景东山橙中生物碱类化学成分

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［摘要］ **目的**：夹竹桃科（Apocynaceae）山橙属（*Melodinus*）植物在我国民间应用的历史悠久，其生物碱类化学成分具有抗肿瘤、抗菌、抗生育等生物活性。该文对夹竹桃科山橙属植物景东山橙（*Melodinus khasianus*）中生物碱类化学成分进行系统研究。**方法**：运用硅胶柱色谱、反相硅胶柱色谱及高效液相制备色谱对景东山橙中生物碱类化学成分进行分离和纯化，并通过其理化性质及波谱数据确证生物碱类化合物的结构。通过噻唑蓝（MTT）比色法，对分离得到的化合物进行前列腺癌细胞（PC-3），肺癌细胞（A549），胃癌细胞（HGC-27）和人白血病细胞（HL-60）的细胞增殖抑制筛选。**结果**：从景东山橙乙醇提取物的三氯甲烷部位分离得到 12 个化合物，分别鉴定为（+）epi-16α-hydro-14, 15vincamine（**1**），16β, 21β-epoxy-vincadine（**2**），melodinines G（**3**），melodinines P（**4**），melodinines N（**5**），16β-hydroxy-19*S*-vindolinine（**6**），16β-hydroxy-19*R*-vindolinine（**7**），15α-hydroxy-14, 15-dihydrovindolinine（**8**），melodinines T（**9**），19-epimeloscandonine（**10**），门洛斯坎刀尼（**11**），攀援山橙碱（**12**）。**结论**：其中化合物**1**～**10** 为首次从景东山橙中分离得到的生物碱。采用 MTT 比色法对化合物进行体外抗肿瘤活性筛选，发现化合物 **4**，**5** 对前列腺癌细胞（PC-3），肺癌细胞（A549），胃癌细胞（HGC-27）和人白血病细胞（HL-60）等肿瘤细胞具有一定的细胞增殖抑制作用。

［关键词］ 山橙属；景东山橙；生物碱；细胞增殖抑制作用
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Chemical Constituents of Alkaloids from *Melodinus khasianus*

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［**Abstract**］ **Objective**: *Melodinus* (Apocynaceae) plants have been used in folks in China for a long time. Alkaloids from *Melodinus* genus have showed significant antitumor, antibacterial and antifertility activities. This study focuses on the chemical constituents of alkaloids from *M. khasianus*. **Method**: The compounds were isolated and purified by silica gel column chromatography, reversed-phase C_{18} column chromatography and preparative liquid chromatography. The compounds were screened out for prostate cancer cells (PC-3), lung cancer cells (A549), gastric cancer cells (HGC-27) and human leukemia cells (HL-60) by MTT method. **Result**: Their chemical structures were established by their physicochemical properties and spectral data. Twelve compounds were isolated and characterized from the chloroform fraction of ethanol extract in *M. khasianus*. Their

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structures were identified as (+) epi-16α-hydro-14, 15vincamine (**1**), 16β, 21β-epoxy-vincadine (**2**), melodinines G (**3**), melodinines P (**4**), melodinines N (**5**), 16β-hydroxy-19*S*-vindolinine (**6**), 16β-hydroxy-19*R*-vindolinine (**7**), 15α-hydroxy-14, 15-dihydrovindolinine (**8**), melodinines T (**9**), 19-epimeloscandonine (**10**), meloscandonine (**11**) and scandine (**12**). **Conclusion**: Compounds **1-10** were isolated from *M. khasianus* for the first time. The cytotoxicity of these compounds was evaluated by MTT method in vitro. Compounds **4** and **5** displayed an inhibitory effect on the proliferation of prostate cancer cells (PC-3), lung cancer cells (A549), gastric cancer cells (HGC-27) and human leukemia cells (HL-60).

[**Key words**] *Melodinus*; *Melodinus khasianus*; alkaloids; cytotoxicity

夹竹桃科山橙属植物为攀援木质藤本,在全球有 53 种,主要分布在亚洲热带、亚热带及大洋洲地区,我国产 11 种,云南、广西、贵州及台湾等省区为主要产地[1]。山橙属植物资源丰富,种类众多,在我国民间应用的历史悠久,常用于治疗小儿疝气、小儿疳疾、消化不良、腹痛、睾丸炎、小儿脑膜炎、骨折和风湿性心脏病等[2]。生物碱类成分是山橙属植物最重要的活性成分,其中 Tabersonine 型生物碱具有较好的活性[3],对多种肿瘤细胞具有显著的细胞毒性,尤其对人白血病细胞(HL-60),肺癌细胞(A549),肝癌细胞(SMMC-7721),乳腺癌细胞(MCF-7)和结肠癌细胞(SW480)等细胞的抑制作用比较明显[4]。景东山橙为木质藤本,生于海拔 1 600 ~ 2 900 m的山地或疏林潮湿地中,主要分布于我国云南省的澄江、大理、景东、镇康、耿马等地[5]。现阶段国内外对该植物化学成分及药理活性的研究还不深入,因此本文基于硅胶柱色谱、反相柱色谱、高效液相制备色谱等方法,对景东山橙中生物碱成分的分离及纯化做了系统性的研究,得到了 12 个生物碱类化合物,分别鉴定为(+)epi-16α-hydro-14, 15vincamine (**1**), 16β, 21β-epoxy-vincadine (**2**), melodinines G (**3**), melodinines P (**4**), melodinines N (**5**), 16β-hydroxy-19*S*-vindolinine (**6**), 16β-hydroxy-19*R*-vindolinine (**7**), l5α-hydroxy-14, 15 dihydro-vindolinine (**8**),为吲哚生物碱;melodinines T (**9**), 19-epimeloscandonine (**10**),门洛斯坎刀尼(**11**),攀援山橙碱(**12**)为喹啉生物碱,其中化合物 **1 ~ 10** 为首次从景东山橙中分离得到,并采用噻唑蓝(MTT)比色法对得到的化合物进行了体外抗肿瘤活性筛选,发现化合物 **4**,**5** 对多种肿瘤细胞株具有较好的细胞增殖抑制作用。为继续研究抗肿瘤先导药物提供了研究基础。

1 材料

Waters e2695-2998 型高效液相色谱系统[配备光电二极管阵列检测器(PDA),美国 Waters 公司];Welch C_{18}分析型色谱柱(4.6 mm × 250 mm, 5 μm);Shim-pack PREP-ODS 半制备型色谱柱(20 mm × 250 mm, 15 μm);色谱硅胶(青岛海洋化工厂);LiChroprep 反相硅胶(德国默克公司);Hei-VAPG3 型旋转蒸发仪(德国海道夫公司);INOVA-600 型核磁共振波谱仪(美国 Varian 公司);1100 Series 6320 ion-trap 型质谱仪(美国 Agilent 公司);BP221S 型 1/1 万电子天平(北京赛多利斯天平有限公司);甲醇、乙腈为色谱纯(瑞典 Oceanpak 公司);实验用水为娃哈哈纯净水。

景东山橙采集自云南普洱,经山东中医药大学李佳教授鉴定为夹竹桃科植物景东山橙 *Melodinus khasianus* 的茎。样品标本保存于山东中医药大学药学院药物化学教研室。

2 提取与分离

取景东山橙茎干燥粉末 20 kg(过 50 目筛),用 90% 乙醇室温回流提取 3 次(分别为 2, 2, 1.5 h),合并提取液减压浓缩得到总浸膏。浸膏加水充分混悬,用 1% 的 HCl 溶液调 pH 1 ~ 2,用石油醚萃取 3 次。水相用 10% 的氨水调 pH 9 ~ 10,三氯甲烷萃取 4 次后得景东山橙总生物碱 221 g。将总生物碱浸膏适量碱性硅胶拌样后,经硅胶柱(100 ~ 200 目)色谱分离,用三氯甲烷-甲醇(100∶1, 80∶1, 60∶1, 40∶1, 20∶1, 10∶1, 4∶1, 1∶1)梯度洗脱,用 TLC 检查,合并得到 23 个部位(Fr. 1 ~ Fr. 23)。Fr. 9 经高效液相制备分离得到化合物(**1**)34.2 mg,(**2**)2.7 mg,(**3**)19.1 mg,(**5**)251.2 mg,(**9**)6.8 mg,(**12**)1 460 mg。Fr. 14 经反相硅胶柱色谱,用甲醇-水洗脱,经高效液相制备分离得到化合物(**4**)8.8 mg,(**10**)45.1 mg,(**11**)36.1 mg。Fr. 19 经反相硅胶柱色谱,用甲醇-水(2∶8, 4∶6, 6∶4, 8∶2, 10∶0)梯度洗脱,得到 9 个部位(A1 ~ A9)。将 A3 用高效液相制备分离,得到了化合物(**6**)105.8 mg,(**7**)16.1 mg,(**8**)2.6 mg。

3 结构鉴定

化合物 **1** ESI-MS *m/z* 353.2 $[M + H]^+$; ^{1}H-NMR($CDCl_3$, 600 MHz) δ: 3.05 (1H, d, J = 7.2 Hz,

H-5a), 2.74(1H, d, J = 7.2 Hz, H-5b), 2.46(1H, d, J = 15.6, H-6a), 2.88(1H, dd, J = 15.6, 1.8 Hz, H-6b), 6.41(1H, d, J = 3.1 Hz, H-9), 7.13(1H, d, J = 8.4 Hz, H-10), 6.40(1H, d, J = 2.4 Hz, H-12), 5.80(1H, d, J = 10.2 Hz, H-14), 5.92(1H, dd, J = 10.2, 4.2 Hz, H-15), 3.21(1H, d, J = 16.2 Hz, H-17a), 3.46(1H, dd, J = 16.2, 4.8 Hz, H-17b), 0.89(3H, s, H-18), 3.35(1H, dd, J = 12.6, 6.0 Hz, H-21), 3.79(6H, s, H-22, H-23), 1.95(3H, s, $COCH_3$); ^{13}C-NMR ($CDCl_3$, 150 MHz) δ: 129.4(C-2), 50.9(C-3), 43.9(C-6), 130.0(C-7), 126.3(C-8), 105.3(C-9), 96.7(C-10), 166.8(C-11), 91.5(C-12), 160.1(C-13), 121.9(C-14), 144.2(C-15), 50.1(C-17), 17.3(C-18), 27.4(C-19), 46.4(C-20), 66.8(C-21), 51.1(C-22), 54.3(C-23), 168.6(C-24), 209.4(C = O)。得到 ESI-MS, ^{1}H-NMR 和 ^{13}C-NMR 数据与文献[6]报道基本一致,故鉴定化合物为(+)epi-16α-hydro-14,15vincamine。

化合物 **2** ESI-MS m/z 325.2 [M + H]$^+$; ^{1}H-NMR($CDCl_3$, 600 MHz) δ: 2.67(2H, m, H-3), 2.93(2H, m, H-5), 3.32(1H, m, H-6a), 2.95(1H, m, H-6b), 7.30(1H, d, J = 7.8 Hz, H-9), 6.99(1H, t, J = 7.8 Hz, H-10), 7.01(1H, t, J = 7.8 Hz, H-11), 7.45(1H, d, J = 7.8 Hz, H-12), 5.56(1H, s, H-14), 5.58(1H, s, H-15), 2.65(1H, d, J = 12 Hz, H-17a), 2.60(1H, d, J = 12 Hz, H-17b), 0.85(3H, t, J = 7.6 Hz, H-18), 1.54(1H, m, H-19a), 1.45(1H, m, H-19b), 4.29(1H, s, H-21), 3.76(1H, s, $COOCH_3$); ^{13}C-NMR ($CDCl_3$, 150 MHz) δ: 137.1(C-2), 53.2(C-3), 52.9(C-5), 24.8(C-6), 110.2(C-7), 128.5(C-8), 119.0(C-9), 119.6(C-10), 121.9(C-11), 111.9(C-12), 138.6(C-13), 125.9(C-14), 130.6(C-15), 86.5(C-16), 51.3(C-17), 9.8(C-18), 29.4(C-19), 51.1(C-20), 98.2(C-21), 173.3($COOCH_3$), 53.1($COOCH_3$)。得到 ESI-MS, ^{1}H-NMR 和 ^{13}C-NMR 数据与文献[7]报道基本一致,故鉴定化合物为 16β, 21β-epoxy-vincadine。

化合物 **3** ESI-MS m/z 295.1 [M + H]$^+$; ^{1}H-NMR($CDCl_3$, 600 MHz) δ: 3.40(1H, m, H-3a), 3.10(1H, d, J = 13.8 Hz, H-3b), 4.06(1H, m, H-5a), 3.87(1H, m, H-5b), 3.12(1H, m, H-6a), 3.06(1H, m, H-6b), 7.45(1H, d, J = 7.2 Hz, H-9), 7.18(1H, t, J = 7.2 Hz, H-10), 7.15(1H, t, J = 7.2 Hz, H-11), 7.26(1H, d, J = 7.2 Hz, H-12), 2.57(1H, m, H-14a), 1.78(1H, br d, J = 10.8 Hz, H-14b), 1.79(1H, br d, J = 10.2 Hz, H-15a), 1.66(1H, d, J = 10.2 Hz, H-15b), 7.10(1H, d, J = 7.8 Hz, H-16), 5.03(1H, d, J = 7.8 Hz, H-17), 1.01(1H, t, J = 7.8 Hz, H-18), 2.44(1H, q, J = 7.8 Hz, H-19a), 1.67(1H, q, J = 7.8 Hz, H-19b), 4.78(1H, s, H-21)。得到 ESI-MS, ^{1}H-NMR 数据与文献[8]报道基本一致,故鉴定化合物为 melodinines G。

化合物 **4** ESI-MS m/z 355.2 [M + H]$^+$; ^{1}H-NMR($CDCl_3$, 600 MHz) δ: 9.11(1H, s, H-1), 3.15(1H, d, J = 10.2 Hz, H-3a), 3.44(1H, ddd, J = 10.2, 4.8, 1.5 Hz, H-3b), 2.70(1H, m, H-5a), 3.00(1H, t, J = 7.8 Hz, H-5b), 1.77(1H, dd, J = 11.4, 3.6 Hz, H-6a), 2.99(1H, m, overlap, H-6b), 6.73(1H, d, J = 2.4 Hz, H-9), 6.65(1H, dd, J = 7.8 Hz, 2.4 Hz, H-11), 6.89(1H, d, J = 7.8 Hz, H-12), 5.74(1H, ddd, J = 9.6, 4.8, 1.5 Hz, H-14), 5.71(1H, d, J = 9.6 Hz, H-15), 2.41(1H, d, J = 15.0 Hz, H-17a), 2.55(1H, d, J = 15.0 Hz, H-17b), 0.62(1H, t, J = 7.8 Hz, H-18), 0.88(1H, m, H-19a), 0.96(1H, m, H-19b), 2.66(1H, s, H-21), 3.61(3H, s, $COOCH_3$); ^{13}C-NMR($CDCl_3$, 150 MHz) δ: 167.5(C-2), 51.6(C-3), 51.9(C-5), 45.1(C-6), 56.5(C-7), 141.7(C-8), 111.5(C-9), 153.1(C-10), 114.6(C-11), 11.3(C-12), 137.4(C-13), 125.4(C-14), 133.9(C-15), 92.1(C-16), 29.8(C-17), 7.1(C-18), 27.1(C-19), 43.6(C-20), 71.3(C-21), 168.4($COOCH_3$), 50.3($COOCH_3$)。得到 ESI-MS, ^{1}H-NMR 和 ^{13}C-NMR 数据与文献[9]报道基本一致,故鉴定化合物为 melodinines P。

化合物 **5** ESI-MS m/z 367.2 [M + H]$^+$; ^{1}H-NMR($CDCl_3$, 600 MHz) δ: 3.03(1H, s, H-3a), 3.45(1H, d, J = 13.2 Hz, H-3b), 2.63(1H, m, H-5a), 3.03(1H, overlap, H-5b), 1.63(1H, dd, J = 10.8, 3.6 Hz, H-6a), 1.93(1H, m, H-6b), 7.10(1H, s, H-9), 6.41(1H, s, H-12), 5.79(1H, dd, J = 9.0, 4.2 Hz, H-14), 5.71(1H, d, J = 9.0 Hz, H-15), 2.56(1H, d, J = 31.8 Hz, H-17a), 2.66(1H, d, J = 31.8 Hz, H-17b), 0.64(3H, t, J = 7.2 Hz, H-18), 0.78(1H, m, H-19a), 0.96(1H, m, H-19b), 2.56(1H, s, H-21), 2.08(1H, s, H-22), 1.95(3H, s, $COCH_3$); ^{13}C-NMR ($CDCl_3$, 150 MHz) δ: 168.9(C-2), 50.5(C-3), 50.9(C-5), 44.5(C-6), 55.5(C-7), 124.8(C-9), 120.7(C-10), 158.0(C-11), 97.6(C-12), 143.3(C-13), 124.8(C-14), 133.1(C-15), 92.0(C-16), 28.3(C-17), 7.4(C-18), 26.8(C-19), 41.3(C-20), 70.1(C-

21), 30.5 (C-22), 169.0 (C=O)。得到 ESI-MS, ^{1}H-NMR 和 ^{13}C-NMR 数据与文献[9]报道基本一致,故鉴定化合物为 melodinines N。

化合物 **6** ESI-MS *m/z* 353.2 [M+H]$^+$; ^{1}H-NMR ($CDCl_3$, 600 MHz) δ 4.10 (1H, dd, *J* = 4.8, 18.0 Hz, H-3a), 3.42 (1H, d, *J* = 17.4 Hz, H-3b), 3.77 (1H, m, H-5a), 3.23 (1H, dd, *J* = 10.2, 17.4 Hz, H-5b), 2.13 (1H, dd, *J* = 15.6, 6.6 Hz, H-6a), 1.94 (1H, m, H-6b), 7.29 (1H, d, *J* = 7.2 Hz, H-9), 6.85 (1H, d, *J* = 7.8 Hz, H-10), 7.05 (1H, t, *J* = 7.8 Hz, H-11), 6.72 (1H, d, *J* = 7.2 Hz, H-12), 5.82 (1H, m, H-14), 6.24 (1H, dd, *J* = 3.0 Hz, 9.6 Hz, H-15), 2.93 (1H, d, *J* = 15.0 Hz, H-17a), 1.62 (1H, d, *J* = 15.0 Hz, H-17b), 0.60 (1H, d, *J* = 7.2 Hz, H-18), 2.93 (1H, q, *J* = 7.2 Hz, H-19), 2.71 (1H, s, H-21), 3.76 (3H, s, OCH_3); ^{13}C-NMR ($CDCl_3$, 150 MHz) δ 48.9 (C-3), 56.9 (C-5), 41.2 (C-6), 57.9 (C-7), 136.5 (C-8), 123.1 (C-9), 121.4 (C-10), 128.2 (C-11), 111.0 (C-12), 148.6 (C-13), 127.7 (C-14), 131.1 (C-15), 84.2 (C-16), 36.2 (C-17), 9.8 (C-18), 51.1 (C-19), 43.3 (C-20), 72.5 (C-21), 172.8 ($COOCH_3$)。得到 ESI-MS, ^{1}H-NMR 和 ^{13}C-NMR 数据与文献[10]报道基本一致,故鉴定化合物为 16β-hydroxy-19*S*-vindolinine。

化合物 **7** ESI-MS *m/z* 353.2 [M+H]$^+$; ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 4.06 (1H, dd, *J* = 4.8, 18.0 Hz, H-3a), 3.50 (1H, d, *J* = 17.4 Hz, H-3b), 3.38 (1H, m, H-5a), 3.22 (1H, m, H-5b), 2.01 (1H, dd, *J* = 15.6, 6.6 Hz, H-6a), 1.87 (1H, m, H-6b), 7.19 (1H, d, *J* = 7.2 Hz, H-9), 6.85 (1H, d, *J* = 7.8 Hz, H-10), 7.06 (1H, t, *J* = 7.2, 7.8 Hz, H-11), 6.72 (1H, d, *J* = 7.2 Hz, H-12), 5.83 (1H, m, H-14), 6.23 (1H, dd, *J* = 4.2, 9.6 Hz, H-15), 2.78 (1H, dd, *J* = 6.6, 12 Hz, H-17a), 1.98 (1H, d, *J* = 12.0 Hz, H-17b), 1.12 (1H, d, *J* = 6.6 Hz, H-18), 2.22 (1H, q, *J* = 5.4 Hz, H-19), 2.15 (1H, s, H-21), 3.60 (3H, s, OCH_3)。得到 ESI-MS, ^{1}H-NMR 数据与文献[10]报道基本一致,故鉴定化合物为 16β-hydroxy-19*R*-vindolinine。

化合物 **8** ESI-MS *m/z* 355.2 [M+H]$^+$; ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 3.20 (1H, m, H-3a), 3.59 (1H, m, H-3b), 3.10 (1H, m, H-5a), 3.50 (1H, m, H-5b), 2.26 (1H, dd, *J* = 7.2 Hz, 14.4 Hz, H-6a), 1.64 (1H, d, *J* = 14.4 Hz, H-6b), 7.64 (1H, d, *J* = 7.2 Hz, H-9), 7.14 (1H, t, *J* = 7.8 Hz, H-10), 6.80 (1H, t, *J* = 7.8 Hz, H-11), 6.70 (1H, d, *J* = 7.8 Hz, H-12), 1.57 (1H, m, H-14a), 2.16 (1H, m, H-14b), 3.94 (1H, t, *J* = 9.0 Hz, H-15), 3.30 (1H, dd, *J* = 24.0, 12.0 Hz, H-16), 1.86 (1H, m, H-17a), 2.70 (1H, m, H-17b), 1.04 (1H, d, *J* = 6.6 Hz, H-18), 2.06 (1H, q, *J* = 7.2 Hz, H-19), 3.80 (1H, s, H-21)。得到 ESI-MS, ^{1}H-NMR 数据与文献[11]报道基本一致,故鉴定化合物为 15α-hydroxy-14, 15-dihydro-vindolinine。

化合物 **9** ESI-MS *m/z* 367.2 [M+H]$^+$; ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 7.90 (1H, s, H-1), 3.28 (1H, m, H-3), 3.10 (1H, dd, *J* = 13.0, 5.5 Hz, H-5a), 3.17 (1H, m, H-5b), 1.99 (1H, m, H-6a), 2.53 (1H, m, H-6b), 7.41 (1H, d, *J* = 7.8 Hz, H-9), 7.08 (1H, t, *J* = 7.8 Hz, H-10), 7.18 (1H, t, *J* = 7.8 Hz, H-11), 6.81 (1H, d, *J* = 7.8 Hz, H-12), 5.94 (1H, dd, *J* = 10.2, 3.6 Hz, H-14), 5.98 (1H, d, *J* = 10.2 Hz, H-15), 2.50 (1H, d, *J* = 13.8 Hz, H-17a), 3.37 (1H, d, *J* = 13.8 Hz, H-17b), 2.24 (1H, s, H-18), 4.20 (1H, s, H-21), 3.56 (3H, s, $OOCCH_3$); ^{13}C-NMR ($CDCl_3$, 150 MHz) δ: 166.1 (C-2), 46.3 (C-3), 52.3 (C-5), 35.9 (C-6), 58.6 (C-7), 128.5 (C-8), 125.9 (C-9), 123.8 (C-10), 127.4 (C-11), 114.9 (C-12), 134.1 (C-13), 127.1 (C-14), 126.9 (C-15), 62.1 (C-16), 41.1 (C-17), 25.6 (C-18), 205.6 (C-19), 55.1 (C-20), 73.9 (C-21), 169.5 ($COOCH_3$), 53.0 ($OOCCH_3$)。得到 ESI-MS, ^{1}H-NMR 和 ^{13}C-NMR 数据与文献[9]报道基本一致,故鉴定化合物为 melodinines T。

化合物 **10** ESI-MS *m/z* 321.2 [M+H]$^+$; ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 7.93 (1H, s, H-1), 3.80 (1H, d, *J* = 18.6 Hz, H-3a), 3.36 (1H, dd, *J* = 18.6, 3.0 Hz, H-3b), 3.28 (1H, dd, *J* = 9.0, 1.2 Hz, H-5a), 3.09 (1H, dd, *J* = 9.0, 7.8 Hz, H-5b), 7.18 (1H, d, *J* = 7.5 Hz, H-9), 7.0 (1H, d, *J* = 7.5 Hz, H-10), 7.17 (1H, d, *J* = 7.5 Hz, H-11), 5.90 (1H, m, H-14), 5.92 (1H, m, H-15), 0.90 (1H, d, *J* = 7.2 Hz, H-18), 3.56 (1H, s, H-21); ^{13}C-NMR ($CDCl_3$, 150 MHz) δ: 168.6 (C-2), 47.1 (C-3), 54.8 (C-5), 39.3 (C-6), 56.7 (C-7), 130.2 (C-8), 124.0 (C-9), 123.8 (C-10), 128.2 (C-11), 116.5 (C-12), 136.6 (C-13), 125.8 (C-14), 127.8 (C-15), 67.7 (C-16), 38.5 (C-17), 8.6 (C-18), 52.9 (C-19), 45.6 (C-20), 61.6 (C-21), 209.6 (C=O)。得到 ESI-MS, ^{1}H-NMR 和 ^{13}C-NMR 数据与文献[10]报道基本一致,故鉴定化合物为 19-epimeloscandonine。

化合物 **11** ESI-MS *m/z* 321.2 $[M+H]^+$; ^{1}H-NMR($CDCl_3$,600 MHz)δ: 3.80(1H,d,*J* = 1.8 Hz,H-3a),3.39(1H,dd,*J* = 1.8,4.8 Hz,H-3b),3.27(1H,m,H-5a),3.13(1H,m,H-5b),2.39 ~ 1.76(5H,m,H-6,H-7,H-19),7.22(1H,d,*J* = 7.8 Hz,H-9),6.99(1H,t,*J* = 7.7 Hz,H-10),7.14(1H,t,*J* = 7.7 Hz,H-11),6.84(1H,d,*J* = 7.8 Hz,H-12),5.90 ~ 5.98(2H,m,H-14,H-15),1.13(1H,d,*J* = 7.2 Hz,H-18),3.36(1H,s,H-21); ^{13}C-NMR($CDCl_3$,150 MHz)δ: 168.9(C-2),47.2(C-3),54.8(C-5),38.0(C-6),54.9(C-7),130.5(C-8),124.0(C-9),123.9(C-10),128.0(C-11),116.2(C-12),136.3(C-13),124.1(C-14),127.4(C-15),67.8(C-16),35.9(C-17),11.1(C-18),51.0(C-19),44.5(C-20),69.8(C-21),210.1(C = O)。得到 ESI-MS,^{1}H-NMR 和 ^{13}C-NMR 数据与文献[12]报道基本一致,故鉴定化合物为 meloscandonine。

化合物 **12** ESI-MS *m/z* 351.2 $[M+H]^+$; ^{1}H-NMR($CDCl_3$,600 MHz)δ: 3.68(1H,m,H-3a),3.32(1H,dd,*J* = 18.6,9.0 Hz,H-3b),3.65(1H,m,H-5a),3.59(1H,m,H-5a),3.90(1H,m,H-6a),3.80(1H,m,H-6b),7.81(1H,d,*J* = 4.8 Hz,H-9),7.21(1H,d,*J* = 7.2 Hz),7.26(1H,d,*J* = 7.8 Hz,H-11),6.88(1H,d,*J* = 7.8 Hz,H-12),5.75(1H,m,H-14),5.93(1H,d,*J* = 10.2 Hz,H-15),3.12(1H,d,*J* = 13.8 Hz,H-17a),2.63(1H,d,*J* = 13.8 Hz,H-17b),5.77(1H,m,H-18a),5.02(1H,dd,*J* = 17.4,10.8 Hz,H-18b),5.65(1H,dd,*J* = 17.4,10.8 Hz,H-19),2.88(1H,s,H-21); ^{13}C-NMR($CDCl_3$,150 MHz)δ: 167.1(C-2),45.2(C-3),53.2(C-5),35.2(C-6),59.2(C-7),128.0(C-8),129.0(C-9),124.5(C-10),132.6(C-11),115.8(C-12),134.5(C-13),122.3(C-14),131.1(C-15),62.3(C-16),44.1(C-17),115.4(C-18),140.3(C-19),45.8(C-20),78.9(C-21),169.0(C = O),52.8(OCH_3)。得到 ESI-MS,^{1}H-NMR 和 ^{13}C-NMR 数据与文献[12]报道基本一致,故鉴定化合物为 Scandine。

4 抗肿瘤活性

采用 MTT 比色法对已得到的化合物进行抗肿瘤活性筛选,将对数生长期的肿瘤细胞用 10% 牛胎血清的 RPMI 1640 培养基配制成 1×10^4 个/mL 的细胞悬液,接种于 96 孔板,每一个孔 100 μL,置 37 ℃,5% CO_2 温箱内培养 24 h 后加入不同浓度的样品 100 μL,样品设 0.1,1,10 mg·L^{-1} 共 3 个质量浓度,每个质量浓度 3 个平行组,对照组中添加等体积溶剂。37 ℃ 5% CO_2 温箱内培养 4 d,去除培养液,每孔加入 MTT 溶液(0.4 g·L^{-1},RPMI 1640 配制)100 μL,37 ℃孵育 4 h。去除上清液,每孔加入 DMSO 150 μL,溶解 Fomazan 颗粒,经轻度振荡后,用 550 型酶标仪在检测波长 540 nm,参考 405 nm 下测定含量。实验得出化合物 **4** 对前列腺癌细胞(PC-3),肺癌细胞(A549),胃癌细胞(HGC-27)和人白血病细胞(HL-60)的半数抑制浓度(IC_{50})分别为 10.12×10^{-6},12.13×10^{-6},11.87×10^{-6},12.73×10^{-6} mol·L^{-1};化合物 **5** 的 IC_{50} 分别为 12.26×10^{-6},12.87×10^{-6},14.13×10^{-6},13.22×10^{-6} mol·L^{-1}。结果表明化合物 **4**,**5** 对肿瘤细胞具有一定的抑制活性。

[参考文献]

[1] FENG T,LI Y,LIU Y P,et al. Melotenine A,a cytotoxic monoterpenoid indole alkaloid from *Melodinus tenuicaudatus*[J]. Org Lett,2010,12(5):968-971.
[2] 中国科学院《中国植物志》编辑委员会. 中国植物志. 第 63 卷. 第 1 册[M]. 北京:科学出版社,2004,63:24-25.
[3] 刘波,刘书剑,李玉擒,等. 狗牙花生物碱成分分析[J]. 中国实验方剂学杂志,2017,23(17):76-80.
[4] FENG T,CAI X H,LI Y,et al. Melohenines A and B,two unprecedented alkaloids from *Melodinus henryi*[J]. Org Lett,2009,11(21):4834-4837.
[5] SHAO S,ZHANG H,YUAN C M,et al. Cytotoxic indole alkaloids from the fruits of *Melodinus cochinchinensis*[J]. Phytochemistry,2015,116(1):367-373.
[6] Baassou S,Mehri H,Plat M. Alcaloideds de *Melodinus aeneus*[J]. Phytochemistry,1978,17(8):1449-1450.
[7] CAI X H,LI Y,LIU Y P,et al. Alkaloids from *Melodinus yunnanensis*[J]. Phytochemistry,2012,83(11):116-124.
[8] FENG T,CAI X H,LIU Y P,et al. Melodinines A-G,monoterpenoid indole alkaloids from *Melodinus henryi*[J]. J Nat Prod,2010,73(1):22-26.
[9] LIU Y P,LI Y,CAI X H,et al. Melodinines M-U,cytotoxic alkaloids from *Melodinus suaveolens*[J]. J Nat Prod,2012,75(2):220-224.
[10] GUO L W,ZHOU Y L. Alkaloids from *Melodinus hemsleyanus*[J]. Phytochemistry,1993,34(2):563-566.
[11] HE Y L,CHEN W M,FENG X Z. The alkaloids of *Melodinus morsei*[J]. Phytochemistry,1994,37(4):1055-1057.
[12] 王世平,李玲,吴艳俊,等. 雷打果中生物碱成分的研究[J]. 时珍国医国药,2011,22(10):2356-2357.

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