文章编号:1001-6880(2016)10-1562-06

球毛壳菌 CIB-160 次生代谢产物的分离鉴定及其免疫活性

徐国波[1,2],刘　俊[1],肖耀华[1],周　孟[1],杨　涛[2],李国友[2*]

[1]贵州医科大学药学院,贵阳 550004;[2] 中国科学院成都生物研究所微生物重点实验室,成都 610041

摘　要:采用硅胶柱层析、HPLC、Sephadex LH-20 凝胶柱层析等分离方法对球毛壳菌 CIB-160 的次生代谢产物进行纯化,得到 10 个化合物。通过 NMR、HR-MS 和旋光数据分析以及文献比对,其结构鉴定为 chaetoglobosins A(**1**)、C(**2**)、E(**3**)、F(**5**)、F_{ex}(**6**)、W(**7**),penochalasin F(**4**),5-(methyl-2-butenyl)-indole-2,3-dione(**8**)、chaetoviridin A(**9**)和 cochliodone A(**10**)。**8** 和 **10** 为首次从野生型球毛壳菌代谢物中分离得到的化合物。体外免疫活性检测显示 **1**~**6** 对小鼠脾细胞的增殖具有抑制作用,IC_{50} 值分别为 0.21、2.8、2.3、2.2、1.7、2.7 μM。同时 **1**~**6** 具有较强的细胞毒性,对静息小鼠脾细胞存活率 IC_{50} 值分别 0.82、7.5、2.3、6.1、4.6、6.7 μM。

关键词:球毛壳菌;次生代谢产物;分离纯化;免疫活性

中图分类号:O629.9　　**文献标识码**:A　　DOI:10.16333/j.1001-6880.2016.10.011

Isolation and Identification of Secondary Metabolites from *Chaetomium globosum* CIB-160 and Their Immunological Activity

XU Guo-bo[1,2],LIU Jun[1],XIAO Yao-hua[1],ZHOU Meng[1],YANG Tao[2],LI Guo-you[2*]

[1]*School of Pharmacy,Guizhou Medical University,Guiyang 550004,China*;[2]*Key Laboratory of Environmental and Applied Microbiology,Chengdu Institute of Biology,Chinese Academy of Sciences,Chengdu 610041,China*

Abstract:Ten compounds were isolated and purified from the metabolites of *Chaetomium globosum* CIB-160 by silica gel column chromatography,HPLC,Sephadex LH-20 chromatography. Their chemical structures were identified as chaetoglobosins A(**1**),C(**2**),E(**3**),F(**5**),F_{ex}(**6**),W(**7**),penochalasin F(**4**),5-(methyl-2-butenyl)-indole-2,3-dione(**8**),chaetoviridin A(**9**),and cochliodone A(**10**) on the basis of NMR,HR-MS and optical rotation data,compound **8** and **10** were first reported from wild strain of *C. globosum*. Compounds **1-6** showed significant inhibition of mouse spleen cell proliferation,their IC_{50} values were 0.21,2.8,2.3,2.2,1.7,2.7 μM,respectively. The IC_{50} values of mice spleen cell survival rates successively were 0.82,7.5,2.3,6.1,4.6,6.7 μM,which indicated compounds **1-6** possessed strong cytotoxicity.

Key words:*Chaetomium globosum*;secondary metabolites;isolation and purification;immunological activity

球毛壳菌(*Chaetomium globosum*)常见于土壤和空气中,是毛壳属(*Chaetomium*)中研究最多的菌种,其代谢物中以细胞松弛素类型的化合物居多,包括 chaetoglobosins A-G、J、Q、R、T、U、V、W、F_{ex}、penochalasin A、cytoglobosins A-G、isochaetoglobosin D。球毛壳菌代谢产物因具有广泛的生物活性如抗肿瘤、抗疟疾、抗菌、免疫调节等而引起药物学家和生物学家的重视[1]。前期研究中,本课题组从毛壳属菌株发现多个具有抗肿瘤[2]、抗菌[3]、抗氧化[4]、调节植物生长[5]等生物活性的新型化合物。为了从毛壳属菌种中寻找更多的活性物质,课题组对毛壳属菌种进行系统研究。尽管文献报道球毛壳菌次级代谢产物研究较多,但基于菌株来源不同,其代谢产物会有较大的差异。本文对从雅安土壤中分离得到的一株球毛壳菌进行了研究,从其次生代谢产物中分离得到了化合物 chaetoglobosins A(**1**)、C(**2**)、E(**3**)、F(**5**)、F_{ex}(**6**)、W(**7**),penochalasin F(**4**),5-(methyl-2-butenyl)-indole-2,3-dione(**8**),chaetoviridin A(**9**)和 cochliodone A(**10**)。其中 **8** 和 **10** 为首次从野生型球毛壳菌代谢物中分离得到的化合物。免疫活性实验测试结果显示化合物 **1**~**6** 细胞松弛素类化合物显示较强的抑制活性。

1　仪器与材料

1.1　材料

菌种球毛壳(*Chaetomium globosum* CIB-160)是

收稿日期:2016-06-01　　接受日期:2016-07-15

基金项目:国家自然科学基金(21272228)

*通讯作者 Tel:86-28-82890415;E-mail:ligy@cib.ac.cn

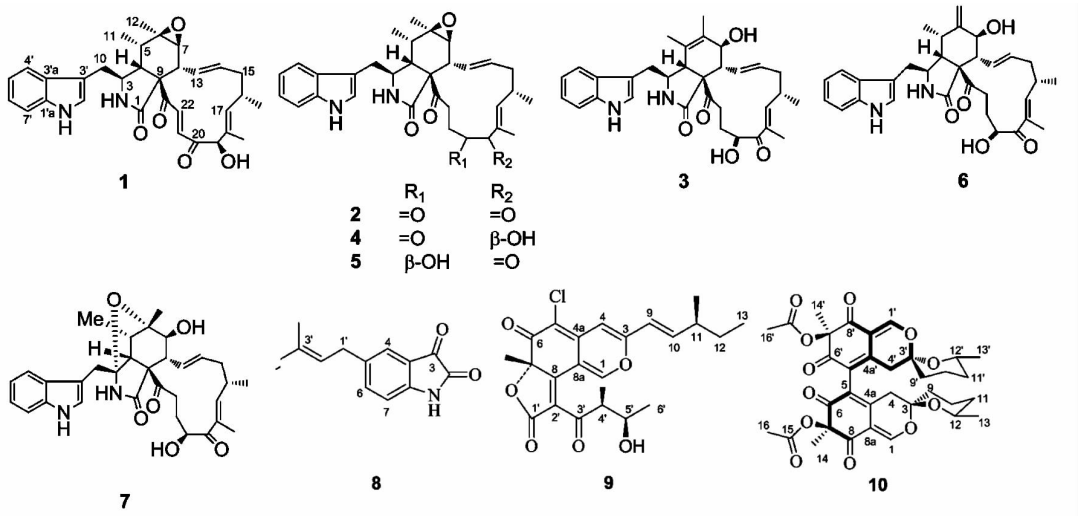

图 1　化合物 1 ~ 10 的化学结构

Fig. 1　Chemical structures of compounds **1-10**

从四川省雅安土壤中采集分离得到,由中国科学院成都生物研究所杨涛副研究员鉴定,以 PDA 斜面培养基 4 ℃转种保存于冰箱中。菌种保藏于中国科学院成都生物研究所。

液体培养基:20% 马铃薯提取物,2% 葡萄糖,0.35 KH_2PO_4,0.15% $MgSO_4 \cdot 7H_2O$,0.15% 酵母膏,用 40% NaOH 水溶液将 pH 调至中性。固体培养基:大米中加入 0.3% 的蛋白胨混合均匀。

Bal b/c 小鼠[四川大学动物实验中心,SCXK(川)2013-026]。

1.2　主要仪器与试剂

Bruker Bio TOF IIIQ 质谱仪(美国 Bruker Daltonic 公司),核磁共振波谱仪 Bruker Avance 600(德国 Bruker 公司),红外光谱仪 Perkin Elmer spectrum one FT-IR(美国 Perkin Elmer 公司),旋光仪 Perkin Elmer 341(美国 Perkin Elmer 公司),半制备 HPLC 仪器(上海伍丰有限公司);硅胶 H(300 ~ 400 和 200 ~ 300 目,青岛海洋化工厂),薄层色谱使用硅胶 GF_{254}(青岛海洋化工);分析级石油醚(60 ~ 90 ℃)、乙酸乙酯、氯仿、甲醇(国药集团化学试剂有限公司),色谱甲醇(美国 Thermo Fisher 公司),氘代甲醇、氘代氯仿、氘代丙酮(瑞士 ARMAR 公司),RPMI 1640 完全培养基(上海宝曼生物科技有限公司),CFSE(美国 Invitrogen Molecular Probes 公司),流式细胞仪(美国 Becton Dickison 公司),Anti-CD3/CD28(上海沪震有限公司),CCK-8(日本 Dojindo 研究所)。

2　实验方法

2.1　发酵

一级种子的发酵:在无菌室中将斜面上的菌种接种到已灭菌的液体培养基中,于恒温箱中培养 3 d。二级发酵:将一级的种子按 10% 的接种量均匀地接种到已灭菌的固体培养基上,并将其放在培养箱中 28 ℃恒温培养 25 d。

2.2　提取与分离

室温下用乙酸乙酯(10 L × 3,一次 2 d)浸提球毛壳霉菌固态发酵物(10.0 kg),提取液减压浓缩得浸膏(67.0 g)。总浸膏经硅胶柱层析(500 g,Φ 60 mm × 500 mm,石油醚-丙酮 5∶1、2∶1、1∶1、0∶1,各 3500 mL),分为四个部分 A(46.4 g)、B(7.2 g)、C(4.8 g)和 D(5.5 g)。A 部分为大量的油脂类以及麦角固醇类物质。B 用硅胶柱进一步分离(200 g,300 ~ 400 目,Φ 50 mm × 350 mm),以石油醚-丙酮(3∶1,800 mL)洗脱得到混合物 B1 和 B2。B1 经 HPLC(45%,乙腈-水)分离得到化合物 **9**(10.0 mg)和 10(25.0 mg)。B2 经 Sephadex LH20 柱层析(氯仿∶甲醇,1∶1)得到化合物 **8**(5.0 mg)。C 部分粗提取物经与对照品对照为嘧啶类化合物。D 部分经 HPLC(45%,乙腈-水)分离得到化合物 **4**(3.0 mg)、**7**(3.4 mg)以及混合物 D1 和 D2。D1 经 HPLC(40%,乙腈-水)分离得到化合物 **1**(60.0 mg)、**2**(40.0 mg)和 **5**(6.0 mg)。D2 经 HPLC(41%,甲醇-水)分离得到化合物 **3**(35.0 mg)和 **6**(2.5 mg)。

2.3　体外免疫活性实验

体外免疫活性实验参考文献方法[6],即刺激后脾细胞增殖检测:取 Bal b/c 小鼠(6 ~ 8 w)脾脏,制

备单细胞悬液,细胞密度调整为 1×10^6 个/mL。使用 2 μmol/L 羧基荧光素乙酰乙酸琥珀酰亚胺酯(CFSE)于 37 ℃避光染色细胞 10 min,加入冰冷的 RPMI 1640 完全培养基终止染色。使用 2 mg/L anti-CD3/1 mg/L anti-CD28 刺激脾细胞(1×10^6 个/mL)。加入不同浓度筛选药物后,于 37 ℃,5% CO_2 中培养 96 h,并以 BD750 为阳性对照[6]。流式细胞仪检测细胞增殖。

静息脾细胞活力检测:取 Bal b/c 小鼠(6 ~ 8 w)脾脏,制备单细胞悬液,细胞密度调整为 1×10^6 个/mL。加入不同浓度筛选药物后,于 37 ℃,5% CO_2 中培养 48 h。使用 CCK-8 检测细胞活力,具体操作参照 CCK-8 检测试剂盒说明书。

3 结果与分析

3.1 发酵产物的鉴定

Chaetoglobosin A (1) 黄色粉末;HR-ESI-MS (positive mode) *m/z* 551.2512 [M + Na]$^+$ (calcd for $C_{32}H_{36}N_2O_5Na^+$, 551.2524);^{1}H NMR (600 MHz, pyridine-d_5):δ 11.90 (1H, s, NH-1′), 9.42 (1H, s, NH-2), 8.04 (1H, d, *J* = 6.5 Hz, H-22), 7.87 (1H, d, *J* = 6.0 Hz, H-4′), 7.58 (1H, overlap, H-7′), 7.33 (1H, s, H-2′), 7.26 (2H, overlap, H-5′, H-6′), 7.06 (1H, d, *J* = 6.5 Hz, H-21), 6.63 (1H, dd, *J* = 15.0, 6.0 Hz, H-13), 5.61 (1H, d, *J* = 9.4 Hz, H-17), 5.44 (1H, s, H-19), 5.29 (1H, m, H-14), 4.15 (1H, s, H-3), 3.29 (1H, s, H-4), 3.13 (2H, overlap, H-8, H-10), 3.05 (1H, dd, *J* = 14.3, 6.6 Hz, H-10), 2.63 (1H, dd, *J* = 9.6, 4.9 Hz, H-7), 2.41 (1H, m, H-16), 2.17 (1H, d like, H-15), 2.06 (1H, m, H-5), 1.93 (1H, m, H-15), 1.54 (3H, s, CH_3-18), 1.28 (3H, d, *J* = 8.1 Hz, CH_3-12), 1.08 (3H, d, *J* = 7.2 Hz, CH_3-11), 0.86 (3H, d, *J* = 6.6 Hz, CH_3-16);^{13}C NMR (150 MHz, acetone-d_6):δ 201.0 (C-20), 197.8 (C-23), 172.8 (C-1), 138.9 (C-17), 136.7 (C-1′a), 135.7 (C-21), 132.7 (C-22), 132.6 (C-14), 132.2 (C-18), 128.9 (C-13), 127.8 (C-3′a), 124.2 (C-5′), 121.2 (C-2′), 118.9 (C-6′), 118.5 (C-4′), 111.4 (C-7′), 109.7 (C-3′), 63.2 (C-9), 62.0 (C-7), 57.2 (C-6), 52.2 (C-3), 48.3 (C-8), 46.7 (C-4), 41.3 (C-15), 36.4 (C-5), 33.2 (C-10), 32.0 (C-16), 20.4 (CH_3-C_{16}), 12.3 (CH_3-11), 9.8 (CH_3-C_{18})。与文献中 chaetoglobosin A 的相关数据一致[7][8]。

Chaetoglobosin C (2) 白色粉末;HR-ESI-MS (positive mode) *m/z* 551.2524 [M + Na]$^+$ (calcd for $C_{32}H_{36}N_2O_5Na^+$, 551.2524);^{1}H NMR (600 MHz, DMSO-d_6):δ 10.92 (1H, s, NH-1′), 8.43 (1H, s, NH-2), 7.53 (1H, d, *J* = 8.1 Hz, H-4′), 7.31 (1H, d, *J* = 8.1 Hz, H-7′), 7.12 (1H, s, H-2′), 7.05 (1H, t, *J* = 8.1 Hz, H-6′), 6.99 (1H, t, *J* = 8.1 Hz, H-5′), 6.06 (1H, dd, *J* = 14.4, 6.0 Hz, H-13), 5.88 (1H, d, *J* = 9.8 Hz, H-17), 4.94 (1H, t, *J* = 11.9 Hz, H-14), 3.74 (1H, s, H-3), 2.83 (1H, dd, *J* = 14.5, 5.5 Hz, H-10), 2.73 (1H, dd, *J* = 14.5, 3.4 Hz, H-10), 2.63 (1H, d, *J* = 6.0, H-8), 2.45 (1H, m, H-4), 2.28 (1H, overlap, H-7 或 H-16), 2.24 (1H, m, H-7 或 H-16), 2.13 (1H, dd, *J* = 9.9, 6.0 Hz, H-15), 1.80 (1H, m, H-5 或 H-15), 1.73 (1H, overlap, H-5 或 H-15), 1.68 (3H, s, CH_3-18), 1.15 (3H, s, CH_3-12), 0.93 (3H, d, *J* = 6.7 Hz, CH_3-11), 0.91 (3H, d, *J* = 7.2 Hz, CH_3-16);^{13}C NMR (150 MHz, DMSO-d_6):δ 208.2 (C-19), 205.3 (C-20), 196.2 (C-23), 173.9 (C-1), 155.8 (C-17), 135.9 (C-1′a), 131.0 (C-18), 128.2 (C-3′a), 127.1 (C-13), 125.2 (C-5′), 121.0 (C-2′), 118.7 (C-6′), 118.4 (C-4′), 111.3 (C-7′), 108.1 (C-3′), 62.3 (C-9), 60.4 (C-7), 56.7 (C-6), 52.3 (C-3), 48.5 (C-4), 48.3 (C-8), 39.6 (C-15), 37.1 (C-22), 36.2 (C-5), 32.6 (C-16), 32.0 (C-10), 31.8 (C-21), 19.4 (CH_3-C_{16}), 19.1 (C-12), 12.4 (C-11), 10.0 (CH_3-C_{18})。与文献中 chaetoglobosin C 的相关数据一致[7]。

Chaetoglobosin E (3) 白色粉末;HR-ESI-MS (positive mode) *m/z* 553.2679 [M + Na]$^+$ (calcd for $C_{32}H_{38}N_2O_5Na^+$, 553.2674);^{1}H NMR (600 MHz, pyridine-d_5):δ 11.90 (1H, s, NH-1′), 9.24 (1H, s, NH-2), 7.85 (1H, d, *J* = 7.8 Hz, H-4′), 7.68 (1H, overlap, H-7′), 7.32 (2H, m, H-2′ 和 H-6′), 7.27 (1H, t, *J* = 7.8 Hz, H-5′), 7.05 (1H, dd, *J* = 15.3, 7.0 Hz, H-13), 6.36 (1H, d, *J* = 9.1 Hz, H-17), 5.50 (1H, *t* like, H-14), 5.33 (1H, t, *J* = 7.8 Hz, H-20), 4.60 (1H, d, *J* = 9.3 Hz, H-8), 4.00 (1H, t, *J* = 7.3 Hz, H-3), 3.46 (1H, overlap, H-4), 3.43 (1H, overlap, H-22), 3.13 (1H, dd, *J* = 14.1, 7.4 Hz, H-10), 3.02 (1H, dd, *J* = 14.1, 7.4 Hz, H-10), 2.72 (1H, t like, H-7), 2.62 (1H, m, H-16), 2.45 (2H, overlap, H-15, H-21), 2.25 (1H, d, *J* = 13.2 Hz, H-15), 1.96 (3H, s, CH_3-18), 1.92 (3H, s, CH_3-12), 1.51 (3H,

s, CH_3-11), 0.84 (3H, d, J = 6.6 Hz, CH_3-16); ^{13}C NMR (150 MHz, pyridine-d_5): δ 210.7 (C-19), 204.8 (C-23), 175.3 (C-1), 148.4 (C-17), 137.5 (C-1′a), 135.4 (C-18), 134.2 (C-14), 134.2 (C-6), 130.0 (C-13), 128.0 (C-3′a), 126.3 (C-5), 124.3 (C-5′), 121.7 (C-2′), 119.2 (C-6′), 118.7 (C-4′), 112.0 (C-7′), 111.3 (C-3′), 71.7 (C-20), 68.8 (C-7), 62.7 (C-9), 58.2 (C-4), 52.6 (C-3), 50.6 (C-8), 41.1 (C-15), 33.3 (C-21), 33.3 (C-10), 32.0 (C-16), 19.6 (CH_3-C_{16}), 17.3 (C-12), 14.9 (C-11), 12.2 (CH_3-C_{18})。与文献中 chaetoglobosin E 的相关数据一致[7]。

Penochalasin F (4) 白色粉末; HR-ESI-MS (positive mode) m/z 553.2676 [M + Na]$^+$ (calcd for $C_{32}H_{38}N_2O_5Na^+$, 553.2674); ^{1}H NMR (600 MHz, pyridine-d_5): δ 12.08 (1 H, s, NH-1′), 9.39 (1 H, s, NH-2), 7.90 (1H, d, J = 7.6 Hz, H-4′), 7.66 (1H, d, J = 7.8 Hz, H-7′), 7.38 (1 H, d, J = 2.1Hz, H-2′), 7.31 ~ 7.25 (2 H, overlap, H-5′, H-6′), 6.68 (1 H, dd, J = 14.3, 10.4 Hz, H-13), 5.47 (1 H, d, J = 8.9 Hz, H-17), 5.32 (1 H, m, H-14), 4.18 (1 H, s, H-19), 3.50 (1 H, m, H-3), 3.20 (1H, dd, J = 14.4, 5.3 Hz, H-10), 3.15 (1H, d, J = 5.5 Hz, H-7), 3.08 (1 H, dd, J = 14.3, 5.6 Hz, H-10), 2.90 (1 H, d, J = 3.7 Hz, H-4), 2.78 ~ 2.67 (3 H, overlap, H-22, H-16 或 H-21), 2.45 ~ 2.39 (2H, overlap, H-5, H-16 或 H-21), 2.16 (1 H, d like, H-15), 2.10 (1 H, q like, H-22), 1.86 (1H, dt, J = 13.5, 11.0 Hz, H-15), 1.59 (3 H, s, CH_3-18), 1.31 (3 H, s, H-12), 1.10 (3 H, d, J = 7.2 Hz, H-11), 0.86 (3 H, d, J = 6.6 Hz, CH_3-16); ^{13}C NMR (150 MHz, pyridine-d_5): δ 210.8 (C-20), 209.2 (C-23), 174.8 (C-1), 138.2 (C-17), 137.3 (C-1′a), 133.4 (C-14), 132.5 (C-18), 129.4 (C-13), 128.4 (C-3′a), 125.2 (C-2′), 121.7 (C-6′), 119.4 (C-5′), 118.9 (C-4′), 112.1 (C-7′), 109.9 (C-3′), 83.1 (C-19), 64.6 (C-9), 62.1 (C-7), 57.1 (C-6), 53.1 (C-3), 49.6 (C-8), 49.3 (C-4), 41.5 (C-15), 37.0 (C-5), 36.2 (C-22), 34.6 (C-21), 34.0 (C-16), 32.1 (C-10), 21.0 (CH_3-C_{16}), 19.5 (C-12), 12.7 (C-11), 10.9 (CH_3-C_{18})。与文献中 penochalasin F 的相关数据一致[9]。

Chaetoglobosin F (5) 白色粉末; HR-ESI-MS (positive mode) m/z 553.2871 [M + Na]$^+$ (calcd for $C_{32}H_{38}N_2O_5Na^+$, 553.2674); ^{1}H NMR (600 MHz, acetone-d_6): δ 10.08 (1H, s, NH-1′), 7.55 (1 H, d, J = 7.9 Hz, H-4′), 7.38 (1 H, d, J = 8.0 Hz, H-7′), 7.30 (1 H, s), 7.15 (1 H, s), 7.09 (1 H, t, J = 7.5 Hz, H-6′), 7.02 (1 H, t, J = 7.5 Hz, H-5′), 6.45 (1 H, dd, J = 15.1, 9.8 Hz, H-13), 6.32 (1 H, d, J = 9.5 Hz, H-17), 5.27 (1 H, m, H-14), 4.73 (1 H, m, H-19), 3.85 (1 H, t like, H-3), 3.79 (1 H, d, J = 7.2 Hz, H-10), 3.31 (1 H, overlap, H-8), 3.02 (1 H, s, H-4), 2.89 (1 H, dd, J = 14.1, 5.6 Hz, H-10), 2.66 (1 H, d, J = 6.0 Hz, H-22), 2.62 (1 H, d, J = 5.1 Hz, H-7), 2.43 (1 H, m, H-15 或 H-16), 2.37 (1 H, m, H-16 或 H-15), 2.24 (1 H, dd, J = 9.7, 5.7 Hz, H-15), 1.80 (3 H, s, CH_3-18), 1.75 ~ 1.62 (3 H, overlap, H-21, H-22), 1.16 (3 H, s, H-12), 1.07 (3 H, d, J = 6.7 Hz, H-11), 0.81 (3 H, d, J = 7.3 Hz, CH_3-16); ^{13}C NMR (150 MHz, acetone-d_6): δ 210.2 (C-19), 205.5 (C-23), 175.5 (C-1), 150.1 (C-17), 138.2 (C-1′a), 136.2 (C-18), 134.4 (C-14), 130.8 (C-13), 129.3 (C-3′a), 125.5 (C-5′), 122.8 (C-2′), 120.3 (C-6′), 119.8 (C-4′), 112.9 (C-7′), 111.6 (C-3′), 72.6 (C-20), 65.7 (C-9), 63.1 (C-7), 58.2 (C-6), 54.0 (C-3), 50.3 (C-8), 49.4 (C-4), 42.2 (C-15), 38.6 (C-22), 38.0 (C-5), 35.0 (C-16), 34.8 (C-21), 32.6 (C-10), 20.7 (CH_3-C_{16}), 20.2 (C-12), 13.4 (C-11), 9.8 (CH_3-C_{18})。与文献中 chaetoglobosin F 的相关数据一致[7]。

Chaetoglobosin F_{ex} (6) 白色粉末; $[\alpha]_D^{20}$ = +62° (c 0.1, MeOH); HR-ESI-MS (positive mode) m/z 553.2674 [M + Na]$^+$ (calcd for $C_{32}H_{38}N_2O_5Na^+$, 553.2674); ^{1}H NMR (600 MHz, acetone-d_6): δ 10.12 (1H, s, NH-1′), 7.53 (1H, d, J = 7.9 Hz, H-4′), 7.37 (1H, d, J = 8.0 Hz, H-7′), 7.19-7.13 (2H, overlap, H-2′, NH-2), 7.08 (1H, t, J = 7.2 Hz, H-6′), 7.01 (1H, t, J = 7.1 Hz, H-5′), 6.26 (1H, d, J = 9.5 Hz, H-13), 6.22 (1H, m, H-17), 5.23 (1H, m, H-14), 5.20 (1H, s, H-12), 4.96 (1H, s, H-12), 4.70 (1H, brs, H-20), 3.86 (2H, overlap, H-7, H-22), 3.45 (1H, m, H-3), 2.87 ~ 2.81 (3H, H-10, H-22, H-21), 2.78 (1H, overlap, H-5 或 H-4), 2.74 ~ 2.69 (2H, H-21, H-4 或 H-5), 2.65 ~ 2.57 (2H, overlap, H-15, H-10 或 H-22), 2.37 (1H, m, H-10 或 H-22), 2.28 ~ 2.19 (1H, m, H-8), 1.79 (3H, s, CH_3-18), 1.69 ~ 1.62 (1H, m, H-15), 1.02 (3H, d, J = 6.7 Hz, H-11), 0.88 (3H, d, J = 7.3 Hz,

CH_3-16);^{13}C NMR (150 MHz, acetone-d_6): δ 208.7 (C-23), 204.0 (C-19), 173.9 (C-1), 150.6 (C-6), 148.7 (C-17), 136.7 (C-1′a), 134.7 (C-14), 133.7 (C-18), 129.0 (C-3′a), 127.9 (C-13), 124.2 (C-2′), 121.3 (C-6′), 118.8 (C-5′), 118.3 (C-4′), 112.0 (C-12), 111.5 (C-7′), 110.1 (C-3′), 71.1 (C-20), 70.3 (C-7), 62.6 (C-9), 52.3 (C-3), 49.2 (C-8), 47.2 (C-4), 40.9 (C-15), 37.0 (C-22), 33.3 (C-10), 32.7 (C-16), 31.9 (C-21), 31.2 (C-5), 19.3 (C-C_{16}), 13.0 (C-11), 11.5 (C-C_{18})。与文献中 chaetoglobosin F_{ex}的相关数据一致[10]。

Chaetoglobosin W (7) 白色粉末;HR-ESI-MS (positive mode) *m/z* 569.2626 [M + Na]$^+$ (calcd for $C_{32}H_{38}N_2O_6Na^+$, 569.2626);^{1}H NMR (600 MHz, pyridine-d_5): δ 11.94 (1H, s, NH-1′), 9.87 (1H, s, NH-2), 7.99 (1H, dd, *J* = 8.9, 3.0 Hz, H-4′), 7.61 (1 H, s, H-2′), 7.54 (1H, dd, *J* = 6.0, 3.0 Hz, H-7′), 7.27 (2H, overlap, H-5′, H-6′), 6.68 (1H, m, H-13), 6.45 (1H, d, *J* = 9.8 Hz, H-17), 6.28 (1H, m, H-14), 5.63 (1H, t like, H-20), 5.32 (1H, t like, H-7), 3.99 (1H, m, H-16), 3.78 (1H, d, *J* = 15.2 Hz, H-10), 3.53 (1H, d, *J* = 15.0 Hz, H-10), 3.45 (1H, s, H-4), 3.19 (1H, m, H-8 或 H-15), 2.99 (2H, overlap, H-22, H-8 或 H-15), 2.67 (1H, m, H-22), 2.52 (1H, m, H-21 或 H-5), 2.25 (2H, overlap, H-21 或 H-5), 2.14 (1H, m, H-15), 1.90 (3H, s, CH_3-18), 1.69 (3H, s, CH_3-12), 1.29 (3H, d, *J* = 7.2 Hz, CH_3-11), 0.81 (3H, d, *J* = 6.7 Hz, CH_3-16);^{13}C NMR (150 MHz, pyridine-d_5): δ 208.2 (C-23), 205.9 (C-19), 175.3 (C-1), 149.7 (C-17), 138.2 (C-1′a), 134.8 (C-14), 132.5 (C-13), 130.0 (C-3′a), 126.1 (C-2′), 122.7 (C-6′), 120.6 (C-4′), 120.3 (C-5′), 112.9 (C-7′), 111.4 (C-3′), 97.5 (C-3), 91.4 (C-6), 78.8 (C-7), 72.8 (C-20), 64.8 (C-9), 54.7 (C-4), 49.6 (C-8), 42.1 (C-15), 40.4 (C-22), 37.4 (C-5), 36.7 (C-10), 34.8 (C-16), 33.7 (C-21), 20.6 (CH_3-C_{16}), 20.0 (C-12), 15.6 (C-11), 13.2 (CH_3-C_{18})。与文献中 chaetoglobosin W 的相关数据一致[11]。

5-(Methyl-2-butenyl)-indole-2,3-dione (8) 黄色粉末;^{1}H NMR (600 MHz, $CDCl_3$): δ 8.85 (1H, s, H-1), 7.40 (1H, s, H-4), 7.35 (1H, d, *J* = 7.8 Hz, H-6), 6.86 (1H, d, *J* = 7.8 Hz, H-7), 5.24 (1H, m, H-2′), 3.28 (2H, overlap, H-1′), 1.75 (3H, s, H-5′), 1.70 (3H, s, H-4′);^{13}C NMR (150 MHz, $CDCl_3$): δ 183.5 (C-3), 160.0 (C-2), 147.5 (C-7a), 138.7 (C-6), 138.0 (C-5), 134.0 (C-3′), 125.3 (C-4), 121.8 (C-2′), 118.2 (C-3a), 112.4 (C-7), 33.4 (C-1′), 25.7 (C-4′), 17.8 (C-5′)。与文献中 5-(methyl-2-butenyl)-indole-2,3-dione 的相关数据一致[12]。

Chaetoviridin A (9) 红色粉末;$[\alpha]_D^{20}$ = +95° (*c* 0.1, $CDCl_3$); HR-ESI-MS (positive mode) *m/z* 455.1030 [M + Na]$^+$ (calcd for $C_{23}H_{25}ClO_6Na^+$, 455.1237);^{1}H NMR (600 MHz, $CDCl_3$): δ 8.75 (1H, s, H-1), 6.59 (1H, dd, *J* = 15.6, 7.8 Hz, H-10), 6.54 (1H, s, H-4), 6.07 (1H, d, *J* = 15.6 Hz, H-9), 3.84 (1H, m, H-5′), 3.62 (1H, m, H-4′), 2.27 (1H, m, H-11), 2.08 (1H, br, OH-5′), 1.43 (2H, m, H-12), 1.14 (3H, d, *J* = 6.0 Hz, H-6′), 1.14 (3H, d, *J* = 6.0 Hz, CH_3-4′), 1.07 (3H, d, *J* = 7.2 Hz, CH_3-11), 0.88 (3H, t, *J* = 7.2 Hz, H-13);^{13}C NMR (150 MHz, $CDCl_3$): δ 201.2 (C-3′), 183.4 (C-6), 167.9 (C-1′), 162.7 (C-8), 157.1 (C-3), 151.5 (C-1), 148.0 (C-10), 139.7 (C-4a), 125.1 (C-2′), 119.8 (C-9), 110.4 (C-8a), 108.9 (C-5), 105.4 (C-4), 87.6 (C-7), 70.9 (C-5′), 51.0 (C-4′), 39.0 (C-11), 29.1 (C-12), 26.2 (CH_3-C_7), 21.4 (CH_3-$C_{4'}$), 19.3 (CH_3-C_{11}), 13.5 (C-6′), 11.7 (C-13)。与文献中 chaetoviridin A 的相关数据一致[13]。

Cochliodone A (10) 白色粉末;$[\alpha]_D^{20}$ = -223° (*c* 0.1, MeOH); HR-ESI-MS (positive mode) *m/z* 661.2281 [M + Na]$^+$ (calcd for $C_{34}H_{38}O_{12}Na^+$, 661.2255);^{1}H NMR (600 MHz, acetone-d_6): δ 7.75 (1H, s, H-1, 1′), 3.88 (1H, m, H-12, 12′), 2.75 (1H, d, *J* = 16.8 Hz, H-4β, 4β′), 2.63 (1H, d, *J* = 16.8 Hz, H-4α, 4α′), 2.06 (3H, s, H-16, 16′), 1.90 (1H, m, H-9, 9′), 1.68 (2H, overlap, H-10, 10′/H-11, 11′), 1.58 (1H, m, H-9, 9′), 1.51 (3H, s, H-14, 14′), 1.22 (1H, m, H-11, 11′), 1.03 (3H, d, *J* = 6.2 Hz, H-13, 13′);^{13}C NMR (150 MHz, acetone-d_6): δ 191.4 (C-6, 6′), 191.1 (C-8, 8′), 168.9 (C-15, 15′), 156.7 (C-1, 1′), 144.7 (C-4a, 4a′), 122.8 (C-5, 5′), 111.5 (C-8a, 8a′), 102.6 (C-3, 3′), 84.7 (C-7, 7′), 68.3 (C-12, 12′), 37.2 (C-4, 4′), 31.8 (C-9, 9′), 31.3 (C-11, 11′), 22.0 (C-14, 14′), 21.0 (C-13, 13′), 19.2 (C-16, 16′), 18.0 (C-10, 10′)。

与文献中 cochliodone A 的相关数据一致[14]。

3.2 体外免疫活性的测试

体外免疫活性实验表明,化合物 **1 ~ 6** 在低浓度情况下对小鼠脾细胞的增殖具有抑制作用,IC_{50}值分别为 0.21、2.8、2.3、2.2、1.7、2.7 μM(阳性 BD750,IC_{50} = 1.0 μM)。化合物 **10** 无免疫抑制活性(c > 50 μM)同时化合物 **1 ~ 6** 对静息小鼠脾细胞存活率 IC_{50} 值分别 0.82、7.5、2.3、6.1、4.6、6.7 μM(阳性 BD750,IC_{50} > 50 μM),表明化合物对细胞具有较强的毒性。

4 讨论

球毛壳次生代谢物大多具有较强的生物活性,如细胞松弛素类化合物 chaetoglobosins A-G,J 对 Hela cell 具有细胞毒活性(IC_{50} = 3.2 ~ 20 μg/mL)[15];Chaetoglobosins(A、C)对苜蓿幼苗具有植物毒素样作用[16];对幽门螺旋杆菌(*Helicobacter pylori*)、金黄色葡萄杆菌(*Staphylococcus aureus*)以及对植物致病菌(*Pythium ultimum*、*Phytophthora capsici*、*Rhizoctonia solani*、*Botrytis cinerea*、*Fusarium oxysporum*)具有一定的抑制作用[17]。随着越来越多的体外药理活性实验模型的建立,毛壳属代谢产物更多的生物活性被人们所发现。本文采用体外检测脾细胞免疫活性旨在探索 Chaetoglobosins 类化合物在低浓度下是否具有免疫抑制活性,以开发免疫抑制新型药物。实验结果表明化合物 **1 ~ 6** 对脾细胞增殖具有较强的抑制作用,但同时在低浓度下对静息脾细胞具有杀伤作用,这可能与该类化合物具有较强的细胞毒性有关。

参考文献

1 Zhang Q, Li HQ, Zong SC, *et al*. Chemical and bioactive diversities of the genus *Chaetomium* secondary metabolites. *Mini Rev Med Chem*, 2012, 12: 127-148.

2 Li GY, Li BG, Yang T, *et al*. Chaetocochins A-C, Epipolythiodioxopiperazines from *Chaetomium cochliodes*. *J Nat Prod*, 2006, 69: 1374-1376.

3 Xu GB, Yang T, Bao JK, *et al*. Isochaetomium A_2, a new bis (naphthodihydropyran-4-one) with antimicrobial and immunological activities from fungus *Chaetomium microcephalum*. *Arch Pharm Res*, 2014, 37: 575-579.

4 Xu GB, He G, Bai HH, *et al*. Indole alkaloids from *Chaetomium globosum*. *J Nat Prod*, 2015, 78: 1479-1485.

5 Xu GB, Li LM, Yang T, *et al*. Chaetoconvosins A and B, alkaloids with new skeleton from fungus *Chaetomium convolutum*. *Org Lett*, 2012, 14: 6052-6055.

6 Liu Y, Yang T, Li H, *et al*. BD750, a benzothiazole derivative, inhibits T cell proliferation by JAK3/STAT5 signal pathway. *Br J Pharmacol*, 2013, 168: 632-643.

7 Sekita S, Yoshihira K, Natori S. Chaetoglobosins, cytotoxic 10-(indol-3-yl)-[13] cytochalasans from *Chaetomium* spp. IV. Carbon-13 nuclear magnetic resonance spectra and their application to a biosynthetic study. *Chem Pharm Bull*, 1983, 31: 490-498.

8 Sekita S, Yoshihira K, Natori S, *et al*. Chaetoglobosins, cytotoxic 10-(indol-3-yl)-[13] cytochalasans from *Chaetomium* spp. III. Structures of chaetoglobosins C, E, F, G, and J. *Chem Pharm Bull*, 1982, 30: 1629-1638.

9 Iwamoto C, Yamada T, Ito Y, *et al*. Cytotoxic cytochalasans from a *Penicillium* species separated from a marine alga. *Tetrahedron*, 2001, 57: 2997-3004.

10 Cui CM, Li XM, Li CS, *et al*. Cytoglobosins A-G, cytochalasans from a marine-derived endophytic fungus, *Chaetomium globosum* QEN-14. *J Nat Prod*, 2010, 73: 729-733.

11 Zhang J, Ge HM, Jiao RH, *et al*. Cytotoxic chaetoglobosins from the endophyte *Chaetomium globosum*. *Planta Med*, 2010, 76: 1910-1914.

12 Breinholt J, Demuth H, Heide M, *et al*. Prenisatin (5-(3-Methyl-2-butenyl) -indole-2, 3-dione): an Antifungal Isatin Derivative from *Cheatomium globosum*. *Acta Chem Scand*, 1996, 50: 443-445.

13 Takahashi M, Koyama K, Natori S. Four new azaphilones from *Chaetomium globosum* var. flavoviridae. *Chem Pharm Bull*, 1990, 38: 625-628.

14 Phonkerd N, Kano*km*edhakul S, Kano*km*edhakul K, *et al*. Bis-spiro-azaphilones and azaphilones from the fungi *Chaetomium cochliodes* VTh01 and *C*. cochliodes CTh05. *Tetrahedron*, 2008, 64: 9636-9645.

15 Sekita S, Yoshihira K, Natori S, *et al*. Chaetoglobosins, cytotoxic 10-(indol-3-yl)-[13] cytochalasans from *Chaetomium* spp. I. production, isolation and some cytological effects of chaetoglobosins A-J. *Chem Pharm Bull*, 1982, 30: 1609-1617.

16 Ichihara A, Katayama K, Teshima H, *et al*. Chaetoglobosin O and other phytotoxic metabolites from *Cylindrocladium floridanum*, a causal fungus of alfalfa black rot disease. *Biosci Biotech Bioch*, 1996, 60: 360-361.

17 Kang J G, Kim K K, Kang K Y. Antagonism and structural identification of antifungal compound from *Chaetomium cochliodes* against phytopathogenic fungi. *Agric Chem Biotech*, 1999, 42: 146-150.