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薄叶山橙中生物碱成分及其抑制肿瘤细胞增殖活性筛选

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摘　要:采用色谱分离手段从薄叶山橙中分离并鉴定了20个化合物,利用波谱解析鉴定了他们结构。分别为水甘草碱(**1**)、11-甲氧基水甘草碱(**2**)、11-羟基水甘草碱(**3**)、19*R*-乙酰基水甘草碱(**4**)、19*R*-羟基水甘草碱(**5**)、11-甲氧基-19*R*-羟基水甘草碱(**6**)、11-羟基-19*R*-乙酰基水甘草碱(**7**)、11,19*R*-二羟基水甘草碱(**8**)、$\triangle^{14}$-长春胺(**9**)、$\triangle^{14}$-长春醇(**10**)、scandine(**11**)、10-hydroxyscandine(**12**)、melodinine T(**13**)、meloscandonine(**14**)、venalstonine(**15**)、19-hydroxyvenalstonine(**16**)、vindolinine(**17**)、melodinine M(**18**)、voaphyline(**19**)、melofusine I(**20**)。生物碱**4**~**6**、**8**、**9**、**13**、**16**和**18**~**20**为首次从该种植物中报道。此外,对分离得到的20个生物碱类化合物进行抑制肿瘤细胞增殖活性筛选,其中化合物**1**、**2**、**4**、**6**、**10**和**17**对**5**种人体肿瘤细胞株具有增殖抑制活性。

关键词:薄叶山橙;生物碱;化学成分;肿瘤细胞增殖

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Alkaloid Constituents from *Melodinus tenuicaudatus* and Their Proliferation Inhibition on Tumor Cells

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Abstract: Twenty compounds were isolated from *Melodinus tenuicaudatus*. Their structures were identified as tabersonine (**1**), 11-methoxytabersonine (**2**), 11-hydroxytabersonine (**3**), 19*R*-acetoxy-tabersonine (**4**), 11, 19*R*-hydroxytabersonine (**5**), 11-methoxy-19*R*-hydroxytabersonine (**6**), 19*R*-acetoxy-11-hydroxytabersonine (**7**), 11-hydroxy-19*R*-hydroxytabersonine (**8**), $\triangle^{14}$-vincamine (**9**), $\triangle^{14}$-vincanol (**10**), scandine (**11**), 10-hydroxyscandine (**12**), melodinine T (**13**), meloscandonine (**14**), venalstonine (**15**), 19-hydroxyvenalstonine (**16**), vindolinine (**17**), melodinine M (**18**), voaphyline (**19**), and melofusine I (**20**). By analysis of NMR, MASS, spectrum and data. Alkaloids **4-6**, **8**, **9**, **13**, **16** and **18-20** were obtained from the plants of *Melodinus tenuicaudatus* for the first time. The isolates were evaluated for the cytotoxic activities by MTT assay. Among them, compounds **1**, **2**, **4**, **6**, **10** and **17** showed growth inhibitory activityagainst the five human tumor cell lines.

Key words: *Melodinus tenuicaudatus*; alkaloids; chemical constituents; tumor cell proliferation

薄叶山橙 *Melodinus tenuicaudatus* 隶属于夹竹桃科(Apocynaceae)山橙属(*Melodinus*)植物。主要产于云南、广西等省区,生于海拔750~1800 m山地密林中或灌木丛中。薄叶山橙树皮具有治腰骨酸痛,病后虚弱,外伤出血等功效[1]。目前国内外对薄叶山橙的研究报道不多,尤其是关于滇南边境地区的薄叶山橙尚未见到有关研究报道。为了进一步研究该属植物的生物碱成分及其活性,本实验对该种植物进行系统深入研究。我们对采自云南省普洱市澜沧县雪岭乡中缅边境的薄叶山橙进行了化学成分及抑制肿瘤细胞增殖活性研究。从中分离得到20个生物碱,分别鉴定为水甘草碱(**1**)、11-甲氧基水甘草碱(**2**)、11-羟基水甘草碱(**3**)、19*R*-乙酰基水甘草碱(**4**)、19*R*-羟基水甘草碱(**5**)、11-甲氧基-19*R*-羟基水甘草碱(**6**)、11-羟基-19*R*-乙酰基水甘草碱(**7**)、11,19*R*-二羟基水甘草碱(**8**)、$\triangle^{14}$-长春胺(**9**)、$\triangle^{14}$-长春醇(**10**)、scandine(**11**)、**10**-hydroxyscandine(**12**)、melodinine T(**13**)、meloscandonine(**14**)、venalstonine

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(**15**)、19-hydroxyvenalstonine(**16**)、vindolinine(**17**)、melodinine M(**18**)、voaphyline(**19**)、melofusine I(**20**)。生物碱**4**~**6**、**8**、**9**、**13**、**16** 和 **18**~**20** 为首次从该种植物中报道。并对分离得到的 20 个生物碱类化合物进行抑制肿瘤细胞增殖活性筛选,其中化合物 **1**、**2**、**4**、**6**、**10** 和 **17** 对 5 种人体肿瘤细胞株具有增殖抑制活性。

1　仪器与材料

1.1　仪器与试剂

薄层色谱硅胶 GF_{254}(青岛海洋化工厂);Sephadex LH-20(Pharmacia 公司);海道夫旋转蒸发仪(德国 Heidolph 公司);SB5200D 超声波清洗仪(宁波新芝生物科技股份有限公司);SHZ-D 型循环水式真空泵(巩义市英峪予华仪器厂);CS101 型号电热鼓风干燥箱(深圳市隆安试验设备有限公司);Agilent G6230 型 HPLC,半制备色谱柱为 Agilent C_{18}(250×10mm);岛津高效液相色谱仪,半制备色谱柱为 Shimadzu C_{18}(250×10mm);Bruker DRX-600 MHz 型核磁共振波谱仪(TMS 为内标,δ 为 ppm,J 为 Hz)。

实验常用试剂如甲醇、丙酮、乙酸乙酯、石油醚、氯仿、正丁醇、乙醇等均为工业级试剂,需重蒸处理。半制备液相和制备液相所用有机试剂为色谱纯。核磁所用氘代试剂为美国 CIL 公司生产。

显色剂:10% 浓硫酸-乙醇溶液、碘粉、改良碘化铋钾试剂。

1.2　细胞株

实验所用的人体白血病细胞 HL-60,肝癌 SMMC7721,肺癌 A-549,乳腺癌 MCF-7 和结肠癌 SW480 细胞株均购于中科院上海生命科学研究院细胞资源中心。

1.3　药材

薄叶山橙地上部分于 2017 年 5 月采自云南省普洱市澜沧县雪岭乡中缅边境。由云南省中医学院中药学院杨竹雅副教授鉴定。

2　实验方法

2.1　提取与分离

薄叶山橙 10 kg 干燥样品,粉碎后用甲醇室温下浸提三次,每次 48 h。过滤并浓缩提取物得到总浸膏为 900 g。浸膏用 0.2% 稀盐酸溶解调 pH 值至 2~3 并用乙酸乙酯萃取 3 次。将萃取后的酸溶液用 5% 氨水溶液调 pH 值至 9~10,边调节边用乙酸乙酯萃取,总共萃取 3 次,得到总碱部分为 95 g。总碱部分用正相硅胶柱划段,用石油醚-丙酮(100∶1-1∶1)洗脱得到六个部分(Fr. A-F)。Fr. A(4.4 g)正相硅胶柱分离,用石油醚-丙酮(20∶1-10∶1)洗脱得到化合物 **1**(30 mg)和 **2**(85 mg)和三个部分;Fr. A-Ⅱ(80 mg)经制备型高效液相色谱洗脱得到化合物 **19**(15 mg)和 **4**(5 mg)。Fr. B(2.3 g)正相硅胶柱分离,用石油醚-丙酮(8∶1~5∶1)洗脱得到三个部分;Fr. B-Ⅱ经反复 RP-18 柱层析得到化合物 **5**(23 mg),Fr. B-Ⅲ经半制备型高效液相色谱洗脱得到化合物 **6**(12 mg)。Fr. C(7.2 g)经中压液相色谱甲醇/水(10∶90~80∶20)梯度洗脱得到三个部分;Fr. C-Ⅱ(1.2 g)采用正相硅胶柱分离,用石油醚-丙酮(6∶1~2∶1)洗脱得到化合物 **14**(11 mg)和 **8**(20 mg);Fr. C-Ⅱ(500 mg)采用制备型高效液相色谱洗脱得到化合物 **3**(300 mg)和 **15**(15 mg);Fr. C-Ⅲ(50 mg)经反复 RP-18 柱层析得到化合物 **7**(11mg)。Fr. D(3.3 g)经中压液相色谱甲醇/水(10∶90~60∶40)梯度洗脱得到二个部分;Fr. D-Ⅰ(500 mg)采用正相硅胶柱分离,采用氯仿:丙酮(10∶1~5∶1)洗脱得到化合物 **13**(5 mg)和 **20**(21 mg);Fr. D-Ⅱ(650 mg)经制备型和半制备型高效液相色谱反复洗脱得到化合物 **11**(18 mg)和 **18**(6 mg)。Fr. E(4 g)经中压液相色谱甲醇/水(10∶90~50∶50)梯度洗脱得到三个部分;Fr. E-Ⅰ(720 mg)采用正相硅胶柱分离,采用氯仿:甲醇(10∶1~5∶1)洗脱得到化合物 **10**(8 mg)和 **16**(50 mg);Fr. E-Ⅱ(100 mg)经制备型高效液相色谱反复洗脱得到化合物 **17**(10 mg)和 **12**(7 mg);Fr. E-Ⅲ(60 mg)经半制备型高效液相色谱反复洗脱得到化合物 **9**(20 mg)。

2.2　体外肿瘤细胞增殖抑制活性实验

采用 MTT[2] 法进行对 5 株人体肿瘤细胞株进行体外细胞毒活性测定。设阴性对照组(DMSO 溶剂对照组)、阳性对照组(顺铂)和 5 个不同浓度的待测样品,每个浓度有三个平行。选择对数生长期细胞,血球计数板计数,按每孔 5×10^4 个癌细胞接种于 96 孔板平底细胞培养板中,置于 37 ℃、5% CO_2 恒温恒湿培养箱中培养。24 h 后加入 200 μL 的不同浓度梯度的样品,继续培养 20 h 后,取出,显微镜下观察每孔细胞形态,记录细胞形态变化情况,接着每孔加入 200 μL,0.5 mg/mL 的 MTT 溶液(溶于平衡盐溶液 PBS),在 37 ℃条件下反应 4 h,小心吸弃孔内培养液,每孔加入 200 μL DMSO 使 Formazane 充分溶解后,用 SpectraMax M5 酶标仪在

用 570 nm 波长下测定各孔的吸光度值(A),按如下公式计算生长抑制率。生长抑制率 = (1-样品组 A 值/对照组 A 值) ×100%。

以样品浓度为横坐标,以抑制率为纵坐标,做图并计算化合物的 IC_{50}值。

3 实验结果

3.1 化合物结构式

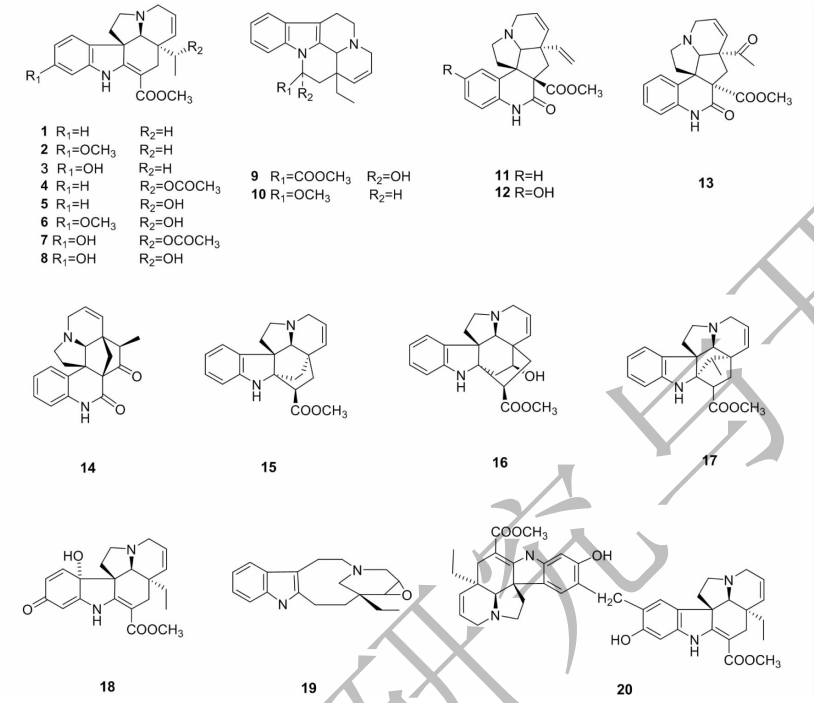

图 1 化合物 1 ~ 20 的化学结构

Fig. 1 The chemical sturctures of compounds **1-20**

3.2 化合物结构鉴定

化合物 1 无色油状;ESI-MS:*m/z* 337 [M + H]$^+$;^{1}H NMR ($CDCl_3$,600 MHz) δ:8.93 (1H,s,-NH),7.20 (1H,d,*J* = 7.3 Hz,H-9),7.07 (1H,t,*J* = 7.3 Hz,H-11),6.80 (1H,t,*J* = 7.3 Hz,H-10),6.75 (1H,d,*J* = 7.3 Hz,H-12),5.74 (1H,dd,*J* = 10.0,3.6 Hz,H-14),5.64 (1H,d,*J* = 10.0 Hz,H-15),0.93 (1H,dq,*J* = 14.5,7.2 Hz,H-19b),0.79 (1H,d,*J* = 14.5,7.2 Hz,H-19a),0.57 (3H,t,*J* = 7.2 Hz,Me-18);^{13}C NMR ($CDCl_3$,150 MHz) δ:169.0 (s,CO_2CH_3),166.8 (s,C-2),143.2 (s,C-13),138.0 (s,C-8),133.1 (d,C-15),127.6 (d,C-11),124.8 (d,C-14),121.4 (d,C-9),120.6 (d,C-10),109.3 (d,C-12),92.1 (s,C-16),70.0 (d,C-21),55.1 (s,C-7),51.0 (q,CO_2CH_3),50.9 (t,C-5),50.6 (t,C-3),44.5 (t,C-6),41.3 (s,C-20),28.4 (t,C-19),26.9 (t,C-17),7.4 (q,C-18)。以上数据与文献[3]报道一致,故鉴定为水甘草碱。

化合物 2 无色油状;ESI-MS:*m/z* 367 [M + H]$^+$;^{1}H NMR ($CDCl_3$,600 MHz) δ:8.98 (1H,s,-NH),7.27 (1H,d,*J* = 8.0 Hz,H-9),6.48 (1H,dd,*J* = 8.0,1.4 Hz,H-10),6.40 (1H,d,*J* = 1.4,Hz,H-12),5.79 (1H,dd,*J* = 10.0,3.3 Hz,H-14),5.68 (1H,d,*J* = 10.0 Hz,H-15),0.95 (1H,dq,*J* = 14.0,7.5 Hz,H-19b),0.81 (1H,dq,*J* = 14.0,7.5 Hz,H-19a),0.64 (3H,t,*J* = 7.5 Hz,Me-18);^{13}C NMR ($CDCl_3$,150 MHz) δ:168.9 (s,CO_2CH_3),167.0 (s,C-2),160.0 (s,C-11),144.4 (s,C-13),133.1 (d,C-15),130.3 (s,C-8),124.7 (d,C-14),121.8 (d,C-9),105.0 (d,C-10),96.7 (d,C-12),92.3 (s,C-16),70.1 (d,C-21),55.4 (q,CO_2CH_3),54.4 (s,C-7),51.0 (t,C-5),50.5 (t,C-3),44.4 (t,C-6),41.3 (s,C-20),28.4 (t,C-17),26.9 (t,C-19),7.5 (q,C-18)。以上数据与文献[4]报道一致,故鉴定为 11-甲氧基水甘草碱。

化合物 3 无色油状,ESI-MS:*m/z* 353 [M + H]$^+$;^{1}H NMR ($CDCl_3$,600 MHz) δ:8.98 (1H,s,-NH),7.05 (1H,d,*J* = 8.0 Hz,H-9),6.41 (1H,d,*J*

=2.0 Hz,H-12),6.34 (1H,dd,*J*=8.0,2.0 Hz,H-10),5.77 (1H,ddd,*J*=10.0,4.6,1.1 Hz,H-14),5.68 (1H,d,*J*=10.0 Hz,H-15),3.75 (3H,s,CO_2CH_3),0.95 (1H,dq,*J*=14.8,7.5 Hz,H-19b),0.86 (1H,dq,*J*=14.8,7.5 Hz,H-19a),0.64 (3H,t,*J*=7.5 Hz,Me-18);^{13}C NMR ($CDCl_3$,150 MHz) *δ*:169.4 (s,CO_2CH_3),167.6 (s,C-2),156.4 (s,C-11),144.2 (s,C-13),133.0 (d,C-15),129.8 (s,C-8),124.7 (d,C-14),121.1 (d,C-9),107.2 (d,C-10),97.7 (d,C-12),92.1 (s,C-16),70.2 (d,C-21),54.5 (s,C-7),51.3 (q,CO_2CH_3),50.9 (t,C-3),50.5 (t,C-5),44.4 (t,C-6),41.3 (s,C-20),28.3 (t,C-17),26.9 (t,C-19),7.5 (q,C-18)。以上数据与文献[4]报道一致,故鉴定为11-羟基水甘草碱。

化合物4　白色无定形粉末;ESI-MS:*m/z* 395 [M+H]$^+$;^{1}H NMR ($CDCl_3$,600 MHz) *δ*:7.79 (1H,s,-NH),7.45 (1H,d,*J*=7.7 Hz,H-9),7.23 (1H,t,*J*=7.7 Hz,H-11),7.08 (1H,d,*J*=7.7 Hz,H-12),6.58 (1H,t,*J*=7.7 Hz,H-10),5.92 (1H,dd,*J*=10.1 Hz,H-14),5.76 (1H,t,*J*=10.1 Hz,H-15),3.30 (1H,dd,*J*=15.5,4.8 Hz,H-3a),3.14 (1H,d,*J*=15.5 Hz,H-3b),2.93 (1H,t,*J*=7.4,1.1 Hz,H-5a),2.23 (dd,*J*=14.3,7.5 Hz,H-7),1.61 (3H,m,*J*=6.4 Hz,Me-18);^{13}C NMR ($CDCl_3$,150 MHz) *δ*:169.9 (s,CO_2CH_3),168.4 (s,$OCOCH_3$),166.0 (s,C-2),143.3 (s,C-13),137.5 (s,C-8),129.6 (d,C-15),127.9 (d,C-11),126.8 (d,C-14),121.2 (d,C-9),120.7 (d,C-10),109.7 (d,C-12),91.7 (s,C-16),69.6 (d,C-21),66.6 (d,C-19),55.4 (s,C-7),50.9 (q,CO_2CH_3),50.9 (t,C-5),50.1 (t,C-3),45.7 (s,C-20),44.3 (t,C-16),27.3 (t,C-17),20.7 (q,$OCOCH_3$),15.3 (q,C-18)。以上数据与文献[5]报道一致,故鉴定为19*R*-乙酰基水甘草碱。

化合物5　无色油状;ESI-MS:*m/z* 353 [M+H]$^+$;^{1}H NMR ($CDCl_3$,600 MHz) *δ*:9.25 (1H,s,-NH),7.29 (1H,d,*J*=7.7 Hz,H-9),7.18 (1H,t,*J*=7.7 Hz,H-11),6.91 (1H,d,*J*=7.7 Hz,H-12),6.85 (1H,t,*J*=7.7 Hz,H-10),5.94 (1H,overlap,H-14),5.82 (1H,overlap,H-15),3.36 (1H,dd,*J*=15.5,4.8 Hz,H-3a),3.25 (1H,d,*J*=15.5 Hz,H-3b),3.13 (1H,t,*J*=7.4 Hz,H-5a),2.91 (1H,m,H-5b),0.90 (3H,d,*J*=6.4 Hz,Me-18);^{13}C NMR ($CDCl_3$,150 MHz) *δ*:168.5 (s,CO_2CH_3),166.4 (s,C-2),143.0 (s,C-13),137.7 (s,C-8),129.4 (d,C-15),127.8 (s,C-11),126.3 (d,C-14),121.5 (d,C-9),120.9 (d,C-10),109.4 (d,C-12),91.2 (s,C-16),67.0 (d,C-21),66.6 (d,C-19),55.5 (s,C-7),51.3 (q,CO_2CH_3),51.0 (t,C-5),50.1 (t,C-3),46.3 (s,C-20),43.9 (t,C-6),27.5 (t,C-17),17.3 (q,C-18)。以上数据与文献[6]报道一致,故鉴定为19*R*-羟基水甘草碱。

化合物6　无色油状;ESI-MS:*m/z* 383 [M+H]$^+$;^{1}H NMR ($CDCl_3$,600 MHz) *δ*:8.90 (1H,s,-NH),7.29 (1H,d,*J*=8.1 Hz,H-9),7.15 (1H,d,*J*=2.0 Hz,H-12),6.43 (1H,dd,*J*=8.1 Hz,H-10),5.82 (1H,overlap,H-14),5.82 (1H,overlap,H-15),3.81 (3H,s,OCH_3),3.24 (1H,dd,*J*=15.8,4.8 Hz,H-3a),3.07 (1H,d,*J*=15.8 Hz,H-3b),2.79 (1H,t,*J*=7.4 Hz,H-5a),2.08 (1H,m,H-5b),0.91 (3H,d,*J*=6.3 Hz,Me-18);^{13}C NMR ($CDCl_3$,150 MHz) *δ*:168.5 (s,CO_2CH_3),166.8 (s,C-2),160.1 (s,C-11),144.2 (s,C-13),130.1 (d,C-15),129.4 (s,C-8),126.3 (d,C-14),121.9 (d,C-9),105.3 (d,C-10),96.7 (d,C-12),91.5 (s,C-16),66.9 (d,C-21),66.7 (d,C-19),55.5 (q,CO_2CH_3),54.9 (s,C-7),51.3 (t,C-5),50.1 (t,C-3),46.4 (s,C-20),44.0 (t,C-6),27.5 (t,C-17),17.4 (q,C-18)。以上数据与文献[7]报道一致,故鉴定为11-甲氧基-19*R*-羟基水甘草碱。

化合物7　无色油状;ESI-MS:*m/z* 411 [M+H]$^+$;^{1}H NMR ($CDCl_3$,600 MHz) *δ*:7.28 (1H,d,*J*=8.1 Hz,H-9),6.40 (1H,d,*J*=1.8 Hz,H-12),6.35 (1H,dd,*J*=8.1,1.8 Hz,H-10),5.94 (1H,dd,*J*=10.0,4.0 Hz,H-14),5.81 (1H,d,*J*=10.0 Hz,H-15),3.81 (3H,s,CO_2CH_3),3.55 (3H,s,CO_2CH_3),0.91 (1H,dq,*J*=14.8,7.5 Hz,H-19b),0.88 (1H,dq,*J*=14.8,7.5 Hz,H-19a),0.62 (3H,t,*J*=7.5 Hz,Me-18);^{13}C NMR ($CDCl_3$,150 MHz) *δ*:170.5 (s,CO_2CH_3),168.5 (s,CO_2CH_3),166.6 (s,C-2),156.4 (s,C-11),144.6 (s,C-13),129.5 (d,C-15),129.4 (s,C-8),127.0 (d,C-14),121.9 (d,C-9),107.0 (d,C-10),98.0 (d,C-12),91.8 (s,C-16),69.8 (d,C-21),66.5 (d,C-19),54.8 (s,C-7),51.0 (q,CO_2CH_3),50.9 (t,C-5),50.1

(t,C-3),45.7 (s,C-20),44.3 (t,C-6),27.4 (t,C-17),20.9 (q,OCOCH_3),15.3 (q,C-18)。以上数据与文献[7]报道一致,故鉴定为 11-羟基-19*R*-乙酰基水甘草碱。

化合物 8 白色无定形粉末;ESI-MS:*m/z* 369 [M + H]$^+$;^{1}H NMR ($CDCl_3$,600 MHz) δ:7.29 (1H,d,*J* = 8.0 Hz,H-9),6.34 (1H,d,*J* = 2.0 Hz,H-12),6.16 (1H,dd,*J* = 8.0,2.0 Hz,H-10),5.95 (1H,dd,*J* = 10.2,3.8 Hz,H-14),5.79 (1H,d,*J* = 10.2 Hz,H-15),0.92 (3H,t,*J* = 6.5 Hz,Me-18);^{13}C NMR ($CDCl_3$,150 MHz) δ:168.7 (s,CO_2CH_3),167.3 (s,C-2),156.4 (s,C-11),144.3 (s,C-13),129.4 (s,C-8),128.9 (d,C-15),126.3 (d,C-14),122.3 (d,C-9),107.3 (d,C-10),97.1 (d,C-12),91.1 (s,C-16),67.2 (d,C-21),66.5 (d,C-19),55.1 (s,C-7),51.6 (q,CO_2CH_3),50.9 (t,C-5),50.1 (t,C-3),46.6 (s,C-20),44.0 (t,C-6),27.3 (t,C-17),17.3 (q,C-18)。以上数据与文献[8]报道一致,故鉴定为 11,19*R*-二羟基水甘草碱。

化合物 9 无色油状;ESI-MS:*m/z* 353 [M + H]$^+$;^{1}H NMR ($CDCl_3$,600 MHz) δ:7.43 (2H,overlap,H-9 and H-12),7.11 (2H,overlap,H-10 and H-11),5.47 (1H,t,*J* = 10.0 Hz,H-14),5.35 (1H,d,*J* = 10.0 Hz,H-15),4.32 (1H,br s,H-16),4.07 (1H,s,H-21),2.01 (1H,m,H-19a),1.60 (1H,m,H-19b),0.98 (3H,t,*J* = 7.5 Hz,Me-18);^{13}C NMR ($CDCl_3$,150 MHz) δ:172.4 (s,CO_2CH_3),136.5 (s,C-13),132.6 (s,C-8),128.8 (s,C-2),126.8 (d,C-15),125.7 (d,C-11),121.6 (d,C-10),120.2 (s,C-9),118.0 (d,C-14),112.5 (d,C-12),106.5 (s,C-7),83.7 (s,C-16),57.0 (d,C-21),52.7 (q,CO_2CH_3),49.8 (t,C-5),46.1 (t,C-3),43.8 (t,C-17),38.4 (s,C-20),35.3 (t,C-19),16.6 (t,C-6),8.4 (q,C-18)。以上数据与文献[9]报道一致,故鉴定为$\triangle^{14}$-长春胺。

化合物 10 白色无定形粉末;ESI-MS:*m/z* 309 [M + H]$^+$;^{1}H NMR ($CDCl_3$,600 MHz) δ:7.40 (1H,d,*J* = 7.5 Hz,H-9),7.25 (1H,t,*J* = 7.5 Hz,H-12),7.12 (1H,t,*J* = 7.5 Hz,H-11),7.07 (1H,t,*J* = 7.5 Hz,H-10),5.60 (1H,dd,*J* = 10.0 Hz,H-15),5.49 (1H,dd,*J* = 10.0,3.3 Hz,H-14),5.47 (1H,dd,*J* = 5.0,3.1 Hz,H-16),4.01 (1H,d,H-21);^{13}C NMR ($CDCl_3$,150 MHz) δ:136.9 (s,C-13),133.2 (s,C-2),128.8 (s,C-8),126.8 (d,C-15),126.7 (d,C-14),121.2 (d,C-10),120.0 (d,C-11),117.9 (d,C-9),112.4 (d,C-12),105.9 (s,C-7),77.6 (d,C-16),57.2 (d,C-21),50.9 (q,OCH_3),49.5 (t,C-5),43.8 (d,C-3),43.2 (s,C-17),39.0 (s,C-20),34.1 (t,C-19),16.5 (t,C-6),8.4 (q,C-18)。以上数据与文献[10]报道一致,故鉴定为$\triangle^{14}$-长春醇。

化合物 11 无色油状;ESI-MS:*m/z* 351 [M + H]$^+$;^{1}H NMR ($CDCl_3$,600 MHz) δ:9.10 (1H,s,-NH),7.40 (1H,d,*J* = 7.8 Hz,H-9),7.08 (1H,t,*J* = 7.8 Hz,H-11),6.81 (1H,t,*J* = 7.8 Hz,H-10),6.61 (1H,d,*J* = 7.8 Hz,H-12),5.74 (1H,dd,*J* = 10.0,3.1 Hz,H-14),5.61 (1H,dd,*J* = 10.0 Hz,H-15),4.94 (1H,d,*J* = 17.5 Hz,H-18a),4.82 (1H,dd,*J* = 10.8,3.3 Hz,H-18b);^{13}C NMR ($CDCl_3$,150 MHz) δ:170.4 (s,C-2),169.2 (s,CO_2CH_3),142.2 (d,C-19),134.2 (s,C-13),131.4 (d,C-15),128.9 (s,C-8),127.5 (d,C-11),127.0 (d,C-9),115.5 (d,C-12),114.6 (t,C-18),83.6 (d,C-21),63.7 (s,C-16),53.3 (t,C-5),52.6 (q,CO_2CH_3),47.7 (t,C-3),46.6 (s,C-20),44.1 (t,C-17),39.9 (t,C-6)。以上数据与文献[11]报道一致,故鉴定为 Scandine。

化合物 12 白色晶体;ESI-MS:*m/z* 367 [M + H]$^+$;^{1}H NMR ($CDCl_3$,600 MHz) δ:9.23 (1H,s,-NH),6.88 (1H,d,*J* = 3.9 Hz,H-9),6.52 (1H,d,*J* = 8.2 Hz,H-12),6.39 (1H,dd,*J* = 8.2,3.9 Hz,H-11),5.79 (2H,m,overlap,H-14,H-15),5.68 (1H,dd,*J* = 17.3,10.8 Hz,H-19),4.97 (1H,d,*J* = 17.3 Hz,H-18a),4.82 (1H,d,*J* = 10.8 Hz,H-18b);^{13}C NMR ($CDCl_3$,150 MHz) δ:170.0 (s,CO_2CH_3),168.3 (s,C-2),152.8 (s,C-10),142.8 (d,C-19),132.6 (d,C-15),128.7 (s,C-13),127.1 (s,C-8),121.5 (d,C-14),116.2 (d,C-11),114.6 (d,C-9),114.5 (d,C-12),113.8 (t,C-18),81.7 (d,C-21),62.1 (s,C-16),58.8 (s,C-7),52.8 (q,CO_2CH_3),52.6 (t,C-5),46.0 (t,C-3),44.4 (s,C-20),43.6 (t,C-17),35.6 (t,C-6)。以上数据与文献[3]报道一致,故鉴定为 10-Hydroxyscandine。

化合物 13 白色无定形粉末;ESI-MS:*m/z* 367 [M + H]$^+$;^{1}H NMR ($CDCl_3$,600 MHz) δ:7.95 (1H,s,-NH),7.42 (1H,d,*J* = 7.8 Hz,H-9),7.19

(1H,t,J = 7.8 Hz,H-11),7.09 (1H,t,J = 7.8 Hz,H-10),6.71 (1H,d,J = 7.8 Hz,H-12),5.99 (1H,d,J = 10.2 Hz,H-15),3.18 (1H,m,H-5b),3.10 (1H,dd,J = 16.8,9.0 Hz,H-5a),2.23 (3H,s,Me-18);^{13}C NMR ($CDCl_3$,150 MHz) δ:207.3 (s,C-19),169.6 (s,CO_2CH_3),166.5 (s,C-2),134.6 (s,C-13),128.6 (s,C-8),127.8 (d,C-14),127.6 (d,C-11),126.5 (d,C-15),126.3 (d,C-9),123.8 (d,C-10),115.3 (d,C-12),74.9 (d,C-21),66.7 (d,C-21),62.2 (s,C-16),58.2 (s,C-7),55.5 (s,C-20),52.8 (q,CO_2CH_3),52.0 (t,C-5),46.1 (t,C-3),41.4 (t,C-17),35.6 (t,C-6),25.2 (q,C-18)。以上数据与文献[12]报道一致,故鉴定为 Melodinine T。

化合物 14 无色油状;ESI-MS:m/z 321[M + H]$^+$;^{1}H NMR ($CDCl_3$,600 MHz) δ:9.30 (1H,s,-NH),7.16 (1H,d,J = 7.5 Hz,H-9),7.04 (1H,t,J = 7.5 Hz,H-11),6.88 (1H,t,J = 7.5 Hz,H-10),6.82 (1H,d,J = 7.5 Hz,H-12),5.90 (1H,d,J = 10.0 Hz,H-15),5.81 (1H,dd,J = 10.0,3.6 Hz,H-14),1.05 (3H,d,J = 7.4 Hz,Me-18);^{13}C NMR ($CDCl_3$,150 MHz) δ:210.3 (s,CO),169.1 (s,C-2),136.5 (d,C-13),130.7 (s,C-8),127.9 (d,C-15),127.6 (d,C-11),124.2 (d,C-14),123.9 (d,C-10),123.8 (d,C-9),116.4 (d,C-12),69.9 (d,C-21),67.9 (s,C-16),54.9 (t,C-5),54.9 (s,C-7),50.9 (d,C-19),47.2 (t,C-3),44.4 (s,C-20),38.1 (t,C-6),36.1(t,C-17),11.1 (t,C-18)。以上数据与文献[13]报道一致,故鉴定为 Meloscandonine。

化合物 15 无色油状;ESI-MS:m/z 337[M + H]$^+$;^{1}H NMR ($CDCl_3$,600 MHz) δ:7.14 (1H,d,J = 7.6 Hz,H-9),7.05 (1H,t,J = 7.8 Hz,H-11),6.78 (2H,overlap,H-10,H-12),5.79 (1H,dd,J = 12.0,4.0 Hz,H-14),5.49 (1H,d,J = 12.0 Hz,H-15),2.93 (3H,s,CO_2CH_3);^{13}C NMR ($CDCl_3$,150 MHz) δ:174.2 (s,CO_2CH_3),149.2 (s,C-13),139.7 (d,C-8),132.8 (s,C-15),126.9 (d,C-11),121.6 (s,C-9),119.5 (d,C-10),110.2 (d,C-12),67.0 (d,C-21),66.7 (s,C-2),56.3 (s,C-7),52.0 (q,CO_2CH_3),50.3 (t,C-5),49.2 (t,C-3),43.6 (d,C-16),36.3 (t,C-6),35.1 (s,C-20),34.1 (t,C-19),31.6 (t,C-18),29.8 (t,C-17)。以上数据与文献[4]报道一致,故鉴定为 Venalstonine。

化合物 16 无色油状;ESI-MS:m/z 353[M + H]$^+$;^{1}H NMR ($CDCl_3$,600 MHz) δ:7.16 (1H,d,J = 7.6 Hz,H-9),6.89 (1H,t,J = 7.6 Hz,H-11),6.85 (2H,overlap,H-10,H-11),5.86 (1H,dd,J = 8.7,10.0 Hz,H-14),3.64 (1H,d,J = 8.7 Hz,H-15);^{13}C NMR ($CDCl_3$,150 MHz) δ:174.0 (s,CO_2CH_3),148.9 (s,C-13),139.7 (s,C-8),129.1 (d,C-11),128.2 (d,C-14),127.0 (d,C-15),121.4 (d,C-9),119.6 (d,C-10),111.5 (d,C-12),71.4 (d,C-21),66.6 (s,C-2),65.3 (d,C-19),55.9 (s,C-7),51.9 (q,CO_2CH_3),50.7 (t,C-5),49.3 (t,C-3),45.8 (t,C-6),42.9 (d,C-16),40.5 (s,C-20),36.3 (t,C-18),26.0 (t,C-17)。以上数据与文献[14]报道一致,故鉴定为 19-Hydroxyvenalstonine。

化合物 17 无色油状;ESI-MS:m/z 337[M + H]$^+$;^{1}H NMR ($CDCl_3$,600 MHz) δ:7.26 (1H,d,J = 7.7 Hz,H-9),7.03 (1H,t,J = 7.7 Hz,H-11),6.77 (1H,d,J = 7.7 Hz,H-12),6.12 (1H,dd,J = 9.9,3.0 Hz,H-14),5.76 (1H,d,J = 9.9 Hz,H-15),4.46 (1H,s,H-21),0.94 (3H,d,J = 6.8 Hz,Me-18);^{13}C NMR ($CDCl_3$,150 MHz) δ:174.4 (s,CO_2CH_3),149.3 (s,C-13),139.7 (s,C-8),131.0 (d,C-15),128.0 (d,C-14),127.4 (d,C-11),123.9 (d,C-9),121.4 (d,C-10),112.7 (d,C-12),81.4 (s,C-2),76.8 (d,C-21),59.8 (s,C-7),57.8 (t,C-3),52.0 (q,CO_2CH_3),49.8 (t,C-5),48.5 (d,C-19),45.9 (s,C-20),39.2 (d,C-16),36.0 (t,C-6),29.0 (t,C-17),7.4 (q,C-18)。以上数据与文献[15]报道一致,故鉴定为 Vindolinine。

化合物 18 黄色针状晶体;ESI-MS:m/z 369[M + H]$^+$;^{1}H NMR (acetone-d_6,600 MHz,) δ:9.82 (1H,s,-NH),7.03 (1H,d,J = 9.6 Hz,H-9),6.10 (1H,d,J = 9.6,1.2 Hz,H-10),5.80 (1H,d,J = 10.2Hz,H-15),5.56 (1H,d,J = 1.2 Hz,H-12),3.77 (1H,ddd,J = 15.6,4,8,1.2 Hz H-3b),3.37 (1H,d,J = 15.6 Hz,H-3a),2.92 (1H,m,H-5a),2.79 (1H,overlap,H-5b),1.44 (q,J = 7.2 Hz,H-19),0.79 (2H,t,J = 7.2 Hz,Me-18);^{13}C NMR (acetone-d_6,150 MHz,) δ:185.0 (s,C-11),167.6 (s,CO_2CH_3),164.5 (s,C-13),159.0 (s,C-2),138.7 (d,C-9),133.7 (d,C-15),132.1 (d,C-10),125.0 (s,C-14),100.3 (d,C-12),96.5 (s,C-16),74.2 (s,C-8),62.7 (d,C-21),57.3 (s,C-7),50.9 (t,C-

5),50.5 (t,C-3),50.4 (q,CO_2CH_3),43.7 (s,C-20),36.6 (t,C-6),29.7 (t,C-17),26.6 (t,C-19),7.4 (q,C-18)。以上数据与文献[12]报道一致,故鉴定为 Melodinine M。

化合物 19 无色油状;ESI-MS:*m/z* 297 [M + H]$^+$;^{1}H NMR ($CDCl_3$,600 MHz) δ:7.29 (1H,d,*J* = 7.7 Hz,H-9),7.08 (1H,d,*J* = 7.7 Hz,H-12),6.93 (1H,t,*J* = 7.7 Hz,H-10),6.76 (1H,t,*J* = 7.7 Hz,H-11),4.07 (1H,t,*J* = 12.9 Hz,H-14),3.11 (1H,t,*J* = 12.9 Hz,H-15),1.10 (2H,m,H-19),0.71 (3H,d,*J* = 7.5 Hz,Me-18);^{13}C NMR ($CDCl_3$,150 MHz) δ:139.3 (s,C-2),135.5 (s,C-13),128.5 (s,C-8),120.6 (d,C-11),118.7 (d,C-10),117.7 (d,C-9),110.0 (d,C-12),109.7 (s,C-7),59.3 (d,C-15),58.5 (t,C-21),53.8(t,C-3),53.4 (t,C-5),52.3 (d,C-14),36.4 (t,C-17),33.6 (s,C-20),32.3 (t,C-19),26.0 (t,C-6),23.2 (t,C-16),7.3 (q,C-18)。以上数据与文献[16]报道一致,故鉴定为 Voaphyline。

化合物 20 黄色油状;ESI-MS:*m/z* 717 [M + H]$^+$;^{1}H NMR ($CDCl_3$,600 MHz) δ:6.92 (s,H-12,12′),6.39 (s,H-9,9′),5.75 (2H,dd,*J* = 10.0,3.5 Hz,H-14,14′),5.65 (2H,d,*J* = 10.0 Hz,H-15,15′),3.77 (2H,s,C-22,22′),2.58 (2H,m,C-5,5′),0.59 (6H,t,*J* = 7.5 Hz,Me-18,18′);^{13}C NMR ($CDCl_3$,150 MHz) δ:169.3 (s,CO_2CH_3),167.4 (s,C-2,2′),152.7 (s,C-11,11′),142.6 (s,C-13,13′),133.2 (d,C-15,15′),131.0 (s,C-8,8′),124.8 (d,C-14,14′),122.9 (d,C-9,9′),119.4 (d,C-10,10′),98.1 (d,C-12,12′),92.1 (s,C-16,16′),69.9 (d,C-21,21′),54.7 (s,C-7),51.2 (q,CO_2CH_3),50.9 (s,C-5,5′),50.4 (t,C-3,3′),44.4 (t,C-6,6′),41.3 (s,C-20,20′),30.3 (t,C-22,22′),28.7 (t,C-17,17′),27.0 (t,C-19,19′),7.5 (q,C-18,18′)。以上数据与文献[17]报道 Melofusine I 相吻合。

3.3 体外肿瘤细胞增殖抑制实验结果

采用 MTT 法对分离得到的 20 个化合物进行体外抑制肿瘤细胞增殖活性测试,结果表明化合物 **1**、**2**、**4**、**6**、**10** 和 **17** 对白血病细胞 HL-60,肝癌 SMMC7721,肺癌 A-549,乳腺癌 MCF-7 和结肠癌 SW480 具有增殖抑制活性,其中化合物 **2** 的增殖抑制活性更加显著,其 IC_{50} 值均小于阳性对照顺铂 (DDP),具体活性结果见表 1。其他的化合物没有抑制肿瘤细胞增殖活性(IC_{50} 值 >40 μM[18])。

表 1 化合物 1、2、4、6、10 和 17 的抗肿瘤活性

Table 1 Cytotoxic activities of compounds **1**,**2**,**4**,**6**,**10** and **17**

化合物 Compound	IC_{50} (μM)				
	HL-60	SMMC-7721	A-549	MCF-7	SW480
1	4.5	16.1	23.7	28.9	11.1
2	0.2	10.6	11.9	2.1	11.9
4	6.0	6.6	20.5	14.2	15.0
6	6.5	15.8	25.1	21.3	10.4
10	15.7	23.7	39.7	14.6	21.9
17	7.1	20.0	27.2	22.3	15.0
DDP	2.5	11.3	17.8	18.2	14.4

4 结论

本研究从薄叶山橙中分离鉴定了 20 个生物碱类化合物。其中包括 19 个单萜吲哚生物碱和一个双吲哚生物碱,化合物 **4**~**6**、**8**、**9**、**13**、**16** 和 **18**~**20** 为首次从该种植物中被分离得到报道。此外,化合物 **1**、**2**、**4**、**6**、**10** 和 **17** 具有一定的抑制肿瘤细胞增殖活性,化合物 **2** 的抑制肿瘤细胞增殖活性更加显著。在目前的山橙属植物研究中均已被报道。但本研究结果为山橙属植物的开发与利用提供了一定的依据,同时也为生物碱类化合物的药物开发利用提供了化学基础,为医药和天然产物开发提供了理论和基础。

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(上接第 1862 页)

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