裕丹参茎叶化学成分研究

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摘要: 采用硅胶、Sephadex LH-20、半制备HPLC等多种色谱方法对裕丹参茎叶50%丙酮提取物乙酸乙酯部位的化学成分进行分离纯化。结合化合物理化性质和波谱特征进行结构鉴定, 得到19个化合物, 分别为: 3′,4′-二羟基-4-(3-甲氧基-3-氧代丙基)-[1,1′-联苯]-3-羧酸 (**1**)、2-羟基-5-(2′-羟基苄基)苯甲酸 (**2**)、脱氢姜酮 (**3**)、3,4-二羟基亚苄基丙酮 (**4**)、苯甲醇-*β*-*D*-葡萄糖苷 (**5**)、脱氢地芰普内酯 (**6**)、rel-5-(3*S*,8*S*-dihydroxy-1*R*,5*S*-dimethyl-7-oxa-6-oxobicyclo[3,2,1]-oct-8-yl)-3-methyl-2*Z*,4*E*-pentadienoic acid (**7**)、6-羟基-3-(2-羟乙基)-2,4,4-三甲基环己-2,5-二烯-1-酮 (**8**)、东莨菪内酯 (**9**)、秦皮乙素 (**10**)、二氢丹参酮I (**11**)、2′-脱氧胸腺嘧啶核苷 (**12**)、槲皮素3-葡萄糖苷 (**13**)、sutchuenmedin A (**14**)、2″-*O*-鼠李糖基淫羊藿次苷II (**15**)、2,2′-氧基双(1,4-二叔丁基苯) (**16**)、1*H*-吲哚-3-甲基醛 (**17**)、3-(2-羰基-丙基)-3-羟基-吲哚啉-2-酮 (**18**)、dysodensiol E (**19**)。其中, 化合物**1**为新化合物, 命名为3′,4′-二羟基-4-(3-甲氧基-3-氧代丙基)-[1,1′-联苯]-3-羧酸。化合物**2**为新天然产物, 化合物**3**～**8**和**14**～**19**为首次从裕丹参中分离得到。采用MTT法对所分离化合物进行抗癌活性测试, 所有化合物均未表现出明显活性。

关键词: 裕丹参; 联苯类; 萜类; 结构鉴定; 抗肝癌活性

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Study on the chemical constituents from the stems and leaves of *Salvia miltiorrhiza* Bunge

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Abstract: Nineteen compounds were isolated from the ethyl acetate fraction of the stems and leaves of *Salvia miltiorrhiza* Bge by using various column chromatographic methods, such as silica gel, Sephadex LH-20 and semi-preparative HPLC. According to their physiochemical properties and spectral data, the structures were further identified as 3′,4′-dihydroxy-4-(3-methoxy-3-oxopropyl)-[1,1′-biphenyl]-3-carboxylic acid (**1**), 2-hydroxy-5-(2′-hydroxybenzyl) benzoic acid (**2**), dehydrozingerone (**3**), 3,4-dihydroxy benzalacetone (**4**), benzyl alcohol-*β*-*D*-glucopyranoside (**5**), dehydrololiolide (**6**), rel-5-(3*S*,8*S*-dihydroxy-1*R*,5*S*-dimethyl-7-oxa-6-oxobicyclo[3,2,1]-oct-8-yl)-3-methyl-2*Z*,4*E*-pentadienoic acid (**7**), 6-hydroxy-3-(2-hydroxyethyl)-2,4,4-trimethylcy clohexa-2,5-dien-1-one (**8**), scopoletin (**9**), esculetin (**10**), dihydrotanshinone I (**11**), 2′-deoxythymidine (**12**), quercetin 3-glucoside (**13**), sutchuenmedin A (**14**), 2″-*O*-rhamnosylicariside-II (**15**), 2,2′-oxybis(1,4-di-tert-butylbenzene) (**16**), 1*H*-indole-3-carbaldehyde (**17**), 3-(2-oxopropyl)-3-hydroxyind-olin-2-one (**18**), dysodensiol E (**19**). Compound **1** was a new biphenyl lignan, named as 3′,4′-dihydroxy-4-(3-methoxy-3-oxopropyl)-[1,1′-biphenyl]-3-carboxylic acid. Compound **2** was a new natural product and compounds **3**−**8** and **4**−**19** were firstly isolated from *S. miltiorrhiza*. MTT method

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was used to screen the anti-hepatoma activity of all the compounds, and none of them showed obvious effect.

Key words: *Salvia miltiorrhiza* Bge.; biphenyls; terpenoids; structural identification; anti-hepatoma activity

丹参 (*Salvia miltiorrhiza* Bge.) 为唇形科鼠尾草属多年生草本植物, 以根及根茎入药[1], 具有活血祛瘀、通经止痛、清心除烦、凉血消痈等功效[2], 临床药用价值极高, 为传统大宗药材。丹参年需求量巨大, 全国丹参种植面积约为1.3万公顷, 以人工种植为主。采收时, 一般只以干燥根及根茎药用, 而丹参的药用部位仅占全草的33%, 地上茎叶全部丢弃, 造成了严重的资源浪费[3]。研究发现, 丹参非传统药用部位含有与根及根茎中结构相同或相似的活性成分[4], 如水溶性酚酸类[5]、黄酮类[6]、三萜类[7]等多种化学成分, 具有抗氧化[8]、抑制脂质过氧化[9]、抑制血小板聚集[10]等作用。裕丹参主产于河南省南阳裕州 (今方城县), 有"丹参之首"的美称。为进一步提高丹参的综合利用率, 本实验以河南方城道地药材裕丹参的茎叶为研究对象, 进行系统的化学成分研究, 并从中分离得到19个化合物, 包括5个酚酸类化合物 (**1**～**5**), 5个萜类化合物 (**6**～**8**、**11**和**19**), 2个香豆素类化合物 (**9**和**10**), 3个生物碱类化合物 (**12**、**17**和**18**), 3个黄酮类化合物 (**13**～**15**), 1个曲地酸类似物 (**16**)。其中, 化合物**1**为新化合物, 化合物**2**为新天然产物, 化合物**3**～**8**和**14**～**19**为首次从裕丹参中分离得到。采用MTT法对所分离化合物进行抗癌活性测试, 均未表现出明显活性。

结果与讨论

化合物**1**为黄色蜡状固体, 易溶于甲醇。HR-ESI-MS $[M+Na]^+$ *m*/*z* 339.084 1 (计算值 $C_{17}H_{16}O_6Na^+$ 339.083 9) 推测分子式为 $C_{17}H_{16}O_6$, 不饱和度为10。IR光谱显示化合物结构中存在羟基 (3 366 cm^{-1})、羰基 (1 697 cm^{-1})。^{1}H NMR (CD_3OD, 500 MHz) 谱 (表1) 中显示6个芳香氢信号 δ_H 8.04 (1H, d, *J* = 1.8 Hz, H-2), 7.60 (1H, dd, *J* = 8.1, 1.8 Hz, H-6), 7.32 (1H, d, *J* = 8.1 Hz, H-5), 7.05 (1H, d, *J* = 1.8 Hz, H-2′), 6.96 (1H, dd, *J* = 8.1, 1.8 Hz, H-6′), 6.83 (1H, d, *J* = 8.1 Hz, H-5′), 两个亚甲基氢信号 δ_H 3.27 (2H, t, *J* = 7.7 Hz, H-7), 2.67 (2H, t, *J* = 7.7 Hz, H-8), 一个甲氧基氢信号 δ_H 3.65 (3H, s, 9-OCH_3); ^{13}C NMR (CD_3OD, 125 MHz) 谱 (表1) 结合HMBC谱共观察到17个碳信号, 包括两个羰基碳信号 δ_C 175.3 (C-9), 171.5 (C-10), 两组1,3,4-三取代苯环碳信号 δ_C 140.1 (C-1), 129.7 (C-2), 132.5 (C-3), 141.2 (C-4), 132.5 (C-5), 130.7 (C-6), 133.1 (C-1′), 114.8 (C-2′), 146.5 (C-3′), 146.7 (C-4′), 116.8 (C-5′), 119.3 (C-6′), 两个亚甲基碳信号 δ_C 30.5 (C-7), 36.7 (C-8), 一个甲氧基碳信号 δ_C 50.0 (9-OCH_3)。在 ^{1}H-^{1}H COSY 谱中出现H-7与H-8的相关信号, 说明存在自旋偶合系统-CH_2-CH_2-。HMBC谱中存在H-8, 9-OCH_3与C-9以及H-7与C-3, C-4, C-5的相关信号, 证明了苯环C-4位丙酸甲酯基片段的存在。H-2与C-10的相关确定了另外一个羰基连在C-3位, H-6与C-1′以及H-2′, H-6′与C-1的相关信号, 确定了两个苯环通过C-1和C-1′相连, 由此推断化合物**1**结构如图1所示, 命名为3′,4′-dihydroxy-4-(3-methoxy-3-oxopropyl) - [1, 1′-biphenyl] -3-carboxylic acid。

化合物**2**为淡黄色粉末, HR-ESI-MS $[M+Na]^+$ *m*/*z* 267.063 0 (计算值 $C_{14}H_{12}O_4Na^+$ 267.062 7) 推测分子式为 $C_{14}H_{12}O_4$。IR光谱显示结构中存在羟基 (3 356 cm^{-1})、羰基 (1 706 cm^{-1})。其中 ^{1}H NMR (CD_3OD, 500 MHz) 谱 (表1) 中显示7个芳香氢信号, 其中 δ_H 7.69 (1H, d, *J* = 1.7 Hz, H-6), 7.31 (1H, dd, *J* = 8.4, 1.7 Hz, H-4), 6.77 (1H, d, *J* = 8.4 Hz, H-3) 为一个1,3,4-三取代苯环上的氢信号, δ_H 6.99 (2H, m, H-4′,6′), 6.75 (1H, m, H-3′), 6.72 (1H, m, H-5′) 为一个1,2-二取代的苯环上的氢信号, δ_H 3.85 (2H, s, H-8) 为一个亚甲基氢信号; ^{13}C NMR (CD_3OD, 125 MHz) 谱 (表1) 结合HMBC谱共观察到14个碳信号, 包括一个羰基信号 δ_C 173.6 (C-7), 两组苯环碳信号 δ_C 113.9 (C-1), 161.2 (C-2), 117.7 (C-3), 137.2 (C-4), 133.4 (C-5), 131.4 (C-6), 128.9 (C-1′), 156.2 (C-2′), 116.0 (C-3′), 128.4 (C-4′), 120.6 (C-5′), 131.3 (C-6′), 其中根据161.2 (C-2), 156.2 (C-2′) 的化学位移值推测均与-OH相连, HMBC谱中存在H-8与C-4, C-5, C-1′, C-2′, C-6′的相关信号确定了-CH_2分别与两个苯环的5位和1′位相连, H-6与C-7呈现远程相关, 确定了羰基连在C-1位, 结合HMBC谱中H-4′, H-6′与C-2′; H-3′与C-5′; H-5′与C-3′、C-1′、C-6′呈现的远程相关信号, 可确定B环氢与碳的数据归属, 化合物**2**鉴定为2-hydroxy-5-(2′-hydroxybenzyl)benzoic acid, 结构如图1所示, 经SciFinder数据库检索发现该化合物目前仅通过合成[11]获得, 本文首次报道了该化合物的核磁和质谱数据。

采用MTT法, 对化合物**1**～**19**进行抗肝癌活性测试, 结果显示, 阳性对照组细胞增殖抑制率为40.50% ± 1.429%, 当 $P < 0.05$ 且细胞增殖抑制率 ≥ 20%时, 可表明该化合物有明显的抗肝癌活性, 因此化合物**1**～**19**均未表现出明显的抗肝癌活性。结果见表2。

Table 1 ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of compounds **1** and **2** (in CD_3OD)

No.	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
1		140.1		113.9
2	8.04 (1H, d, *J* = 1.8 Hz)	129.7		161.2
3		132.4	6.77 (1H, d, *J* = 8.4 Hz)	117.7
4		141.2	7.31 (1H, dd, *J* = 8.4, 1.7 Hz)	137.2
5	7.32 (1H, d, *J* = 8.1 Hz)	132.5		133.4
6	7.60 (1H, dd, *J* = 8.1, 1.8 Hz)	130.7	7.69 (1H, d, *J* = 1.7 Hz)	131.4
7	3.27 (2H, t, *J* = 7.7 Hz)	30.5		173.6
8	2.67 (2H, t, *J* = 7.7 Hz)	36.7	3.85 (2H, s)	35.7
9		175.3		
10		171.5		
9-OCH_3	3.65 (3H, s)	50.0		
1′		133.1		128.9
2′	7.05 (1H, d, *J* = 1.8 Hz)	114.8		156.2
3′		146.5	6.75 (1H, m)	116.0
4′		146.7	6.99 (1H, m)	128.4
5′	6.83 (1H, d, *J* = 8.1 Hz)	116.8	6.72 (1H, m)	120.6
6′	6.96 (1H, dd, *J* = 8.1, 1.8 Hz)	119.3	6.99 (1H, m)	131.3

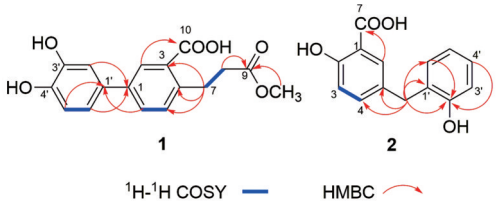


Figure 1 Key ^{1}H-^{1}H COSY and HMBC correlations of compounds **1** and **2**

Table 2 Effects of compounds on proliferation of HepG2 cells. $n = 3$, $\bar{x} \pm s$. $^{*}P < 0.05$, $^{**}P < 0.01$ *vs* control group (Con)

Group	Inhibition rate/%	Group	Inhibition rate/%
Con	0.058 51 ± 0.007 907	**10**	−9.821 ± 3.900
5-Fluorouracil	40.50 ± 1.429**	**11**	−1.966 ± 0.697 6
1	13.42 ± 3.715**	**12**	−10.47 ± 11.00
2	11.16 ± 0.830 0**	**13**	−0.635 1 ± 2.435
3	−18.32 ± 12.06	**14**	−3.091 ± 2.721
4	1.740 ± 2.643	**15**	−19.97 ± 4.682
5	9.566 ± 2.906*	**16**	3.509 ± 3.254
6	14.61 ± 2.489**	**17**	−5.428 ± 1.757
7	10.92 ± 1.312**	**18**	19.46 ± 3.755**
8	−5.658 ± 0.697 8	**19**	12.48 ± 5.440**
9	11.86 ± 1.155**		

实验部分

赛谱锐思LC52型高压制备液相色谱仪 (赛谱锐思北京科技有限公司); Thermo Nicolet IS10红外光谱仪 (美国Thermo Fisher公司); Bruker AVANCE III 500型核磁共振仪、Bruker maxis HD型飞行时间质谱 (德国Bruker公司); 色谱柱为COSMOSIL C18-MS-II (250 mm × 20 mm, 5 μm) (日本COSMOSIL公司); 薄层色谱硅胶GF 254 (青岛海洋化工厂); Sephadex LH-20葡聚糖凝胶 (美国GE公司); MCI Gel CHP-20柱色谱材料 (日本三菱化学公司); Toyopearl HW-40C (日本TOSOH公司); 柱层析所用硅胶G (200～300目, 青岛海洋化工厂); 所用色谱纯试剂和分析纯试剂均为天津市富宇精细化工有限公司生产; 实验细胞: 人肝癌细胞 (HepG2) 购自武汉塞维尔生物科技有限公司; 实验药物: 5-氟尿嘧啶购自上海麦克林生化科技有限公司。

实验用裕丹参茎叶于2020年9月采自河南南阳市方城县, 经河南中医学大学陈随清教授及董诚明教授鉴定为唇形科植物裕丹参 *Salvia miltiorrhiza* Bge 的干燥茎叶, 标本 (20200924B) 存在于河南中医药大学中药化学研究室。

1 提取分离

裕丹参干燥茎叶30.0 kg, 经组织破碎后用50%含水丙酮提取2次, 提取液减压浓缩得总浸膏 (10.8 kg), 加水分散溶解, 依次用石油醚、乙酸乙酯、正丁醇萃取, 得到石油醚部位 (30.2 g), 乙酸乙酯部位 (239.5 g), 正丁醇部位 (456.1 g) 和水部位。

乙酸乙酯部位 (239.5 g) 用甲醇溶解, 经硅胶柱 (200～300目) 色谱分离, 依次用二氯甲烷−甲醇 (100∶0→0∶100) 梯度洗脱, 薄层检识合并得到6个组分 (Fr. A.1～Fr.A.6)。Fr.A.4 (57.3 g) 经MCI中压柱分离, 用甲醇−水梯度溶剂 (10∶90→100∶0) 洗脱以获得7个组分 (Fr.A.4.1～Fr.A.4.7)。Fr.A.4.1 (2.1 g) 上Sephadex LH-20凝胶柱, 纯甲醇洗脱, 薄层检识合并得到6个组分 (Fr.A.4.1.1～Fr.A.4.1.6)。Fr.A.4.1.1经半制备高效液相色谱 (甲醇−水55∶45) 制备得到化合物**2** (5.28 mg, t_R = 30.5 min) 和化合物**7** (1.62 mg, t_R = 35.8 min)。Fr.A.4.1.4上Toyopearl HW-40C凝胶柱, 纯甲醇洗脱, 薄层检识合并得到6个组分 (Fr.A.4.1.4.1～Fr.A.4.1.4.6), Fr.A.4.1.4.1经半制备高效液相色谱 (甲醇−水24∶76) 制备得到化合物**9** (2.21 mg, t_R = 40.2 min) 和化合物**1** (2.18 mg, t_R = 42.5 min)。Fr.A.4.1.4.2经半制备高效液相色谱 (乙腈−水60∶40) 制备得到化合物**3** (6.37 mg, t_R = 36.8 min) 和化合物**6** (5.02 mg, t_R = 39.6 min)。Fr.A.4.1.4.4经半制备高效液相色谱 (甲醇−水24∶76) 制备得到化合物**4** (2.17 mg, t_R = 25.4 min) 和化合物**10** (3.82 mg, t_R = 43.5 min)。Fr.A.4.1.4.5经半制备高效液相色谱 (甲醇−水34∶66) 制备得到化合物**5** (16.2 mg, t_R = 18.9 min) 和化合物**11** (2.0 mg, t_R = 27.5 min)。Fr.A.4.1.4.6经半制备高效液相色谱 (乙腈−水25∶75) 制备得到化合物**8** (14.0 mg, t_R = 30.5 min) 和化合物**12** (9.82 mg, t_R = 24.5 min)。Fr.A.4.1.6上硅胶柱, 依次用

石油醚−乙酸乙酯 (10∶1→0∶1), 乙酸乙酯−甲醇 (10∶1→0∶1) 梯度洗脱, 薄层检识合并得到 8 个组分 (Fr. A.4.1.6.1～Fr.A.4.1.6.8), Fr.A.4.1.6.2 经半制备高效液相色谱 (乙腈−水 60∶40) 制备得到化合物 **15** (302 mg, t_R = 45.4 min) 和化合物 **17** (5.02 mg, t_R = 27.4 min)。Fr. A.4.1.6.4 经半制备高效液相色谱 (甲醇−水 24∶76) 制备得到化合物 **16** (7.8 mg, t_R = 15.8 min)、化合物 **18** (16.1 mg, t_R = 34.5 min) 和化合物 **19** (2.85 mg, t_R = 24.5 min)。Fr.A.4.1.6.5 经半制备高效液相色谱 (甲醇−水 45∶55) 制备得到化合物 **14** (5.2 mg, t_R = 28.5 min) 和化合物 **13** (24.13 mg, t_R = 27.5 min)。

2 结构鉴定

化合物 **1** 黄色蜡状固体, 溶于甲醇, 紫外 254 nm 下显示为暗斑, 10% 浓硫酸乙醇溶液加热不显色 (105 ℃), UV (CH_3OH) λ_{max} (log ε): 219.0 (3.044), IR (CH_3OH) ν_{max} 3 366、1 697 cm^{-1}, HR-ESI-MS $[M+Na]^+$ *m/z* 339.084 1 (计算值 $C_{17}H_{16}O_6Na^+$ 339.083 9), ^1H NMR 和 ^{13}C NMR 数据见表 1。

化合物 **2** 浅黄色粉末, 溶于甲醇, 紫外 254 nm 下显示为暗斑, 10% 浓硫酸乙醇溶液加热不显色 (105 ℃), UV (CH_3OH) λ_{max} (log ε): 212.0 (2.569), IR (CH_3OH) ν_{max} 3 356、1 706 cm^{-1}, HR-ESI-MS $[M+Na]^+$ *m/z* 267.063 0 (计算值 $C_{14}H_{12}O_4Na^+$ 267.062 7), ^1H NMR 和 ^{13}C NMR 数据见表 1。

化合物 **3** 浅黄色粉末 (甲醇、氯仿)。ESI-MS *m/z*: 193.09 $[M+H]^+$; 分子式为 $C_{11}H_{12}O_3$。^1H NMR ($CDCl_3$, 500 MHz) δ_H: 7.46 (1H, d, *J* = 15.9 Hz, H-7), 7.10 (1H, dd, *J* = 8.2, 1.8 Hz, H-6), 7.07 (1H, d, *J* = 1.8 Hz, H-2), 6.94 (1H, d, *J* = 8.2 Hz, H-5), 6.60 (1H, d, *J* = 15.9 Hz, H-8), 3.94 (3H, s, 3-OCH_3), 2.38 (3H, s, H-10); ^{13}C NMR ($CDCl_3$, 125 MHz) δ_C: 126.9 (C-1), 109.3 (C-2), 148.3 (C-3), 146.9 (C-4), 114.8 (C-5), 123.5 (C-6), 143.8 (C-7), 125.0 (C-8), 198.5 (C-9), 27.3 (C-10), 56.0 (3-OCH_3)。以上数据与文献[12]报道对照基本一致, 故鉴定化合物 **3** 为 dehydrozingerone。

化合物 **4** 黄色油状物 (甲醇、氯仿)。ESI-MS *m/z*: 179.07 $[M+H]^+$; 分子式为 $C_{10}H_{10}O_3$。^1H NMR (CD_3OD, 500 MHz) δ_H: 7.48 (1H, d, *J* = 15.9 Hz, H-7), 7.08 (1H, d, *J* = 1.8 Hz, H-2), 6.95 (1H, dd, *J* = 8.2, 1.8 Hz, H-6), 6.74 (1H, d, *J* = 8.2 Hz, H-5), 6.50 (1H, d, *J* = 15.9 Hz, H-8), 2.29 (3H, s, H-10); ^{13}C NMR (CD_3OD, 125 MHz) δ_C: 127.7 (C-1), 116.6 (C-2), 146.9 (C-3), 149.9 (C-4), 115.3 (C-5), 124.7 (C-6), 146.9 (C-7), 123.5 (C-8), 201.6 (C-9), 27.0 (C-10)。以上数据与文献[13]报道对照基本一致, 故鉴定化合物 **4** 为 3,4-dihydroxy benzalacetone。

化合物 **5** 无色蜡状固体 (二甲基亚砜)。ESI-MS *m/z*: 271.12 $[M+H]^+$; 分子式为 $C_{13}H_{18}O_6$。^1H NMR (DMSO-d_6, 500 MHz) δ_H: 7.39 (2H, t, *J* = 7.3 Hz, H-4,6), 7.33 (2H, t, *J* = 7.5 Hz, H-3, 7), 7.27 (1H, t, *J* = 7.2 Hz, H-5), 5.09 (1H, d, *J* = 4.8 Hz, -OH), 4.94 (1H, d, *J* = 4.7 Hz, -OH), 4.89 (1H, d, *J* = 4.9 Hz, -OH), 4.81 (1H, d, *J* = 12.3 Hz, H-1a), 4.56 (1H, d, *J* = 12.3 Hz, H-1b), 4.52 (1H, t, J = 5.9 Hz, -OH), 4.22 (1H, d, *J* = 7.8 Hz, H-1′), 3.69 (1H, m, H-6′b), 3.46 (1H, d, *J* = 11.6, 5.7 Hz, H-6′a); ^{13}C NMR (DMSO-d_6, 125 MHz) δ_C: 70.1 (C-1), 127.3 (C-2), 128.1 (C-3, 7), 127.6 (C-4, 6), 138.1 (C-5), 102.1 (C-1′), 73.5 (C-2′), 76.7 (C-3′), 69.4 (C-4′), 77.0 (C-5′), 61.1 (C-6′)。以上数据与文献[14]报道对照基本一致, 故鉴定化合物 **5** 为苯甲醇-*β*-*D*-葡萄糖苷。

化合物 **6** 无色固体 (甲醇、氯仿)。ESI-MS *m/z*: 195.10 $[M+H]^+$; 分子式为 $C_{11}H_{14}O_3$。^1H NMR (CD_3OD, 500 MHz) δ_H: 6.04 (1H, brs, H-7), 2.85 (1H, d, *J* = 14.5 Hz, H-4a), 2.55 (1H, d, *J* = 14.5 Hz, H-4b), 2.45 (1H, dd, *J* = 14.3, 1.9 Hz, H-2a), 2.32 (1H, dd, *J* = 14.3, 1.9 Hz, H-2b), 1.57 (1H, s, H-9), 1.41 (3H, s, H-11), 1.29 (3H, s, H-10); ^{13}C NMR (CD_3OD, 125 MHz) δ_C: 37.0 (C-1), 54.9 (C-2), 207.1 (C-3), 54.9 (C-4), 88.2 (C-5), 181.3 (C-6), 115.6 (C-7), 173.6 (C-8), 30.2 (C-9), 26.5 (C-10), 27.5 (C-11)。以上数据与文献[15]报道对照基本一致, 故鉴定化合物 **6** 为脱氢地芰普内酯。

化合物 **7** 无定形粉末 (甲醇、氯仿)。ESI-MS *m/z*: 297.13 $[M+H]^+$; 分子式为 $C_{15}H_{20}O_6$。^1H NMR (CD_3OD, 500 MHz) δ_H: 8.01 (1H, d, *J* = 16.0 Hz, H-4), 6.49 (1H, s, H-2), 6.43 (1H, d, *J* = 16.0 Hz, H-5), 3.84 (1H, m, H-4′), 2.09 (3H, s, 3-CH_3), 1.34 (3H, m, 1′-CH_3), 1.07 (3H, m, 5′-CH_3); ^{13}C NMR (CD_3OD, 125 MHz) δ_C: 171.0 (C-1), 122.2 (C-2), 148.6 (C-3), 133.5 (C-4), 131.3 (C-5), 89.8 (C-1′), 42.3 (C-2′), 65.2 (C-3′), 41.0 (C-4′), 53.5 (C-5′), 181.1 (C-6′), 82.8 (C-8′), 20.9 (3-CH_3), 18.5 (1′-CH_3), 14.5 (5′-CH_3)。以上数据与文献[16]报道对照基本一致, 故鉴定化合物 **7** 为 rel-5-(3*S*,8*S*-dihydroxy-1*R*, 5*S*-dimethyl-7-oxa-6-oxobicyclo[3, 2, 1] -oct-8-yl) -3-methyl-2*Z*,4*E*-pentadienoic acid。

化合物 **8** 黄色蜡状固体 (甲醇、氯仿)。ESI-MS *m/z*: 197.12 $[M+H]^+$; 分子式为 $C_{11}H_{16}O_3$。^1H NMR (CD_3OD, 500 MHz) δ_H: 5.98 (1H, s, H-5), 3.63 (2H, m, H-8), 2.69 (2H, m, H-7), 1.94 (3H, s, H-11), 1.27 (6H, s, H-9, 10); ^{13}C NMR (CD_3OD, 125 MHz) δ_C: 183.8 (C-1), 132.3 (C-2), 161.6 (C-3), 41.2 (C-4), 128.4 (C-5), 146.6 (C-6), 35.2 (C-7), 61.0 (C-8), 26.9 (C-9, 10), 12.0 (C-11)。以上数

据与文献[17]报道对照基本一致, 故鉴定化合物**8**为6-羟基-3-(2-羟乙基)-2,4,4-三甲基环己-2,5-二烯-1-酮。

化合物**9** 无定形粉末 (甲醇、氯仿)。ESI-MS *m*/*z*: 193.05 $[M+H]^+$; 分子式为$C_{10}H_8O_4$。^{1}H NMR (CD_3OD, 500 MHz) δ_H: 7.84 (1H, d, *J* = 9.6 Hz, H-4), 7.09 (1H, s, H-5), 6.76 (1H, s, H-8), 6.19 (1H, d, *J* = 9.6 Hz, H-3), 3.89 (3H, s, 6-OCH_3); ^{13}C NMR (CD_3OD, 125 MHz) δ_C: 164.0 (C-2), 112.6 (C-3), 146.1 (C-4), 109.9 (C-5), 147.1 (C-6), 152.9 (C-7), 103.9 (C-8), 151.4 (C-9), 112.5 (C-10), 56.8 (6-OCH_3)。以上数据与文献[18]报道对照基本一致, 故鉴定化合物**9**为东莨菪内酯。

化合物**10** 黄色蜡状固体 (甲醇、氯仿)。ESI-MS *m*/*z*: 179.04 $[M+H]^+$; 分子式为$C_9H_6O_4$。^{1}H NMR (CD_3OD, 500 MHz) δ_H: 7.78 (1H, d, *J* = 9.6 Hz, H-4), 6.93 (1H, s, H-5), 6.74 (1H, s, H-8), 6.17 (1H, d, *J* = 9.6 Hz, H-3); ^{13}C NMR (CD_3OD, 125 MHz) δ_C: 164.3 (C-2), 112.5 (C-3), 146.0 (C-4), 113.0 (C-5), 144.6 (C-6), 152.0 (C-7), 103.6 (C-8), 150.5 (C-9), 112.8 (C-10)。以上数据与文献[19]报道对照基本一致, 故鉴定化合物**10**为秦皮乙素。

化合物**11** 红色粉末 (甲醇、氯仿)。ESI-MS *m*/*z*: 279.10 $[M+H]^+$; 分子式为$C_{18}H_{14}O_3$。^{1}H NMR ($CDCl_3$, 500 MHz) δ_H: 9.20 (1H, d, *J* = 8.9 Hz, H-1), 8.15 (1H, d, *J* = 8.7 Hz, H-6), 7.62 (1H, d, *J* = 8.7 Hz, H-7), 7.51 (1H, dd, *J* = 8.9, 7.0 Hz, H-2), 7.34 (1H, d, *J* = 7.0 Hz, H-3), 4.97 (1H, m, H-15a), 4.42 (1H, m, H-15b), 3.63 (1H, m, H-16), 2.62 (3H, s, H-18), 1.41 (3H, d, *J* = 6.8 Hz, H-17); ^{13}C NMR ($CDCl_3$, 125 MHz) δ_C: 124.9 (C-1), 130.3 (C-2), 128.8 (C-3), 135.0 (C-4), 124.6 (C-5), 131.9 (C-6), 120.2 (C-7), 128.1 (C-8), 125.8 (C-9), 131.8 (C-10), 184.1 (C-11), 175.5 (C-12), 118.3 (C-13), 170.5 (C-14), 81.6 (C-15), 34.7 (C-16), 18.8 (C-17), 19.8 (C-18)。以上数据与文献[20]报道对照基本一致, 故鉴定化合物**11**为二氢丹参酮I。

化合物**12** 白色蜡状固体 (甲醇、氯仿)。ESI-MS *m*/*z*: 243.10 $[M+H]^+$; 分子式为$C_{10}H_{14}N_2O_5$。^{1}H NMR (CD_3OD, 500 MHz) δ_H: 7.81 (1H, d, *J* = 1.2 Hz, H-3), 6.27 (1H, t, *J* = 6.27 Hz, H-2′), 4.40 (1H, dt, *J* = 6.6, 3.5 Hz, H-5′), 3.90 (1H, t, *J* = 3.4 Hz, H-4′), 3.79 (1H, dd, *J* = 12.0, 3.2 Hz, H-6′a), 3.73 (1H, dd, *J* = 12.0, 3.2 Hz, H-6′b), 2.23 (2H, m, H-3′), 1.87 (3H, s, H-7); ^{13}C NMR (CD_3OD, 125 MHz) δ_C: 152.4 (C-2), 111.5 (C-3), 138.2 (C-4), 166.4 (C-6), 12.4 (C-7), 88.8 (C-2′), 41.2 (C-3′), 72.2 (C-4′), 86.2 (C-5′), 62.8 (C-6′)。以上数据与文献[21]报道对照基本一致, 故鉴定化合物**12**为2′-脱氧胸腺嘧啶核苷。

化合物**13** 黄色粉末 (甲醇、氯仿)。ESI-MS *m*/*z*: 465.10 $[M+H]^+$; 分子式为$C_{21}H_{20}O_{12}$。^{1}H NMR (CD_3OD, 500 MHz) δ_H: 7.71 (1H, d, *J* = 1.8 Hz, H-2′), 7.58 (1H, dd, *J* = 8.5, 1.8 Hz, H-6′), 6.87 (1H, d, *J* = 8.5 Hz, H-5′), 6.37 (1H, d, *J* = 1.4 Hz, H-8), 6.19 (1H, d, *J* = 1.4 Hz, H-6), 5.24 (1H, d, *J* = 8.5 Hz, H-1″), 3.70 (1H, dd, *J* = 12.0, 1.9 Hz, H-6″a), 3.58 (1H, dd, *J* = 12.0, 5.3 Hz, H-6″b), 3.49 (1H, m, H-2″), 3.44 (1H, t, *J* = 8.9 Hz, H-3″), 3.36 (1H, dd, *J* = 11.0, 7.3 Hz, H-4″), 3.21 (1H, ddd, *J* = 9.0, 5.4, 2.4 Hz, H-5″); ^{13}C NMR (CD_3OD, 125 MHz) δ_C: 158.7 (C-2), 135.6 (C-3), 179.5 (C-4), 163.1 (C-5), 99.9 (C-6), 166.0 (C-7), 94.7 (C-8), 159.0 (C-9), 105.7 (C-10), 123.2 (C-1′), 116.0 (C-2′), 149.9 (C-3′), 145.9 (C-4′), 117.5 (C-5′), 123.1 (C-6′), 104.3 (C-1″), 75.7 (C-2″), 78.1 (C-3″), 71.2 (C-4″), 78.4 (C-5″), 62.6 (C-6″)。以上数据与文献[22]报道对照基本一致, 故鉴定化合物**13**为槲皮素3-葡萄糖苷。

化合物**14** 白色蜡状固体 (甲醇、氯仿)。ESI-MS *m*/*z*: 659.23 $[M+H]^+$; 分子式为$C_{33}H_{38}O_{14}$。^{1}H NMR (CD_3OD, 500 MHz) δ_H: 7.89 (2H, d, *J* = 8.8 Hz, H-2′,6′), 7.10 (2H, dd, *J* = 8.8 Hz, H-3′, 5′), 6.69 (1H, d, *J* = 10.0 Hz, H-5″), 6.73 (1H, d, *J* = 10.0 Hz, H-1″), 6.18 (1H, brs, H-6), 5.56 (1H, d, *J* = 1.2 Hz, H-1‴), 4.99 (1H, brs, H-1⁗), 4.28 (1H, brss, H-2‴), 3.89 (3H, s, 4′-OCH_3), 3.35～4.00 (7H, m, H-3‴,4‴,5‴,2⁗,3⁗,4⁗, 5⁗), 1.45 (3H, s, H-4″), 1.43 (3H, s, H-5″), 1.21 (3H, d, *J* = 6.2 Hz, H-6‴), 0.91 (3H, d, *J* = 5.5 Hz, H-6⁗); ^{13}C NMR (CD_3OD, 125 MHz) δ_C: 158.7 (C-2), 136.7 (C-3), 179.8 (C-4), 160.9 (C-5), 100.6 (C-6), 162.6 (C-7), 106.6 (C-8), 152.4 (C-9), 102.3 (C-10), 123.7 (C-1′), 131.8 (C-2′, 6′), 163.6 (C-4′), 115.4 (C-3′, 5′), 56.0 (4′-OCH_3), 115.3 (C-1″), 130.0 (C-2″), 79.0 (C-3″), 28.4 (C-4″), 28.4 (C-5″), 102.5 (C-1‴), 79.5 (C-2‴), 72.1 (C-3‴), 74.0 (C-1‴), 70.6 (C-5‴), 17.9 (C-6‴), 102.1 (C-1⁗), 70.7 (C-2⁗), 72.0 (C-3⁗), 73.5 (C-4⁗), 70.4 (C-5⁗), 17.9 (C-6⁗)。以上数据与文献[23]报道对照基本一致, 故鉴定化合物**14**为sutchuenmedin A。

化合物**15** 黄色粉末 (甲醇、氯仿)。ESI-MS *m*/*z*: 661.25 $[M+H]^+$; 分子式为$C_{33}H_{40}O_{14}$。^{1}H NMR (CD_3OD, 500 MHz) δ_H: 7.83 (2H, d, *J* = 8.8 Hz, H-2′, 6′), 7.04 (2H, dd, *J* = 8.8 Hz, H-3′, 5′), 6.24 (1H, s, H-6), 5.55 (1H, brs, H-1‴), 5.16 (1H, t, *J* = 6.6 Hz, H-2″), 5.06 (1H, brs, H-1⁗), 4.36 (1H, brs, H-2‴), 3.87 (3H, s, 4′-OCH_3), 3.35～4.00 (7H, m, H-3‴,4‴,5‴,2⁗,3⁗,4⁗, 5⁗), 3.31 (1H, m, 1″), 1.69 (3H, s, H-4″), 1.64 (3H, s,

H-5″), 1.26 (3H, d, *J* = 5.6 Hz, H-6‴), 0.93 (3H, d, *J* = 5.6 Hz, H-6⁗); ^{13}C NMR (CD_3OD, 125 MHz) δ_C: 158.4 (C-2), 136.3 (C-3), 179.6 (C-4), 160.7 (C-5), 99.3 (C-6), 163.3 (C-7), 107.8 (C-8), 155.6 (C-9), 105.6 (C-10), 124.0 (C-1′), 131.8 (C-2′,6′), 163.1 (C-4′), 115.1 (C-3′, 5′), 56.0 (4′-OCH_3), 22.4 (C-1″), 123.7 (C-2″), 132.4 (C-3″), 25.9 (C-4″), 18.2 (C-5″), 103.6 (C-1‴), 78.8 (C-2‴), 70.3 (C-3‴), 74.0 (C-1‴), 72.0 (C-5‴), 17.9 (C-6‴), 102.2 (C-1⁗), 72.2 (C-2⁗), 72.0 (C-3⁗), 73.5 (C-4⁗), 71.9 (C-5⁗), 17.8 (C-6⁗)。以上数据与文献[24]报道对照基本一致, 故鉴定化合物**15**为*2″-O*-鼠李糖基淫羊藿次苷II。

化合物**16** 白色粉末 (甲醇、氯仿)。ESI-MS *m*/*z*: 395.33 $[M+H]^+$; 分子式为$C_{28}H_{42}O$。^{1}H NMR ($CDCl_3$, 500 MHz) δ_H: 7.54 (2H, d, *J* = 8.1 Hz, H-6, 6′), 7.37 (2H, d, *J* = 1.8 Hz, H-3, 3′), 7.14 (2H, d, *J* = 8.6, 2.5 Hz, H-5,5′), 1.34 (18H, s, H-12,13,14,12′,13′,14′), 1.29 (18H, s, H-8,9,10,8′,9′,10′); ^{13}C NMR ($CDCl_3$, 125 MHz) δ_C: 147.1 (C-1,1′), 147.6 (C-2,2′), 124.5 (C-3,3′), 138.5 (C-4, 4′), 123.2 (C-5,5′), 119.1 (C-6,6′), 34.9 (C-7,7′), 31.4 (C-8,9,10,8′,9′,10′), 34.5 (C-11,11′), 30.2 (C-12,13,14,12′, 13′,14′)。以上数据与文献[25]报道对照基本一致, 故鉴定化合物**16**为2,2′-氧基双(1,4-二叔丁基苯)。

化合物**17** 红棕色粉末 (甲醇、氯仿)。ESI-MS *m*/*z*: 146.06 $[M+H]^+$; 分子式为C_9H_7NO。^{1}H NMR (CD_3OD, 500 MHz) δ_H: 9.87 (1H, s, H-8), 8.15 (1H, d, *J* = 8.15 Hz, H-4), 8.08 (1H, s, H-2), 7.47 (1H, td, *J* = 7.8 Hz, H-7), 7.27 (H, t, *J* = 7.2 Hz, H-6), 7.22 (H, d, *J* = 7.7 Hz, H-5); ^{13}C NMR (CD_3OD, 125 MHz) δ_C: 139.7 (C-2), 120.1 (C-3), 125.7 (C-3a), 125.0 (C-4), 122.4 (C-5), 123.6 (C-6), 113.1 (C-7), 138.9 (C-7a), 187.4 (C-8)。以上数据与文献[26]报道对照基本一致, 故鉴定化合物**17**为1*H*-吲哚-3-甲基醛。

化合物**18** 深黄色油状物 (甲醇、丙酮)。ESI-MS *m*/*z*: 206.08 $[M+H]^+$; 分子式为$C_{11}H_{11}NO_3$。^{1}H NMR (acetone-d_6, 500 MHz) δ_H: 7.30 (1H, d, *J* = 7.4 Hz, H-4), 7.20 (1H, td, *J* = 7.8, 1.2 Hz, H-6), 6.99 (H, td, *J* = 7.8, 1.2 Hz, H-6), 6.87 (1H, d, *J* = 7.8 Hz, H-7), 3.33 (1H, d, *J* = 16.6 Hz, H-8a), 3.10 (1H, d, *J* = 16.6 Hz, H-8b), 2.05 (1H, s, H-10); ^{13}C NMR (acetone-d_6, 125 MHz) δ_C: 181.1 (C-2), 74.8 (C-3), 130.7 (C-4), 123.4 (C-5), 124.8 (C-6), 111.2 (C-7), 51.1 (C-8), 207.4 (C-9), 30.4 (C-10), 132.3 (C-11), 143.7 (C-12)。以上数据与文献[27]报道对照基本一致, 故鉴定化合物**18**为3-(2-羰基-丙基)-3-羟基-吲哚啉-2-酮。

化合物**19** 白色蜡状固体 (甲醇、丙酮)。ESI-MS *m*/*z*: 253.18 $[M+H]^+$; 分子式为$C_{15}H_{24}O_3$。^{1}H NMR (acetone-d_6, 500 MHz) δ_H: 6.88 (1H, d, *J* = 4.6 Hz, H-5), 3.37 (1H, dd, *J* = 10.1, 5.8 Hz, H-9), 2.72 (1H, dd, *J* = 13.9, 5.8 Hz, H-7a), 2.18 (1H, m, H-7b), 2.15 (1H, m, H-4), 1.84 (1H, m, H-8a), 1.81 (1H, m, H-2a), 1.79 (1H, m, H-3), 1.67 (1H, m, H-1a), 1.57 (1H, m, H-12), 1.53 (1H, m, H-8b), 1.43 (1H, m, H-2b), 1.43 (1H, m, H-1a), 1.02 (3H, s, H-11), 0.91 (3H, d, *J* = 6.6 Hz, H-13), 0.90 (3H, d, *J* = 6.6 Hz, H-14); ^{13}C NMR (acetone-d_6, 125 MHz) δ_C: 40.0 (C-1), 27.5 (C-2), 55.9 (C-3), 52.8 (C-4), 151.4 (C-5), 129.8 (C-6), 22.4 (C-7), 27.1 (C-8), 76.1 (C-9), 47.0 (C-10), 19.8 (C-11), 33.2 (C-12), 20.1 (C-13), 22.0 (C-14), 171.6 (C-15)。以上数据与文献[28]报道对照基本一致, 故鉴定化合物**19**为dysodensiol E。

3 抗肝癌活性筛选

HepG2细胞经含10%胎牛血清的DMEM培养1周, 取对数生长期细胞, 以每毫升2×10^4个细胞接种于96孔板, 每孔200 μL, 待细胞贴壁后, 换为含药培养基。分为对照组 (Con)、给药组 (10 μmol·L^{-1}) 和阳性对照组 (5-氟尿嘧啶, 10 μmol·L^{-1}), 培养24 h后, 每孔加入20 μL MTT溶液 (5 mg·mL^{-1}), 继续培养4 h, 小心吸净培养液, 每孔再加入150 μL DMSO, 震荡10 min, 使其完全溶解。继续培养24 h, MTT法检测各化合物对细胞活力的影响, 结果以吸光度 (*A*) 值和增殖抑制率 (PR, %) 表示, PR = $(1-A_{给药}/A_{对照})\times100\%$。实验独立平行重复3次。

采用GraphPad Prism 5统计软件进行数据分析, 数据以均数 ± 标准差 ($\bar{x} \pm s$) 表示。进行正态性及方差齐性检验, 差异满足正态性及方差齐性则进行多组间比较, 采用单因素方差分析 (one-way ANOVA), 以 $P < 0.05$为差异有统计学意义。

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利益冲突: 所有作者均声明不存在利益冲突。

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