以 VEGFR 为靶点的齐墩果酸衍生物的设计、合成及抗肿瘤活性研究

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摘要: 以齐墩果酸 (oleanolic acid, OA) 为先导化合物进行结构优化, 通过引入乙二胺结构单元, 并以乙二胺为连接臂拼合多种生物活性片段, 设计并合成了 20 个新的衍生物, 其结构经 ^{1}H NMR、^{13}C NMR 和 HR-MS 确证。对所合成的目标化合物以人肝癌细胞 (HepG2) 和人胃癌细胞 (SGC7901) 进行体外抗肿瘤活性测试, 结果表明目标化合物对两种肿瘤细胞的抑制活性均明显强于 OA, 其中 **I$_6$**、**I$_8$** 和 **I$_9$** 对 HepG2 细胞显示出较强的活性 (IC_{50} = 16.7、9.8 和 6.3 μmol·L^{-1})。分子模拟对接研究表明, 目标化合物 **I$_6$**～**I$_9$** 和血管内皮生长因子受体 (vascular endothelial growth factor receptor, VEGFR) 蛋白具有较好的结合能力。对化合物 **I$_6$**～**I$_9$** 进行 VEGFR-2 的抑制活性测试, 结果表明化合物 **I$_9$** 对 VEGFR-2 具有较强的抑制作用 (IC_{50} = 0.56 μmol·L^{-1})。

关键词: 齐墩果酸衍生物; 合成; 分子对接; 抗肿瘤活性

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Design, synthesis and anti-tumor activity studies of oleanolic acid derivatives using VEGFR as target

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Abstract: In this study, twenty containing ethylenediamine groups derivatives of oleanolic acid (OA) were synthesized, their structures were determined by ^{1}H NMR, ^{13}C NMR and HR-MS. The anti-tumor activities in HepG2 and SGC7901 cells were evaluated by MTT assay. The results showed that all compounds exhibited anti-tumor activity, compounds **I$_6$**, **I$_8$** and **I$_9$** exhibited significant anti-tumor activities with IC_{50} values of 16.7, 9.8 and 6.3 μmol·L^{-1}, respectively. Molecular docking studies showed that compounds **I$_6$**–**I$_9$** produce higher combining ability with VEGFR. Compound **I$_6$**–**I$_9$** were further evaluated for the inhibitory activity against VEGFR-2, the result showed **I$_9$** had a strong inhibitory effect on VEGFR with IC_{50} values of 0.56 μmol·L^{-1}.

Key words: oleanolic acid derivative; synthesis; molecular docking; anti-tumor activity

长期以来, 恶性肿瘤治疗一直是临床上面临的最大难题。分子靶向治疗凭借其针对性、特异性和有效性强且毒副作用低等优点, 已成为国内外肿瘤治疗领域的研究热点[1]。天然产物凭借其结构的多样性, 较高的生物活性和较小的毒副作用成为靶向药物开发的重点[2]。齐墩果酸 (oleanolic acid, OA) 是一种重要的五环三萜类天然产物, 具有多种生物活性, 尤其是抗肿瘤活性, 包括抑制人肺癌细胞、人宫颈癌 HeLa 细胞、人乳腺癌 MCF 细胞以及肝癌细胞株 HepG2 等, 但分子作用机制尚不完全明确[3, 4]。以血管内皮生长因子 (vascular endothelial growth factor, VEGF) 及其受体 VEGFR 为靶点的药物可以减少血管内皮生长因子和血管内皮生长因子受体的表达, 消耗肿瘤细胞产生的血管内皮生长因子, 从而抑制肿瘤血管的生成, 遏制肿瘤生长[5]。已有研究表明 OA 能够抑制肿瘤细胞中 VEGFR 的表达, 因此 VEGFR 可能是

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齐墩果酸抗肿瘤作用的重要靶点[6−9]。

舒尼替尼 (sunitinib) 是由辉瑞公司研制的 VEGFR 抑制剂 (VEGFR-2, IC_{50}=9 nmol·L^{-1})[10]。运用分子模拟对接方法研究舒尼替尼与 VEGFR 靶蛋白 (PDB 编号: 5ew3) 的相互作用 (图 1), 结果表明舒尼替尼结构中的乙二胺片段可以和 VEGFR 蛋白的氨基酸残基 (Asp1046) 形成牢固的氢键, 与靶蛋白产生较大结合力, 说明乙二胺基团可能是其抗肿瘤作用的重要药效基团。此外, 文献报道多个小分子 VEGFR-2 抑制剂能与靶蛋白狭长空腔的氨基酸残基 Asp1046 产生氢键作用[11,12], 进一步说明 Asp1046 氨基酸残基可能是小分子与 VEGFR 蛋白作用的重要结合位点。本文将乙二胺基团引入到 OA-28 位, 并以乙二胺作为连接臂, 利用拼合法引入多种生物活性基团, 设计并合成了 20 个衍生物 **I_1**～**I_{10}** 和 **II_1**～**II_{10}** (图 2), 目标化合物的合成见合成路线 1。同时对目标化合物进行了体外抗肿瘤活性测试和分子模拟对接研究。

结果与讨论

1 化学部分

以齐墩果酸为起始原料, 经过 C-3 位氧化或 C-3 位乙酰化反应、C-28 位酰氯化反应、取代反应和酰胺化反应共 4 步反应合成了 20 个齐墩果酸衍生物。目标化合物经 ^{1}H NMR、^{13}C NMR 和 HR-MS 确证其结构, 数据见实验部分。

2 生物活性评价

2.1 目标化合物对人癌细胞株的体外增殖抑制实验

采用 MTT 法测试目标化合物对人肝癌细胞 (HepG2) 和人胃癌细胞 (SGC7901) 的体外细胞毒活性, 以舒尼替尼 (sunitinib) 为阳性对照物, 结果见表 1。结果表明, 目标化合物对两种肿瘤细胞的抑制活性均明显强于 OA, 其中 **I_6**、**I_8** 和 **I_9** 对 HepG2 细胞显示出较强的活性, IC_{50} 值分别是 16.7、9.8 和 6.3 μmol·L^{-1}。

2.2 目标化合物与 VEGFR 的对接

以 PDB 数据库中 VEGFR 为靶点 (PDB 编号: 5ew3), 作为分子对接受体模型。将 5ew3 导入计算机辅助药物设计软件 Molegro Virtual Docker (MVD 6.0), 根据 cavity 的探测选择最佳对接区域 (center x: 18.57 y: 8.63 z: 12.57, radius: 15)。将受体蛋白中原有配体替换为目标配体分子, 利用 Docking wizard 向导设定配体的对接运算次数后, 选择打分函数 MolDock Score [GRID] 与构象搜寻 MolDock SE[13], 分别对化合物 (**I_1**～**I_{10}** 和

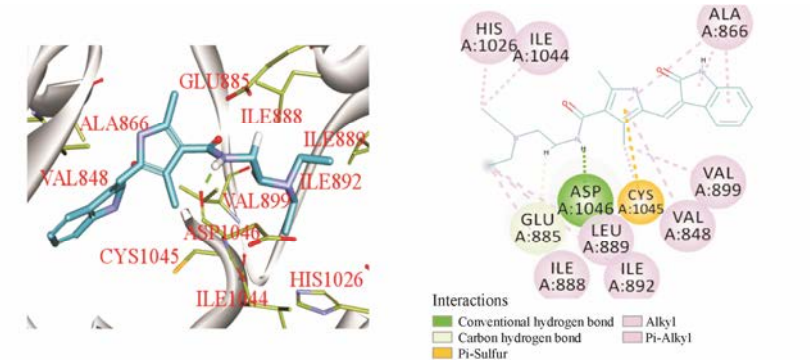


Figure 1 Binding of sunitinib to the active site of vascular endothelial growth factor receptor (VEGFR)

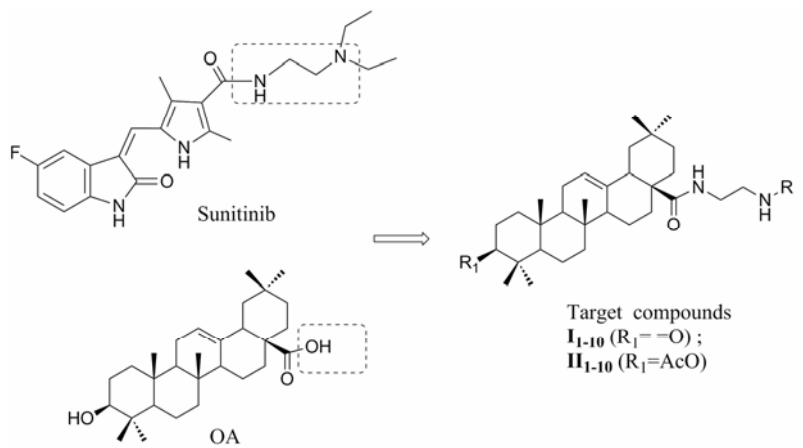
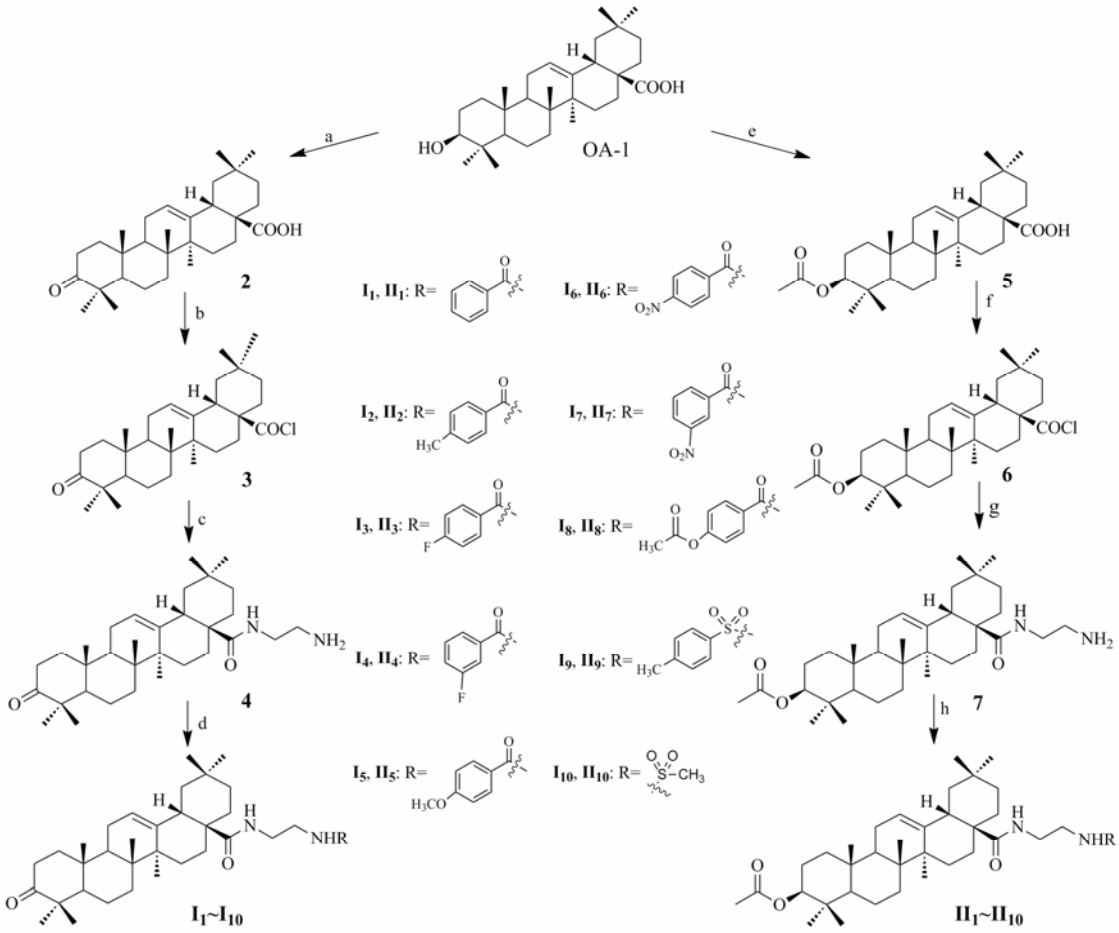


Figure 2 The design of oleanolic acid (OA) derivatives



Reagent and conditions: (a) Jone's reagent, 0 ℃, 2 h; (b) $(COCl)_2$, CH_2Cl_2, 40 ℃, 2 h; (c) $NH_2CH_2CH_2NH_2$, $CHCl_3$, Py, DMAP, rt, 3 h; (d) RCOCl, $CHCl_3$, Py, DMAP, 60 ℃, 3 h; (e) $(CH_3CO)_2O$, Py, DMAP, rt, overnight; (f) $(COCl)_2$, CH_2Cl_2, 40 ℃, 2 h; (g) $NH_2CH_2CH_2NH_2$, $CHCl_3$, Py, DMAP, 60 ℃, 3 h; (h) RCOCl, $CHCl_3$, Py, DMAP, 60 ℃, 3 h

Scheme1　Synthetic route of target compounds

II_1～II_{10}) 与受体对接的结合能进行评分，评分数值的绝对值越大表明配体与受体亲和力越好[14]。选取 MD score 绝对值最高的分子构象作为与靶点的模拟对接模型，利用 Discovery studio 4.0 分析得到配体与氨基酸残基作用的图像。利用打分函数评价受体与配体的结合情况，同时考虑氢键作用以及靶点关键氨基酸的结合情况[14−16]，化合物 **I_1**～**I_{10}** 和 **II_1**～**II_{10}** 的对接结果见表 2。结果表明，目标化合物均能较好地插入到靶点的活性口袋中，利用氢键、疏水键、π-σ 键、π-alkyl 等与靶蛋白的多个氨基酸残基相互作用 (Asp1046、Glu885、Cys1045、Val899、Val848、Ala866、Ile892、Ile1044、His1026、Leu889 等)。I 系列化合物结合能明显高于 II 系列，**I_2**～**I_9** 有较高的分值，**I_5**～**I_9** 具有氢键作用，其中化合物 **I_6**、**I_7**、**I_9** 结构中的乙二胺片段与 VEGFR 靶蛋白的关键氨基酸 Asp1046 通过氢键紧密结合。通过对比化合物 **I_9** 和 sunitini 与靶蛋白的对接图 (图 3 和图 1) 发现，sunitinib 和化合物 **I_9** 与靶蛋白连有相同的疏水氨基酸残基 (Cys1045、Ala866、Ile892、Ile1044、His1026、Leu889 等)，乙二胺片段均与 Asp1046 产生氢键作用，初步判断化合物 **I_9** 和 sunitinib 与靶蛋白具有相同的结合位点[11,17]。

2.3　VEGFR-2 抑制实验　选取化合物 **I_6**～**I_9** 进行 VEGFR-2 抑制实验，实验由 Invitrogen 公司完成。**I_6**～**I_9** 对 VEGFR-2 的 IC_{50} 值为 10.15、12.56、4.83 和 0.56 μmol·L^{-1}，结果表明化合物 **I_9** 对 VEGFR-2 蛋白具有较好的抑制作用。

3　小结

本文以 OA 为起始原料，通过在 OA-28 位引入乙二胺基团，并以此为连接臂，进一步通过拼合的方法引入多个生物活性片段，设计并合成了 20 个新的 OA 衍生物，目标化合物结构经 ^{1}H NMR、^{13}C NMR 和 HR-MS 确证。采用 MTT 法考察目标化合物的体外抗肿瘤活性，结果表明化合物对人肝癌细胞 (HepG2) 和人胃癌细胞 (SGC7901) 具有抑制活性。初步的构

Table 1 Anti-tumor activity of the target compounds on HepG2 and SGC7901 cell lines. [a]Inhibitory percentage of cells treated with each compound at a concentration of 10 μmol·L^{-1} for 72 h; [b]The agent concentration that inhibited SGC7901 and HepG2 cells growth by 50%

Compd.	Inhibition rate/%[a]		IC_{50}/μmol·L^{-1}[b]	
	HepG2	SGC7901	HepG2	SGC7901
OA	17.5	12.6	>50	>50
I_1	29.6	19.3	35.3	>50
I_2	32.5	22.3	30.4	>50
I_3	40.3	32.6	26.1	35.6
I_4	36.5	26.3	30.3	40.0
I_5	35.7	22.5	33.2	>50
I_6	46.2	37.9	16.7	23.6
I_7	41.9	26.8	24.1	32.5
I_8	51.1	39.6	9.8	20.8
I_9	59.9	46.7	6.3	23.5
I_{10}	24.9	17.8	41.5	>50
II_1	27.1	19.9	>50	>50
II_2	29.5	23.2	45.0	>50
II_3	38.5	33.7	38.7	>50
II_4	34.5	28.1	48.0	>50
II_5	31.5	32.1	42.1	>50
II_6	44.6	35.5	29.0	32.5
II_7	38.6	31.5	35.6	45.5
II_8	48.6	37.5	21.8	29.5
II_9	50.6	41.5	20.8	27.6
II_{10}	22.9	17.1	>50	>50
Sunitinib	62.4	59.7	31.5	17.5

效关系研究表明 OA-3 位羰基化合物活性优于 OA-3 位乙酰基 (**I_n** > **II_n**), OA-28 位末端 N 上引入苯磺酰基 (**I_9**) 活性优于取代苯甲酰基 (**I_1**～**I_8**) 和磺酰基衍生物 (**I_{10}**), 苯环上取代基 R 的电性效应及体积大小均可能影响化合物活性 ($CH_3OCO > NO_2 > F > CH_3O > CH_3$), 苯环上对位取代优于邻位取代 (**$I_3$** > **$I_4$**, **$I_6$** > **$I_7$**)。其中化合物 **$I_6$**、**$I_8$** 和 **$I_9$** 对 HepG2 细胞表现出显著的活性,

Table 2 Energy scores for different compounds with VEGFR (PDB: 5ew3)

Compd.	MolDock Score /kcal·mol^{-1}	Number of hydrogen bonds	Hydrogen-interacting residues
I_1	−138.526	0	–
I_2	−145.263	0	–
I_3	−145.046	0	–
I_4	−147.628	0	–
I_5	−149.803	2	Cys1045, Glu885
I_6	−151.513	1	Asp1046, Cys1045
I_7	−154.871	1	Asp1046
I_8	−164.439	1	Glu885
I_9	−142.675	2	Asp1046
I_{10}	−121.375	2	Ile1025
II_1	−121.398	2	Arg1027
II_2	−128.294	2	Ala881, Agr1032
II_3	−131.439	2	Arg1027, Ile1025
II_4	−130.004	2	Glu885, Arg1027
II_5	−137.42	1	Glu885
II_6	−134.693	2	Arg1027
II_7	−132.446	1	Arg1027
II_8	−141.63	2	Glu885, Arg1027
II_9	−126.145	2	Asp1046, Arg1027
II_{10}	−120.029	1	Arg1027
Sunitinib	−154.827	2	Asp1046
OA	−93.4356	1	Ile1044

IC_{50} = 16.7、9.8 和 6.3 μmol·L^{-1}。通过计算机辅助设计分子对接方法预测目标化合物和 VEGFR 靶点的结合能, 结果表明 OA-3 位羰基化合物的结合能均高于 OA-3 位乙酰基化合物。选取可以通过氢键与 VEGFR 蛋白氨基酸残基 (Asp1046) 相互作用的化合物 **I_6**～**I_9** 进行 VEGFR 抑制活性测试, 结果表明其具有较强的 VEGFR 抑制活性。实测活性与分子模拟对接预测的结果相关性较好, 本研究结果对进一步优化设计齐墩果酸衍生物作为VEGFR抑制剂的研究具有参考价值。

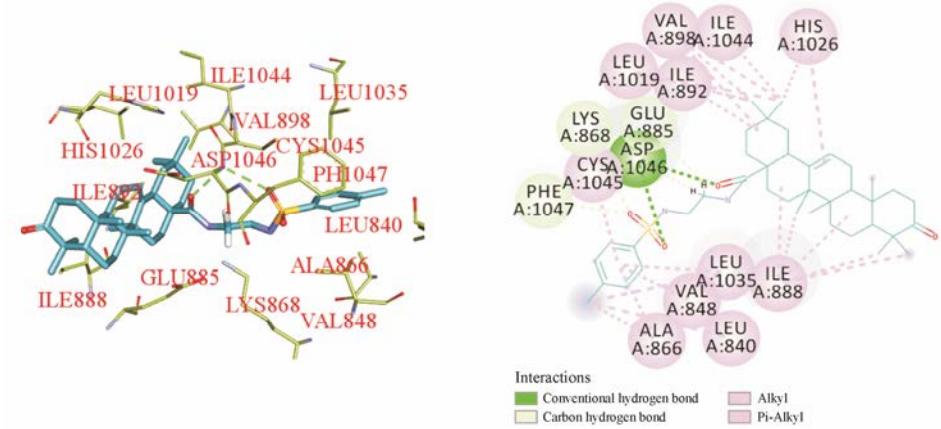


Figure 3 Binding of **I_9** to the active site of VEGFR

实验部分

Büchi B-540 熔点测定仪 (温度计未经校正); ARX-400 型核磁共振仪 (TMS 为内标); Autospec Ultima-TOF 质谱测定仪; WZZ-1S (2s) 自动旋光仪; 齐墩果酸 (质量分数>98%) 购于陕西慈缘生物技术有限公司; 薄层色谱硅胶 GF254 (青岛海洋化工厂); 显色剂为 10%硫酸乙醇溶液; RPMI-1640 培养基 (含 10%胎牛血清, 100 μg·mL^{-1}青霉素, 100 μg·mL^{-1}链霉素)、溴化四氮唑盐 (MTT)、胰蛋白酶 (Trypsin) 和标准胎牛血清 (FBS)、SGC7901 细胞和 HepG2 细胞由沈阳药科大学药理教研室提供; 实验所用试剂均为市售分析纯。

1 化学合成

1.1 3-羰基齐墩果酸 (2) 的制备 将化合物 **1** (OA, 2.5 g, 5.47 mmol) 溶于 25 mL 丙酮中, 冰盐浴冷却至−10 ℃, 缓慢滴加 28.9 mmol Jones 试剂, 直至反应液橘红色不再消失, 滴毕, 室温下继续反应 2 h, TLC 监测反应进程。反应完毕, 加入 75 mL 异丙醇, 淬灭反应 30 min。减压旋蒸, 除去部分溶剂, 加入适量水稀释, 乙酸乙酯萃取, 水洗, 合并有机相, 无水 Na_2SO_4 干燥过夜, 抽滤, 洗涤, 减压旋蒸, 得淡绿色固体, 50 ℃真空干燥。甲醇/二氯甲烷 (1∶10) 重结晶, 得白色晶体化合物 **2** 1.92 g, 收率 97%, mp 218.1～220.7 ℃ (文献值 mp 220.3～222.1 ℃)[18]。

1.2 3-羰基齐墩果酸酰氯 (3) 的制备 将化合物 **2** (3.50 g, 7.69 mmol) 溶于 40 mL 无水二氯甲烷中, 加热至 40 ℃, 缓慢滴加草酰氯 (4.2 mL, 49.63 mmol), 滴毕, 回流反应 2 h, TLC 监测反应, 反应完全后, 减压蒸干溶剂得浅棕色固体粉末, 密闭备用。

1.3 化合物 (4) 的制备 将溶于 25 mL 无水三氯甲烷中的化合物 **3** (1.5 g, 3.17 mmol) 缓慢滴加到乙二胺 (2.12 mL, 31.7 mmol) 溶液中, 加入 DMAP (0.019 g, 0.16 mmol) 和无水吡啶 (1.27 mL, 15.85 mmol)。室温反应 3 h, TLC 监测反应, 反应结束后, 用 10 mL 稀盐酸 (5%) 洗涤, 二氯甲烷萃取, 水洗, 合并有机相, 无水 Na_2SO_4 干燥过夜, 抽滤, 洗涤, 旋蒸除去溶剂, 得棕色固体, 50 ℃真空干燥。硅胶柱色谱分离, 甲醇/二氯甲烷 (1∶150) 洗脱, 得白色粉末化合物 **4** 1.26 g, 收率 80%。mp 149.5～150.3 ℃; HR-MS *m/z*: 497.402 8 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.60 (1H, d, *J* = 5.1 Hz, CONH), 5.40 (1H, d, *J* = 15.1 Hz, H-12), 3.51 (1H, dd, *J* = 13.7, 5.8 Hz, $CONHCH_2CH_2$), 3.22 (1H, dd, *J* = 13.9, 5.6 Hz, $CONHCH_2CH_2$), 3.07 (2H, s, $CONHCH_2CH_2$), 2.94 (2H, t, *J* = 5.4 Hz, NH_2), 2.63 (1H, d, *J* = 9.4 Hz, H-18), 2.55 (1H, m, H-2), 2.37 (1H, m, H-12), 2.55 (1H, ddd, *J* = 15.9, 11.1, 7.3 Hz, H-2), 2.37 (1H, ddd, *J* = 15.7, 6.5, 3.5 Hz, H-2), 1.17, 1.09, 1.05, 0.92, 0.91, 0.81 (3H, s, CH_3)。

1.4 化合物 I_1～I_{10} 的制备

化合物 I_1 的制备 将化合物 **4** (0.497 g, 1.0 mmol) 溶于 10 mL 无水三氯甲烷中, 加入苯甲酰氯 (0.71 g, 5.0 mmol)、无水吡啶 (0.41 mL, 5.0 mmol) 和 DMAP (0.006 g, 0.05 mmol), 升温至 60 ℃, 持续回流反应 3 h。TLC 监测反应, 反应结束后, 用 10 mL 稀盐酸 (5%) 洗涤, 加入适量水稀释, 二氯甲烷萃取, 水洗, 合并有机相, 无水 Na_2SO_4 干燥, 抽滤, 洗涤, 旋蒸除去溶剂, 得淡黄色油状物质, 50 ℃真空干燥。硅胶柱色谱分离, 甲醇/二氯甲烷 (0～10%) 洗脱, 得白色固体化合物 $\mathbf{I_1}$ 0.42 g, 收率 70%。mp110.2～112.0 ℃; $[\alpha]_D^{25}$ +36.5 (*c* 0.40, $CHCl_3$); HR-MS *m/z*: 601.429 0 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86～7.80 (2H, m, ph-H-2', H-6'), 7.60 (1H, dd, *J* = 15.1, 7.6 Hz, $CONHCH_2CH_2NH$), 7.45～7.40 (2H, m, ph-H-3', H-5'), 6.48 (1H, d, *J* = 5.7 Hz, CONH), 5.42 (1H, t, *J* = 3.5 Hz, H-12), 3.66～3.35 (4H, m, $CONHCH_2CH_2$), 2.63～2.57 (1H, m, H-18), 2.54 (1H, ddd, *J* = 15.9, 11.3, 7.Hz, H-2), 2.35 (1H, ddd, *J* = 15.8, 6.6, 3.Hz, H-2), 1.17, 1.07, 1.02, 0.97, 0.89, 0.87, 0.75 (each 3H, s, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 217.63, 180.55, 166.46, 165.92, 163.52, 144.45, 132.61, 130.29, 129.40, 115.67, 115.58, 115.12, 115.36, 55.24, 47.46, 46.73, 46.59, 46.42, 42.28, 41.90, 39.54, 39.20, 39.23, 36.76, 33.09, 32.95, 32.67, 31.85, 30.60, 29.65, 27.25, 26.36, 25.60, 23.68, 23.37, 21.48, 19.39, 16.68, 14.97 。

同法合成 I_2～I_{10}

$\mathbf{I_2}$, 白色固体, 收率 70%。mp 99.5～101.7 ℃; $[\alpha]_D^{25}$ +42.3 (*c* 0.55, $CHCl_3$); HR-MS *m/z*: 615.444 8 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (2H, d, *J* = 8.1 Hz, ph-H-2', H-6'), 7.46 (1H, s, $CONHCH_2CH_2NH$), 7.22 (2H, d, *J* = 8.0 Hz, ph-H-3', H-5'), 6.50 (1H, t, *J* = 5.3 Hz, CONH), 5.42 (1H, t, *J* = 3.5 Hz, H-12), 3.65～3.37 (4H, m, $CONHCH_2CH_2NH$), 2.62～2.58 (1H, m, H-18), 2.57～2.49 (1H, m, H-2), 2.39 (3H, s, ph-CH_3), 2.37～2.32 (1H, m, H-2), 1.15, 1.09, 1.03, 0.97, 0.89, 0.87, 0.75 (each 3H, s, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 217.56, 180.68, 166.68, 166.12, 163.45, 144.28, 141.15, 132.65, 130.03, 129.38, 115.58, 115.36, 55.64, 48.46, 47.83, 46.59, 45.37, 42.08, 41.95, 39.44, 39.29, 39.11, 36.66, 34.08, 32.92, 32.67, 31.81, 30.69, 29.70, 27.20, 26.46, 25.65, 23.72, 23.28, 21.58, 20.93,

19.41, 16.70, 14.95。

I_3, 白色固体, 收率 61%。mp 102.3～103.5 ℃; $[\alpha]_D^{25}$ +40.6 (*c* 0.45, $CHCl_3$); HR-MS *m/z*: 619.419 8 $[M+H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 8.13 (2H, dd, *J* = 8.3, 5.5 Hz, ph-H-2', H-6'), 7.89 (1H, dd, *J* = 8.2, 5.7 Hz, $CONHCH_2CH_2NH$), 7.13 (2H, dt, *J* = 16.7, 8.5 Hz, ph-H-3', H-5'), 6.56 (1H, d, *J* = 5.3 Hz, CONH), 5.43 (1H, s, H-12), 3.54 (4H, m, $CONHCH_2CH_2NH$), 2.60 (1H, dd, *J* = 15.9, 8.7 Hz, H-18), 2.37 (1H, m, H-2), 1.16, 1.09, 1.04, 0.99, 0.91, 0.87, 0.76 (each 3H, s, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 217.68, 180.99, 166.77, 166.02, 163.52, 144.40, 132.69, 130.00, 129.48, 115.70, 115.58, 115.48, 115.36, 55.24, 47.46, 46.73, 46.59, 46.47, 42.08, 41.95, 39.44, 39.29, 39.11, 36.66, 34.08, 32.92, 32.67, 31.81, 30.69, 29.70, 27.22, 26.36, 25.64, 23.73, 23.49, 21.48, 19.47, 16.72, 14.97 。

I_4, 白色粉末状固体, 收率 56%。mp 98.5～102.2 ℃; $[\alpha]_D^{25}$ +45.6 (*c* 0.55, $CHCl_3$); HR-MS *m/z*: 619.419 8 $[M+H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 8.20 (1H, ph-H-2'), 7.60 (1H, dd, *J* = 15.1, 7.6 Hz, $CONHCH_2CH_2NH$), 7.38～7.12 (3H, m, ph-H-4', 5', 6'), 6.56 (1H, d, *J* = 5.3 Hz, CONH), 5.43 (1H, s, H-12), 3.54 (4H, m, $CONHCH_2CH_2NH$), 2.60 (1H, dd, *J* = 15.9, 8.7 Hz, H-18), 2.37 (1H, ddd, *J* = 15.7, 6.2, 3.3 Hz, H-2), 1.16, 1.09, 1.04, 0.99, 0.89, 0.85, 0.75 (each 3H, s, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 217.65, 180.86, 166.67, 166.01, 163.50, 144.42, 132.67, 130.05, 129.46, 115.72, 115.58, 115.48, 115.38, 55.25, 47.46, 46.74, 46.60, 46.47, 42.10, 41.93, 39.45, 39.29, 39.11, 37.66, 34.10, 32.93, 32.68, 31.81, 30.65, 29.72, 27.23, 26.35, 25.64, 23.74, 23.47, 21.46, 19.45, 16.74, 14.95 。

I_5, 白色粉末固体, 收率 91%。mp 159.6～162.8 ℃; $[\alpha]_D^{25}$ +41.8 (*c* 0.50, $CHCl_3$); HR-MS *m/z*: 631.439 8 $[M+H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 7.82 (2H, d, *J* = 8.7 Hz, ph-H-2', H-6'), 7.63 (1H, d, *J* = 12.0 Hz, $CONHCH_2CH_2NH$), 6.91 (2H, dd, *J* = 8.5, 2.9 Hz, ph-H-3', H-5'), 6.63 (1H, s, CONH), 5.41 (1H, s, H-12), 3.85 (3H, dd, *J* = 10.8, 3.1 Hz, ph-OCH_3), 3.56～3.11 (4H, m, $CONHCH_2CH_2NH$), 2.70～2.59 (1H, m, H-18), 2.53 (1H, dt, *J* = 11.8, 7.8Hz, H-2), 2.40～2.28 (1H, m, H-2), 1.14, 1.07, 1.02, 0.96, 0.92, 0.83, 0.74 (each 3H, s, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 217.69, 180.43, 167.50, 162.15, 144.35, 128.94, 126.23, 122.81, 113.63, 55.38, 55.22, 47.43, 46.75, 46.59, 46.37, 45.91, 42.03, 41.82, 41.46, 39.82, 39.27, 39.10, 36.64, 34.10, 32.96, 32.74, 31.80, 30.69, 29.69, 27.26, 26.38, 25.66, 23.64, 23.52, 21.47, 19.49, 16.71, 14.96 。

I_6, 白色粉末固体, 收率 91%。mp 167.2～170.5 ℃; $[\alpha]_D^{25}$ +38.6 (*c* 0.45, $CHCl_3$); HR-MS *m/z*: 646.414 2 $[M+H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 8.40 (1H, s, $CONHCH_2CH_2NH$), 8.06 (2H, d, *J* = 8.4 Hz, ph-H-2', H-6'), 7.82 (2H, t, *J* = 7.5 Hz, ph-H-3', H-5'), 6.56 (1H, s, CONH), 5.45 (1H, s, H-12), 3.7～3.41 (4H, m, $CONHCH_2CH_2NH$), 2.61 (1H, d, *J* = 9.7 Hz, H-18), 2.42～2.31 (1H, m, H-2), 1.18, 1.09, 1.04, 0.99, 0.93, 0.89, 0.76 (each 3H, s, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 217.50, 181.77, 165.33, 148.45, 144.47, 144.41, 137.33, 130.91, 128.32, 124.29, 123.71, 123.43, 123.03, 55.21, 47.45, 46.69, 46.57, 43.15, 42.16, 42.09, 39.30, 39.20, 39.10, 36.65, 34.09, 34.00, 32.87, 32.66, 31.80, 30.69, 27.18, 26.35, 25.61, 23.83, 23.53, 23.46, 21.48, 19.45, 16.75, 14.97 。

I_7, 白色粉末状固体, 收率 88%。mp 158.5～160.4 ℃。$[\alpha]_D^{25}$ +32.8 (*c* 0.35, $CHCl_3$); HR-MS *m/z*: 646.414 2 $[M+H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 8.42 (1H, s, $CONHCH_2CH_2NH$), 8.25 (1H, m, ph-H-2'), 7.82～7.39 (3H, m, ph-H-3', 4', 5'), 6.76 (1H, s, CONH), 5.39 (1H, s, H-12), 3.60～3.28 (4H, m, $CONHCH_2CH_2NH$), 2.76 (1H, d, *J* = 9.7 Hz, H-18), 2.39～2.18 (1H, m, H-2), 1.18, 1.09, 1.04, 0.99, 0.94, 0.90, 0.76 (each 3H, s, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 217.49, 181.76, 165.31, 147.45, 145.46, 143.42, 137.34, 130.95, 128.35, 124.25, 123.73, 123.45, 123.05, 55.23, 47.43, 46.68, 46.57, 43.14, 42.16, 41.95, 39.32, 39.20, 39.08, 36.64, 34.09, 34.02, 32.85, 32.65, 31.80, 30.65, 27.18, 26.34, 25.62, 23.82, 23.54, 23.45, 21.48, 19.46, 16.76, 14.96 。

I_8, 白色粉末固体, 收率 73%。mp 180.2～182.1 ℃; $[\alpha]_D^{25}$ +39.8 (*c* 0.45, $CHCl_3$); HR-MS *m/z*: 659.434 6 $[M+H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 7.81 (2H, d, *J* = 8.5 Hz, ph-H-2', H-6'), 7.72 (1H, s, -$CONHCH_2CH_2NH$), 7.10 (2H, dd, *J* = 16.5, 8.4 Hz, ph-H-3', H-5'), 6.52 (1H, s, CONH), 5.34 (1H, s, H-12), 3.58～3.29 (4H, m, -$CONHCH_2CH_2NH$), 2.58～2.50 (1H, m, H-18), 2.25 (3H, d, *J* = 7.0 Hz, ph-OAc), 1.07 (3H, s, CH_3), 1.00 (3H, s, CH_3), 0.95 (3H, s, CH_3), 0.89 (3H, s, CH_3), 0.83 (3H, s, CH_3), 0.79 (3H, s, CH_3), 0.67 (3H, s, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 217.85, 180.90, 179.58, 169.00, 153.19, 144.35, 131.72, 131.28, 128.60, 122.97, 121.68, 55.25, 47.47, 46.75, 46.60, 46.47, 42.08, 41.95, 41.89～41.85, 39.60, 39.30, 39.10, 36.66, 34.14, 34.03, 32.93, 32.68, 31.80, 30.69, 29.70, 27.24, 26.37, 25.66, 23.69, 23.49, 21.49, 21.16, 19.48, 16.73, 14.96 。

I_9, 白色粉末固体, 收率 69%。mp 124.2～125.9 ℃;

$[\alpha]_D^{25}$ +46.8 (*c* 0.55, $CHCl_3$); HR-MS *m/z*: 651.411 8 $[M+H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 7.74 (2H, d, *J* = 8.3 Hz, ph-H-2', H-6'), 7.30 (2H, d, *J* = 8.0 Hz, ph-H-3', H-5'), 6.33 (1H, t, *J* = 5.4 Hz, CONH), 5.44 (1H, t, *J* = 3.5 Hz, H-12), 5.31 (1H, d, *J* = 8.6 Hz, $CONHCH_2CH_2NH$), 3.51～3.00 (4H, m, $CONHCH_2CH_2NH$), 2.55 (1H, m, H-18), 2.55 (1H, m, H-2), 2.42 (1H, s, ph-CH_3), 2.37 (1H, m, H-2), 1.17 (3H, s, CH_3), 1.09 (3H, s, CH_3), 1.05 (6H, s, $2CH_3$), 0.93 (3H, s, CH_3), 0.89 (3H, s, CH_3), 0.76 (3H, s, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 217.75, 180.63, 166.58, 166.42, 163.39, 142.38, 140.19, 132.63, 130.83, 129.35, 115.53, 115.38, 55.64, 48.46, 47.83, 46.59, 45.37, 42.08, 41.95, 39.44, 39.29, 39.11, 36.66, 34.08, 32.92, 32.67, 31.81, 30.69, 29.70, 27.20, 26.46, 25.65, 23.72, 23.28, 21.58, 20.67, 19.41, 16.70, 14.95。

I_{10}, 白色粉末固体, 收率 64%。mp 123.4～125.2 ℃; $[\alpha]_D^{25}$ +34.5 (*c* 0.35, $CHCl_3$); HR-MS *m/z*: 575.380 4 $[M+H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 6.35 (1H, d, *J* = 5.3 Hz, CONH), 5.44 (1H, t, *J* = 3.5 Hz, H-12), 5.05 (1H, s, 1H, $CONHCH_2CH_2NH$), 3.60～3.16 (4H, m, $CONHCH_2CH_2$), 2.98～2.95 (3H, m, S-CH_3), 2.67 (1H, m, H-18), 2.56 (1H, m, H-2), 2.36 (1H, m, H-2), 1.24, 1.17, 1.13, 1.09, 1.01, 0.91, 0.82 (each 3H, s, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 217.65, 180.65, 166.56, 163.39, 129.35, 115.53, 55.64, 48.46, 47.83, 46.59, 45.37, 42.08, 41.95, 39.44, 39.29, 39.11, 36.66, 34.08, 32.92, 32.67, 31.81, 30.69, 29.70, 27.20, 26.46, 25.65, 23.72, 23.28, 21.58, 20.67, 19.41, 16.70, 14.95 。

1.5 3-乙酰氧基齐墩果酸的制备 (5) 的制备 将化合物 **1** OA (4.16 g, 11.12 mmol) 溶于 30 mL 无水吡啶中, 冰浴冷却至 0 ℃, 缓慢滴加乙酸酐 (10.52 mL, 111.2 mmol), 滴毕, 加入 DMAP (0.052 g, 0.42 mmol), 室温反应过夜, TLC 监测反应。反应结束后, 将反应液倾入 200 mL 冰水中, 不断搅拌直至固体全部析出, 二氯甲烷萃取, 稀盐酸 (5%) 洗, 水洗, 无水 Na_2SO_4 干燥, 抽滤, 洗涤, 蒸干溶剂, 得深黄色油状物, 50 ℃真空干燥。用甲醇/二氯甲烷重结晶得白色针状晶体化合物 5 4.9 g, 收率 88%, mp 268.1～270.8 ℃。(文献值: mp 269～271 ℃)[19]。

1.6 3-乙酰氧基齐墩果酸酰氯 (6) 的制备 将化合物 **5** (2.5 g, 5.02 mmol) 溶于 25 mL 无水二氯甲烷中, 加热至 40 ℃, 搅拌下缓慢滴加草酰氯 (2.5 mL, 29.25 mmol), 回流反应 2 h, TLC 监测反应, 反应完全后, 减压蒸干溶剂, 减压蒸干得浅棕色固体粉末, 密闭备用。

1.7 化合物 (7) 的制备 将溶于 30 mL 无水三氯甲烷中的化合物 **6** (2.5 g, 4.84 mmol) 缓慢滴加到乙二胺 (2.5 mL, 37.4 mmol) 溶液中, 加入 DMAP (0.02 g, 0.16 mmol) 和无水吡啶 (1.5 mL, 18.63 mmol), 升温至 60 ℃, 持续回流反应 3 h。室温反应 3 h, TLC 监测反应, 反应结束后, 用 10 mL 稀盐酸 (5%) 洗涤, 二氯甲烷萃取, 水洗, 合并有机相, 无水 Na_2SO_4 干燥过夜, 抽滤, 洗涤, 旋蒸除去溶剂, 得棕色固体粉末, 50 ℃真空干燥。硅胶柱色谱分离, 甲醇/二氯甲烷 (1∶50) 洗脱, 得白色粉末化合物 **7** (2.35 g, 4.35 mmol), 收率 90%。mp 198.7～201.3 ℃; HR-MS *m/z*: 541.429 0 $[M+H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 6.38 (1H, t, *J* = 5.4 Hz, CONH), 5.39 (1H, t, *J* = 3.4 Hz, H-12), 4.48 (1H, dd, *J* = 10.0, 5.9 Hz, H-3), 3.45 (1H, dq, *J* = 12.0, 6.0 Hz, $NHCH_2$), 3.15～3.04 (1H, m, $NHCH_2$), 3.02 (2H, s, NH_2), 2.85～2.78 (2H, m, $NHCH_2CH_2$), 2.56 (1H, dd, *J* = 12.8, 3.3 Hz, H-18), 2.05 (3H, s, 3-$COCH_3$), 0.91 (6H, s, $2CH_3$), 1.16, 0.93, 0.87, 0.85, 0.78 (each 3H, s, CH_3)。

1.8 化合物 II_1～II_{10} 的制备

化合物 II_1 的制备 将化合物 **7** (0.50 g, 0.925 mmol) 溶于 10 mL 无水三氯甲烷中, 加入苯甲酰氯 (0.63 g, 4.65 mmol)、无水吡啶 (0.37 mL, 4.65 mmol) 和 DMAP (0.011 g, 0.093 mmol), 升温至 60 ℃, 持续回流反应 3 h。TLC 监测反应, 反应结束后, 用 10 mL 稀盐酸 (5%) 洗涤, 加入适量水稀释, 二氯甲烷萃取, 水洗, 合并有机相, 无水 Na_2SO_4 干燥, 抽滤, 洗涤, 旋蒸除去溶剂, 得淡黄色油状物质, 50 ℃真空干燥。硅胶柱色谱分离, 甲醇/二氯甲烷 (0～10%) 洗脱, 得白色粉末固体化合物 **II_1** 0.423 g, 收率 71%。mp 167.1～168.9 ℃; $[\alpha]_D^{25}$ +62.8 (*c* 0.95, $CHCl_3$); HR-MS *m/z*: 645.455 4 $[M+H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 7.88～7.78 (1H, m, 1H, ph-H-2', H-6'), 7.61 (1H, d, *J* = 14.8 Hz, $CONHCH_2CH_2NH$), 7.51～7.40 (3H, m, ph-H-3', H-4', H-5'), 6.47 (1H, d, *J* = 5.7 Hz, CONH), 5.40 (1H, t, *J* = 3.4 Hz, H-12), 4.47 (1H, dd, *J* = 10.3, 5.9 Hz, H-3), 3.75～3.21 (4H, m, $CONHCH_2CH_2$), 2.56 (1H, dd, *J* = 12.8, 3.3 Hz, H-18), 2.04 (3H, s, 3-$OCOCH_3$), 1.14, 0.89, 0.87, 0.86, 0.85, 0.84, 0.70 (each 3H, s, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.55, 169.25, 165.92, 163.52, 162.59, 147.45, 133.63, 131.23, 129.40, 115.67, 115.58, 115.12, 114.59, 55.24, 47.46, 46.73, 46.59, 46.42, 42.28, 41.90, 39.54, 39.20, 37.23, 36.76, 33.09, 32.95, 32.67, 31.85, 30.60, 29.65, 27.25, 26.36, 25.60, 23.68, 23.37, 22.32, 21.48, 19.39, 17.38, 16.68, 14.97。

II_2, 白色粉末固体, 收率 70%。mp 161.1～163.7 ℃; $[\alpha]_D^{25}$ +40.6 (*c* 0.52, $CHCl_3$); HR-MS *m*/*z*: 659.471 2 $[M+H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 7.72 (2H, d, *J* = 8.2 Hz, ph-H-2', H-6'), 7.49 (1H, d, *J* = 8.1 Hz, $CONHCH_2CH_2NH$), 7.23 (2H, dd, *J* = 14.3, 7.9 Hz, ph-H-3', H-5'), 6.26 (1H, t, *J* = 5.4 Hz, CONH), 5.38 (1H, dt, *J* = 12.6, 3.3 Hz, H-12), 4.48 (1H, dt, *J* = 8.1, 6.8 Hz, H-3), 3.71～3.16 (4H, m, $CONHCH_2CH_2$), 2.54 (1H, dd, *J* = 10.1, 3.4 Hz, H-18), 2.46 (3H, s, ph-CH_3), 2.05 (3H, s, 3-$OCOCH_3$), 1.15, 0.92, 0.90, 0.86, 0.84, 0.73, 0.70 (each 3H, s, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.54, 169.26, 165.93, 163.48, 162.59, 148.35, 132.96, 131.35, 129.45, 115.61, 115.25, 115.22, 114.59, 57.14, 49.46, 47.78, 46.59, 45.42, 42.38, 41.95, 39.56, 39.25, 38.25, 36.83, 33.15, 32.75, 32.53, 31.85, 30.44, 29.65, 28.15, 26.56, 25.36, 23.65, 23.35, 22.34, 21.56, 21.02, 19.16, 17.37, 16.57, 14.65 。

II_3, 白色粉末状固体, 收率 75%, mp 170.8～173.2 ℃; $[\alpha]_D^{25}$ +58.6 (*c* 0.85, $CHCl_3$); HR-MS *m*/*z*: 663.445 8 $[M+H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 7.91～7.84 (4H, m, ph-H-2', 3', 5', 6'), 6.48 (1H, t, *J* = 5.8 Hz, CONH), 5.40 (1H, t, *J* = 3.3 Hz, H-12), 4.47 (1H, dd, *J* = 10.8, 5.3 Hz, H-3), 3.39 (4H, m, $CONHCH_2CH_2$), 2.63～2.51 (1H, m, H-18), 2.05 (3H, s, 3-$OCOCH_3$), 1.14, 0.91, 0.88, 0.87, 0.85, 0.76, 0.65 (each 3H, s, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.56, 169.45, 165.76, 163.52, 147.37, 134.58, 131.23, 129.40, 119.05, 116.59, 115.12, 55.24, 47.46, 46.73, 46.59, 46.42, 42.28, 41.90, 39.54, 39.20, 38.13, 36.76, 33.09, 32.95, 32.67, 31.85, 30.60, 29.65, 27.25, 26.36, 25.60, 23.68, 23.37, 19.11, 17.45, 16.65, 14.95 。

II_4, 白色粉末状固体, 收率 65%。mp 162.1～164.2 ℃; $[\alpha]_D^{25}$ +30.6 (*c* 0.43, $CHCl_3$); HR-MS *m*/*z*: 663.445 8 $[M+H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 8.03～7.64 (4H, m, ph-H-2', 4', 5', 6'), 6.40 (1H, t, *J* = 5.8 Hz, CONH), 5.36 (1H, t, *J* = 3.3 Hz, H-12), 4.51 (1H, dd, *J* = 10.8, 5.3 Hz, H-3), 3.45 (4H, m, $CONHCH_2CH_2$), 2.76～2.41 (1H, m, H-18), 2.15 (3H, s, 3-$OCOCH_3$), 1.10, 0.89, 0.87, 0.86, 0.85, 0.76, 0.67 (each 3H, s, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.57, 169.44, 165.77, 163.54, 147.35, 137.28, 135.21, 130.40, 121.25, 116.28, 115.02, 54.24, 47.46, 46.28, 46.53, 46.19, 42.18, 41.56, 39.53, 39.58, 39.23, 36.76, 33.09, 32.95, 32.67, 31.85, 30.60, 29.65, 27.25, 26.36, 25.60, 23.68, 23.37, 19.23, 17.47, 16.68, 14.96 。

II_5, 白色固体粉末状固体, 收率 75%。mp 153.2～155.1 ℃; $[\alpha]_D^{25}$ +42.5 (*c* 0.50, $CHCl_3$); HR-MS *m*/*z*: 675.465 8 $[M+H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 7.79 (2H, d, *J* = 8.8 Hz, ph-H-2', H-6'), 7.48 (1H, s, $CONHCH_2CH_2NH$), 6.91 (2H, d, *J* = 8.8 Hz, ph-H-3', H-5'), 6.48 (1H, d, *J* = 5.7 Hz, CONH), 5.40 (1H, t, *J* = 3.4 Hz, H-12), 4.47 (1H, dd, *J* = 10.4, 5.8 Hz, H-3), 3.82 (3H, s, ph-OCH_3), 3.40～3.33 (4H, m, $CONHCH_2CH_2$), 2.67 (1H, dd, *J* = 10.2, 3.4 Hz, H-18), 2.05 (3H, s, 3-$OCOCH_3$), 1.14, 0.89, 0.87, 0.86, 0.85, 0.84, 0.70 (each 3H, s, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.65, 169.37, 165.86, 162.42, 148.57, 135.26, 132.25, 127.44, 118.09, 115.65, 114.12, 56.28, 53.87, 47.46, 46.73, 46.59, 46.12, 42.38, 41.95, 39.55, 39.20, 38.13, 36.76, 33.09, 32.95, 32.67, 31.85, 30.60, 29.60, 27.23, 26.35, 25.60, 23.18, 22.37, 19.13, 17.45, 16.65, 14.95 。

II_6, 白色固体粉末 g, 产率 80%。mp 161.6～163.7 ℃; $[\alpha]_D^{25}$ +29.6 (*c* 0.40, $CHCl_3$); HR-MS *m*/*z*: 690.440 2 $[M+H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 8.39 (1H, s, $CONHCH_2CH_2NH$), 8.28 (2H, d, *J* = 8.8 Hz, ph-H-2', H-6'), 8.03 (2H, d, *J* = 8.8 Hz, ph-H-3', H-5'), 6.51 (1H, t, *J* = 5.8 Hz, CONH), 5.41 (1H, t, *J* = 3.3 Hz, H-12), 4.47 (1H, dd, *J* = 10.8, 5.3 Hz, H-3), 3.57 (2H, dd, *J* = 10.0, 4.3 Hz, $CONHCH_2CH_2$), 3.44 (2H, dd, *J* = 10.4, 5.3 Hz, $CONHCH_2CH_2$), 2.59 (1H, dd, *J* = 10.2, 3.4 Hz, H-18), 2.05 (3H, s, 3-$OCOCH_3$), 1.15, 0.91, 0.88, 0.84, 0.86, 0.69, 0.58 (each 3H, s, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.75, 169.45, 165.33, 148.45, 144.47, 144.41, 137.33, 130.91, 128.32, 124.29, 123.71, 123.43, 123.03, 55.21, 47.45, 46.69, 46.57, 43.15, 42.16, 42.09, 39.30, 39.20, 39.10, 36.65, 34.09, 34.00, 32.87, 32.66, 31.80, 30.69, 27.18, 26.35, 25.61, 24.13, 23.53, 23.16, 21.48, 19.45, 16.75, 14.97 。

II_7, 白色粉末状固体, 收率 70%。mp 155.2～157.1 ℃; $[\alpha]_D^{25}$ +30.4 (*c* 0.45, $CHCl_3$); HR-MS *m*/*z*: 690.4402 $[M+H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 8.41 (1H, s, $CONHCH_2CH_2NH$), 8.33 (1H, m, ph-H-2'), 7.98～7.82 (3H, m, ph-H-3', 4', 5'), 6.65 (1H, t, *J* = 5.8 Hz, CONH), 5.39 (1H, t, *J* = 3.3 Hz, H-12), 4.49 (1H, dd, *J* = 10.8, 5.3 Hz, H-3), 3.52 (2H, dd, *J* = 10.0, 4.3 Hz, $CONHCH_2CH_2$), 3.45 (2H, dd, *J* = 10.4, 5.3 Hz, $CONHCH_2CH_2$), 2.52 (1H, dd, *J* = 10.2, 3.4 Hz, H-18), 2.10 (3H, s, 3-$OCOCH_3$), 1.16, 1.10, 0.93, 0.89, 0.86, 0.72, 0.60 (each 3H, s, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.76, 169.25, 165.31, 147.45, 145.46, 143.42, 137.34, 130.95, 128.35, 124.25, 123.73, 123.45, 123.05, 55.23, 47.43, 46.68, 46.57, 43.14, 42.16, 41.95, 39.32, 39.20, 39.08, 36.64, 34.09, 34.02, 32.85, 32.65, 31.80, 30.65, 27.18, 26.34, 25.62, 24.82, 23.64, 23.13, 21.48, 19.16, 16.80, 14.86 。

II$_8$, 白色固体粉末, 收率 65%。mp 130.3～131.4 ℃; $[\alpha]_D^{25}$ +48.6 (*c* 0.55, $CHCl_3$); HR-MS *m*/*z*: 703.460 8 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89～7.84 (2H, m, ph-H-2', H-6'), 7.16～7.14 (2H, m, ph-H-3', H-5'), 6.49 (1H, t, *J* = 5.6 Hz, CONH), 5.40 (1H, t, *J* = 3.4 Hz, H-12), 4.47 (1H, dd, *J* = 10.0, 6.1 Hz, H-3), 3.66～3.30 (4H, m, $CONHCH_2CH_2$), 2.58 (1H, dd, *J* = 10.2, 3.4 Hz, H-18), 2.36 (3H, s, ph-OAc), 2.05 (3H, s, 3-$OCOCH_3$), 1.143 (3H, s, -CH_3), 0.87 (6H, s, 2CH_3), 0.90, 0.85, 0.84, 0.71 (each 3H, s, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.54, 179.62, 169.20, 165.92, 163.52, 153.19, 147.35, 131.75, 131.28, 128.60, 122.97, 121.68, 147.45, 133.63, 131.23, 129.40, 115.67, 115.58, 115.12, 55.24, 47.46, 46.73, 46.59, 46.42, 42.28, 41.90, 39.54, 39.20, 39.23, 36.76, 33.09, 32.95, 32.67, 31.85, 30.60, 29.65, 27.25, 26.36, 25.60, 23.68, 23.37, 21.48, 19.39, 17.38, 16.68, 14.97。

II$_9$, 白色粉末状固体, 收率 60%。mp 127.3～129.6 ℃; $[\alpha]_D^{25}$ +52.1 (*c* 0.65, $CHCl_3$); HR-MS *m*/*z*: 695.437 8 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (2H, d, *J* = 8.2 Hz, ph-H-2', H-6'), 7.29 (2H, d, *J* = 8.1 Hz, ph-H-3', H-5'), 6.32 (1H, t, *J* = 5.5Hz, $CONHCH_2CH_2NH$), 5.41 (1H, d, *J* = 3.4 Hz, H-12), 4.49 (1H, dd, *J* = 10.2, 5.7 Hz, H-3), 3.46～3.05 (4H, $CONHCH_2CH_2$), 2.51 (1H, d, *J* = 9.8 Hz, H-18), 2.42 (3H, s, ph-CH_3), 2.05 (3H, s, 3-$OCOCH_3$), 1.15, 0.93, 0.91, 0.90, 0.87, 0.86, 0.68 (each 3H, s, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.65, 169.25, 166.59, 163.52, 142.45, 132.63, 131.23, 129.40, 115.67, 115.58, 115.12, 55.24, 47.46, 46.73, 46.59, 46.42, 42.28, 41.90, 39.54, 39.20, 39.23, 36.76, 33.09, 32.95, 32.67, 31.85, 30.60, 29.65, 27.25, 26.36, 25.60, 23.68, 23.37, 21.48, 19.39, 17.38, 16.68, 14.95。

II$_{10}$, 白色固体, 收率 51%。mp 127.2～130.2 ℃; $[\alpha]_D^{25}$ +48.3 (*c* 0.55, $CHCl_3$); HR-MS *m*/*z*: 619.406 4 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.35 (1H, d, *J* = 5.3 Hz, CONH), 5.45～5.37 (1H, d, *J* = 3.4 Hz, H-12), 4.49 (1H, dd, *J* = 10.2, 5.7 Hz, H-3), 3.57～3.10 (4H, m, $CONHCH_2CH_2$), 2.96 (3H, m, S-CH_3), 2.54 (1H, m, H-18), 2.15 (3H, s, CH_3), 1.83～1.66 (1H, m, H-2), 1.65～1.31 (1H, m, H-2), 1.19, 1.11, 0.96, 0.92, 0.88, 0.75 (each 3H, s, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 181.55, 169.25, 165.92, 163.52, 147.45, 129.40, 115.67, 55.24, 47.46, 46.73, 46.59, 46.42, 42.28, 41.90, 39.54, 39.20, 38.25, 36.76, 33.12, 32.75, 32.67, 31.25, 30.45, 29.65, 27.25, 26.36, 25.60, 24.12, 23.52, 21.58, 19.52, 18.02, 16.85, 14.97。

2 初步体外细胞毒活性测试

取对数生长期的各株细胞以每孔 5×10^3 个接种于 96 孔板内, 每组 3 个复孔, CO_2 培养箱中培养 24 h 后备用。待细胞贴壁后, 分别加入待测化合物和舒尼替尼, 药品浓度为 10 μmol·L^{-1}, 空白组为阴性对照组, 舒尼替尼为阳性对照组。培养箱中继续培养 72 h。加入 MTT 溶液 (5 mg·mL^{-1}, 20 μL), 继续培养 4 h, 吸去每孔上清液, 加入二甲基亚砜 200 μL, 震荡器上震荡 5 min, 然后用酶标仪于 570 nm 处测 OD 值, 重复 3 次实验, 取平均值, 计算各组细胞的 IC_{50}, 抑制率 = (对照组 OD 值−药物组 OD 值)/对照组 OD 值×100%。

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