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思茅山橙根中生物碱类成分及其抗肿瘤活性研究

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摘　要:采用多种色谱法从思茅山橙根部分的生物碱粗提物分离得到 10 个生物碱类化合物,利用波谱等方法对其结构进行鉴定,包括 3 种不同的生物碱骨架结构,分别为喹啉型生物碱:melodinhenine D(**1**)、melaxilline A(**2**)、11-methoxytabersonine(**3**)、meloscandonine(**4**)、melodinine T(**5**);白坚木型生物碱:venalstonidine(**6**)、vandrikidine(**7**);长春曼胺型生物碱:14,15-dehydrovincine(**8**)、14,15-dehydro-16-*epi*-vincamine(**9**)、△^{14}-vincanol(**10**)。除化合物 **1**,**5** 和 **8** 外,其余化合物均首次从思茅山橙中分离得到。采用 MTT 法对分离所得的化合物进行体外抗人乳腺癌细胞 MCF-7 活性测试,结果显示化合物 **3** 表现出显著的细胞毒活性,其 IC_{50} 值为 23.4 μM。

关键词:夹竹桃科;思茅山橙;11-methoxytabersonine;细胞毒活性;MCF-7

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Chemical Constituents and Antitumor Activity of *Melodinus henryi* Roots

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Abstract: Ten alkaloids were isolated from the roots of *Melodinus henryi* by a combination of various chromatographic techniques, including column chromatography over silica gel, pH-zone-refining high-speed counter-current chromatography, and reversed-phase HPLC, etc. The structures of these alkaloids were determined on the basis of spectroscopic analysis including MS and NMR data, which were divided into three types, including quinine-type alkaloids: melodinhenine D (**1**), melaxilline A (**2**), **11**-methoxytabersonine (**3**), meloscandonine (**4**), melodinine T (**5**); aspidospermidine-type alkaloids: venalstonidine (**6**), vandrikidine (**7**); vincadine-type alkaloids: 14,15-dehydrovincine (**8**), 14,15-dehydro-16-*epi*-vincamine (**9**), △^{14}-vincanol (**10**). All the compounds except **1**, **5** and **8** were isolated from *Melodinus henryi* for the first time. All the compounds were tested for their cytotoxicities against human breast cancer MCF-7 cells using the MTT method, and compound **3** exhibited significant cytotoxic effect with IC_{50} value of 23.4 μM.

Key words: Apocynaceae; *Melodinus henryi*; 11-methoxytabersonine; cytotoxicity; MCF-7

思茅山橙 *Melodinus henryi* Craib 为夹竹桃科(Apocynaceae)山橙属(*Melodinus*)植物,广泛分布于中印半岛、马来西亚西部、喜马拉雅山脉东部以及我国的云南南部。其成熟果实可食,亦可以作为药用,主要治疗小儿脑膜炎、骨折等[1]。目前国内外对思茅山橙的研究报道主要集中在其果实、叶、茎部位化学成分的研究[2-6],还未有对思茅山橙根部位化学成分的系统研究。为探索思茅山橙根中所含活性成分,扩大思茅山橙药用部位,本实验对采自云南的思茅山橙根部分进行系统的化学成分研究,从中分离得到 10 个生物碱类化合物,包括 3 种不同类型的生物碱骨架结构,分别为喹啉型生物碱:melodinhenine D(**1**)、melaxilline A(**2**)、11-methoxytabersonine(**3**)、meloscandonine(**4**)、melodinine T(**5**);白坚木型生物碱:venalstonidine(**6**)、vandrikidine(**7**);长春曼胺型生物碱:14,15-dehydrovincine(**8**)、14,15-dehydro-16-*epi*-vincamine(**9**)、△^{14}-vincanol(**10**)。化合物 **2**~**4**,**6**~**7**,**9**~**10** 为首次从该植物中分离得到,其中化合

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物 **3** 对人乳腺癌细胞 MCF-7 具有显著的细胞毒活性,IC_{50}值为 23.4 μM。

1　仪器与材料

Bruker-400 核磁共振波谱仪(布鲁克公司,瑞士);Varian INOVA-600 核磁共振波谱仪(美国 Varian 公司);pH 区带高速逆流色谱(上海同田生物技术股份有限公司);半制备高效液相色谱仪(普源 L-3000);3111 型 CO_2 培养箱(美国 Thermo 公司);Enspire 全波长酶标仪(美国 Perkins Elmer 公司);OLYMPUS-CK 光学显微镜(日本 OLYMPUS 公司);96 孔细胞培养板(美国 Corning 公司产品);薄层硅胶 GF_{254},柱色谱硅胶(200 ~ 300 目)(青岛海洋化工厂);碳酸铵(天津光复精细化工研究所);半制备高效液相色谱用乙腈为色谱纯(美国 Tedia 公司),其余试剂均为分析纯;人乳腺癌细胞 MCF-7 细胞株(中国科学院上海细胞生化所)。

思茅山橙的根部位药材采于云南省,经山东中医药大学周凤琴教授鉴定为夹竹桃科(Apocynaceae)山橙属(*Melodinus*)植物思茅山橙 *Melodinus henryi* Craib 的根。

2　提取与分离

20 kg 干燥的思茅山橙根,粗粉后用 95% 乙醇回流提取 3 次,每次 2 h,乙醇提取液减压浓缩至无醇味,得浸膏 1.96 kg,加适量水溶解成混悬液,用石油醚萃取至澄清后加入浓盐酸调节 pH = 2,用乙酸乙酯萃取至澄清,然后用氨水调节 pH = 10,用氯仿萃取 6 次并合并,氯仿相回收溶剂后得生物碱粗提物。氯仿相(234 g)采用硅胶柱进行分离,以二氯甲烷-甲醇(100∶0、50∶1、25∶1、15∶1、10∶1、5∶1、3∶1、1∶1)梯度洗脱,经薄层色谱检识合并得 16 个组分(Fr. 1 ~ 16)。Fr. 1 采用 pH 区带高速逆流色谱进行分离(石油醚-乙酸乙酯-甲醇-水(5∶5∶1∶9,v/v),将各溶剂按比例加于分液漏斗中,静置分层,分出上下相,在上相加入 10 mmol/L 的三乙胺作固定相,下相加入 10 mmol/L 的盐酸作流动相),得到化合物 **1**(0.5 g)及其他流份,合并后利用半制备高效液相色谱(55% 乙腈-5 mmol/L 碳酸铵)得到化合物 **3**(7 mg),**6**(23.5 mg)和 **7**(5 mg),各化合物的制备溶液在 60 ℃加热减压条件下蒸干,其中所含碳酸铵在此过程中分解而除去;Fr. 5 经中压-Flash 液相以甲醇-水(55%、65%、75%、85%、100%)进行洗脱,分别得到 5 个组分(Fr. 5-1 ~ 5)。Fr. 5-2(甲醇-水 = 65%)经半制备高效液相色谱(48% 乙腈-5mmol/L 碳酸铵)得到化合物 **8**(24 mg),制备溶液蒸干条件同上。Fr. 7 经中压-Flash 液相以甲醇-水(40%、60%、70%、80%、100%)进行洗脱,得到 5 个组分(Fr. 7-1 ~ 5)。Fr. 7-1(甲醇-水 = 40%)经半制备高效液相色谱(55% 乙腈-5 mmol/L 碳酸铵)得到化合物 **9**(14.9 mg)和 **10**(36.9 mg),制备溶液蒸干条件同上;Fr. 7-2(甲醇-水 = 60%)经半制备高效液相色谱(48% 乙腈-5 mmol/L 碳酸铵),得到化合物 **2**(17.4 mg)、**4**(54.3 mg)和 **5**(14.6 mg),制备溶液蒸干条件同上。

3　结果

3.1　结构鉴定

化合物 1　白色结晶(CH_2Cl_2);ESI-MS: *m/z* 351.2 $[M+H]^+$($C_{21}H_{22}N_2O_3$);^{1}H NMR (Methanol-d_4, 600 MHz) δ: 7.43 (1H, d, J = 7.6 Hz, H-9), 7.35 (1H, t, J = 7.6 Hz, H-11), 7.17 (1H, t, J = 7.6 Hz, H-10), 6.96 (1H, d, J = 7.6 Hz, H-12), 6.12 (1H, d, J = 9.6 Hz, H-15), 5.85 (1H, m, H-14), 5.82 (1H, m, H-19), 5.17 (1H, m, H-18b), 5.03 (1H, m, H-18a), 4.06 (1H, s, H-21), 3.57 (3H, s, -COOMe), 3.71 (1H, m, H-3a), 3.66 (1H, m, H-3a), 3.63 (1H, m, H-5a), 3.03 (1H, m, H-5b), 2.77 (1H, overlapped, H-6b), 2.77 (1H, overlapped, H-17b), 2.34 (1H, m, H-6a), 2.16 (1H, m, H-17a); ^{13}C NMR (Methanol-d_4, 150 MHz) δ: 168.1 (-COOMe), 169.5 (C-2), 141.7 (C-19), 137.4 (C-13), 135.4 (C-15), 130.1 (C-11), 127.1 (C-9), 125.1 (C-8, 10), 117.0 (C-12), 116.6 (C-14), 115.2 (C-18), 80.0 (C-21), 62.3 (C-16), 60.8 (C-7), 54.3 (C-5), 53.0 (-COOMe), 46.7 (C-3), 45.5 (C-20), 44.1 (C-17), 33.5 (C-6)。以上波谱数据与文献[5]报道一致,故鉴定化合物 **1** 为 Melodinhenine D。

化合物 2　白色无定型粉末;ESI-MS: *m/z* 375.4 $[M+Na]^+$($C_{21}H_{20}N_2O_4$);^{1}H NMR ($CDCl_3$, 600 MHz) δ: 8.74 (1H, s, -NH), 7.56 (1H, d, J = 7.2 Hz, H-9), 7.23 (1H, t, J = 7.2 Hz, H-11), 7.16 (1H, t, J = 7.2 Hz, H-10), 6.87 (1H, d, J = 7.2 Hz, H-12), 6.17 (1H, m, H-14), 5.86 (1H, d, J = 9.6 Hz, H-15), 4.29 (1H, s, H-21), 3.56 (3H, s, -

COOMe),3.20 (2H,m,H-3),3.13 (1H,m,H-5a),3.04 (1H,m,H-5b),2.83 (1H,dd,J = 12.6,7.2 Hz,H-16),2.43 (1H,q,J = 7.2 Hz,H-19),2.14 (1H,dd,J = 12.6,7.2 Hz,H-17a),2.08 (1H,m,H-6a),2.01 (1H,t,J = 12.6 Hz,H-17b),1.86 (1H,m,H-6b),2.37 (3H,q,J = 7.2 Hz,H-19);^{13}C NMR ($CDCl_3$,150 MHz) δ:174.5 (-COOMe),171.4 (C-2),134.8 (C-13),131.9 (C-15),129.3 (C-14),127.8 (C-9),127.7 (C-11),126.5 (C-8),124.4 (C-10),115.6 (C-12),76.3 (C-21),58.1 (C-7),53.0 (C-5),51.4 (-COOMe),48.9 (C-16),47.5 (C-19),46.1 (C-20),45.3 (C-3),42.0 (C-17),40.1 (C-6),12.9 (C-18)。以上波谱数据与文献[7]报道一致,故鉴定化合物**2**为 Melaxilline A。

化合物 3 白色无定型粉末;ESI-MS: m/z 367.9 $[M+H]^+$ ($C_{22}H_{26}N_2O_3$);^{1}H NMR (DMSO-d_6,400 MHz) δ:8.97 (1H,s,-NH),7.10 (1H,d,J = 8.2 Hz,H-9),6.39 (1H,dd,J = 8.2,2.2 Hz,H-10),6.41 (1H,d,J = 2.2 Hz,H-12),5.80 (1H,m,H-14),5.72 (1H,d,J = 9.9 Hz,H-15),3.72 (3H,s,-COOMe),2.62 (1H,s,H-21),0.65 (3H,t,J = 7.5 Hz,H-18);^{13}C NMR (DMSO-d_6,100 MHz) δ:169.0 (-COOMe),167.2 (C-2),160.0 (C-11),144.4 (C-13),133.1 (C-15),130.5 (C-8),124.9 (C-14),121.8 (C-9),105.0 (C-10),96.7 (C-12),92.4 (C-16),70.2 (C-21),54.5 (C-7),51.0 (-COOMe),50.9 (C-5),50.6 (C-3),44.6 (C-6),41.4 (C-20),28.4 (C-17),26.9 (C-19),7.51 (C-18)。以上数据与文献[8]报道一致,故鉴定化合物**3**为 11-Methoxytabersonine。

化合物 4 白色无定型粉末;ESI-MS: m/z 319.3 $[M-H]^-$ ($C_{20}H_{20}N_2O_2$);^{1}H NMR ($CDCl_3$,600 MHz) δ:8.30 (1H,s,-NH),7.22 (1H,d,J = 7.5 Hz,H-9),7.14 (1H,t,J = 7.6 Hz,H-11),6.98 (1H,t,J = 7.6 Hz,H-10),6.84 (1H,d,J = 7.8 Hz,H-12),5.91-5.98 (2H,m,H-14,H-15),3.80 (1H,d,J = 18.7 Hz,H-3a),3.39 (1H,dd,J = 18.7,3.0 Hz,H-3b),3.36 (1H,s,H-21),3.27 (1H,m,H-5a),3.13 (1H,m,H-5b),1.75-2.39 (5H,m,H-6,H-17,H-19),1.13 (3H,d,J = 7.0 Hz,H-18);^{13}C NMR ($CDCl_3$,150 MHz) δ:210.1 (C = O),168.9 (C-2),136.2 (C-13),130.4 (C-8),128.0 (C-11),127.5 (C-15),124.2 (C-14),124.0 (C-9),123.8 (C-10),116.3 (C-12),67.8 (C-16),54.7 (C-5),51.1 (C-19),47.3 (C-3),44.5 (C-20),38.5 (C-6),36.0 (C-17),11.2 (C-18)。以上数据与文献[9]报道一致,故鉴定化合物**4**为 Meloscandonine。

化合物 5 白色无定型粉末;ESI-MS: m/z 389.5 $[M+Na]^+$ ($C_{21}H_{22}N_2O_4$);^{1}H NMR ($CDCl_3$,600 MHz) δ:7.81 (1H,s,-NH),7.42 (1H,d,J = 7.8 Hz,H-9),7.18 (1H,t,J = 7.8 Hz,H-11),7.09 (1H,t,J = 7.8 Hz,H-10),6.82 (1H,d,J = 7.8 Hz,H-12),6.00 (1H,d,J = 10.0 Hz,H-15),5.93 (1H,dd,J = 10.2,3.6 Hz,H-14),4.20 (1H,s,H-21),3.56 (3H,s,-COOMe),3.38 (1H,m,H-3a),3.37 (1H,d,J = 13.8 Hz,H-17b),3.20 (1H,dd,J = 17.2,5.6 Hz,H-3b),3.18 (1H,m,H-5b),3.10 (1H,dd,J = 16.8,9.0 Hz,H-5a),2.52 (1H,m,H-6b),2.51 (1H,d,J = 13.8 Hz,H-17a),2.24 (3H,s,H-18),1.99 (1H,m,H-6a);^{13}C NMR ($CDCl_3$,150 MHz) δ:205.4 (C-19),170.0 (-COOMe),166.1 (C-2),134.0 (C-13),128.5 (C-8),127.1 (C-14),127.4 (C-11),126.7 (C-15),126.0 (C-9),123.8 (C-10),115.0 (C-12),73.9 (C-21),62.1 (C-16),58.7 (C-7),55.1 (C-20),53.0 (-COOMe),52.3 (C-5),46.3 (C-3),41.3 (C-17),36.0 (C-6),24.5 (C-18)。以上数据与文献[10]报道一致,故鉴定化合物**5**为 Melodinine T。

化合物 6 白色无定型粉末;ESI-MS: m/z 375.4 $[M+Na]^+$ ($C_{21}H_{24}N_2O_3$);^{1}H NMR (DMSO-d_6,400 MHz) δ:6.92 (2H,overlapped,H-9,11),6.58 (2H,overlapped,H-10,12),5.42 (1H,s,-NH),3.65 (3H,s,-COOMe),3.43 (1H,dd,J = 12.6,5.2 Hz,H-3a),3.02 (1H,d,J = 12.6 Hz,H-3b),2.75 (1H,t,J = 8.4 Hz,H-5a),2.50 (1H,overlapped,H-5b),2.42 (1H,m,H-6a),0.88 (1H,m,H-6b),3.33 (1H,overlapped,H-14),2.97 (1H,t,J = 9.6 Hz,H-16),2.79 (1H,d,J = 4.0 Hz,H-15),2.64 (1H,m,H-17a),2.45 (1H,s,H-21),1.68 (1H,m,H-19a),1.54 (1H,m,H-18a),1.51 (1H,m,H-17b),1.40 (1H,m,H-19b),1.28 (1H,m,H-18b);^{13}C NMR (DMSO-d_6,100 MHz) δ:174.6 (-COOMe),150.7 (C-13),139.2 (C-8),127.0 (C-11),121.0 (C-9),118.1 (C-10),110.6 (C-12),67.1 (C-2),62.2 (C-21),57.6 (C-15),55.6 (C-7),53.2 (C-14),52.0 (-COOMe),49.2 (C-5),

48.2 (C-3), 43.2 (C-16), 36.4 (C-6), 35.6 (C-20), 32.7 (C-18), 27.6 (C-17), 27.0 (C-19)。以上数据与文献[11]报道一致,故鉴定化合物 **6** 为 Venalstonidine。

化合物 7　白色无定型粉末; ESI-MS: *m/z* 381.2 $[M-H]^-$ ($C_{22}H_{26}N_2O_4$); ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 9.56 (1H, s, -NH), 7.13 (1H, d, J = 8.0 Hz, H-9), 6.70 (1H, d, J = 2.0 Hz, H-12), 6.35 (1H, dd, J = 8.0, 2.0 Hz, H-10), 5.82 (1H, m, H-14), 5.77 (1H, d, J = 10.0 Hz, H-15), 3.71 (3H, s, OMe-C11), 3.66 s (3H, s, -COOMe), 3.38 (1H, m, H-5b), 3.13 (1H, m, H-5a), 3.12 (1H, m, H-19), 2.95 (1H, m, H-3a), 2.92 (1H, m, H-17a), 2.65 (1H, s, H-21), 2.50 (1H, m, H-3b), 2.27 (1H, d, J = 14.4 Hz, H-17b), 1.87 (1H, m, H-6a), 1.60 (1H, m, H-6b), 0.74 (3H, d, J = 6.4 Hz, H-18); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 167.9 (-COOMe), 166.1 (C-2), 159.9 (C-11), 145.5 (C-13), 130.6 (C-15), 130.0 (C-8), 126.3 (C-14), 121.7 (C-9), 105.1 (C-10), 97.6 (C-12), 91.8 (C-16), 66.7 (C-21), 64.5 (C-19), 55.5 (OMe-C11), 54.7 (C-7), 50.9 (-COOMe), 50.63 (C-5), 50.64 (C-3), 46.6 (C-20), 45.7 (C-6), 27.9 (C-17), 19.2 (C-18)。以上数据与文献[12]报道一致,故鉴定化合物 **7** 为 Vandrikidine。

化合物 8　白色无定型粉末; ESI-MS: *m/z* 383.8 $[M+H]^+$ ($C_{22}H_{26}N_2O_4$); ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.24 (1H, d, J = 8.4 Hz, H-9), 6.67 (1H, dd, J = 8.4, 2.0 Hz, H-10), 6.54 (1H, s, H-12), 5.65 (1H, d, J = 10.4 Hz, H-15), 5.38 (1H, m, H-14), 3.93 (1H, s, H-21), 3.72 (3H, s, -COOMe), 3.69 (3H, s, OMe-C11), 3.24 (2H, m, H-5), 3.02 (1H, m, H-6a), 2.95 (1H, m, H-3a), 2.82 (1H, d, J = 17.2 Hz, H-3b), 2.40 (1H, dd, J = 16.0, 4.8 Hz, H-6b), 2.33 (1H, d, J = 14.4 Hz, H-17a), 2.13 (1H, d, J = 14.4 Hz, H-17b), 1.82 (1H, m, H-19a), 1.50 (1H, m, H-19b), 0.91 (3H, t, J = 7.6 Hz, H-18); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 173.3 (-COOMe), 155.48 (C-11), 135.2 (C-13), 131.3 (C-2), 129.2 (C-15), 123.8 (C-14), 123.2 (C-8), 118.5 (C-9), 108.8 (C-10), 104.6 (C-7), 96.1 (C-12), 82.3 (C-16), 57.5 (C-21), 55.7 (OMe-C11), 53.5 (-COOMe), 49.4 (C-5), 43.9 (C-3), 43.5 (C-17), 36.7 (C-20), 34.7 (C-19), 16.6 (C-6), 8.9 (C-18)。以上数据与文献[13]报道一致,故鉴定化合物 **8** 为 14,15-Dehydrovincine。

化合物 9　白色无定型粉末; ESI-MS: *m/z* 353 $[M+H]^+$ ($C_{21}H_{24}N_2O_3$); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.50 (1H, d, J = 7.2 Hz, H-12), 7.36 (1H, d, J = 7.2 Hz, H-9), 6.99 (2H, m, H-10, 11), 5.47 (1H, m, H-14), 5.22 (1H, d, J = 10.4 Hz, H-15), 3.96 (1H, s, H-21), 3.45 (3H, s, -COOMe), 2.64 (1H, d, J = 14.0 Hz, H-17a), 2.11 (1H, d, J = 14.0 Hz, H-17b), 1.75 (1H, m, H-19a), 1.52 (1H, m, H-19b), 0.92 (1H, t, J = 7.6 Hz, H-20); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 170.4 (COOMe), 137.1 (C-13), 133.2 (C-2), 128.8 (C-8), 127.0 (C-14), 126.6 (C-15), 115.5 (C-5), 120.8 (C-11), 119.5 (C-10), 117.9 (C-9), 114.2 (C-12), 105.4 (C-7), 84.5 (C-16), 57.2 (C-21), 52.4 (-COOMe), 49.6 (C-5), 45.1 (C-17), 43.9 (C-3), 38.4 (C-20), 34.8 (C-19), 16.6 (C-6), 9.0 (C-18)。以上数据与文献[14]报道一致,故鉴定化合物 **9** 为 14,15-Dehydro-16-*epi*-vincamine。

化合物 10　白色无定型粉末; ESI-MS: *m/z* 295.3 $[M+H]^+$ ($C_{19}H_{22}N_2O$); ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.68 (1H, dd, J = 7.2, 1.2 Hz, H-12), 7.36 (1H, dd, J = 7.2, 2.0 Hz, H-9), 7.00 (2H, overlapped, H-10, 11), 5.51 (1H, d, J = 10.0 Hz, H-15), 5.46 (1H, m, H-14), 5.17 (1H, td, J = 9.2, 4.4 Hz, H-16), 3.94 (1H, s, H-21), 3.25 (2H, m, H-5), 3.03 (1H, m, H-6a), 2.42 (1H, dd, J = 16.0, 6.0 Hz, H-6b), 2.92 (1H, m, H-17a), 2.67 (1H, d, J = 17.2 Hz, H-17b), 2.22 (1H, dd, J = 13.6, 4.4 Hz, H-3a), 1.84 (1H, dd, J = 13.6, 10.0 Hz, H-3b), 1.76 (1H, m, H-19a), 1.56 (1H, s, H-19b), 0.94 (3H, t, J = 7.2 Hz, H-18); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 136.8 (C-13), 133.9 (C-2), 128.8 (C-8), 127.8 (C-15), 126.8 (C-14), 120.7 (C-11), 119.6 (C-10), 117.9 (C-9), 112.9 (C-12), 105.0 (C-7), 76.7 (C-16), 57.2 (C-21), 49.3 (C-5), 44.0 (C-3), 42.4 (C-17), 39.6 (C-20), 34.1 (C-19), 16.6 (C-6), 9.0 (C-18)。以上数据与文献[15]报道一致,故鉴定化合物 **10** 为△^{14}-Vincanol。

3.2　对 MCF-7 细胞毒活性的测定

取 96 孔细胞培养板,每孔加入浓度为 3×10^5

个/mL 的细胞悬液 200 μL,37 ℃、5% CO_2 培养箱中贴壁培养 24 h 后,加入不同浓度的受试药物(溶解在含 0.3% DMSO 的培养基中)200 μL/孔,终浓度分别为 90 μg/mL(C1)、60 μg/mL(C2)、30 μg/mL(C3)、15 μg/mL(C4)、7.5 μg/mL (C5),每浓度 3 个复孔,正常对照组加入等量含 0.3% DMSO 的培养基 (C0),同时设阳性对照组(顺铂 100 μg/mL)和阴性对照组(只加培养基),37 ℃ 5% CO_2 培养箱中培养 24 h。随后,吸弃 50 μL 培养液,每孔加入 50 μL MTT 溶液(5 mg/mL),培养箱中放置 4 h。弃去培养液和 MTT,每孔加入 200 μL DMSO,置摇床上低速振荡 10 min,使结晶物充分溶解。酶联免疫检测仪 570 nm 处测量各孔的吸光值,计算抑制率和 IC_{50}。通过测定发现仅化合物 **3** 具有明显的细胞毒活性(IC_{50}值为 23.4 μM),其余化合物无细胞毒活性(IC_{50} > 100 μM),阳性对照药顺铂的 IC_{50}值为 4.6 μM。

4 讨论

本实验在对思茅山橙根部位生物碱类成分系统研究的基础上,对所得化合物进行人乳腺癌细胞株 MCF-7 的细胞毒活性实验,发现化合物 **3** 具有较好的细胞毒活性,为思茅山橙根部位药用价值的开发利用提供实验依据。

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