′), 21.3 (C-5′), 21.3 (C-6′), 16.7 (C-7′), 97.0 (C-1″),

化合物**11** 白色无定形粉末, 分子式为$C_{41}H_{64}O_{13}$; ^{1}H NMR (CD_3OD, 400 MHz, *J* in Hz) δ_H: 5.45 (1H, s, H-2′), 5.25 (1H, d, *J* = 2.6 Hz, H-6), 4.87 (1H, dd, *J* = 5.2, 10.2 Hz, H-12), 4.86 (1H, m, H-1″), 4.86 (1H, dd, *J* = 2.0, 9.9 Hz, H-1‴), 4.13 (1H, m, H-3″), 4.12 (1H, m, H-5″), 4.12 (1H, m, H-3‴), 3.67 (1H, m, H-3), 3.50 (1H, m, H-5‴), 3.50 (3H, s, OCH_3), 3.21 (1H, m, H-4″), 3.12 (1H, d, *J* = 9.0 Hz, H-4‴), 2.24 (1H, m, H-4′), 2.14 (3H, s, H-21), 2.12 (3H, s, H-7′), 1.60 (3H, s, H-18), 1.20 (3H, d, *J* = 6.3 Hz, H-6″), 1.19 (3H, d, *J* = 6.2 Hz, H-6‴), 1.14 (3H, s, H-19), 1.10 (3H, dd, *J* = 6.8, 8.7 Hz, H-5′/H-6′); ^{13}C NMR (CD_3OD, 150 MHz) δ_C: 39.8 (C-1), 30.1 (C-2), 79.3 (C-3), 39.8 (C-4), 140.2 (C-5), 119.7 (C-6), 35.2 (C-7), 75.0 (C-8), 45.2 (C-9), 38.1 (C-10), 25.4 (C-11), 73.3 (C-12), 58.7 (C-13), 89.9 (C-14), 34.2 (C-15), 33.2 (C-16), 93.0 (C-17), 10.4 (C-18), 18.4 (C-19), 211.6 (C-20), 27.6 (C-21), 167.5 (C-1′), 114.3 (C-2′), 167.5 (C-3′), 39.3 (C-4′), 21.3 (C-5′), 21.3 (C-6′), 16.7 (C-7′), 97.0 (C-1″),

36.6 (C-2″), 78.5 (C-3″), 83.9 (C-4″), 69.8 (C-5″), 18.6 (C-6″), 100.6 (C-1‴), 37.4 (C-2‴), 76.9 (C-3‴), 81.7 (C-4‴), 73.2 (C-5‴), 18.5 (C-6‴), 57.4 (OCH_3)。以上波谱数据与文献[24]报道对比, 确定化合物 **11** 为 caudatin-3-*O*-*β*-*D*-cymaropyranosyl-(1→4)-*β*-*D*-digitoxopyranoside。

化合物 **12** 白色无定形粉末, 分子式为 $C_{49}H_{78}O_{16}$; ^{1}H NMR (CD_3OD, 400 MHz, *J* in Hz) δ_H: 5.54 (1H, s, H-2′), 5.34 (1H, d, *J* = 2.0 Hz, H-6), 4.86 (1H, dd, *J* = 1.6, 9.6 Hz, H-1″), 4.79 (1H, dd, *J* = 1.8, 9.6 Hz, H-1‴), 4.60 (1H, d, *J* = 9.2 Hz, H-1⁗), 4.48 (1H, dd, *J* = 4.1, 11.6 Hz, H-12), 3.89 (1H, m, H-5‴), 3.86 (1H, m, H-3″), 3.85 (1H, m, H-3‴), 3.82 (1H, m, H-5″), 3.54 (3H, s, OCH_3), 3.53 (1H, m, H-3), 3.50 (1H, m, H-3⁗), 3.42 (3H, s, OCH_3), 3.42 (3H, s, OCH_3), 3.30 (1H, m, H-5⁗), 3.26 (1H, m, H-4″), 3.23 (1H, m, H-4‴), 3.18 (1H, m, H-4⁗), 2.40 (1H, m, H-4′), 2.17 (3H, s, H-21), 2.11 (3H, s, H-7′), 1.50 (3H, s, H-18), 1.30 (3H, d, *J* = 6.0 Hz, H-6⁗), 1.29 (3H, d, *J* = 6.4 Hz, H-6‴), 1.18 (3H, d, *J* = 6.3 Hz, H-6″), 1.14 (3H, s, H-19), 1.07 (3H, dd, *J* = 6.8, 8.7 Hz, H-5′/H-6′); ^{13}C NMR (CD_3OD, 150 MHz) δ_C: 39.8 (C-1), 30.2 (C-2), 79.3 (C-3), 39.8 (C-4), 140.2 (C-5), 119.7 (C-6), 35.2 (C-7), 75.0 (C-8), 45.2 (C-9), 38.1 (C-10), 25.4 (C-11), 73.3 (C-12), 58.7 (C-13), 89.9 (C-14), 34.2 (C-15), 33.2 (C-16), 93.0 (C-17), 10.4 (C-18), 18.4 (C-19), 211.6 (C-20), 27.6 (C-21), 167.4 (C-1′), 114.3 (C-2′), 167.4 (C-3′), 39.3 (C-4′), 21.3 (C-5′), 21.3 (C-6′), 16.7 (C-7′), 97.2 (C-1″), 36.6 (C-2″), 78.5 (C-3″), 83.9 (C-4″), 69.8 (C-5″), 18.6 (C-6″), 58.5(OCH_3), 101.2 (C-1‴), 36.4 (C-2‴), 78.6 (C-3‴), 83.9 (C-4‴), 70.0 (C-5‴), 18.6 (C-6‴), 58.4 (OCH_3), 102.8 (C-1⁗), 37.4 (C-2⁗), 76.9 (C-3⁗), 81.7 (C-4⁗), 73.2 (C-5⁗), 18.5 (C-6⁗), 57.4 (OCH_3)。以上波谱数据与文献[25]报道对比, 确定化合物 **12** 为 otophylloside B。

化合物 **13** 白色无定形粉末, 分子式为 $C_{48}H_{76}O_{16}$; ^{1}H NMR (CD_3OD, 400 MHz, *J* in Hz) δ_H: 5.54 (1H, s, H-2′), 5.37 (1H, d, *J* = 2.0 Hz, H-6), 4.86 (1H, dd, *J* = 1.6, 9.6 Hz, H-1″), 4.79 (1H, dd, *J* = 1.8, 9.6 Hz, H-1‴), 4.60 (1H, d, *J* = 9.2 Hz, H-1⁗), 4.48 (1H, dd, *J* = 4.1, 11.6 Hz, H-12), 4.26 (1H, m, H-3‴), 3.87 (1H, m, H-3″), 3.87 (1H, m, H-5‴), 3.81 (1H, m, H-5″), 3.54 (1H, m, H-3), 3.46 (3H, s, OCH_3), 3.43 (3H, s, OCH_3), 3.40 (1H, m, H-5⁗), 3.26 (1H, m, H-4″), 3.22 (1H, m, H-4‴), 3.20 (1H, m, H-3⁗), 3.19 (1H, m, H-4⁗), 2.40 (1H, m, H-4′), 2.17 (3H, s, H-21), 2.12 (3H, s, H-7′), 1.51 (3H, s, H-18), 1.39 (3H, d, *J* = 6.0 Hz, H-6⁗), 1.29 (3H, d, *J* = 6.4 Hz, H-6‴), 1.18 (3H, d, *J* = 6.3 Hz, H-6″), 1.15 (3H, s, H-19), 1.08 (3H, dd, *J* = 6.8, 8.7 Hz, H-5′/H-6′); ^{13}C NMR (CD_3OD, 150 MHz) δ_C: 39.8 (C-1), 30.2 (C-2), 79.3 (C-3), 39.8 (C-4), 140.2 (C-5), 119.7 (C-6), 35.2 (C-7), 74.9 (C-8), 45.2 (C-9), 38.1 (C-10), 25.4 (C-11), 73.3 (C-12), 58.7 (C-13), 89.9 (C-14), 34.2 (C-15), 33.2 (C-16), 93.0 (C-17), 10.4 (C-18), 18.4 (C-19), 211.6 (C-20), 27.6 (C-21), 167.5 (C-1′), 114.3 (C-2′), 167.4 (C-3′), 39.3 (C-4′), 21.3 (C-5′), 21.3 (C-6′), 16.7 (C-7′), 97.2 (C-1″), 36.4 (C-2″), 78.5 (C-3″), 83.8 (C-4″), 69.8 (C-5″), 18.6 (C-6″), 58.7 (OCH_3), 100.6 (C-1‴), 36.4 (C-2‴), 78.6 (C-3‴), 83.9 (C-4‴), 70.0 (C-5‴), 18.5 (C-6‴), 102.8 (C-1⁗), 37.4 (C-2⁗), 76.9 (C-3⁗), 81.7 (C-4⁗), 73.2 (C-5⁗), 18.5 (C-6⁗), 57.4 (OCH_3)。以上波谱数据与文献[26]报道对比, 确定化合物 **13** 为 otophylloside E。

化合物 **14** 白色无定形粉末, 分子式为 $C_{48}H_{76}O_{16}$; ^{1}H NMR (CD_3OD, 400 MHz, *J* in Hz) δ_H: 5.54 (1H, s, H-2′), 5.35 (1H, d, *J* = 2.0 Hz, H-6), 4.96 (1H, d, *J* = 9.6 Hz, H-1″), 4.85 (1H, dd, *J* = 1.8, 9.6 Hz, H-1‴), 4.60 (1H, d, *J* = 9.2 Hz, H-1⁗), 4.48 (1H, dd, *J* = 4.1, 11.6 Hz, H-12), 4.23 (1H, d, *J* = 2.8 Hz, H-3″), 3.85 (1H, m, H-3‴), 3.83 (1H, m, H-5‴), 3.82 (1H, m, H-5″), 3.54 (3H, s, OCH_3), 3.53 (1H, m, H-3), 3.51 (1H, m, H-3⁗), 3.43 (3H, s, OCH_3), 3.34 (1H, m, H-5⁗), 3.28 (1H, m, H-4‴), 3.20 (1H, m, H-4″), 3.20 (1H, m, H-4⁗), 2.40 (1H, m, H-4′), 2.16 (3H, s, H-21), 2.12 (3H, s, H-7′), 1.51 (3H, s, H-18), 1.30 (3H, d, *J* = 6.0 Hz, H-6⁗), 1.21 (3H, d, *J* = 6.0 Hz, H-6‴), 1.19 (3H, d, *J* = 6.3 Hz, H-6″), 1.15 (3H, s, H-19), 1.07 (3H, dd, *J* = 6.8, 8.7 Hz, H-5′/H-6′); ^{13}C NMR (CD_3OD, 150 MHz) δ_C: 39.8 (C-1), 30.1 (C-2), 79.3 (C-3), 39.8 (C-4), 140.2 (C-5), 119.7 (C-6), 35.2 (C-7), 75.0 (C-8), 45.2 (C-9), 38.0 (C-10), 25.4 (C-11), 73.3 (C-12), 58.7 (C-13), 89.9 (C-14), 34.2 (C-15), 33.2 (C-16), 93.1 (C-17), 10.4 (C-18), 18.4 (C-19), 211.6 (C-20), 27.6 (C-21), 167.5 (C-1′), 114.3 (C-2′), 167.5 (C-3′), 39.3 (C-4′), 21.3 (C-5′), 21.3 (C-6′), 16.7 (C-7′), 97.0 (C-1″), 36.6 (C-2″), 78.5 (C-3″), 83.9 (C-4″), 69.8 (C-5″), 18.6 (C-6″), 100.6 (C-1‴), 36.4 (C-2‴), 78.6 (C-3‴), 83.8 (C-4‴), 70.0 (C-5‴), 18.5 (C-6‴), 58.7 (OCH_3), 102.8 (C-1⁗), 37.4 (C-2⁗), 76.9 (C-3⁗), 81.7 (C-4⁗), 73.2 (C-5⁗), 18.4 (C-6⁗), 58.5 (OCH_3)。以上波谱数据与文献[26]报道对比, 确定化合物 **14** 为 otophylloside F。

3 化合物 1 的酸水解实验

取 2 mg 化合物 **1**, 溶解在 2 mL 甲醇与二氧六环混合液中 (甲醇−二氧六环, 1∶1), 加入 2 mL HCl (2 mol·L^{-1}),

在60 ℃水浴中加热搅拌2 h, TLC检测确认反应完全后, 加入4 mL水稀释, 并用饱和$NaHCO_3$调节pH到中性。接着用2倍体积的氯仿进行萃取, 得到水部位 (糖) 和氯仿部位 (苷元), 分别浓缩得到相应反应产物。对于水部位的反应产物, 用氯仿−甲醇 (10∶1) 溶解, 以加拿大麻吡喃糖标准品和毛地黄毒吡喃糖标准品为对照, 进行TLC鉴别。当展开剂为氯仿−甲醇 (9∶1) 时, 加拿大麻吡喃糖和毛地黄毒吡喃糖的R_f值分别为: 0.45和0.22, 当展开剂为丙酮−石油醚 (2∶3) 时, 其R_f值分别为: 0.32和0.16。显色剂为10%的硫酸−乙醇溶液。对于氯仿部位 (苷元) 则通过HPLC进行分离纯化, 最后通过1D NMR和2D NMR验证苷元的结构。

4 细胞毒活性测试

采用MTS法测定化合物对人肝癌细胞株 (HepG2) 的细胞毒性。取对数生长期的细胞, 用含10%胎牛血清的DMEM培养液配成单个细胞悬液, 调整细胞密度为每毫升1×10^4个后, 将细胞接种到96孔板, 每孔体积100 μL, 培养于37 ℃、5% CO_2的培养箱内。培养24 h后加入待测化合物溶液, 化合物用DMSO溶解, 分设空白组、化合物组和阳性对照组, 每孔终体积200 μL, 每种处理均设3个复孔。培养48 h后, 弃培养上清, 每孔加MTS溶液20 μL和培养液100 μL, 设3个空白复孔 (MTS溶液20 μL和培养液100 μL的混合液), 继续孵育2～4 h, 使反应充分进行。选择492 nm波长, 多功能酶标仪读取各孔光吸收值, 记录结果。实验设顺铂 (DDP) 为阳性化合物, 数据处理后以浓度为横坐标, 细胞存活率为纵坐标绘制细胞生长曲线, 应用两点法 (Reed and Muench法) 计算化合物的IC_{50}值。

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