密花樫木根化学成分及其蛋白酪氨酸磷酸酶 1B 抑制活性研究

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摘　要:为研究密花樫木(*Dysoxylum densiflorum*)根的化学成分及其蛋白酪氨酸磷酸酶 1B(PTP-1B)抑制活性。利用多种色谱方法从密花樫木根乙酸乙酯萃取物中分离得到 23 个化合物,并运用现代波谱技术鉴定其结构,分别为 5(10),13*E*-halimadiene-3α,15-diol(**1**)、(-)-agbanindiol A(**2**)、polylauioid H(**3**)、2-oxopopulifolic acid(**4**)、dysoxydenone C(**5**)、2-oxo-ent-cleroda-3,13*Z*-dien-15-oic acid(**6**)、nakamurol B(**7**)、methyl (13*E*)-2-oxoneocleroda-3,13-dien-15-oate(**8**)、15-acetoxy-ent-3,13*E*-clerodadien-2-one(**9**)、(3α,4β,13*E*)-neoclerod-13-ene-3,4,15-triol(**10**)、5(10),14-halimadiene-3α,13ξ-diol(**11**)、dysokusone A(**12**)、14,15-dinorclerod-3-ene-2,13-dione(**13**)、3,4-epoxyclerodan-13*E*-en-15-oic acid(**14**)、dysokusone G(**15**)、15-acetyloxyl-3α,4β-dihydroxy-neoclerod-13*Z*-ene(**16**)、[1α(*E*),2β,4aβ,8aα]-5-(decahydro-4a-hydroy-1,2,5,5-tetra-methyl-1-naphthalenyl)-3-methl-2-penten-1-ol(**17**)、kolavenol(**18**)、(13*E*)-2-oxoneocleroda-3,13-dien-15-ol(**19**)、2β-hydroxykolavenol(**20**)、ent-3β,4β-epoxyclerod-13*E*-en-15-ol(**21**)、2-oxodihydrokolavenol acetate(**22**)和(3α,4β,13*E*)-4-ethoxyneoclerod-13-ene-3,15-diol(**23**)。化合物 **2**、**4**、**6** ~ **9**、**11**、**13**、**14**、**17** ~ **20**、**22** ~ **23** 为首次从樫木属植物中分离得到。采用体外 PTP-1B 抑制活性评价所得化合物的生物活性,发现化合物 **3**、**12**、**16**、**21** 具有抑制 PTP-1B 作用,其 IC_{50} 值分别为 31.25 ± 0.64、0.30 ± 0.56、0.64 ± 0.51 和 78.50 ± 0.59 μmol/L。

关键词:密花樫木;化学成分;PTP-1B 抑制活性

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Study on chemical constituents from the roots of *Dysoxylum densiflorum* and their protein tyrosine phosphatase 1B inhibitory activity

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Abstract:In order to study the chemical constituents from the roots of *Dysoxylum densiflorum* and their protein tyrosine phosphatase 1B(PTP-1B) inhibitory activity. Twenty-three compounds were isolated from the *D. densiflorum* applying various chromatographic techniques. Their structures were elucidated and identified as 5(10),13*E*-halimadiene-3α,15-diol (**1**),(-)-agbanindiol A (**2**),polylauioid H (**3**),2-oxopopulifolic acid (**4**),dysoxydenone C (**5**),2-oxo-ent-cleroda-3,13*Z*-dien-15-oic acid (**6**),nakamurol B (7),methyl (13*E*)-2-oxoneocleroda-3,13-dien-15-oate (**8**),15-acetoxy-ent-3,13*E*-clerodadien-2-one (**9**),(3α,4β,13*E*)-neoclerod-13-ene-3,4,15-triol (**10**),5(10),14-halimadiene-3α,13ξ-diol (**11**),dysokusone A (**12**),14,15-dinorclerod-3-ene-2,13-dione (**13**),3,4-epoxyclerodan-13*E*-en-15-oic acid (**14**),dysokusone G (**15**),15-acetyloxyl-3α,4β-dihydroxy-neoclerod-13*Z*-ene (**16**),[1α(*E*),2β,4aβ,8aα]-5-(decahydro-4a-hydroy-1,2,5,5-tetra-methyl-1-naphthalenyl)-3-methl-2-penten-1-ol (**17**), kolavenol (**18**), (13*E*)-2-oxoneocleroda-3, 13-dien-15-ol (**19**), 2β-hydroxykolavenol (**20**), ent-3β, 4β-epoxyclerod-13*E*-en-15-ol (**21**), 2-oxodihydrokolavenol acetate (**22**), and (3α, 4β, 13*E*)-4-ethoxyneoclerod-13-ene-3,15-diol (**23**). Compounds **2**,**4**,**6-9**,**11**,**13**,**14**,**17-20**,**22-23** are obtained from *Dysoxylum* for the first time. Compounds **3**,**12**,**16** and **21** had PTP-1B inhibitory activity with the IC_{50} values were 31.25 ± 0.64,0.30 ± 0.56,0.64 ± 0.51 and 78.50 ± 0.59 μmol/L,respectively.

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Key words: *Dysoxylum densiflorum*; chemical composition; PTP-1B inhibitory activity

密花楔木(*Dysoxylum densiflorum*)产于我国云南西双版纳(勐腊、景洪),生于海拔 520 ~ 800 m 的湿性热带季节性谷边、河边坡地的雨林中[1]。现代研究表明,密花楔木中含有丰富的倍半萜、三萜、二萜、柠檬苦素类等化学成分,部分化合物具有良好的抗菌、抗肿瘤、镇痛等多种药理活性[2-5]。蛋白质酪氨酸磷酸酶(proteintyrosine phosphatase, PTPs)是一组催化蛋白质酪氨酸脱磷酸化的酶。蛋白质酪氨酸磷酸酶 1B(proteintyrosine phosphatase 1B, PTP-1B)通过激活的胰岛素受体和下游底物蛋白去磷酸化,在调节胰岛素作用中发挥关键作用[6-7]。因此 PIP-1B 是胰岛素信号转导通路中的关键的负调节蛋白,PTP-1B 抑制剂是开发抗糖尿病药物的重要选择。课题组前期发现楝科楔木属植物多脉楔木中的二萜类成分表现出对 PTP-1B 抑制作用[8],同属其他植物尚未见此活性研究报道。因此,为进一步探究密花楔木的活性成分,本实验通过硅胶柱色谱、半制备色谱、制备色谱结合核磁共振波谱技术对密花楔木根提取物乙酸乙酯萃取部位进行研究,以期为抑制 PTP-1B 活性成分的发现提供依据。

1　材料与方法

1.1　仪器与材料

Bruker Avance Ⅲ 600 型核磁共振仪(德国 Bruker 公司);LC-3000 型制备高效液相色谱仪(北京创新通恒科技有限公司);制备型色谱柱(250 mm × 21.2 mm, 10 μm)(北京慧得易科技有限责任公司);半制备型色谱柱(Luna C-18, 250 mm × 10 mm, 10 μm)(美国 Phenomenex 公司);硅胶 G(100 ~ 200, 200 ~ 300 目,青岛海洋化工厂);酶标仪(赛默飞世尔科技(中国)有限公司);重组人 PTP-1B 蛋白(Abcam 公司);氯化钠(国药集团化学试剂有限公司);三羟甲基氨基甲烷 Tris(Gen-view 科技有限公司);硝基苯基磷酸二钠(*p*-nitrophenyl phosphate, *p*-NPP)(上海麦克林生化科技有限公司);乙二胺四乙酸二钠(ethylene diamine tetraacetic acid, EDTA)(北京索莱宝科技有限公司);二硫苏糖醇(*DL*-dithiothreitol, DTT)(北京索莱宝科技有限公司);半制备及制备液相所用试剂为色谱纯,其他均为分析纯。

密花楔木根于 2020 年 5 月采自云南勐海,经江西中医药大学江西民族传统药现代科技与产业发展协同创新中心张亚梅副教授鉴定为楝科葱臭木属植物密花楔木根(*Dysoxylum densiflorum*)。凭证标本(2020-05)存放于江西中医药大学江西民族传统药现代科技与产业发展协同创新中心。

1.2　方法

密花楔木根 10 kg,粉碎,采用回流提取法用 95% 乙醇提取 3 次,每次 1 h,合并提取液,过滤后减压浓缩得到总浸膏 2400 g,将总浸膏用乙酸乙酯-水(1∶1)萃取,减压浓缩后得到乙酸乙酯萃取物 491.35 g。取乙酸乙酯浸膏 150 g 用硅胶色谱柱(15 cm × 15 cm)分离,以石油醚-丙酮(10∶1、7∶1、4∶1、1∶1)和 95% 乙醇为洗脱剂,得到 3 个流分(MG1 ~ MG3)。

MG1 部位分别经两次制备 HPLC(乙腈-水 80∶20→95∶5、乙腈-水 80∶20)得到 MZ1a 和 MZ1b,MZ1a 分别经正向半制备 HPLC(石油醚-丙酮 8∶1)、半制备 HPLC(乙腈-水 80∶20)得到化合物 **19**(4.8 mg, t_R = 12 min)、**20**(1.1 mg, t_R = 17 min)。MZ1b 分别经正向半制备 HPLC(石油醚-丙酮 8∶1)、半制备 HPLC(乙腈-水 70∶30)得到化合物 **18**(1 mg, t_R = 40 min)。

MG2 部位分别经两次制备 HPLC(乙腈-水 60∶40→95∶5、乙腈-水 50∶50)得到化合物 **10**(91.7 mg, t_R = 25 min)、**22**(30.3 mg, t_R = 36 min)和 MZ2a ~ MZ2f, MZ2a 分别经正向半制备 HPLC(石油醚-丙酮 6∶1)、半制备 HPLC(乙腈-水 60∶40→95∶5)得到化合物 **7**(1 mg, t_R = 29 min);MZ2b 分别经正向半制备 HPLC(石油醚-丙酮 8∶1)、半制备 HPLC(乙腈-水 60∶40→95∶5)得到化合物 **5**(1 mg, t_R = 13 min);MZ2c 分别经正向半制备 HPLC(石油醚-丙酮 8∶1)、半制备 HPLC(乙腈-水 60∶40→95∶5)得到化合物 **11**(1 mg, t_R = 18 min);MZ2d 分别经正向半制备 HPLC(石油醚-丙酮 10∶1)、半制备 HPLC(乙腈-水 60∶40)得到化合物 **12**(5.1 mg, t_R = 24 min)、**14**(7 mg, t_R = 23 min)、**15**(9.2 mg, t_R = 26 min)、**21**(4.5 mg, t_R = 26 min);MZ2e 分别经正向半制备 HPLC(石油醚-丙酮 10∶1)、半制备 HPLC(乙腈-水 60∶40)得到化合物 **6**(1 mg, t_R = 32 min);MZ2f 分别经正向半制备 HPLC(石油醚-丙酮 10∶1)、半制备 HPLC(乙腈-水 60∶40)得到化合物 **8**(45.1 mg, t_R = 50 min)、**9**(2.4 mg, t_R = 50 min)、**13**(1 mg, t_R = 17 min)。

MG3 部位分别经两次制备 HPLC(乙腈-水 50∶50→80∶20、乙腈-水 30∶70→60∶40)得到 MZ3a ~

MZ3c,MZ3a 分别经正向半制备 HPLC(石油醚-丙酮 3∶1)、半制备 HPLC(乙腈-水 40∶60)得到化合物 **17**(3.3 mg,t_R = 26 min);MZ3b 分别经正向半制备 HPLC(石油醚-丙酮 6∶1)、半制备 HPLC(乙腈-水 45∶55)得到化合物 **1**(1 mg,t_R = 17 min)、**2**(1 mg,t_R = 19 min)、**3**(1.5 mg,t_R = 22 min)、**4**(1 mg,t_R = 29 min)、**16**(1 mg,t_R = 34 min);MZ3c 分别经正向半制备 HPLC(石油醚-丙酮 3∶1)、半制备 HPLC(乙腈-水 55∶45)得到化合物 **23**(11.4 mg,t_R = 13 min)。

1.3 PTP-1B 抑制活性测定

以对 *p*-NPP 作为底物,苏拉明钠为阳性对照,根据 PTP-1B 水解 *p*-NPP 的磷酸基团而产生颜色反应来测定 PTP-1B 的活性。测试样品用 DMSO 溶解,配制为质量浓度 10 mg/mL 的母液,取母液 10 μL 加缓冲液至质量浓度梯度为 0、0.0125、0.025、0.05、0.1、0.2 mg/mL,在 96 孔板中加入缓冲液(180 μL 缓冲体系组成为 11 mmol/L Tris-HCl pH 7.5、55 mmol/L DTT、1.1 mmol/L EDTA、33 mmol/L *p*-NPP),37 ℃再回温 5 min 后加入 *p*-NPP 50 μL,37 ℃再回温 5 min 后加入样品,酶 20 μL,37 ℃孵育 30 min 后,加入 mol/L NaOH 10 μL 终止反应。以不含样品的系统为空白对照,利用 SpectraMax MD5 酶标仪测定 405 nm 处吸光度(A)[9]。应用 Graphpad 软件计算半抑制浓度(IC_{50})。

2 结果

2.1 结构鉴定

化合物 1 淡黄色胶状;分子式为 $C_{20}H_{34}O_2$。^{1}H NMR(600 MHz,$CDCl_3$)δ:5.38(1H,t,*J* = 6.9 Hz,H-14),4.14(2H,d,*J* = 7.1 Hz,H-15),3.45(1H,d,*J* = 11.0 Hz,H-3),1.67(3H,s,H-16),1.04(3H,s,H-18),0.96(3H,s,H-19),0.84(3H,d,*J* = 6.8 Hz,H-20),0.82(3H,s,H-17);^{13}C NMR(150 MHz,$CDCl_3$)δ:25.2(C-1),27.4(C-2),76.3(C-3),34.1(C-4),136.3(C-5),25.5(C-6),27.1(C-7),33.3(C-8),40.5(C-9),131.7(C-10),39.8(C-11),34.5(C-12),140.9(C-13),122.7(C-14),59.4(C-15),16.0(C-16),16.5(C-17),24.0(C-18),20.1(C-19),21.1(C-20)。以上数据与文献[10]报道基本一致,故鉴定化合物 **1** 为 5(10),13*E*-halimadiene-3α,15-diol。

化合物 2 白色粉末;分子式为 $C_{20}H_{34}O_2$。^{1}H NMR(600 MHz,$CDCl_3$)δ:5.38(1H,tq,*J* = 6.9,1.3 Hz,H-14),4.83(2H,dd,*J* = 36.5,1.5 Hz,H-18),4.33(1H,t,*J* = 3.1 Hz,H-3),4.13(2H,d,*J* = 6.9 Hz,H-15),1.67(3H,d,*J* = 1.3 Hz,H-16),1.24(3H,s,H-20),0.81(3H,d,*J* = 6.4 Hz,H-17),0.76(3H,s,H-19);^{13}C NMR(150 MHz,$CDCl_3$)δ:16.5(C-1),27.0(C-2),74.6(C-3),160.8(C-4),39.7(C-5),38.1(C-6),32.8(C-7),36.7(C-8),39.1(C-9),48.5(C-10),36.4(C-11),34.6(C-12),140.6(C-13),122.8(C-14),59.4(C-15),16.5(C-16),16.0(C-17),109.4(C-18),22.9(C-19),18.2(C-20)。以上数据与文献[11]报道基本一致,故鉴定化合物 **2** 为(-)-agbanindiol A。

化合物 3 白色结晶;分子式为 $C_{21}H_{36}O_4$。^{1}H NMR(600 MHz,$CDCl_3$)δ:5.67(1H,q,*J* = 1.2 Hz,H-14),3.68(3H,s,15-OCH_3),3.58(1H,m,H-3),2.16(3H,d,*J* = 1.2 Hz,H-16),1.25(3H,s,H-18),1.12(3H,s,H-19),0.79(3H,d,*J* = 6.2 Hz,H-17),0.74(3H,s,H-20);^{13}C NMR(150 MHz,$CDCl_3$)δ:16.5(C-1),30.6(C-2),76.6(C-3),76.5(C-4),41.5(C-5),32.5(C-6),26.6(C-7),36.4(C-8),38.9(C-9),41.0(C-10),36.9(C-11),34.9(C-12),161.9(C-13),115.0(C-14),167.4(C-15),19.3(C-16),16.1(C-17),21.7(C-18),17.4(C-19),18.5(C-20),51.0(15-OCH_3)。以上数据与文献[12]报道基本一致,故鉴定化合物 **3** 为 polylauioid H。

化合物 4 白色结晶;分子式为 $C_{20}H_{34}O_2$。^{1}H NMR(600 MHz,$CDCl_3$)δ:5.68(1H,d,*J* = 1.5 Hz,H-3),3.61(2H,m,H-15),1.85(3H,d,*J* = 1.3 Hz,H-18),1.08(3H,s,H-19),0.86(3H,d,*J* = 6.6 Hz,H-20),0.79(3H,m,H-16),0.76(3H,s,H-17);^{13}C NMR(150 MHz,$CDCl_3$)δ:35.5(C-1),200.7(C-2),172.7(C-3),125.3(C-4),39.5(C-5),34.8(C-6),26.8(C-7),35.7(C-8),38.4(C-9),45.5(C-10),29.9(C-11),34.7(C-12),29.3(C-13),39.7(C-14),60.7(C-15),19.6(C-16),15.6(C-17),18.2(C-18),18.9(C-19),17.9(C-20)。以上数据与文献[13]报道基本一致,故鉴定化合物 **4** 为 2-oxopopulifolic acid。

化合物 5 透明胶状;分子式为 $C_{20}H_{32}O_3$。^{1}H NMR(600 MHz,$CDCl_3$)δ:5.89(1H,m,H-3),1.98(3H,d,*J* = 1.4 Hz,H-20),1.13(3H,s,H-19),0.91(9H,dt,*J* = 6.8,4.2 Hz,H-16,17,18);^{13}C NMR(150 MHz,$CDCl_3$)δ:48.3(C-1),199.0(C-2),

128.9(C-3),159.6(C-4),74.6(C-5),34.2(C-6),40.3(C-7),25.3(C-8),35.9(C-9),40.1(C-10),33.5(C-11),48.1(C-12),210.7(C-13),52.7(C-14),24.9(C-15),22.9(C-16),23.0(C-17),17.2(C-18),21.4(C-19),19.7(C-20)。以上数据与文献[14]报道基本一致,故鉴定化合物 **5** 为 dysoxydenone C。

化合物 6　白色结晶;分子式为 $C_{21}H_{32}O_3$。^{1}H NMR(600 MHz,$CDCl_3$)δ:5.73(1H,m,H-3),5.62(1H,d,J = 1.4 Hz,H-13),3.67(3H,s,15-OCH_3),1.88(3H,d,J = 1.3 Hz,H-16),1.87(3H,d,J = 1.4 Hz,H-18),1.11(3H,m,H-19),0.89(3H,d,J = 6.7 Hz,H-17),0.81(3H,s,H-20);^{13}C NMR(150 MHz,$CDCl_3$)δ:34.8(C-1),200.2(C-2),125.6(C-3),172.4(C-4),39.8(C-5),35.5(C-6),26.9(C-7),35.9(C-8),38.9(C-9),45.4(C-10),35.8(C-11),26.8(C-12),160.5(C-13),115.7(C-14),166.6(C-15),25.3(C-16),15.7(C-17),18.9(C-18),18.4(C-19),17.7(C-20),50.9(15-OCH_3)。以上数据与文献[15]报道基本一致,故鉴定化合物 **6** 为 2-oxo-ent-cleroda-3,13*Z*-dien-15-oic acid。

化合物 7　透明胶状;分子式为 $C_{20}H_{32}O_2$。^{1}H NMR(600 MHz,$CDCl_3$)δ:5.86(1H,m,H-14),5.71(1H,m,H-3),5.19(1H,dt,J = 17.3,1.8 Hz,H-15a),5.07(1H,dd,J = 10.8,1.8 Hz,H-15b),1.87(3H,d,J = 1.3 Hz,H-18),1.26(3H,s,H-16),1.10(3H,s,H-19),0.82(3H,d,J = 6.2 Hz,H-17),0.80(3H,s,H-20);^{13}C NMR(150 MHz,$CDCl_3$)δ:34.9(C-1),200.4(C-2),125.5(C-3),172.5(C-4),39.8(C-5),35.5(C-6),26.8(C-7),35.9(C-8),38.3(C-9),45.6(C-10),31.2(C-11),34.7(C-12),73.1(C-13),144.7(C-14),112.1(C-15),30.0(C-16),15.6(C-17),18.9(C-18),18.3(C-19),18.0(C-20)。以上数据与文献[16]报道基本一致,故鉴定化合物 **7** 为 nakamurol B。

化合物 8　白色粉末;分子式为 $C_{21}H_{32}O_3$。^{1}H NMR(600 MHz,$CDCl_3$)δ:5.68(1H,t,J = 1.2 Hz,H-3),5.61(1H,q,J = 1.3 Hz,H-14),3.63(3H,s,15-OCH_3),2.10(3H,d,J = 1.2 Hz,H-16),1.85(3H,d,J = 1.2 Hz,H-18),1.07(3H,s,H-19),0.80(3H,d,J = 6.1 Hz,H-17),0.78(3H,s,H-20);^{13}C NMR(150 MHz,$CDCl_3$)δ:34.8(C-1),199.8(C-2),125.3(C-3),172.2(C-4),39.7(C-5),35.4(C-6),26.7(C-7),35.9(C-8),38.6(C-9),45.5(C-10),35.4(C-11),33.9(C-12),160.2(C-13),115.0(C-14),166.9(C-15),19.0(C-16),15.5(C-17),18.8(C-18),18.2(C-19),17.7(C-20),50.6(15-OCH_3)。以上数据与文献[17]报道基本一致,故鉴定化合物 **8** 为 methyl (13*E*)-2-oxoneocleroda-3,13-dien-15-oate。

化合物 9　白色结晶;分子式为 $C_{22}H_{34}O_3$。^{1}H NMR(600 MHz,$CDCl_3$)δ:5.72(1H,q,J = 1.1 Hz,H-3),5.31(1H,td,J = 7.3,1.6 Hz,H-14),4.52(1H,m,H-15),2.04(3H,s,OAc),1.88(3H,s,H-18),1.74(3H,m,H-16),1.11(3H,s,H-19),0.85(3H,d,J = 6.3 Hz,H-17),0.80(3H,s,H-20);^{13}C NMR(150 MHz,$CDCl_3$)δ:36.3(C-1),200.4(C-2),125.9(C-3),172.7(C-4),40.2(C-5),35.4(C-6),27.2(C-7),36.5(C-8),39.2(C-9),45.9(C-10),35.9(C-11),25.5(C-12),143.5(C-13),119.2(C-14),61.1(C-15),24.1(C-16),16.1(C-17),19.3(C-18),18.7(C-19),18.2(C-20),21.5(CH_3COO),171.4(CH_3COO)。以上数据与文献[18]报道基本一致,故鉴定化合物 **9** 为 15-acetoxy-ent-3,13*E*-clerodadien-2-one。

化合物 10　白色结晶;分子式为 $C_{20}H_{34}O_3$。^{1}H NMR(600 MHz,CD_3OD)δ:5.34(1H,m,H-14),4.06(2H,d,J = 6.8 Hz,H-15),3.48(1H,q,J = 3.2 Hz,H-3),1.85(1H,dd,J = 12.4,2.0 Hz,H-10),1.67(3H,d,J = 1.3 Hz,H-16),1.19(3H,s,H-18),1.11(3H,s,H-19),0.79(3H,d,J = 5.9 Hz,H-17),0.74(3H,s,H-20);^{13}C NMR(150 MHz,CD_3OD)δ:17.6(C-1),31.0(C-2),76.8(C-3),77.3(C-4),42.5(C-5),33.5(C-6),27.9(C-7),37.3(C-8),39.7(C-9),41.7(C-10),38.4(C-11),34.1(C-12),140.7(C-13),124.2(C-14),59.4(C-15),16.6(C-16),16.5(C-17),21.3(C-18),17.9(C-19),19.0(C-20)。以上数据与文献[17]报道基本一致,故鉴定化合物 **10** 为(3α,4β,13*E*)-neoclerod-13-ene-3,4,15-triol。

化合物 11　透明胶状;分子式为 $C_{20}H_{34}O_2$。^{1}H NMR(600 MHz,$CDCl_3$)δ:5.89(1H,dd,J = 17.4,10.8 Hz,H-14),5.20(1H,dd,J = 17.4,1.2 Hz,H-15a),5.05(1H,dd,J = 10.8,1.2 Hz,H-15b),1.27(3H,s,H-16),1.04(3H,s,H-18),0.95(3H,s,H-19),0.82(6H,d,J = 6.4 Hz,H-17,20);^{13}C NMR

(150 MHz, $CDCl_3$) δ: 25.4 (C-1), 27.7 (C-2), 76.5 (C-3), 40.1 (C-4), 136.4 (C-5), 27.4 (C-6), 27.6 (C-7), 33.5 (C-8), 40.4 (C-9), 111.9 (C-10), 30.1 (C-11), 36.6 (C-12), 73.5 (C-13), 145.4 (C-14), 100.1 (C-15), 24.1 (C-16), 16.1 (C-17), 25.8 (C-18), 20.4 (C-19), 21.5 (C-20)。以上数据与文献[10]报道基本一致,故鉴定化合物 **11** 为 5(10),14-halimadiene-3α,13ξ-diol。

化合物 12 白色结晶;分子式为 $C_{20}H_{32}O_2$。^{1}H NMR (600 MHz, $CDCl_3$) δ: 5.87 (1H, m, H-3), 1.89 (3H, d, J = 1.5 Hz, H-20), 0.91 (9H, m, H-12, 17, 18), 0.84 (3H, s, H-19); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 54.6 (C-1), 199.4 (C-2), 127.0 (C-3), 163.3 (C-4), 47.9 (C-5), 26.1 (C-6), 43.5 (C-7), 24.5 (C-8), 40.2 (C-9), 37.8 (C-10), 33.8 (C-11), 16.9 (C-12), 48.0 (C-13), 210.9 (C-14), 52.6 (C-15), 24.7 (C-16), 22.8 (C-17), 22.7 (C-18), 17.2 (C-19), 22.1 (C-20)。以上数据与文献[19]报道基本一致,故鉴定化合物 **12** 为 dysokusone A。

化合物 13 淡黄色胶状;分子式为 $C_{18}H_{28}O_2$。^{1}H NMR (600 MHz, $CDCl_3$) δ: 5.72 (1H, s, H-3), 2.12 (3H, m, H-16), 1.88 (3H, d, J = 2.0 Hz, H-18), 1.11 (3H, d, J = 2.6 Hz, H-19), 0.83 (6H, m, H-17, 20); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 35.1 (C-1), 200.3 (C-2), 125.7 (C-3), 172.6 (C-4), 40.0 (C-5), 35.7 (C-6), 27.0 (C-7), 36.4 (C-8), 38.4 (C-9), 46.2 (C-10), 30.9 (C-11), 37.2 (C-12), 208.6 (C-13), 30.3 (C-16), 15.9 (C-17), 18.4 (C-18), 19.1 (C-19), 17.9 (C-20)。以上数据与文献[20]报道基本一致,故鉴定化合物 **13** 为 14,15-dinorclerod-3-ene-2,13-dione。

化合物 14 透明胶状;分子式为 $C_{20}H_{32}O_3$。^{1}H NMR (600 MHz, $CDCl_3$) δ: 5.67 (1H, s, H-14), 2.93 (1H, d, J = 1.8 Hz, H-3), 2.16 (3H, s, H-16), 1.17 (3H, s, H-18), 1.04 (3H, s, H-19), 0.79 (3H, d, J = 6.3 Hz, H-17), 0.66 (3H, s, H-20); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 19.8 (C-1), 28.2 (C-2), 62.3 (C-3), 66.6 (C-4), 37.4 (C-5), 35.2 (C-6), 28.4 (C-7), 37.2 (C-8), 39.3 (C-9), 48.1 (C-10), 36.2 (C-11), 36.5 (C-12), 164.0 (C-13), 115.2 (C-14), 171.9 (C-15), 17.0 (C-16), 16.1 (C-18), 15.5 (C-17), 18.7 (C-19), 19.5 (C-20)。以上数据与文献[21]报道基本一致,故鉴定化合物 **14** 为 3,4-epoxyclerodan-13E-en-15-oic acid。

化合物 15 透明胶状;分子式为 $C_{20}H_{30}O_2$。^{1}H NMR (600 MHz, $CDCl_3$) δ: 6.03 (1H, s, H-13), 5.87 (1H, m, H-3), 2.27 (3H, s, H-12), 2.12 (1H, m, H-16), 1.86 (3H, d, J = 1.6 Hz, H-20), 0.93 (3H, s, H-17), 0.92 (3H, s, H-18), 0.90 (3H, s, H-19); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 53.8 (C-1), 199.4 (C-2), 126.9 (C-3), 163.2 (C-4), 47.5 (C-5), 24.8 (C-6), 40.8 (C-7), 27.6 (C-8), 39.7 (C-9), 37.5 (C-10), 161.5 (C-11), 21.3 (C-12), 124.6 (C-13), 201.0 (C-14), 54.5 (C-15), 25.2 (C-16), 22.8 (C-17), 22.8 (C-18), 16.9 (C-19), 22.0 (C-20)。以上数据与文献[22]报道基本一致,故鉴定化合物 **15** 为 dysokusone G。

化合物 16 透明胶状;分子式为 $C_{22}H_{38}O_4$。^{1}H NMR (600 MHz, $CDCl_3$) δ: 5.32 (1H, t, J = 7.4 Hz, H-14), 4.54 (2H, m, H-15), 3.58 (1H, t, J = 2.9 Hz, H-3), 2.04 (3H, s, OAc), 1.24 (3H, s, H-18), 1.12 (3H, s, H-19), 0.80 (3H, d, J = 6.0 Hz, H-17), 0.72 (3H, s, H-20); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 16.6 (C-1), 21.3 (C-2), 76.6 (C-3), 76.5 (C-4), 41.5 (C-5), 32.5 (C-6), 26.6 (C-7), 36.3 (C-8), 39.0 (C-9), 40.9 (C-10), 37.4 (C-11), 26.0 (C-12), 144.4 (C-13), 118.4 (C-14), 61.1 (C-15), 23.9 (C-16), 16.1 (C-17), 21.6 (C-18), 17.4 (C-19), 18.4 (C-20), 171.3 (CH_3COO), 30.6 (CH_3COO)。以上数据与文献[23]报道基本一致,故鉴定化合物 **16** 为 15-acetyloxyl-3α,4β-dihydroxy-neoclerod-13Z-ene。

化合物 17 透明胶状;分子式为 $C_{20}H_{36}O_2$。^{1}H NMR (600 MHz, $CDCl_3$) δ: 5.40 (1H, t, J = 7.0 Hz, H-14), 4.14 (2H, d, J = 6.9 Hz, H-15), 1.68 (3H, s, H-16), 1.28 (3H, s, H-18), 1.02 (3H, s, H-19), 0.79 (3H, d, J = 5.4 Hz, H-20), 0.72 (3H, s, H-17); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 27.3 (C-1), 32.0 (C-2), 33.1 (C-3), 42.1 (C-4), 76.2 (C-5), 37.1 (C-6), 37.1 (C-7), 23.5 (C-8), 43.2 (C-10), 38.9 (C-11), 36.8 (C-12) 123.2 (C-13), 40.9 (C-14), 59.6 (C-15), 21.2 (C-16), 18.4 (C-17), 16.7 (C-18), 16.2 (C-19), 14.9 (C-20)。以上数据与文献[24]报道基本一致,故鉴定化合物 **17** 为[1α(E),2β,4aβ,8aα]-5-(decahydro-4a-hydroy-1,2,5,5-tetra-methyl-1-naphthalenyl)-3-methl-2-penten-1-ol。

化合物 18 淡黄色胶状;分子式为 $C_{20}H_{34}O$。^{1}H

NMR(600 MHz,$CDCl_3$)δ:5.38(1H,tq,J = 6.9,1.3 Hz,H-14),5.17(1H,ddt,J = 4.2,2.7,1.2 Hz,H-3),4.12(2H,d,H-15),1.66(3H,m,H-16),1.57(3H,d,J = 3.1 Hz,H-20),0.98(3H,d,J = 0.7 Hz,H-19),0.79(3H,d,J = 6.4 Hz,H-17),0.70(3H,s,H-18);^{13}C NMR(150 MHz,$CDCl_3$)δ:36.8(C-1),27.0(C-2),120.5(C-3),144.6(C-4),38.3(C-5),36.9(C-6),27.6(C-7),36.4(C-8),38.7(C-9),46.5(C-10),18.4(C-11),32.9(C-12),140.9(C-13),123.0(C-14),59.5(C-15),16.6(C-16),18.5(C-17),18.1(C-18),20.0(C-19),16.1(C-20)。以上数据与文献[25]报道基本一致,故鉴定化合物 **18** 为 kolavenol。

化合物 19　无色结晶;分子式为 $C_{20}H_{32}O_2$。^{1}H NMR(600 MHz,$CDCl_3$)δ:5.71(1H,m,H-3),5.37(1H,tq,J = 6.9,1.3 Hz,H-14),4.12(2H,m,H-15),1.88(3H,d,J = 1.3 Hz,H-18),1.66(3H,m,H-16),1.11(3H,d,J = 0.7 Hz,H-19),0.83(3H,dd,J = 4.2,1.9 Hz,H-17),0.80(3H,s,H-20);^{13}C NMR(150 MHz,$CDCl_3$)δ:35.1(C-1),200.6(C-2),125.6(C-3),172.8(C-4),40.0(C-5),35.9(C-6),27.0(C-7),36.1(C-8),138.9(C-9),45.8(C-10),35.7(C-11),32.4(C-12),40.0(C-13),123.5(C-14),59.5(C-15),16.8(C-16),15.9(C-17),19.1(C-18),18.5(C-19),18.1(C-20)。以上数据与文献[17]报道基本一致,故鉴定化合物 **19** 为(13E)-2-oxoneocleroda-3,13-dien-15-ol。

化合物 20　透明胶状;分子式为 $C_{20}H_{34}O_2$。^{1}H NMR(600 MHz,$CDCl_3$)δ:5.42(1H,tq,J = 7.0,1.3 Hz,H-3),5.35(1H,dt,J = 4.6,1.5 Hz,H-14),4.15(1H,s,H-2),4.13(2H,d,J = 7.0 Hz,H-15),1.64(3H,s,H-16),0.94(3H,s,H-19),0.81(3H,d,J = 6.6 Hz,H-18),0.73(3H,s,H-17),0.73(3H,s,H-20);^{13}C NMR(150 MHz,$CDCl_3$)δ:27.6(C-1),65.5(C-2),150.1(C-3),122.2(C-4),38.5(C-5),36.4(C-6),36.6(C-7),36.4(C-8),38.9(C-9),41.0(C-10),28.1(C-11),32.4(C-12),141.2(C-13),122.9(C-14),59.6(C-15),16.7(C-16),16.0(C-17),18.2(C-18),18.7(C-19),18.5(C-20)。以上数据与文献[26]报道基本一致,故鉴定化合物 **20** 为 2β-hydroxykolavenol。

化合物 21　透明胶状;分子式为 $C_{20}H_{34}O_2$。^{1}H NMR(600 MHz,$CDCl_3$)δ:5.39(1H,ddq,J = 7.0,5.5,1.3 Hz,H-14),4.14(2H,d,J = 6.9 Hz,H-15),2.92(1H,d,J = 1.9 Hz,H-3),1.67(3H,d,J = 1.3 Hz,H-16),1.17(3H,s,H-18),1.04(3H,s,H-19),0.78(3H,m,H-17),0.64(3H,s,H-20);^{13}C NMR(150 MHz,$CDCl_3$)δ:15.5(C-1),28.4(C-2),62.4(C-3),66.6(C-4),37.4(C-5),37.3(C-6),28.3(C-7),36.2(C-8),39.2(C-9),48.0(C-10),37.0(C-11),33.2(C-12),140.7(C-13),123.1(C-14),59.6(C-15),16.7(C-16),16.1(C-17),17.0(C-18),19.9(C-19),18.8(C-20)。以上数据与文献[27]报道基本一致,故鉴定化合物 **21** 为 ent-3β,4β-epoxyclerod-13E-en-15-ol。

化合物 22　透明胶状;分子式为 $C_{22}H_{36}O_3$。^{1}H NMR(600 MHz,$CDCl_3$)δ:5.69(1H,p,J = 1.1 Hz,H-3),4.05(2H,m,H-15),2.02(3H,s,OAc),1.86(3H,d,J = 1.4 Hz,H-16),1.08(3H,m,H-18),0.87(3H,d,J = 6.5 Hz,H-20),0.79(3H,d,J = 6.2 Hz,H-17),0.77(3H,s,H-19);^{13}C NMR(150 MHz,$CDCl_3$)δ:35.6(C-1),200.5(C-2),125.6(C-3),171.3(C-4),39.9(C-5),35.0(C-6),27.0(C-7),35.6(C-8),38.6(C-9),45.7(C-10),34.7(C-11),30.3(C-12),29.5(C-13),35.9(C-14),62.9(C-15),19.4(C-16),15.8(C-17),18.5(C-18),19.1(C-19),18.1(C-20),172.7(CH_3COO),21.1(CH_3COO)。以上数据与文献[13]报道基本一致,故鉴定化合物 **22** 为 2-oxodihydrokolavenol acetate。

化合物 23　透明胶状;分子式为 $C_{22}H_{40}O_3$。^{1}H NMR(600 MHz,$CDCl_3$)δ:5.41(1H,m,H-14),4.13(2H,d,J = 7.0 Hz,H-15),3.79(1H,t,J = 2.9 Hz,H-3),3.34(2H,2dq,J = 11.0,7.0 Hz,4-O$CH_2$$CH_3$),3.11(3H,d,$J$ = 7.0 Hz,4-OCH_2CH_3),1.68(3H,s,H-16),1.15(3H,s,H-18),1.09(3H,s,H-19),0.76(3H,d,J = 6.3 Hz,H-17),0.70(3H,s,H-20);^{13}C NMR(150 MHz,$CDCl_3$)δ:16.2(C-1),30.7(C-2),72.0(C-3),79.4(C-4),42.4(C-5),32.0(C-6),26.9(C-7),36.1(C-8),38.6(C-9),39.5(C-10),37.3(C-11),33.2(C-12),141.5(C-13),123.0(C-14),59.6(C-15),16.3(C-16),16.6(C-17),14.5(C-18),17.7(C-19),18.5(C-20),57.0(4-OCH_2CH_3),16.3(4-OCH_2CH_3)。以上数据与文献[17]报道基本一致,故鉴定化合物 **23** 为(3α,4β,13E)-4-ethoxyneoclerod-13-ene-3,15-diol。

2.2 PTP-1B 抑制活性筛选结果

本文对分离得到的23个化合物进行PTP-1B抑制活性筛选，结果见表1，与阳性药苏拉明钠对比（抑制率62.31%，IC_{50}值为134.81±8.36 μmol/L），化合物**3**、**12**、**16**、**21**表现出PTP-1B抑制活性，IC_{50}值分别为31.25±0.64、0.30±0.56、0.64±0.51、78.50±0.59 μmol/L。

表1 化合物对PTP-1B的影响（$\bar{x}\pm s, n=6$）

Table 1 Effects of the compounds on PTP-1B activity（$\bar{x}\pm s, n=6$）

化合物 Compound	抑制率 Inhibition ratio(%)	IC_{50} (μmol/L)
3	54.64±1.67	31.25±0.64***
12	64.28±2.94	0.30±0.56***
16	70.95±2.32	0.64±0.51***
21	76.99±3.28	78.50±0.59***
苏拉明钠 Suraminsodium	62.31±3.97	134.81±8.36

注：与苏拉明钠组比较，***$P<0.001$。

Note: Compared with suraminsodium, ***$P<0.001$.

3 讨论与结论

本项研究从密花楔木根乙酸乙酯萃取物中共分离得到23个成分，并且进行PTP-1B抑制活性筛选，在此基础上对构效关系进行初步探讨。化合物**3**、**16**、**21**均为clerodane型，化合物**12**为prenyleudesmane型，且抑制效果最好，提示prenyleudesmane型二萜抑制活性强于clerodane型；化合物**3**、**21**均为*E*构型，化合物**16**为*Z*构型，而化合物**16**表现出较强的抑制活性，仅次于化合物**12**，提示*Z*型二萜类化合物抑制活性较强；化合物**3**、**16**结构中含有乙酰基，且抑制活性均较好，乙酰基是否与抑制活性有关还需进一步探讨。

二萜类成分在小分子天然PTP-1B抑制剂开发中发挥了不错的潜力，楔木属植物是二萜类成分的丰富来源[28]，从该属植物中挖掘天然来源的抗糖尿病候选药物是值得进一步探索。

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