佛波醇制备工艺优化及其衍生物的合成表征与细胞毒活性研究

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摘 要:目前佛波醇制备过程比较繁琐,本研究首先对制备工艺进行优化,使制备周期缩短至3天。然后以佛波醇、二十碳五烯酸、二十二碳六烯酸、花生酸为原料,设计合成了18个新化合物,运用^{1}H NMR,^{13}C NMR,HR-MS对化合物进行结构表征,并测试了这些化合物对人正常胚肺成纤维细胞(MRC-5)的毒性。结果显示13个化合物对正常细胞毒性较大(IC_{50} < 38.12 μmol/L),5个化合物毒性较小(IC_{50} > 100 μmol/L)。佛波醇的12位羟基、13位羟基分别与长链饱和脂肪酸形成单酯时毒性较低,实验结果为佛波醇的结构修饰提供参考。

关键词:佛波醇;工艺优化;结构修饰;毒性研究

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Optimization preparation process of phorbol and synthesis, characterization and cytotoxicity of its derivatives

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Abstract: The process of phorbol preparation is complicated, in this study, the preparation process was optimized and the preparation cycle was shorten to 3 days. 18 new compounds were designed and synthesized using phorbol, eicosapentaenoic acid, docosahexaenoic acid and arachidic acid. The compounds were characterized by ^{1}H NMR, ^{13}C NMR and HR-MS, and the toxicity of these compounds to hunan cells (MRC-5) was tested. There were 13 compounds with high cytotoxicity (IC_{50} < 38.12 μmol/L) and 5 compounds with low cytotoxicity (IC_{50} > 100 μmol/L) in the cytotoxicity asssy. The phorbol-12-monoester and phorbol-13-monoester, which were esterified with long-chain saturated fatty acids had low cytotoxicity. The results of the test could provide guidance for phorbol structural modification.

Key words: phorbol; process optimization; structural modification; cytotoxicity

佛波醇酯(phorbol ester)——二萜类化合物,广泛存在于大戟科和瑞香科植物中,具有多种生物活性,如抗肿瘤、抗HIV、抗炎、抗菌等[1-4]。其中,12-*O*-十四烷酰佛波醇-13-乙酯(12-*O*-tetradecanoylphorbol-13-acetate,TPA)在进行白血病治疗的临床II期试验[5],12-去氧佛波醇-13-乙酯(prostratin)在进行抗艾滋病(human immunodeficiency virus,HIV)的临床I期试验[6]。佛波醇酯化合物结构相近,化合物的分离具有工作繁琐、分离难度大、量微等缺点,这就对佛波醇酯的生物活性测试和构效关系研究带来困难;佛波醇酯生物活性多样,但也具有一定的毒性,但其毒性与化合物结构关系研究较少,本研究首先对巴豆油水解工艺进行优化,制备佛波醇(phorbol);并对其进行结构修饰,以二十碳五烯酸(eicosapentaenoic acid,EPA)和二十二碳六烯酸(docosahexaenoic acid,DHA)为代表的长链不饱和脂肪酸和以花生酸为代表的长链饱和脂肪酸对佛波醇进行酯化反应;考察了佛波醇酯化衍生物对人正常胚肺成纤维细胞(MRC-5)的毒性;实验结果将为佛波醇酯类药物制备提供参考。

1 试剂与方法

1.1 仪器与试剂

Rudolph AutopolII 旋光仪;Bruker VERTEX 70 红外光谱仪;Shimadzu UV-2600 紫外可见分光光度

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计;Agilent 1260 高效液相色谱仪;Agilent 6540 超高解析度四级杆-飞行时间质谱仪;Unity Inova 400、600MHz 超导核磁共振谱仪;TECAN INFINITE M1000 多功能酶标仪。

佛波醇(phorbol)和二十碳五烯酸(EPA)为实验室自制,二十二碳六烯酸(购于 MedChemExpress 公司)。其他试剂与溶剂为国产分析纯。

1.2 佛波醇制备工艺优化

佛波醇制备工艺目前比较成熟[7,8],但其制备周期需一周左右,为了缩短制备周期,本研究从水解时碱的浓度、水解时间、调节 pH 的节点、柱层析的选择四个方面对制备过程进行优化。

1.3 佛波醇衍生物合成线路

佛波醇结构修饰过程主要包括羟基的选择性保护、选择性酯化、去保护等[9,10]。

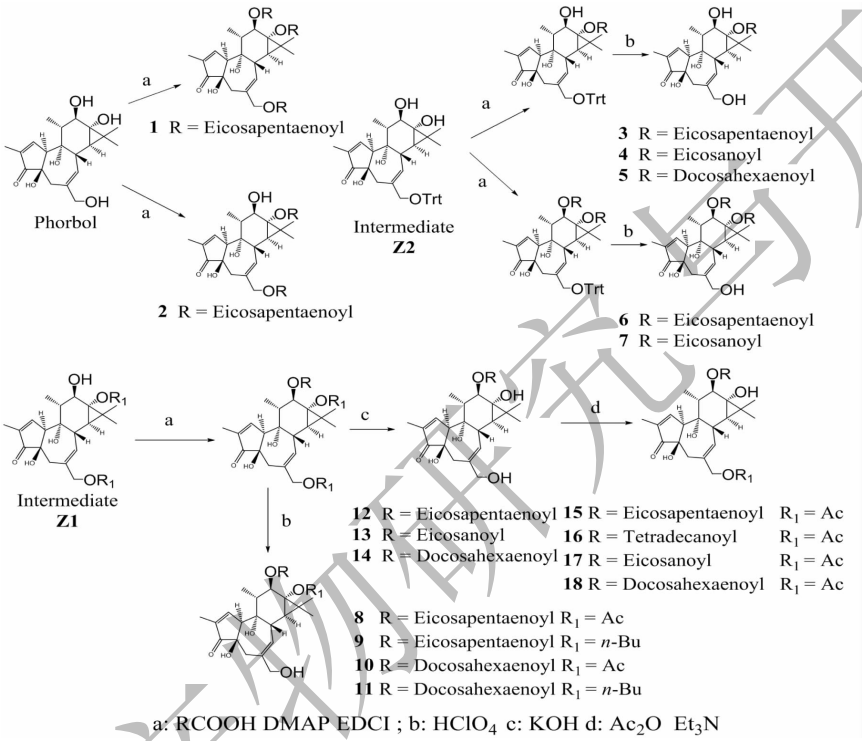

图 1 佛波醇衍生物合成路线

Fig. 1 Synthesis of phorbol derivatives

1.4 化学合成

1.4.1 中间体(Z1、Z2)

参照文献[9,11]制备得到中间体佛波醇-13,20-二乙酯(Z1)和佛波醇-20-三苯基甲醚(Z2)。

^{1}H NMR ($CDCl_3$,600 MHz) δ:7.56 (1H,s,H-1),5.66 (1H,m,H-7),4.45 (2H,m,H-20),3.98 (1H,d,J=9.8 Hz,H-12),3.20 (1H,s,H-8),3.12 (1H,s,H-10),2.51/2.38 (2H,dd,J=19.2 Hz,H-5),2.10 (3H,s,COCH_3),2.04 (3H,s,COCH_3),1.98 (1H,m,H-11),1.22 (3H,s,H-16),1.21 (3H,s,H-17),1.02 (1H,d,J=5.7 Hz,H-14),1.01 (1H,d,J=6.5 Hz,H-18)。(中间体 Z1,白色固体,1.90 g,产率 80%)。

^{1}H NMR ($CDCl_3$,400 MHz) δ:7.51 (1H,s,H-1),7.39 (6H,m,Tr),7.28 (6H,m,Tr),7.22 (3H,m,Tr),5.55 (1H,m,H-7),4.11 (1H,d,J=10.8 Hz,H-12),3.54 (2H,m,H-20),3.03 (1H,s,H-8),2.91 (1H,s,H-10),2.41/2.34 (2H,dd,J=19.2 Hz,H-5),1.85 (1H,m,H-11),1.73 (3H,m,H-19),1.27 (3H,s,H-16),1.14 (3H,s,H-17),1.00 (1H,d,J=6.2 Hz,H-18),0.76 (1H,d,J=5.2 Hz,H-14)。(中间体 Z2,淡黄色固体,1.946 g,产率 58%)。

1.4.2 佛波醇-12,13,20-三二十碳五烯酸酯(**1**),佛波醇-13,20-二二十碳五烯酸酯(**2**)

在 100 mL 圆底烧瓶中投入佛波醇(150 mg,0.41 mmol),二十碳五烯酸(457 mg,1.5 mmol),DMAP(153 mg,1.23 mmol)和 EDCI(485 mg,2.46

mmol),用 20 mL 二氯甲烷溶解,室温搅拌过夜。待反应完毕,经后处理用硅胶柱层析得产物 **1**(石油醚∶乙酸乙酯 =20∶1,300 mg,60%)。

参照产物 **1** 的合成,不同的是所用原料、催化剂、反应时间均减小一半,得产物 **2**(石油醚∶乙酸乙酯 =5∶1,247 mg,68%)。

1.4.3　佛波醇-13-二十碳五烯酸酯(**3**),佛波醇-13-花生酸酯(**4**),佛波醇-13-二十二碳六烯酸酯(**5**),佛波醇-12,13-二二十碳五烯酸酯(**6**),佛波醇-12,13-二花生酸酯(**7**)

在 100 mL 圆底烧瓶中投入中间体 Z2(200 mg,0.33 mmol),二十碳五烯酸(150 mg,0.5 mmol),DMAP(60 mg,0.5 mmol)和 EDCI(190 mg,1 mmol),用 20 mL 二氯甲烷溶解,室温搅拌过夜。待反应完毕,经后处理用硅胶柱柱层析洗脱得粗品(石油醚∶乙酸乙酯 =3∶1,174 mg,33.7%),将粗品加入 10 mL 0.35 % 高氯酸甲醇溶液反应 60 min,加入适量 $NaHCO_3$ 中和反应,回收溶剂。加入水和二氯甲烷分层,经后处理用硅胶柱层析得产物 **3**(石油醚∶乙酸乙酯 =1∶1,105 mg,80%)。

参照产物 **3** 的合成,得产物 **4**(石油醚∶乙酸乙酯 =3∶2,160 mg,88%),产物 5(石油醚∶乙酸乙酯 =1∶1,86mg,74%),产物 6(石油醚∶乙酸乙酯 =5∶1,180 mg,78%)和产物 7(石油醚∶乙酸乙酯 =5∶1,200 mg,70%)。

1.4.4　12-*O*-二十碳五烯酰佛波醇-13-乙酯(**8**),12-*O*-二十碳五烯酰佛波醇-13-丁酯(**9**),12-*O*-二十二碳六烯酰佛波醇-13-乙酯(**10**),12-*O*-二十二碳六烯酰佛波醇佛波醇-13-丁酯(**11**)

在 100 mL 圆底烧瓶中投入中间体 Z1(216 mg,0.48 mmol),参照产物 **1** 的合成得到 12-*O*-二十碳五烯酰佛波醇-13,20-二乙酯(石油醚∶乙酸乙酯 =4∶1,250 mg,70%),直接加入 10 mL 0.35% 高氯酸甲醇溶液水解 12 h。参照产物 **3** 的后处理方法得到产物 **8**(石油醚∶乙酸乙酯 =3∶2,150 mg,64%)。

参照产物 **8** 的合成,得到产物 **9**(石油醚∶乙酸乙酯 =3∶2,95 mg,52%),产物 **10**(石油醚∶乙酸乙酯 =3∶2,150 mg,79%)和产物 **11**(石油醚∶乙酸乙酯 =3∶2,78 mg,85%)。

1.4.5　佛波醇-12-二十碳五烯酸酯(**12**),佛波醇-12-花生酸酯(**13**),佛波醇-12-二十二碳六烯酸酯(**14**)在 100 mL 圆底烧瓶中投入 12-*O*-二十碳五烯酰佛波醇-13,20-二乙酯(550 mg,0.67 mmol),10 mL 0.05 M 氢氧化钾甲醇溶液,室温反应 60 min。待反应完毕加入适量稀盐酸中和反应,回收溶剂,加入水和二氯甲烷分层。经后处理用硅胶柱层析得到产物 **12**(石油醚∶乙酸乙酯 =1∶1,340 mg,76%)。

参照产物 **12** 的合成,得到产物 **13**(石油醚∶乙酸乙酯 =1∶1,200 mg,75%)和产物 **14**(石油醚∶乙酸乙酯 =2∶3,203 mg,76%)。

1.4.6　12-*O*-二十碳五烯酰佛波醇-20-乙酯(**15**),12-*O*-十四烷酰佛波醇-20-乙酯(**16**),12-*O*-花生酰佛波醇-20-乙酯(**17**),12-*O*-二十二碳六烯酰佛波醇-20-乙酯(**18**)

在 100 mL 圆底烧瓶中投入化合物 **12**(290 mg,0.45 mmol),乙酸酐(275 mg,2.7 mmol)和三乙胺(227 mg,2.25 mmol),用 20 mL 二氯甲烷溶解,室温反应 12 h。待反应完毕,经后处理用硅胶柱层析得到产物 **15**(石油醚∶乙酸乙酯 =3∶2,150 mg,48.7%)。

参照产物 **15** 的合成,得到产物 **16**(石油醚∶乙酸乙酯 =3∶1,156 mg,68%),产物 **17**(石油醚∶乙酸乙酯 =3∶1,352 mg,84%)和产物 **18**(石油醚∶乙酸乙酯 =3∶1,100 mg,86%)。

1.5　对正常胚肺成纤维细胞的毒性测试

取对数生长期的正常胚肺成纤维细胞(MRC-5,5 × 10^4/mL)接种于 96 孔板,每孔 100 μL 细胞,培养 24 h 后进行药物处理。药物最小终浓度 1 μmol/L,最大 100 μmol/L,按等比设置 5 个浓度。每个浓度设置 3 个重复孔。给药 48 h 后,每孔加入 10 μL CCK-8 试剂,并放置细胞培养箱中继续培养 1 h。最后用酶标仪检测每孔中的吸光度(450 nm),并计算 IC_{50}。

2　结果与讨论

2.1　佛波醇水解工艺优化结果

传统的水解工艺以 11.0 g 氢氧化钡溶于 400 mL 甲醇(2.75 mg/mL),对 100 mL 巴豆油水解 24h,此过程中并未对碱的浓度和水解时间进行考察。本研究在其他条件不变的情况下,将碱的浓度提高到 4.13 mg/mL(1.5 倍)、4.95 mg/mL(1.8 倍)和 5.50 mg/mL(2.0 倍)分别对 100 mL 巴豆油进行水解,每 2 h 取一次样对水解情况进行监测,连续取 7 次样,经高效液相检测佛波醇的含量。水解曲线见图 2。

如图所示,佛波醇的含量大致是从增加到下降再到平衡的一个过程。碱的浓度为 4.13 mg/mL

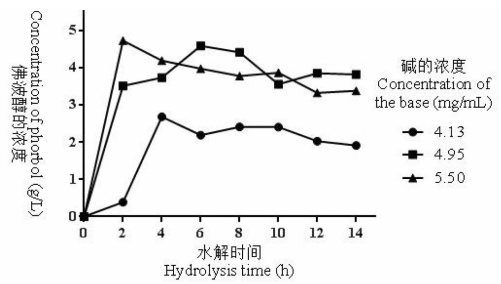

图 2 佛波醇水解曲线

Fig. 2 Hydrolysis curve of phorbol

时,佛波醇含量较低;碱的浓度为 5.50 mg/mL 时,佛波醇含量在 2 h 达到最高,但考虑到碱的浓度过高会破坏佛波醇母核,同时会产生大量粘稠物质使搅拌停止,所以碱的浓度不宜过高。而碱的浓度为 4.95 mg/mL 时,佛波醇含量较高,水解时间较短,所以碱浓度最优选择是 4.95 mg/mL。

同时可见,搅拌 6 h 后佛波醇含量开始下降,所以选定 6 h 为终止时间。

传统工艺水解完成后,未经调节 pH 直接减压旋蒸除去甲醇,但佛波醇长时间处于碱性条件下会发生构型变化,由佛波醇转化为异佛波醇[12],所以水解完毕应该立即调节 pH 至弱酸性,这在目前的水解工艺中被忽视。本研究将水解完毕的水解液分为等体积的两份(各 100 mL),一份调节 pH 至弱酸性,一份未调节。经减压旋蒸除去甲醇,然后加入等体积水,分别取样经液相分析。分析结果见表 1。

表 1 调节 pH 至弱酸性对佛波醇和异佛波醇含量的影响

Table 1 The effect of adjusting pH to weakly acidic on the concentration of phorbol and isophorbol

pH	含量 Concentration(g/L)	
	佛波醇 Phorbol	异佛波醇 Isophorbol
弱酸性 Weakly acidic	6.67	1.70
碱性 Alkaline	5.86	2.61

如表 1 所示,水解液调 pH 至弱酸性时,佛波醇含量较未调的高,同时异佛波醇含量较未调的低。说明提前调节 pH,可有效防止佛波醇向异佛波醇转化。

此外,不同于硅胶柱分离,本研究对佛波醇的分离纯化采用减压 ODS 柱,用 15% ~20% 的甲醇水溶液冲洗 10 个柱体积即可富集大部分佛波醇(纯度 > 90%),产率 1.2% 左右。

水解工艺优化的结果:氢氧化钡的用量为文献报道的 1.8 倍(4.95 mg/mL);水解时间为 6 h;水解完毕立即调 pH 至弱酸性;分离纯化采用减压 ODS 柱。整个制备周期可缩短至 3 天,能够为佛波醇的结构修饰快速提供原料。

2.2 化合物理化与波谱数据

化合物 1 无色油状液体;$[\alpha]_D^{25}$ 10 (c 0.2, MeOH);UV (MeOH) λmax (log ε) 234 (3.92) nm;IR (KBr) λmax 3410, 2962, 1734, 1717, 1653, 1628 cm^{-1};^{1}H NMR ($CDCl_3$, 600 MHz) δ:7.60 (1H, s, H-1), 5.72 (1H, m, H-7), 5.65 (1H, s, C9-O*H*), 5.41-5.29 (m, 31H, H-12 and C*H*=C*H* ×15), 4.46 (2H, m, H-20), 3.25 (1H, s, H-8), 3.23 (1H, s, H-10), 2.84-2.80 (m, 24H, =CHCH_2CH= ×12), 2.32 (6H, m, COCH_2CH_2 ×3), 2.13-2.05 (13H, m, H-11, CH_2CH_2CH= ×3 and =CHC$H_2$$CH_3$ ×3), 1.78 (3H, m, H-19), 1.73 ~ 1.66 (6H, m, COCH_2CH_2 ×3), 1.23 (3H, s, H-16), 1.21 (3H, s, H-17), 1.04 (1H, d, J = 5.1 Hz, H-14), 0.97 (9H, t, J = 7.6 Hz, CH_3 ×3), 0.88 (1H, d, J = 6.4 Hz, H-18);^{13}C NMR ($CDCl_3$, 151 MHz) δ:208.75, 176.27, 173.39, 173.37, 160.89, 135.80, 132.99, 132.95, 132.20, 132.18 (2C), 129.78, 129.06 (3C), 129.00, 128.95, 128.75, 128.71 (2C), 128.68, 128.45, 128.42, 128.40, 128.37 (3C), 128.32, 128.28, 128.26, 128.24, 128.17, 128.04, 128.02, 127.97, 127.17 (2C), 127.14, 78.20, 76.80, 73.68, 69.47, 65.58, 56.25, 43.16, 39.54, 39.16, 36.38, 34.13, 33.90, 33.76, 27.01, 26.72 (2C), 26.68, 26.66, 25.93 (6C), 25.79 (2C), 25.77, 25.70, 25.19, 24.91, 24.52, 24.02, 20.71 (3C), 16.96, 14.58, 14.43 (3C), 10.26; HR-ESI-MS: m/z 1239.8204 [M + Na]$^+$ (Calcd 1239.8204)。

化合物 2 无色油状液体;[α]25 D 24 (c 0.2, MeOH);UV (MeOH) λmax (log ε) 234 (3.80) nm;IR (KBr) λmax 3414, 2961, 1711, 1653, 1628 cm^{-1};^{1}H NMR ($CDCl_3$, 600 MHz) δ:7.58 (1H, s, H-1), 5.68 (1H, m, H-7), 5.42-5.30 (m, 20H C*H*=C*H* ×10), 4.45 (2H, m, H-20), 3.97 (1H, d, J = 9.8 Hz, H-12), 3.20 (1H, s, H-8), 3.15 (1H, s, H-10), 2.84-2.80 (m, 16H, =CHCH_2CH= ×8), 2.57/2.51 (2H, dd, J = 19.3 Hz, H-5), 2.37-2.31 (3H, t, J = 7.6 Hz, 4H, COC$H_2$$CH_2$ ×2), 2.14-2.05 (8H, m, CH_2CH_2CH= ×2 and =CHC$H_2$$CH_3$ ×2), 1.99

(1H, m, H-11), 1.78 (3H, m, H-19), 1.73-1.67 (4H, m, $COCH_2CH_2\times2$), 1.25 (3H, s, H-16), 1.22 (3H, s, H-17), 1.06 (1H, d, J = 6.4 Hz, H-18), 1.02 (1H, d, J = 5.5 Hz, H-14), 0.97 (6H, t, J = 7.6 Hz, $CH_2CH_3\times2$); ^{13}C NMR ($CDCl_3$, 151 MHz) δ: 208.69, 176.68, 173.38, 160.53, 136.21, 133.23, 132.71, 132.20, 132.18, 129.28, 129.04, 129.01, 128.78, 128.74, 128.71, 128.46, 128.43, 128.41, 128.36, 128.31, 128.24 (2C), 128.18, 128.03, 127.99, 127.16, 127.14, 78.38, 77.51, 73.49, 69.47, 68.01, 56.81, 45.22, 39.51, 39.18, 35.54, 33.84, 33.76, 26.71 (2C), 26.66, 25.79 (6C), 25.69 (2C), 24.91, 24.74, 23.87, 20.71 (2C), 17.01, 15.22, 14.43 (2C), 10.28; HR-ESI-MS: m/z 955.6068 [M + Na]$^+$ (Calcd 955.6064)。

化合物 3 无色油状液体; $[\alpha]_D^{25}$ 26 (c 0.1, MeOH); UV (MeOH) λmax (log ε) 234 (3.67) nm; IR (KBr) λmax 3 365, 2 922, 1 733, 1 707, 1 653, 1 623 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.55 (1H, s, H-1), 5.62 (1H, m, H-7), 5.42-5.32 (10H, m, $CH=CH\times5$), 4.00 (2H, m, H-20), 3.97 (1H, d, J = 9.5 Hz, H-12), 3.19 (1H, s, H-8), 3.13 (1H, s, H-10), 2.84-2.80 (8H, m, $=CHCH_2CH=\times4$), 2.51/2.45 (2H, dd, J = 19.3 Hz, H-5), 2.36 (3H, t, J = 7.6 Hz, 2H, $COCH_2CH_2$), 2.12-2.06 (4H, m, $CH_2CH_2CH=$ and $=CHCH_2CH_3$), 1.99 (1H, m, H-11), 1.75 (3H, m, H-19), 1.71 (2H, m, $COCH_2CH_2$), 1.23 (3H, s, H-16), 1.20 (3H, s, H-17), 1.02-1.01 (4H, m, H-14 and H-18), 0.97 (3H, t, J = 7.6 Hz, CH_2CH_3); ^{13}C NMR ($CDCl_3$, 151 MHz) δ: 209.04, 176.60, 160.49, 140.89, 133.04, 132.02, 129.09, 129.01, 128.62, 128.57, 128.28, 128.24, 128.07, 128.01, 127.82, 126.97, 78.36, 77.33, 73.46, 67.89, 56.66, 44.88, 38.97, 38.45, 35.50, 33.69, 26.49, 25.61 (3C), 25.52, 24.58, 23.72, 20.53, 16.90, 15.02, 14.25, 10.11; HR-ESI-MS m/z 671.3902 [M + Na]$^+$ (Calcd 671.3924)。

化合物 4 白色蜡状物; $[\alpha]_D^{25}$ 51 (c 0.5, MeOH); UV (MeOH) λmax (log ε) 234 (3.71) nm; IR (KBr) λmax 3393, 2916, 2849, 1734, 1717, 1653 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.50 (1H, s, H-1), 5.57 (1H, m, H-7), 3.97-3.91 (3H, m, H-20 and H-12), 3.15 (1H, s, H-8), 3.08 (1H, s, H-10), 2.46/2.41 (2H, dd, J = 19.3 Hz, H-5), 2.32 (2H, m, $COCH_2$), 1.96 (1H, m, H-11), 1.70 (3H, m, H-19), 1.59 (2H, m, $COCH_2CH_2$), 1.23 (32H, m, $CH_2\times16$), 1.18 (3H, s, H-16), 1.17 (3H, s, H-17), 0.9-0.95 (4H, m, H-14 and H-18), 0.85 (3H, t, J = 6.6 Hz, CH_2CH_3); ^{13}C NMR ($CDCl_3$, 151 MHz) δ: 209.43, 177.08, 160.73, 141.16, 133.14, 129.25, 78.57, 77.40, 73.57, 67.95, 67.83, 56.76, 44.76, 38.98, 38.30, 35.58, 34.44, 32.01, 29.80-29.73 (9C), 29.56, 29.45 (2C), 29.36, 29.26, 26.50, 24.89, 23.81, 22.78, 17.12, 15.11, 14.21, 10.23; HR-ESI-MS: m/z 681.4709 [M + Na]$^+$ (Calcd 681.4706)。

化合物 5 无色油状液体; $[\alpha]_D^{25}$ 67 (c 0.3, MeOH); UV (MeOH) λmax (log ε) 234 (3.83) nm; IR (KBr) λmax 3 365, 2 961, 1 698, 1 627 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.53 (1H, s, H-1), 5.61 (1H, m, H-7), 5.45-5.27 (12H, m, $CH=CH\times6$), 3.98 (2H, m, H-20), 3.95 (1H, d, J = 10.2 Hz, H-12), 3.17 (1H, s, H-8), 3.11 (1H, s, H-10), 2.84-2.80 (10H, m, $=CHCH_2CH=\times5$), 2.49/2.43 (2H, dd, J = 19.0 Hz, H-5), 2.40 (4H, m, $COCH_2CH_2$), 2.10-2.03 (2H, m, $=CH_2CH_2CH_3$), 1.99 (1H, m, H-11), 1.73 (3H, m, H-19), 1.21 (3H, s, H-16), 1.19 (3H, s, H-17), 0.99-0.98 (4H, m, H-14 and H-18), 0.96 (3H, t, J = 7.5 Hz, CH_2CH_3); ^{13}C NMR ($CDCl_3$, 151 MHz) δ: 209.30, 176.31, 160.66, 141.13, 133.16, 132.12, 129.80, 129.17, 128.66, 128.52, 128.39 (2C), 128.14 (2C), 127.96 (2C), 127.54, 127.10, 78.51, 77.41, 73.55, 68.20, 67.94, 56.79, 44.82, 39.03, 38.44, 35.59, 34.30, 26.52, 25.74-25.71 (4C), 25.64, 23.85, 22.69, 20.65, 17.08, 15.12, 14.38, 10.23; HR-ESI-MS: m/z 697.4069 [M + Na]$^+$ (Calcd 697.4080)。

化合物 6 无色油状液体; $[\alpha]_D^{25}$ 6 (c 0.1, MeOH); UV (MeOH) λmax (log ε) 234 (3.51) nm; IR (KBr) λmax 3411, 2961, 1734, 1710, 1653, 1628 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.58 (1H, s, H-1), 5.68 (1H, m, H-7), 5.66 (1H, s, C9-OH), 5.41-5.29 (21H, m, H-12 and $CH=CH\times10$), 4.03 (2H, m, H-20), 3.24 (2H, m, H-8 and H-10), 2.84-2.80 (16H, m, $=CHCH_2CH=\times8$), 2.53/2.50

(2H, dd, J = 19.3 Hz, H-5), 2.33 (4H, m, COCH_2CH$_2$ × 2), 2.14-2.06 (9H, m, H-11, CH$_2$CH_2CH = × 2 and = CHCH_2CH$_3$ × 2), 1.77 (3H, m, H-19), 1.70 (4H, m, COCH$_2$CH_2 × 2), 1.24 (3H, s, H-16), 1.20 (3H, s, H-17), 1.06 (1H, d, J = 5.2 Hz, H-14), 0.97 (6H, dt, J = 7.6 Hz, CH$_2$CH_3 × 2), 0.89 (1H, d, J = 6.4 Hz, H-18); ^{13}C NMR (CDCl$_3$, 151 MHz) δ: 209.06, 176.28, 173.41, 160.89, 140.61, 132.99, 132.18, 129.33, 129.05 (2C), 129.00, 128.94, 128.73, 128.71, 128.44, 128.41, 128.35 (2C), 128.30, 128.27, 128.24, 128.16, 128.01, 127.97, 127.16, 127.13, 78.33, 76.88, 73.81, 68.18 (2C), 65.65, 56.28, 43.11, 39.21, 38.75, 36.53, 34.13, 33.89, 27.00, 26.67, 26.64, 25.92, 25.78-25.76 (4C), 25.68 (2C), 25.19, 24.52, 24.02, 20.70 (2C), 16.98, 14.59, 14.42 (2C), 10.24; HR-ESI-MS: m/z 955.6066 [M + Na]$^+$ (Calcd 955.6064)。

化合物 7 无色油状液体; $[\alpha]_D^{25}$ 30 (c 0.3, CDCl$_3$); UV (CDCl$_3$) λmax (log ε) 234 (3.99) nm; IR (KBr) λmax 3365, 2916, 2849, 1734, 1714, 1653 cm^{-1}; ^{1}H NMR (CDCl$_3$, 600 MHz) δ: 7.57 (1H, s, H-1), 5.72 (1H, s, C9-OH), 5.67 (1H, m, H-7), 5.40 (1H, d, J = 10.3 Hz, H-12), 4.02 (2H, m, H-20), 3.25 (1H, s, H-8), 3.23 (1H, s, H-10), 2.56/2.50 (2H, dd, J = 19.3 Hz, H-5), 2.31 (4H, m, COCH_2 × 2), 2.14 (1H, m, H-11), 1.74 (3H, m, H-19), 1.61 (4H, m, COCH$_2$CH_2 × 2), 1.24 (67H, m, H-16 and CH_2 × 32), 1.19 (3H, s, H-17), 1.05 (1H, d, J = 5.04 Hz, H-14), 0.87 (9H, m, H-18 and CH_3 × 2); ^{13}C NMR (CDCl$_3$, 151 MHz) δ: 209.24, 176.39, 173.56, 160.87, 140.56, 132.79, 129.24, 78.31, 76.51, 73.64, 67.97, 65.37, 56.03, 42.86, 38.95, 38.42, 36.29, 34.54, 34.37, 31.89 (2C), 29.67-29.62 (19C), 29.59, 29.51, 29.43, 29.32 (2C), 29.28, 29.23, 29.08, 28.98, 25.69, 25.20, 24.50, 23.81, 22.65 (2C), 16.79, 14.38, 14.07 (2C), 10.04; HR-ESI-MS: m/z 975.7627 [M + Na]$^+$ (Calcd 975.7629)。

化合物 8 无色油状液体; $[\alpha]_D^{25}$ 236 (c 0.2, CDCl$_3$); UV (CDCl$_3$) λmax (log ε) 234 (3.82) nm; IR (KBr) λmax 3 410, 2 923, 1 717, 1 628 cm^{-1}; ^{1}H NMR (CDCl$_3$, 600 MHz) δ: 7.58 (1H, s, H-1), 5.67 (1H, m, H-7), 5.57 (1H, s, C9-OH), 5.36 (11H, m, H-12 and CH = CH × 5), 4.02 (2H, m, H-20), 3.25 (1H, s, H-8), 3.23 (1H, m, H-10), 2.80 (8H, m, = CHCH_2CH = × 4), 2.54/2.50 (2H, dd, J = 19.3 Hz, H-5), 2.33 (2H, m, COCH_2), 2.16 (1H, m, H-11), 2.12 (4H, m, CH$_2$CH_2CH = and = CHCH_2CH$_3$), 2.08 (3H, s, COCH_3), 1.76 (3H, m, H-19), 1.71 (2H, m, COCH$_2$CH_2), 1.23 (3H, s, H-16), 1.20 (3H, s, H-17), 1.09 (1H, d, J = 5.2 Hz, H-14), 0.96 (3H, t, J = 7.5 Hz, = CHCH$_2$CH_3), 0.89 (1H, d, J = 6.4 Hz, H-18); ^{13}C NMR (CDCl$_3$, 151 MHz) δ: 209.15, 173.91, 173.55, 160.84, 140.71, 133.01, 132.18, 129.26, 129.06, 128.93, 128.73, 128.43, 128.35, 128.28, 128.16, 127.97, 127.13, 78.40, 76.83, 73.81, 68.12, 65.80, 56.22, 43.00, 39.13, 38.67, 36.37, 34.11, 26.63, 25.80, 25.75 (3C), 25.68, 25.15, 23.97, 21.22, 20.70, 16.93, 14.57, 14.41, 10.23; HR-ESI-MS: m/z 713.4016 [M + Na]$^+$ (Calcd 713.4029)。

化合物 9 无色油状液体; $[\alpha]_D^{25}$ 36 (c 0.2, MeOH); UV (MeOH) λmax (log ε) 234 (3.67) nm; IR (KBr) λmax 3 404, 2 962, 1 734, 1 709, 1 628 cm^{-1}; ^{1}H NMR (CDCl$_3$, 600 MHz) δ: 7.59 (1H, s, H-1), 5.69-5.68 (2H, m, C9-OH, H-7), 5.42-5.30 (11H, m, H-12 and CH = CH × 5), 4.03 (2H, m, H-20), 3.25 (1H, s, H-8), 3.24 (1H, s, H-10), 2.84-2.80 (8H, m, = CHCH_2CH = × 4), 2.53/2.50 (2H, dd, J = 19.3 Hz, H-5), 2.37-2.27 (4H, m, COCH_2-^{EPA}, COCH_2-^{Bu}), 2.15-2.05 (5H, m, CH$_2$CH_2CH = , = CHCH_2CH$_3$ and H-11), 1.77 (3H, m, H-19), 1.74-1.66 (4H, m, COCH$_2$CH_2-^{EPA} and COCH$_2$CH_2-^{Bu}), 1.24 (3H, s, H-16), 1.21 (3H, s, H-17), 1.06 (1H, d, J = 5.2 Hz, H-14), 0.98-0.94 (6H, m, CH$_2$CH_3-^{EPA} and CH$_2$CH_3-^{Bu}), 0.89 (1H, d, J = 6.4 Hz, H-18); ^{13}C NMR (CDCl$_3$, 151 MHz) δ: 209.08, 176.37, 173.44, 160.91, 140.57, 132.98, 132.19, 129.30, 129.04, 128.95, 128.72, 128.43, 128.34, 128.28, 128.17, 127.96, 127.12, 78.33, 76.91, 73.81, 68.16, 65.51, 56.27, 43.12, 39.20, 38.75, 36.53, 36.36, 34.14, 26.64, 25.91, 25.75 (3C), 25.68, 25.19, 24.01, 20.70, 18.21, 16.99, 14.60, 14.43, 13.80, 10.26; HR-ESI-MS: m/z 741.4330 [M + Na]$^+$ (Calcd 741.4342)。

化合物 10 无色油状液体;$[\alpha]_D^{25}$ 20 (*c* 0.2, MeOH);UV (MeOH) λmax (log ε) 234 (3.69) nm;IR (KBr) λmax 3 411, 2 961, 1 717, 1 628 cm^{-1};^1H NMR ($CDCl_3$, 600 MHz) δ:7.56 (1H, s, H-1), 5.67 (1H, m, H-7), 5.59 (1H, s, C9-O*H*), 5.40-5.26 (13H, m, H-12 and C*H*=C*H* ×6), 4.01 (2H, m, H-20), 3.25 (1H, s, H-8), 3.22 (1H, s, H-10), 2.83-2.80 (10H, m, =CHCH_2CH= ×5), 2.56/2.50 (2H, dd, *J* = 19.0 Hz, H-5), 2.38 (4H, m, COCH_2CH_2), 2.14 (1H, m, H-11), 2.07 (3H, s, COCH_3), 2.06-2.02 (2H, m, =CH_2CH_2CH_3), 1.74 (3H, m, H-19), 1.23 (3H, s, H-16), 1.20 (3H, s, H-17), 1.09 (1H, d, *J* = 4.9 Hz, H-14), 0.96 (3H, t, *J* = 7.5 Hz, =CHCH_2CH_3), 0.88 (1H, d, *J* = 6.2 Hz, H-18);^{13}C NMR ($CDCl_3$, 151 MHz) δ: 209.25, 173.89, 173.12, 160.85, 140.76, 132.97, 132.14, 129.53, 129.20, 128.68, 128.40 (2C), 128.37, 128.16, 128.15, 128.10, 127.95, 127.81, 127.11, 78.45, 77.01, 73.79, 68.07, 65.76, 56.14, 42.95, 39.05, 38.57, 36.34, 34.51, 25.81, 25.74 (4C), 25.65, 23.94, 23.04, 21.20, 20.67, 16.89, 14.57, 14.38, 10.20; HR-ESI-MS: *m/z* 739.417 9 [M + Na]$^+$ (Calcd 739.418 6)。

化合物 11 无色油状液体;$[\alpha]_D^{25}$ 15 (*c* 0.1, MeOH);UV (MeOH) λmax (log ε) 234 (3.92) nm;IR (KBr) λmax 3 407, 2 962, 1 735, 1 709, 1 628 cm^{-1};^1H NMR ($CDCl_3$, 600 MHz) δ:7.57 (1H, s, H-1), 5.74 (1H, s, C9-O*H*), 5.67 (1H, m, H-7), 5.41-5.31 (13H, m, H-12 and C*H*=C*H* ×6), 4.02 (2H, m, H-20), 3.26 (1H, s, H-8), 3.24 (1H, s, H-10), 2.84 (10H, m, =CHCH_2CH= ×5), 2.56/2.50 (2H, dd, *J* = 19.0 Hz, H-5), 2.38-2.35 (4H, m, COCH_2CH_2-^{DHA}), 2.33-2.28 (2H, m, COCH_2-^{Bu}), 2.15 (1H, m, H-11), 2.06 (2H, m, =CH_2CH_2CH_3), 1.74 (3H, m, H-19), 1.65 (2H, m, COCH_2CH_2^{Bu}), 1.24 (3H, s, H-16), 1.20 (3H, s, H-17), 1.07 (1H, d, *J* = 5.0 Hz, H-14), 0.98-0.92 (6H, m, CH_2CH_3-^{DHA} and CH_2CH_3-^{Bu}), 0.89 (3H, d, *J* = 6.2 Hz, H-18);^{13}C NMR ($CDCl_3$, 151 MHz) δ: 209.33, 176.38, 173.02, 160.95, 140.70, 132.96, 132.14, 129.51, 129.29, 128.68, 128.39 (2C), 128.37, 128.15 (2C), 128.10, 127.95, 127.82, 127.10, 78.45, 77.06, 73.77, 68.10, 65.47, 56.15, 43.03, 39.07, 38.58, 36.48, 36.36, 34.52, 25.92, 25.73 (4C), 25.64, 23.96, 23.05, 20.67, 18.19, 16.95, 14.59, 14.39, 13.78, 10.22; HR-ESI-MS: *m/z* 767.448 9 [M + Na]$^+$ (Calcd 767.449 9)。

化合物 12 无色油状液体;$[\alpha]_D^{25}$ 52 (*c* 0.2, MeOH);UV (MeOH) λmax (log ε) 234 (3.79) nm;IR (KBr) λmax 3 397, 2 922, 1 699, 1 653, 1 628 cm^{-1};^1H NMR ($CDCl_3$, 600 MHz) δ:7.55 (1H, s, H-1), 5.63 (1H, m, H-7), 5.40-5.28 (10H, m, C*H*=C*H* ×5), 4.82 (1H, d, *J* = 9.72 Hz, H-12), 4.01 (2H, m, H-20), 3.15 (1H, s, H-8), 3.09 (1H, s, H-10), 2.82-2.79 (8H, m, =CHCH_2CH= ×4), 2.53/2.45 (2H, dd, *J* = 19.3 Hz, H-5), 2.34 (2H, m, COCH_2-), 2.10 (4H, m, CH_2CH_2CH= and =CHCH_2CH_3), 2.05 (1H, m, H-11), 1.77 (3H, m, H-19), 1.69 (2H, m, COCH_2CH_2), 1.17 (3H, s, H-16), 1.03 (3H, s, H-17), 1.00 (1H, d, *J* = 6.5 Hz, H-18), 0.95 (3H, t, *J* = 7.6 Hz, =CHCH_2CH_3), 0.91 (1H, d, *J* = 6.1Hz, H-14);^{13}C NMR ($CDCl_3$, 151 MHz) δ: 209.10, 176.63, 160.42, 141.03, 133.53, 132.19, 129.35, 129.19, 128.79, 128.72, 128.44, 128.37, 128.23, 128.17, 127.98, 127.14, 87.89, 79.17, 73.61, 68.00, 60.95, 57.04, 43.44, 39.10, 38.79, 35.41, 33.94, 27.73, 26.71, 25.78 (3C), 25.68, 25.00, 22.51, 20.70, 17.35, 16.12, 14.42, 10.30; HR-ESI-MS: *m/z* 671.390 8 [M + Na]$^+$ (Calcd 671.392 4)。

化合物 13 无色油状液体;$[\alpha]_D^{25}$ 108 (*c* 0.7, MeOH);UV (MeOH) λmax (log ε) 234 (3.73) nm;IR (KBr) λmax 3447, 2915, 2843, 1735, 1699, 1653, 1628 cm^{-1};^1H NMR ($CDCl_3$, 600 MHz) δ:7.57 (1H, s, H-1), 5.64 (1H, m, H-7), 4.83 (1H, d, *J* = 9.78 Hz, H-12), 4.02 (2H, m, H-20), 3.17 (1H, s, H-8), 3.10 (1H, s, H-10), 2.54/2.47 (2H, dd, *J* = 19.3 Hz, H-5), 2.33 (2H, m, COCH_2), 2.13 (1H, m, H-11), 1.78 (3H, m, H-19), 1.61 (2H, m, COCH_2CH_2), 1.25 (32H, m, CH_2 ×16), 1.18 (3H, s, H-16), 1.04 (3H, s, H-17), 1.02 (1H, d, *J* = 6.5 Hz, H-18), 0.92 (1H, d, *J* = 6.1 Hz, H-14), 0.87 (3H, t, *J* = 6.9 Hz, CH_2CH_3);^{13}C NMR ($CDCl_3$, 151 MHz) δ: 209.00, 176.81, 160.32, 140.79, 133.36, 129.24, 87.54, 79.01, 73.44, 67.87, 60.82, 56.86, 43.26, 38.92, 38.60, 35.17, 34.34, 31.89, 29.66-

29.61 (9C), 29.56, 29.45, 29.33, 29.23, 29.13, 27.56, 24.98, 22.66, 22.31, 17.14, 15.91, 14.09, 10.11; HR-ESI-MS: *m/z* 681.470 7 [M + Na]$^+$ (Calcd 681.470 7)。

化合物 14 无色油状液体;[α]$_D^{25}$ 73 (*c* 0.3, MeOH);UV (MeOH) λmax (log ε) 234 (3.76) nm;IR (KBr) λmax 3 417, 1 729, 1 696, 1 649, 1 628 cm^{-1};^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.56 (1H, s, H-1), 5.64 (1H, m, H-7), 5.41-5.30 (12H, m, C*H* = C*H* ×6), 4.84 (1H, d, *J* = 10.3 Hz, H-12), 4.81 (1H, s, C9-O*H*), 4.02 (2H, m, H-20), 3.16 (1H, s, H-8), 3.09 (1H, s, H-10), 2.84-2.74 (10H, m, = CHCH_2CH = ×5), 2.54/2.47 (2H, dd, *J* = 19.1 Hz, H-5), 2.41-2.39 (4H, m, COCH_2CH_2), 2.13 (1H, m, H-11), 2.07 (2H, m, = CH_2CH_2CH$_3$), 1.78 (3H, m, H-19), 1.18 (3H, s, H-16), 1.04 (3H, s, H-17), 1.02 (1H, d, *J* = 6.2 Hz, H-18), 0.97 (3H, t, *J* = 7.6 Hz, = CHCH$_2$CH_3), 0.92 (1H, d, *J* = 5.8 Hz, H-14);^{13}C NMR ($CDCl_3$, 151 MHz) δ: 209.11, 176.15, 160.42, 141.04, 133.53, 132.18, 129.80, 129.36, 128.72, 128.49, 128.43, 128.41, 128.18 (2C), 128.04, 127.99, 127.72, 127.14, 87.97, 79.16, 73.61, 67.99, 60.97, 57.06, 43.43, 39.10, 38.79, 35.40, 34.33, 27.74, 25.77 (4C), 25.68, 22.85, 22.49, 20.70, 17.33, 16.11, 14.42, 10.30; HR-ESI-MS: *m/z* 697.407 1 [M + Na]$^+$ (Calcd 697.408 0)。

化合物 15 无色油状液体;[α]$_D^{25}$ 49 (*c* 0.2, MeOH);UV (MeOH) λmax (log ε) 234 (3.70) nm;IR (KBr) λmax 3447, 2919, 1734, 1705, 1653, 1628 cm^{-1};^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.59 (1H, s, H-1), 5.67 (1H, m, H-7), 5.43-5.29 (11H, m, H-12 and C*H* = C*H* ×5), 4.83 (1H, d, *J* = 9.8 Hz, H-12), 4.46 (2H, m, H-20), 3.16 (1H, s, H-8), 3.10 (1H, s, H-10), 2.84-2.80 (8H, m, = CHCH_2CH = ×4), 2.52/2.42 (2H, dd, *J* = 19.3 Hz, H-5), 2.36 (2H, m, COCH_2), 2.13-2.07 (5H, m, CH$_2$CH_2CH =, = CHCH_2CH$_3$ and H-11), 2.05 (3H, s, COCH_3), 1.80 (3H, m, H-19), 1.71 (2H, m, COCH$_2$CH_2), 1.20 (3H, s, H-16), 1.05 (3H, s, H-17), 1.02 (1H, d, *J* = 6.5 Hz, H-18), 0.98 (3H, t, *J* = 7.6 Hz, = CHCH$_2$CH_3), 0.90 (1H, d, *J* = 6.3 Hz, H-14);^{13}C NMR ($CDCl_3$, 151 MHz) δ: 208.67, 176.53, 170.88, 160.33, 136.04, 133.64, 132.69, 132.19, 129.21, 128.78, 128.73, 128.44, 128.38, 128.22, 128.16, 127.98, 127.14, 87.85, 79.17, 73.42, 69.58, 60.86, 56.83, 43.58, 39.41, 39.17, 35.27, 33.90, 27.95, 26.71, 25.78 (3C), 25.68, 24.99, 22.39, 21.10, 20.70, 17.33, 16.15, 14.42, 10.29; HR-ESI-MS: *m/z* 713.401 9 [M + Na]$^+$ (Calcd 713.403 0)。

化合物 16 无色油状液体;[α]$_D^{25}$ 55 (*c* 0.2, MeOH);UV (MeOH) λmax (log ε) 234 (3.83) nm;IR (KBr) λmax 3447, 2922, 2853, 1734, 1700, 1653, 1628 cm^{-1};^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.59 (1H, s, H-1), 5.66 (1H, m, H-7), 4.83 (1H, d, *J* = 9.8 Hz, H-12), 4.46 (2H, m, H-20), 3.15 (1H, s, H-8), 3.10 (1H, s, H-10), 2.53/2.38 (2H, dd, *J* = 19.3 Hz, H-5), 2.34-2.31 (2H, m, COCH_2), 2.10 (1H, m, H-11), 2.05 (3H, s, COCH_3), 1.79 (3H, m, H-19), 1.61 (2H, m, COCH$_2$CH_2), 1.29-1.24 (20H, m, CH_2 ×10), 1.19 (3H, s, H-16), 1.04 (3H, s, H-17), 1.02 (1H, d, *J* = 6.5 Hz, H-18), 0.87 (4H, m, H-14 and CH_3);^{13}C NMR ($CDCl_3$, 151 MHz) δ: 208.69, 176.73, 170.76, 160.32, 135.83, 133.45, 132.63, 87.48, 78.95, 73.27, 69.45, 60.76, 56.74, 43.38, 39.23, 38.93, 35.07, 34.33, 31.89, 29.63, 29.60 (2C), 29.55, 29.44, 29.32, 29.22, 29.13, 27.69, 24.98, 22.66, 22.25, 20.93, 17.12, 15.92, 14.09, 10.12; HR-ESI-MS: *m/z* 639.387 7 [M + Na]$^+$ (Calcd 639.387 3)。

化合物 17 无色油状液体;[α]$_D^{25}$ 24 (*c* 0.2, MeOH);UV (MeOH) λmax (log ε) 234 (3.54) nm;IR (KBr) λmax 3447, 2921, 2852, 1734, 1705, 1653, 1628 cm^{-1};^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.59 (1H, s, H-1), 5.67 (1H, m, H-7), 4.83 (1H, d, *J* = 9.8 Hz, H-12), 4.46 (2H, m, H-20), 3.15 (1H, s, H-8), 3.10 (1H, s, H-10), 2.52/2.38 (2H, dd, *J* = 19.3 Hz, H-5), 2.33 (2H, m, COCH_2), 2.05 (3H, s, COCH_3), 1.80 (3H, m, H-19), 1.61 (2H, m, COCH$_2$CH_2), 1.24 (32H, m, CH_2 ×16), 1.19 (3H, s, H-16), 1.04 (3H, s, H-17), 1.02 (1H, d, *J* = 6.5 Hz, H-18), 0.90 (1H, d, *J* = 6.2 Hz, H-14), 0.87 (3H, t, *J* = 7.1 Hz, CH$_2$CH_3);^{13}C NMR ($CDCl_3$, 151 MHz) δ: 208.84, 176.91, 170.95, 160.47, 135.99, 133.62, 132.79, 87.66, 79.13, 73.43, 69.61, 60.91, 56.89, 43.55, 39.39, 39.11, 35.22, 34.49, 32.07, 29.84-29.78 (9C), 29.73, 29.62, 29.50, 29.40,

29.31, 27.88, 25.15, 22.83, 22.40, 21.10, 17.29, 16.10, 14.26, 10.28; HR-ESI-MS: *m/z* 723.480 5 [M + Na]$^+$ (Calcd 723.481 2)。

化合物 18　无色油状液体; $[\alpha]_D^{25}$ 44 (*c* 0.2, MeOH); UV (MeOH) λmax (log ε) 234 (3.73) nm; IR (KBr) λmax 3 445, 2 961, 1 698, 1 628 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.59 (1H, s, H-1), 5.66 (1H, m, H-7), 5.45-5.27 (13H, m, H-12 and C*H*=C*H* ×6), 4.84 (1H, d, *J* = 9.7 Hz, H-12), 4.69 (1H, s, C9-O*H*), 4.46 (2H, m, H-20), 3.15 (1H, s, H-8), 3.09 (1H, s, H-10), 2.83-2.80 (10H, m, =CHC*H*$_2$CH= ×5), 2.52/2.41 (2H, dd, *J* = 18.7 Hz, H-5), 2.39 (4H, m, COC*H*$_2$C*H*$_2$), 2.12-2.08 (3H, m, H-11 and =CH$_2$C*H*$_2$CH$_3$), 2.05 (3H, s, COC*H*$_3$), 1.79 (3H, m, H-19), 1.19 (3H, s, H-16), 1.04 (3H, s, H-17), 1.02 (1H, d, *J* = 6.3 Hz, H-18), 0.96 (3H, t, *J* = 7.5 Hz, =CHCH$_2$C*H*$_3$), 0.90 (1H, d, *J* = 6.0 Hz, H-14); ^{13}C NMR ($CDCl_3$, 151 MHz) δ: 208.68, 176.03, 170.86, 160.33, 136.03, 133.64, 132.71, 132.17, 129.81, 128.71, 128.49, 128.43, 128.41, 128.17 (2C), 128.02, 127.98, 127.70, 127.13, 87.89, 79.13, 73.42, 69.58, 60.88, 56.87, 43.56, 39.41, 39.13, 35.27, 34.31, 27.93, 25.77 (4C), 25.68, 22.85, 22.37, 21.09, 20.69, 17.30, 16.11, 14.41, 10.28; HR-ESI-MS: *m/z* 739.417 7 [M + Na]$^+$ (Calcd 739.418 6)。

2.3　佛波醇衍生物对正常细胞的毒性研究

表 2　佛波醇衍生物对正常细胞毒性结果($n=3$, $\bar{x} \pm s$)

Table 2　Results of cytotoxicity of phorbol derivatives ($n=3$, $\bar{x} \pm s$)

细胞株/半数抑制浓度 Cell line/ IC_{50} (μmol/L)			
化合物 Compounds	人胚肺成纤维细胞 MRC-5	化合物 Compounds	人胚肺成纤维细胞 MRC-5
1	>100	10	8.10 ± 0.41
2	32.17 ± 1.61	11	8.31 ± 0.42
3	26.97 ± 1.35	12	5.39 ± 0.27
4	>100	13	>100
5	7.40 ± 0.37	14	1.52 ± 0.08
6	>100	15	5.60 ± 0.28
7	>100	16	11.84 ± 0.59
8	8.50 ± 0.43	17	38.12 ± 1.91
9	16.15 ± 0.81	18	5.01 ± 0.25

如表 2 所示,13 个化合物毒性较高(IC_{50} < 38.12 μmol/L),5 个化合物毒性较低(IC_{50} > 100 μmol/L)。佛波醇与长链饱和、不饱和脂肪酸形成三酯、二酯衍生物时,毒性较低(化合物 **1**, **6** 和 **7**, IC_{50} > 100 μmol/L);佛波醇的 13 位羟基成乙酯或丁酯,12 位羟基与长链不饱和脂肪酸成酯时毒性较高(化合物 **8**, **9**, **10** 和 **11** 的 IC_{50} 8.10 → 16.15 μmol/L);佛波醇 13 位羟基与长链不饱和脂肪酸成单酯时毒性较大(化合物 **3** 和 **5** 的 IC_{50} 分别为 26.97 μmol/L 和 7.40 μmol/L),与长链饱和脂肪酸成单酯时毒性较小(化合物 **4**, IC_{50} > 100 μmol/L);佛波醇 12 位羟基形成单酯化衍生物与佛波醇 13 位羟基形成单酯化衍生物的结果类似(化合物 **12** 和 **14** 的 IC_{50} 分别为 5.39 μmol/L 和 1.52 μmol/L,而化合物 **13** 的 IC_{50} > 100 μmol/L);佛波醇-12,20-二酯化衍生物普遍具有细胞毒性(化合物 **15**, **16**, **17** 和 **18** 的 IC_{50} 5.01 → 38.12 μmol/L)。

3　结论

本研究对佛波醇制备工艺进行优化,使制备周期缩短至 3 天,可快速获得佛波醇;对佛波醇进行结构修饰,合成了 18 个新化合物,包括佛波醇单酯、二酯、三酯化衍生物;考察了佛波醇衍生物对正常细胞的毒性,结果表明:长链不饱和脂肪酸(EPA, DHA)的引入使大部分衍生物具有毒性,以佛波醇酯作为抗肿瘤药物开发时,应该避免引入长链不饱和脂肪酸;佛波醇-12,20-二酯化衍生物同样具有毒性,在佛波醇结构修饰时应该避免合成这类产物;而佛波醇 12 位羟基、13 位羟基分别与长链饱和脂肪酸形

成单酯时毒性较低。实验结果可为佛波醇的结构修饰提供参考。

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