熊果酸–三氮唑衍生物的合成及抗肿瘤活性研究

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摘要: 以熊果酸为母体, 对其C-3和C-28位进行结构修饰, 引入1,2,3-三氮唑, 合成了10个新型熊果酸衍生物, 结构经MS、^{1}H NMR和^{13}C NMR确证。通过MTT法, 选用高表达人癌细胞 (乳腺癌MCF-7和胃癌SGC-7901细胞) 对化合物进行初步体外抗肿瘤活性筛选, 结果表明, 所有化合物对MCF-7和SGC-7901肿瘤细胞的抑制活性均明显高于熊果酸。其中化合物**II**$_4$对MCF-7和SGC-7901细胞具有较强的抗肿瘤作用, 活性与阳性对照药尼洛替尼相当, 分子对接也显示其与c-Kit具有较高的结合能力, 值得进一步研究。

关键词: 熊果酸; 分子对接; c-Kit; 结构修饰; 抗肿瘤活性

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Synthesis and anti-tumor activity of ursolic acid-triazole derivatives

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Abstract: Ten ursolic acid derivatives were designed from the lead compound ursolic acid by introducing 1, 2, 3-triazole at C-3 and C-28. The target compounds were synthesized and characterized by ^{1}H NMR and ^{13}C NMR. MTT assay was used to study the antitumor activity of these compounds in human cancer cells with high expression (MCF-7 and SGC-7901). The results showed that the antitumor activity of all compounds on MCF-7 and SGC-7901 tumor cells was significantly higher than that of ursolic acid. The compound **II**$_4$ exhibited significant antitumor activity which was equivalent to the positive control drug nilotinib, molecular docking showed that the compound **II**$_4$ have high binding ability with c-Kit, which deserves further research.

Key words: ursolic acid; molecular docking; c-Kit; structural modification; anti-tumor activity

熊果酸 (ursolic acid, UA) 别名乌索酸, 是一种天然的乌苏烷型五环三萜类化合物 (图1)。熊果酸具有抗菌、抗病毒、抗肿瘤、抗氧化、治疗糖尿病、保肝等药理活性[1-4]。熊果酸已被公认具有良好的抗肿瘤活性和广泛的抗肿瘤途径[5], 如诱导肿瘤细胞分化及凋亡[6], 抑制肿瘤细胞生成和增殖等[7], 为了改善其水溶性差, 生物利用度低的缺点, 研究人员对熊果酸结构进行修饰, 多集中在C-3位羟基和C-28位羧基[8], 得到的衍生物活性及药动学性质都得到增强和改善。

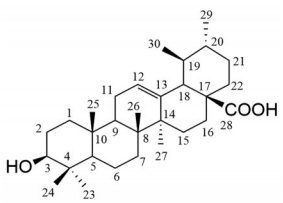

Figure 1 Chemical structure of ursolic acid (UA)

在药物及天然产物的衍生物设计中, 拼合原理[9]的应用尤具潜力, 即在同一分子中将两种或多种药物的结构或药效团进行拼合, 达到提高相关生物活性或降低毒副作用的效果。含氮唑环作为常见的药物结构活性基团[10], 具有抗炎、抗菌、抗肿瘤等作用[11], 独特结

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构可与目标靶点结合形成范德华力或氢键等偶极作用, 且化学性质稳定, 作为极性基团也可提高化合物的溶解度从而改善生物利用度[12]。三氮唑基团是唑类基团中热点药效团[13], 具有1,2,3-三氮唑和1,2,4-三氮唑两种异构体形式, 其中前者的结构具有较强的偶极矩, 作为可能的氢键供给体, 在与靶点结合亲和力方面表现更优[14]。Kim等[15]通过将神经酰胺的酰胺键替换为1,2,3-三氮唑, 设计出了具有更高细胞毒性的新型化合物, 一些基于1,2,3-三氮唑结构的衍生物也进入临床评估其在癌症治疗中的潜在用途[16]。

结合课题组前期利用分子对接技术及化学合成方法对五环三萜化合物的设计和结构修饰研究[17-19], 拼合含氮杂环可提高化合物与靶点蛋白的结合能力, 细胞实验显示相较于母体, 获得了更显著的抗肿瘤活性。本研究在前期工作基础上, 选择在多个恶性肿瘤中具有高表达的III型受体酪氨酸激酶 (c-Kit) 作为靶点蛋白[20], 将设计出的熊果酸衍生物与受体c-Kit晶体结构 (PDB: 1T46) 进行分子对接[21], 以UA为起始原料, 对其C-3位和C-28位分别引入酯键和1,2,3-三氮唑结构, 并通过连接碳链的长短初步探究构效关系 (图2)。

为了验证设计合理性, 使用分子对接软件Molegro virtual docker (MVD) 评估目标化合物与c-Kit靶蛋白的结合能力, 目标化合物与蛋白结合均优于母体, 可进行下一步合成。

结果

1 化学合成

如合成路线1所示, 以UA为母体, 将1,2,3-三氮唑结构分别引入其C-3和C-28位, 设计并合成了10种UA衍生物, 目标化合物$\mathbf{I}_1$～$\mathbf{I}_5$、$\mathbf{II}_1$～$\mathbf{II}_5$的收率、熔点、质谱数据列于表1, 中间体**3-1**～**3-5**、**5-1**～**5-5**及目标化合物$\mathbf{I}_1$～$\mathbf{I}_5$、$\mathbf{II}_1$～$\mathbf{II}_5$的^{1}H NMR、^{13}C NMR数据列于表2。

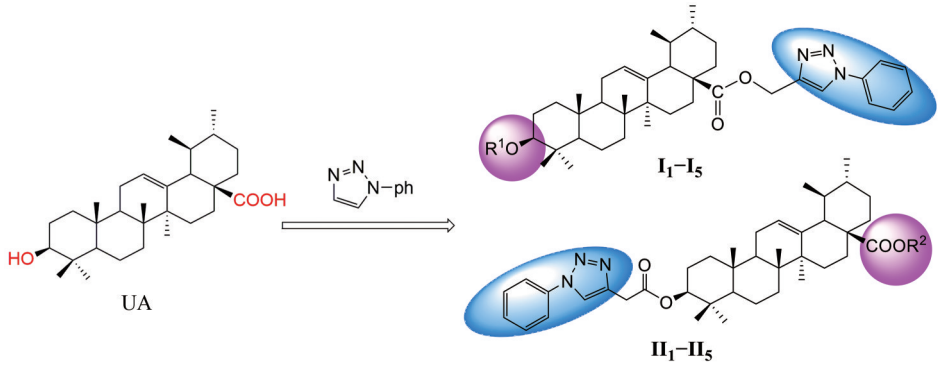
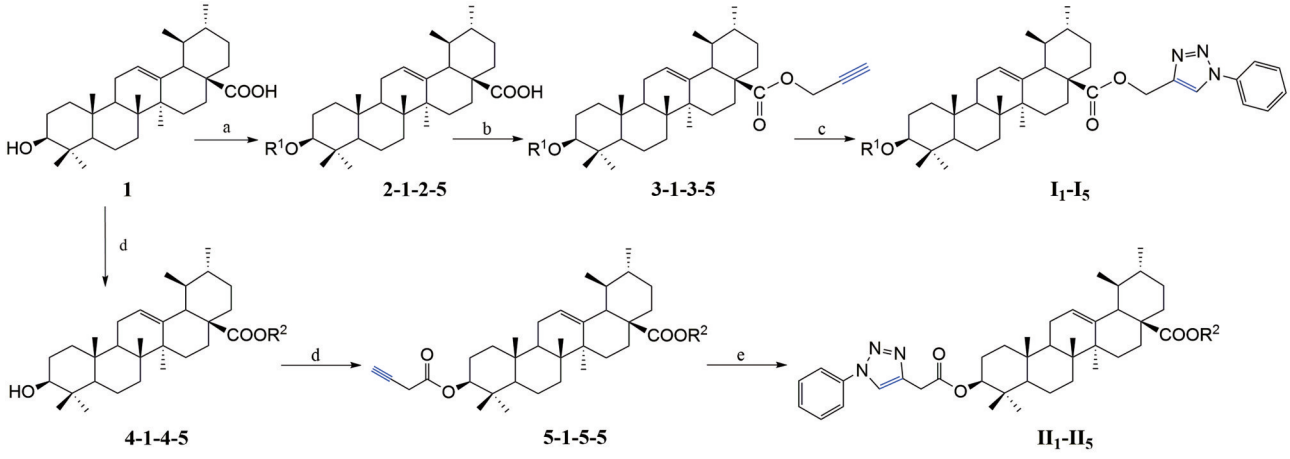


Figure 2 Design of new ursolic acid derivatives



$\mathbf{I}_1$ R^1= $-COCH_3$ $\mathbf{I}_2$ R^1= $-COCH_2CH_3$ $\mathbf{I}_3$ R^1= $-COCH_2CH_2CH_3$ $\mathbf{I}_4$ R^1= $-COCH_2CH_2CH_2CH_3$ $\mathbf{I}_5$ R^1= $-COCH_2CH_2CH_2CH_2CH_3$

$\mathbf{II}_1$ R^2= $-CH_2CH_3$ $\mathbf{II}_2$ R^2= $-CH_2CH_2CH_3$ $\mathbf{II}_3$ R^2= $-CH_2CH_2CH_2CH_3$ $\mathbf{II}_4$ R^2= $-CH_2CH_2CH_2CH_2CH_3$ $\mathbf{II}_5$ R^2= $-CH_2CH_2CH_2CH_2CH_2CH_3$

Scheme 1 Synthesis route of the target compound $\mathbf{I}_1$–$\mathbf{I}_5$ and $\mathbf{II}_1$–$\mathbf{II}_5$. Reagents and conditions: (a) appropriate anhydride, DMAP, THF, rt; (b) propargyl bromide, K_2CO_3, DMF, 60 ℃; (c) R^2Br, K_2CO_3, DMF, rt; (d) 3-butynoic acid, DCC, DMAP, CH_2Cl_2, rt; (e) aromatic azide, sodium ascorbate, $CuSO_4·5H_2O$, EtOH, rt

Table 1 Yield, melting point and MS data of the target compounds

Compd.	Yield/%	Melting/℃	ESI-MS *m/z* $[M+H]^+$
$\mathbf{I}_1$	50.4	176.3−177.7	656.29
$\mathbf{I}_2$	49.2	175.0−176.3	671.24
$\mathbf{I}_3$	55.6	171.4−172.7	684.51
$\mathbf{I}_4$	57.5	172.8−174.2	698.62
$\mathbf{I}_5$	58.1	168.5−170.0	712.52
$\mathbf{II}_1$	48.2	169.5−170.4	670.52
$\mathbf{II}_2$	50.6	170.2−171.1	684.37
$\mathbf{II}_3$	47.2	167.8−168.5	698.79
$\mathbf{II}_4$	50.8	165.2−166.3	712.64
$\mathbf{II}_5$	46.1	162.7−163.8	726.82

2 生物活性评价

以尼洛替尼[22]为阳性对照, 采用四甲基偶氮唑盐法 (MTT 法), 在癌细胞 (人乳腺癌细胞 MCF-7 和人胃癌细胞 SGC-7901) 中检测熊果酸及其衍生物的抗肿瘤活性, 实验结果见表 3, 化合物 $\mathbf{II}_4$ 对 MCF-7 和 SGC-7901 细胞的 IC_{50} 值分别为 14.28 和 8.46 μmol·L^{-1}, 接近阳性对照药尼洛替尼, 具有较强的抑制活性。

3 分子对接

选择 c-Kit 抑制剂尼洛替尼和母体熊果酸作对照, 进行分子对接研究, 评分绝对值越大, 结合亲和力越强, 结果如表 4 所示。衍生物结构中拼合 1,2,3-三氮唑结构, 对接得分明显高于母体, 与尼罗替尼相近, 其中化合物 $\mathbf{II}_4$ 对接得分最高。为了明确化合物 $\mathbf{II}_4$ 与 c-Kit 的相互作用情况, 利用 Discovery Studio 2019 Client 软件, 分析结合位点可能的结合方式 (图 3)。化合物 $\mathbf{II}_4$ 能够进入蛋白的活性口袋, 并与 c-Kit 蛋白的多个氨基酸

Table 2 Spectral data of the compounds **3-1**−**3-5**, **5-1**−**5-5**, $\mathbf{I}_1$−$\mathbf{I}_5$, $\mathbf{II}_1$−$\mathbf{II}_5$

Compd.	^{1}H NMR (600 MHz, $CDCl_3$)	^{13}C NMR (150 MHz, $CDCl_3$)
3-1	δ_H 5.28 (s, 1H, 12-H), 4.40 (t, *J* = 7.0 Hz, 1H, 3-H), 2.89 (dd, *J* = 11.2, 4.2 Hz, 1H, H-18), 2.97(s, 1H), 2.06 (s, 3H), 1.03−2.04 (m, 20H), 0.90−1.07 (m, 2 H), 1.05 (s, 3H), 0.93 (s, 3H), 0.91 (s, 3H), 0.90 (s, 3H), 0.85 (s, 3H), 0.82 (s, 3H), 0.79 (s, 3H)	δ_C 179.5, 172.6, 143.3, 123.8, 83.1, 81.0, 55.2, 52.0, 47.6, 46.4, 46.1, 43.0, 42.4, 40.2, 39.8, 38.2, 37.8, 35.7, 32.4, 31.5, 30.4, 29.2, 27.2, 25.0, 24.3, 23.8, 23.5, 23.4, 22.0, 19.6, 17.3, 16.7, 16.2
3-2	δ_H 5.30 (s, 1H, 12-H), 4.45 (t, *J* = 7.2 Hz, 1H, 3-H), 2.85 (dd, *J* = 12.0, 4.8 Hz, 1H, H-18), 2.95 (s, 1H), 2.27−2.30 (m, 2H), 1.01−2.07 (m, 20H), 0.97−1.09 (m, 2 H), 1.19 (s, 3H), 1.02 (s, 3H), 0.92 (s, 3H), 0.91 (s, 3H), 0.90 (s, 3H), 0.87 (s, 3H), 0.84 (s, 3H), 0.81 (s, 3H)	δ_C 179.0, 174.3, 143.9, 124.3, 82.4, 80.3, 55.6, 52.4, 47.3, 46.7, 46.5, 42.7, 42.1, 39.7, 39.1, 37.9, 37.6, 36.2, 32.9, 31.2, 29.4, 28.3, 26.7, 24.7, 24.0, 23.5, 23.4, 23.1, 21.6, 19.0, 17.7, 16.9, 16.4, 9.7
3-3	δ_H 5.27 (s, 1H, 12-H), 4.42 (t, *J* = 7.2 Hz, 1H, 3-H), 2.85 (dd, *J* = 10.6, 4.0 Hz, 1H, H-18), 2.98 (s, 1H), 1.83−2.35 (m, 4H), 1.05−2.02 (m, 20H), 0.95−1.12 (m, 2 H), 1.05 (s, 3H), 1.02 (s, 3H), 1.00 (s, 3H), 0.95 (s, 3H), 0.92 (s, 3H), 0.88 (s, 3H), 0.87 (s, 3H), 0.84 (s, 3H)	δ_C 179.7, 174.0, 140.1, 123.1, 83.5, 81.1, 55.0, 52.1, 47.5, 46.2, 43.1, 42.3, 40.1, 39.2, 38.7, 38.5, 37.2, 37.0, 36.2, 36.0, 33.2, 30.8, 28.8, 28.0, 27.2, 24.2, 23.8, 23.5, 23.0, 22.6, 21.6, 18.5, 18.1, 16.1, 14.0
3-4	δ_H 5.32 (s, 1H, 12-H), 4.38 (t, *J* = 7.0 Hz, 1 H, 3-H), 2.85 (dd, *J* = 12.2, 4.6 Hz, 1H, H-18), 2.92 (s, 1H), 1.40−2.32 (m, 6H), 1.10−2.13 (m, 20H), 0.97−1.15 (m, 2H), 1.08 (s, 3H), 1.05 (s, 3H), 0.97 (s, 3H), 0.93 (s, 3H), 0.92 (s, 3H), 0.89 (s, 3H), 0.87 (s, 3H), 0.82 (s, 3H)	δ_C 180.2, 173.2, 139.5, 123.4, 83.7, 80.6, 55.6, 52.4, 47.1, 46.6, 42.8, 41.7, 41.2, 39.9, 38.2, 37.4, 37.0, 36.8, 36.4, 36.2, 34.2, 31.2, 29.3, 28.5, 26.9, 24.5, 23.8, 23.6, 23.1, 22.3, 21.2, 18.4, 17.7, 16.9, 16.6, 13.5
3-5	δ_H 5.26 (s, 1H, 12-H), 4.38 (t, *J* = 7.0 Hz, 1H, 3-H), 2.85 (dd, *J* = 10.4, 4.0 Hz, 1H, H-18), 2.95 (s, 1H), 1.25−2.36 (m, 8H), 1.12−2.21 (m, 20H), 0.94−1.14 (m, 2H), 1.10 (s, 3H), 1.04 (s, 3H), 1.02 (s, 3H), 0.97 (s, 3H), 0.93 (s, 3H), 0.92 (s, 3H), 0.88 (s, 3H), 0.87 (s, 3H)	δ_C 180.0, 173.5, 138.2, 123.0, 84.0, 81.2, 55.2, 52.0, 47.7, 46.2, 43.1, 42.0, 40.6, 39.7, 39.2, 37.8, 37.5, 37.0, 36.4, 36.0, 33.7, 31.6, 31.2, 29.0, 28.9, 26.3, 24.1, 24.0, 23.8, 23.4, 22.1, 21.6, 18.1, 17.2, 16.7, 16.0, 14.2
5-1	δ_H 5.32 (s, 1H, 12-H), 4.45 (t, *J* = 7.4 Hz, 1H, 3-H), 4.15−4.21 (m, 2H), 2.82 (s, 1H), 2.55 (dd, *J* = 10.8, 4.0 Hz, 1H, H-18), 1.10−2.11 (m, 20H), 1.23 (s, 3H), 1.10 (s, 3H), 1,02 (s, 3H), 0.90 (s, 3H), 0.89 (s, 3H), 0.88 (s, 3H), 0.85 (s, 3H), 0.82 (s, 3H)	δ_C 177.5, 160.2, 140.2, 123.3, 84.1, 75.1, 74.6, 61.3, 55.7, 52.8, 48.6, 47.1, 42.8, 40.5, 39.5, 39.0, 38.9, 37.8, 37.5, 37.0, 33.2, 30.7, 29.1, 26.5, 24.6, 24.0, 23.8, 23.6, 23.2, 21.4, 18.9, 17.6, 17.0, 16.5, 15.1
5-2	δ_H 5.28 (s, 1H, 12-H), 4.42 (t, *J* = 8.0 Hz, 1H, 3-H), 4.10−4.20 (m, 2H), 2.74 (s, 1H), 2.52 (dd, *J* = 9.4, 4.2 Hz, 1H, H-18), 1.06−2.14 (m, 20H), 1.68−1.74 (m, 2H), 1.10 (s, 3H), 1,02 (s, 3H), 0.98 (s, 3H), 0.90 (s, H), 0.88 (s, 3H), 0.86 (s, 3H), 0.85 (s, 3H), 0.83 (s, 3H)	δ_C 177.0, 155.6, 139.1, 123.0, 83.5, 74.9, 73.8, 65.7, 55.7, 53.2, 48.6, 47.5, 42.2, 40.2, 39.1, 38.7, 38.5, 37.6, 37.3, 36.8, 33.5, 30.5, 29.4, 26.0, 24.3, 23.9, 23.7, 23.5, 23.0, 21.9, 21.4, 18.5, 17.6, 17.2, 16.2, 10.4
5-3	δ_H 5.21 (s, 1H, 12-H), 4.40 (t, *J* = 8.2 Hz, 1H, 3-H), 4.05−4.12 (m, 2H), 2.70 (s, 1H), 2.50 (dd, *J* = 10.8, 4.4 Hz, 1H, H-18), 1.10−2.18 (m, 20H), 1.37−1.55 (m, 4H), 1.07 (s, 3H), 1,03 (s, 3H), 1.01 (s, 3H), 0.92 (s, H), 0.90 (s, 3H), 0.88 (s, 3H), 0.86 (s, 3H), 0.81 (s, 3H)	δ_C 177.5, 154.0, 138.3, 123.4, 84.3, 74.5, 73.9, 65.3, 55.2, 53.7, 48.4, 47.2, 42.6, 40.5, 39.4, 39.2, 38.5, 37.8, 37.6, 35.9, 34.7, 32.4, 30.2, 28.8, 27.2, 24.5, 24.0, 23.5, 22.6, 22.3, 21.6, 21.5, 18.2, 18.1, 17.5, 16.4, 14.0
5-4	δ_H 5.30 (s, 1H, 12-H), 4.42 (t, *J* = 7.6 Hz, 1H, 3-H), 4.02−4.10 (m, 2H), 2.74 (s, 1H), 2.56 (dd, *J* = 12.0, 4.4 Hz, 1H, H-18), 1.05−2.13 (m, 20H), 1.39−1.61 (m, 6H), 1.02 (s, 3H), 0.99 (s, 3H), 0.98 (s, 3H), 0.90 (s, H), 0.89 (s, 3H), 0.88 (s, 3H), 0.86 (s, 3H), 0.84 (s, 3H)	δ_C 177.2, 153.5, 138.0, 124.0, 83.8, 74.2, 73.9, 65.8, 55.6, 53.3, 49.2, 48.5, 44.1, 41.2, 40.2, 39.5, 39.2, 37.2, 36.4, 35.3, 34.2, 30.1, 29.0, 28.4, 27.9, 27.0, 24.3, 23.9, 22.8, 22.5, 22.1, 21.6, 21.4, 18.9, 18.8, 18.2, 16.2, 14.2
5-5	δ_H 5.23 (s, 1H, 12-H), 4.46 (t, *J* = 7.0 Hz, 1H, 3-H), 4.02−4.08 (m, 2H), 2.70 (s, 1H), 2.56 (dd, *J* = 12.2, 4.8 Hz, 1H, H-18), 1.12−2.25 (m, 20H), 1.36−1.65 (m, 8H), 1.04 (s, 3H), 0.98 (s, 3H), 0.97 (s, 3H), 0.89 (s, H), 0.87 (s, 3H), 0.85 (s, 3H), 0.80 (s, 3H), 0.78 (s, 3H)	δ_C 177.6, 154.0, 138.5, 123.5, 84.2, 74.5, 74.0, 66.0, 56.2, 54.2, 50.1, 48.3, 45.4, 42.1, 41.8, 40.5, 39.7, 39.2, 38.2, 36.2, 35.1, 31.7, 29.3, 29.2, 28.4, 28.0, 27.5, 24.1, 23.6, 23.4, 22.7, 22.3, 21.5, 21.4, 19.3, 19.2, 18.6, 17.0, 12.7

Continued

Compd.	^{1}H NMR (600 MHz, $CDCl_3$)	^{13}C NMR (150 MHz, $CDCl_3$)
I_1	δ_H 7.79 (s, 1H, triazole-H), 7.05−7.53 (m, 5H), 5.31 (s, 1H, 12-H), 5.20 (d, *J* = 2.0 Hz, 2H), 4.49 (t, *J* = 7.2 Hz, 1 H, 3-H), 2.93 (dd, *J* = 11.0, 4.6 Hz, 1H, H-18), 2.06 (s, 3H), 1.07−2.01 (m, 20H), 0.94−1.05 (m, 2H), 1.02 (s, 3H), 0.91 (s, 3H), 0.90 (s, 3H), 0.89 (s, 3H), 0.86 (s, 3H), 0.85 (s, 3H), 0.82 (s, 3H)	δ_C 179.5, 174.2, 144.7, 134.4, 131.2, 128.4, 128.4, 127.0, 127.0, 122.9, 80.4, 63.1, 55.1, 47.3, 46.7, 46.4, 43.1, 42.0, 39.3, 38.0, 37.7, 36.1, 35.8, 34.1, 32.8, 32.7, 32.2, 30.6, 29.8, 28.0, 27.9, 25.6, 25.6, 23.7, 23.5, 23.5, 21.1, 18.0, 16.9, 16.6, 15.3
I_2	δ_H 7.81 (s, 1H, triazole-H), 7.02−7.50 (m, 5H), 5.32 (s, 1H, 12-H), 5.19 (d, *J* = 3.2 Hz, 2H), 4.50 (t, *J* = 7.9 Hz, 1H, 3-H), 2.89 (dd, *J* = 9.2, 4.0 Hz, 1H, H-18), 2.31−2.27 (m, 2H), 1.04−2.05 (m, 20H), 1.22 (s, 3H), 0.94−1.07 (m, 2H), 1.01 (s, 3H), 0.91 (s, 3H), 0.90 (s, 3H), 0.87 (s, 3H), 0.87 (s, 3H), 0.85 (s, 3H), 0.80 (s, 3H)	δ_C 178.5, 171.0, 144.8, 132.3, 132.2, 126.1 126.1, 126.0, 126.0, 122.8, 115.8, 115.6, 80.8, 63.9, 55.1, 53.2, 47.4, 46.7, 46.5, 42.1, 42.0, 39.3, 38.8, 38.1, 37.7, 36.7, 34.1, 33.0, 32.6, 32.2, 28.0, 27.3, 25.7, 23.8, 23.6, 23.5, 23.4, 21.3, 18.1, 16.8, 16.6, 15.2
I_3	δ_H 7.84 (s, 1H, triazole-H), 7.05−7.58 (m, 5H), 5.32 (s, 1H, 12-H), 5.23 (d, *J* = 3.0 Hz, 2H), 4.52 (t, *J* = 7.2 Hz, 1H, 3-H), 2.85 (dd, *J* = 8.0, 4.0 Hz, 1H, H-18), 2.35−2.29 (m, 2H), 1.06−2.01 (m, 20H), 1.61−1.68 (m, 2H), 1.60 (m, 2H), 1.23 (s, 3H), 1.15 (s, 3H), 0.95−1.07 (m, 2H), 0.94 (s, 3H), 0.89 (s, 3H), 0.87 (s, 3H), 0.85 (s, 3H), 0.84 (s, 3H), 0.78 (s, 3H)	δ_C 178.4, 170.9, 144.7, 132.7, 132.7, 132.7 132.2, 132.1, 127.7, 125.7, 125.7, 125.4, 125.4, 80.7, 55.0, 47.3, 46.6, 46.3, 42.0, 41.9, 39.2, 38.7, 38.0, 37.7, 37.5, 36.6, 32.8, 32.5, 32.1, 30.6, 27.9, 27.1, 26.8, 25.6, 23.7, 23.4, 23.4, 23.2, 21.2, 18.0, 16.7, 16.5, 15.1
I_4	δ_H 7.82 (s, 1H, triazole-H), 7.01−7.50 (m, 5H), 5.32 (s, 1H, 12-H), 5.21 (d, *J* = 3.4 Hz, 2H), 4.51 (t, *J* = 7.5 Hz, 1 H, 3-H), 2.83 (dd, *J* = 8.0, 4.0 Hz, 1H, H-18), 2.33−2.25 (m, 2H), 1.01−2.05 (m, 20H), 1.35−1.80 (m, 4H), 1.25 (s, 3H), 1.16 (s, 3H), 0.92−1.04 (m, 2 H), 1.02 (s, 3H), 0.92 (s, 3H), 0.89 (s, 3H), 0.87 (s, 3H), 0.86 (s, 3H), 0.79 (s, 3H)	δ_C 178.5, 174.3, 144.7, 138.7, 134.9, 132.7, 131.2, 128.0, 128.0, 127.2, 127.2, 122.9, 80.5, 64.6, 55.1, 47.4, 46.7, 46.4, 42.1, 42.1, 42.0, 39.3, 38.6, 38.1, 37.8, 36.8, 34.1, 33.0, 32.6, 32.2, 32.2, 30.7, 28.1, 28.0, 27.3, 23.8, 23.8, 23.5, 23.4, 21.2, 18.1, 16.8, 16.7, 15.3
I_5	δ_H 7.87 (s, 1H, triazole-H), 7.03−7.55 (m, 5H), 5.29 (s, 1H, 12-H), 5.19 (d, *J* = 3.2 Hz, 2H), 4.49 (t, *J* = 7.2 Hz, 1 H, 3-H), 2.82 (dd, *J* = 8.4, 4.3 Hz, 1H, H-18), 2.34−2.26 (m, 2H), 1.04−2.08 (m, 20H), 1.26−1.78 (m, 6H), 1.21 (s, 3H), 1.18 (s, 3H), 0.90−1.06 (m, 2 H), 0.96 (s, 3H), 0.93 (s, 3H), 0.89 (s, 3H), 0.86 (s, 3H), 0.85(s, 3H), 0.76 (s, 3H)	δ_C 179.7, 174.4, 144.9, 134.7, 131.4, 128.7, 128.7, 127.2, 127.2, 123.1, 80.6, 63.3, 55.3, 47.6, 46.9, 46.6, 42.2, 42.2, 39.5, 38.3, 37.9, 36.3, 36.0, 34.3, 33.1, 33.0, 32.4, 32.4, 30.8, 30.1, 28.2, 28.1, 27.5, 27.5, 25.8, 25.8, 23.9, 23.7, 23.6, 18.3, 17.1, 16.8, 16.8, 15.5, 9.5
II_1	δ_H 7.88 (s, 1H, triazole-H), 7.10−7.55 (m, 5H), 5.43 (s, 1H, 12-H), 4.41 (t, *J* = 8.0 Hz, 1H, 3-H), 4.25−4.19 (m, 2H), 3.63 (t, *J* = 7.2 Hz, 2H), 2.58 (dd, *J* = 12.0, 4.0 Hz, 1H, H-18), 1.01−2.02 (m, 20H), 1.20 (s, 3H), 1.15 (s, 3H), 0.92−1.08 (m, 2H), 0.98 (s, 3H), 0.91 (s, 3H), 0.89 (s, 3H), 0.84 (s, 3H), 0.80 (s, 3H), 0.71 (s, 3H)	δ_C 179.5, 174.2, 144.7, 133.5, 131.2, 128.4, 128.4, 127.0, 127.0, 125.9, 125.9, 80.4, 64.4, 55.1, 47.4, 46.7, 46.4, 42.1, 42.0, 42.0, 39.3, 38.0, 37.7, 36.8, 36.1, 35.8, 32.9, 32.8, 32.2, 30.6, 29.9, 28.0, 27.9, 25.6, 23.7, 23.5, 23.4, 21.3, 21.1, 18.6, 16.9, 15.3
II_2	δ_H 7.83 (s, 1H, triazole-H), 7.03−7.53 (m, 5H), 5.21 (s, 1H, 12-H), 4.29 (t, *J* = 8.2 Hz, 1H, 3-H), 4.24−4.17 (m, 2H), 3.60 (t, *J* = 7.0 Hz, 2H), 2.55 (dd, *J* = 12.4, 3.5 Hz, 1H, H-18), 1.03−2.06 (m, 20H), 1.72 (m, 2 H), 1.19 (s, 3H), 1.14 (s, 3H), 0.90−1.10 (m, 2H), 0.98 (s, 3H), 0.93 (s, 3H), 0.91 (s, 3H), 0.89 (s, 3H), 0.87 (s, 3H), 0.85 (s, 3H)	δ_C 178.6, 171.2, 144.9, 132.4, 132.4 132.3, 132.3, 126.2, 126.2, 126.1, 125.9, 121.9, 121.9, 121.8, 80.9, 64.0, 55.2, 47.5, 46.9, 46.6, 42.2, 42.1, 39.4, 39.0, 37.8, 36.9, 34.2, 33.1, 32.7, 32.3, 30.8, 28.1, 27.3, 26.7, 25.8, 23.9, 23.6, 23.6, 23.5, 18.2, 16.9, 15.3, 9.33
II_3	δ_H 7.86 (s, 1H, triazole-H), 7.09−7.45 (m, 5H), 5.24 (s, 1H, 12-H), 4.23 (t, *J* = 8.2 Hz, 1 H, 3-H), 4.20−4.10 (m, 2H), 3.58 (t, *J* = 7.2 Hz, 2H), 2.52 (dd, *J* = 12.0, 4.5 Hz, 1H, H-18), 1.05−2.10 (m, 20H), 1.32−1.59 (m, 4H), 1.15 (s, 3H), 1.10 (s, 3H), 0.91−1.07 (m, 2H), 0.95 (s, 3H), 0.93 (s, 3H), 0.90 (s, 3H), 0.89 (s, 3H), 0.87 (s, 3H), 0.83 (s, 3H)	δ_C 179.4, 171.0, 144.7, 136.4, 134.2, 131.8, 130.7, 130.0, 130.0, 127.4, 127.4, 122.9, 121.4, 119.7, 80.8, 65.0, 55.2, 47.5, 46.7, 46.4, 42.1, 39.4, 39.4, 38.1, 37.7, 36.9, 36.5, 34.1, 30.7, 29.9, 29.9, 28.0, 27.3, 25.7, 25.7, 24.8, 23.7, 23.6, 23.6, 21.3, 18.2, 17.0, 16.7, 15.4
II_4	δ_H 7.84 (s, 1H, triazole-H), 7.13−7.50 (m, 5H), 5.31 (s, 1H, 12-H), 4.26 (t, *J* = 8.0 Hz, 1H, 3-H), 4.23−4.12 (m, 2H), 3.55 (t, *J* = 7.2 Hz, 2H), 2.50 (dd, *J* = 12.0, 4.0 Hz, 1H, H-18), 1.02−2.14 (m, 20H), 1.35−1.69 (m, 6H),1.12 (s, 3H), 0.95−1.05 (m, 2H), 0.99 (s, 3H), 0.98 (s, 3H), 0.94 (s, 3H), 0.91 (s, 3H), 0.89 (s, 3H), 0.79 (s, 3H), 0.77 (s, 3H)	δ_C 179.6, 174.3, 144.8, 134.6, 131.3, 128.5, 128.5, 127.1, 127.1, 123.0, 80.5, 65.2, 55.2, 47.5, 46.8, 46.5, 42.1, 42.1, 39.4, 39.4, 38.1, 37.8, 36.9, 36.2, 35.9, 33.0, 32.9, 30.7, 30.0, 30.0, 28.1, 27.4, 26.8, 25.7, 25.7, 23.8, 23.6, 23.5, 18.2, 18.2, 17.0, 16.7, 15.4, 15.4, 9.4
II_5	δ_H 7.91 (s, 1H, triazole-H), 7.10−7.59 (m, 5H), 5.43 (s, 1H, 12-H), 4.32 (t, *J* = 8.2 Hz, 1H, 3-H), 4.30−4.16 (m, 2H), 3.58 (t, *J* = 7.2 Hz, 2H), 2.55 (dd, *J* = 12.4, 4.2 Hz, 1H, H-18), 1.01−2.07 (m, 20H), 1.28−1.65 (m, 8H),1.10 (s, 3H), 0.94−1.06 (m, 2H), 0.99 (s, 3H), 0.98 (s, 3H), 0.97 (s, 3H), 0.91 (s, 3H), 0.90 (s, 3H), 0.79 (s, 3H), 0.75 (s, 3H)	δ_C 179.4, 171.1, 144.7, 136.4, 134.2, 131.8, 130.7, 130.7, 130.0, 130.0, 127.4, 127.4, 122.9, 80.9, 66.2, 55.2, 47.5, 46.7, 46.4, 42.1, 42.1, 42.0, 39.4, 38.1, 37.7, 36.9, 36.5, 34.1, 33.0, 32.9, 32.9, 30.7, 30.0, 28.0, 27.3, 25.7, 24.8, 23.7, 21.3, 21.3, 18.2, 17.0, 16.7, 15.4, 15.4, 9.4

残基形成氢键、疏水等相互作用。对比c-Kit抑制剂尼洛替尼的对接结果, 发现二者都与氨基酸残基Asp810形成氢键, 且化合物**II_4**连接氢键的为C-28位拼合的1,2,3-三氮唑结构, 有文献[23]报道, c-Kit抑制剂能与Asp810产生氢键作用, 推测氨基酸残基Asp810是c-Kit蛋白与抑制剂结合的关键氨基酸。初步判断化合物**II_4**和尼洛替尼与靶蛋白具有相同的结合位点。

4 结论

以UA为母体, 设计并合成了10种UA衍生物, 结构经MS和NMR表征。体外细胞实验表明, 化合物对

Table 3 Antitumor activity of different derivatives on cancer cells MCF-7 and SGC-7901

Compd.	IC_{50}/μmol·L^{-1}		Compd.	IC_{50}/μmol·L^{-1}	
	MCF-7	SGC-7901		MCF-7	SGC-7901
I$_1$	40.22 ± 0.11	37.02 ± 0.07	**II**$_1$	39.27 ± 0.19	30.18 ± 0.22
I$_2$	31.48 ± 0.10	20.13 ± 0.13	**II**$_2$	33.32 ± 0.15	21.45 ± 0.08
I$_3$	30.15 ± 0.15	19.21 ± 0.13	**II**$_3$	23.13 ± 0.20	20.28 ± 0.15
I$_4$	18.22 ± 0.09	12.72 ± 0.15	**II**$_4$	14.28 ± 0.14	8.46 ± 0.10
I$_5$	38.65 ± 0.16	25.12 ± 0.08	**II**$_5$	20.68 ± 0.19	19.54 ± 0.17
UA	> 50	> 50	Nilotinib	15.40 ± 0.10	10.15 ± 0.08

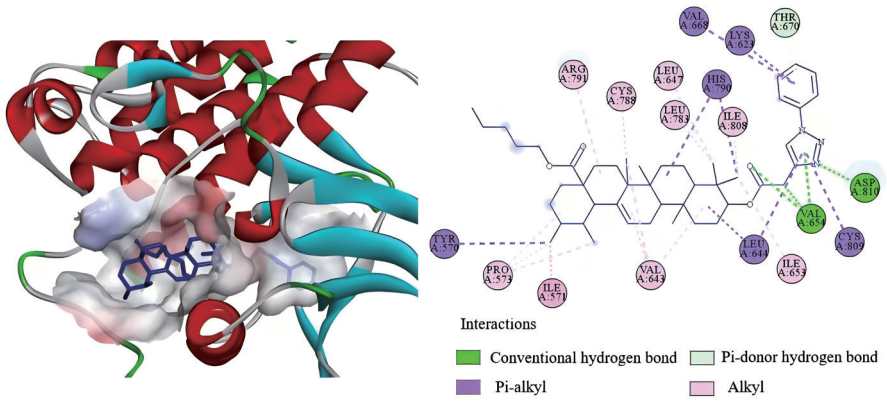


Figure 3 The binding mode of derivative **II**$_4$ with c-Kit protein predicted by molecular docking

Table 4 Docking analysis of ursolic acid derivatives and c-Kit protein

Compd.	Docking score	Compd.	Docking score
I$_1$	−154.697	**II**$_1$	−155.338
I$_2$	−162.983	**II**$_2$	−160.921
I$_3$	−162.793	**II**$_3$	−161.544
I$_4$	−167.873	**II**$_4$	−172.941
I$_5$	−161.868	**II**$_5$	−165.844
Nilotinib	−170.459	UA	−120.341

癌细胞 MCF-7 和 SGC-7901 具有抑制作用, 初步的构效关系研究表明, C-3 位引入 1,2,3-三氮唑结构的化合物活性优于 C-28 位, 且引入碳链的长短会影响衍生物活性, 两类化合物酯键碳数目为 5 时, 活性最优, 其中化合物 **II**$_4$对 c-Kit 的抑制活性与阳性对照药尼洛替尼相当, 分子对接结果显示化合物中的三氮唑作为酰胺基团的生物电子等排体, 具有提高化合物亲和力的作用, 分析化合物 **II**$_4$和尼洛替尼均与 Asp810 氨基酸残基形成氢键, 具有相同的结合位点。总体而言, UA 结构中引入三氮唑结构, 有利于增强其抗肿瘤活性。这对熊果酸进一步结构修饰及优化具有一定的参考意义。

实验部分

1 主要仪器与试剂

Büchi B-540 熔点测定仪 (瑞士 BUCHI 公司); BrukerARX-600 型核磁共振分析仪 (瑞士 Bruker 公司), $CDCl_3$为溶剂, TMS 为内标; 热电−菲尼根 LCQ 型质谱仪 (美国 San Francisco 公司); SPECTRA MAX plus 型酶标仪 (美国 MDC 公司); XDS-1B 倒置显微镜 (重庆留辉科技有限公司); TGL-16B 低速台式离心机 (上海安亭科学仪器厂)。熊果酸 (质量分数 > 98%) 购于中山市远洋生物医药技术有限公司; 显色剂为 10% (体积分数) 硫酸−乙醇溶液; 活性测试所用的 RPMI-1640 培养基 (含 10% 胎牛血清, 100 μmol·L^{-1}青霉素, 100 μmol·L^{-1}链霉素)、溴化四偶氮唑盐 (MTT) 购于南京绿合生化科技有限公司。所有试剂均为分析纯或化学纯。

2 化学合成

2.1 3*β*-乙酰氧基-乌苏烷型-12-烯-28-羧酸(2-1) 的合成 参照课题组前期合成方法[17-19], 向起始原料 UA (500 mg, 1.10 mmol) 的 THF (50 mL) 溶液中缓慢滴加乙酸酐 (0.565 mL, 6.00 mmol), 室温搅拌, 加入 DMAP (10 mg, 0.09 mmol), 室温搅拌 4 h, TLC 跟踪反应完成。用饱和氯化钠溶液 (20 mL) 洗涤, 后用乙酸乙酯 (10 mL) 萃取 3 次, 有机相合并后经无水硫酸钠干燥, 过滤, 减压蒸除溶剂, 得到的粗产物经硅胶柱层析纯化, 洗脱液 [石油醚∶乙酸乙酯 = 10∶1 (*v*/*v*)], 得到 450 mg 白色固体 **2-1**, 产率 82%, m.p. 184.6～185.7 ℃。

2.2 3*β*-乙酰氧基-乌苏烷型-12-烯-28-羧酸炔丙酯 (3-1) 的合成 将化合物 **2-1** (400 mg, 0.80 mmol) 加入 DMF (20 mL) 中, 再依次加入炔丙溴 (350 mg,

2.32 mmol) 和碳酸钾 (110 mg, 0.80 mmol), 控温 60 ℃反应 8 h 后降至室温, 用饱和氯化钠溶液 (20 mL) 洗涤, 后用二氯甲烷 (10 mL) 萃取 3 次, 有机相合并后经无水硫酸钠干燥, 过滤, 减压蒸除溶剂, 得到的粗产物经硅胶柱层析纯化, 洗脱液 [石油醚∶乙酸乙酯 = 10∶1 (*v*/*v*)], 得到 343 mg 类白色固体 **3-1**, 产率 80%, m.p. 191.6～193.1 ℃。

2.3 3*β*-羟基-乌苏烷型-12-烯-28-羧酸乙酯 (4-1) 的合成 将起始原料 UA (500 mg, 1.10 mmol)、碳酸钾 (300 mg, 2.17 mmol) 加入 DMF (20 mL) 中, 缓慢滴加溴乙烷 (0.24 mL, 5.02 mmol), 室温搅拌 5 h, TLC 跟踪反应完成。减压蒸发除去溶剂, 用饱和氯化钠溶液 (20 mL) 洗涤, 后用乙酸乙酯 (10 mL) 萃取 3 次, 合并有机相, 经无水硫酸钠干燥, 过滤, 减压蒸除溶剂, 得到的粗产物经硅胶柱层析纯化, 洗脱液 [石油醚∶乙酸乙酯 = 15∶1 (*v*/*v*)], 得到 353 mg 白色固体 **4-1**, 产率 66%, m.p. 177.2～178.7 ℃。

2.4 3*β*-(3-炔基丁酰氧)-乌苏烷型-12-烯-28-羧酸乙酯 (5-1) 的合成 将 3-丁炔酸 (84 mg, 1.00 mmol) 溶解于二氯甲烷 (10 mL) 中, 搅拌加入 DCC (206 mg, 1.00 mmol), 10 mn 后缓慢加入 DMAP (10 mg, 0.09 mmol) 和已溶解于二氯甲烷 (20 mL) 中的化合物 **4-1** (480 mg, 1.00 mmol), 室温搅拌 3 h, TLC 跟踪反应完成。用饱和氯化钠溶液 (20 mL) 洗涤, 后用二氯甲烷 (10 mL) 萃取 3 次, 有机相合并后经无水硫酸钠干燥, 过滤, 减压蒸除溶剂, 得到的粗产物经硅胶柱层析纯化, 洗脱液 [石油醚∶乙酸乙酯 = 8∶2 (*v*/*v*)], 得到 321 mg 白色固体 **5-1**, 产率 58%, m.p. 173.2～173.9 ℃。

2.5 3*β*-乙酰氧基-乌苏烷型-12-烯-28-酸(1-苯基三氮唑-4-)甲酯 (I_1) 的合成 将化合物 **3-1** (376 mg, 0.70 mmol) 加入到无水乙醇 (10 mL) 中, 再依次加入叠氮苯 (143 mg, 1.20 mmol)、抗坏血酸钠 (36 mg, 0.18 mmol) 和五水合硫酸铜 (100 mg, 0.40 mmol) 室温搅拌 4 h, TLC 跟踪反应完成。减压蒸发除去溶剂, 用饱和氯化钠溶液 (20 mL) 洗涤, 后用乙酸乙酯 (10 mL) 萃取 3 次, 合并有机相, 经无水硫酸钠干燥, 过滤, 减压蒸除溶剂, 得到的粗产物经硅胶柱层析纯化, 洗脱液 [石油醚∶乙酸乙酯 = 20∶1 (*v*/*v*)], 得到白色固体 $\mathbf{I_1}$。

2.6 3*β*-丙酰氧基-乌苏烷型-12-烯-28-酸(1-苯基三氮唑-4-)甲酯 (I_2) 的合成 按照制备 $\mathbf{I_1}$ 的方法, 由母体 UA (1.10 mmol) 与丙酸酐 (6.00 mmol) 反应。最终粗品经硅胶柱色谱纯化, 洗脱液 [石油醚∶乙酸乙酯 = 20∶1 (*v*/*v*)], 得到白色固体 $\mathbf{I_2}$。

2.7 3*β*-丁酰氧基-乌苏烷型-12-烯-28-酸(1-苯基三氮唑-4-)甲酯 (I_3) 的合成 按照制备 $\mathbf{I_1}$ 的方法, 由母体 UA (1.10 mmol) 与丁酸酐 (6.00 mmol) 反应。最终粗品经硅胶柱色谱纯化, 洗脱液 [石油醚∶乙酸乙酯 = 20∶1 (*v*/*v*)], 得到白色固体 $\mathbf{I_3}$。

2.8 3*β*-戊酰氧基-乌苏烷型-12-烯-28-酸(1-苯基三氮唑-4-)甲酯 (I_4) 的合成 按照制备 $\mathbf{I_1}$ 的方法, 由母体 UA (1.10 mmol) 与戊酸酐 (6.00 mmol) 反应。最终粗品经硅胶柱色谱纯化, 洗脱液 [石油醚∶乙酸乙酯 = 20∶1 (*v*/*v*)], 得到白色固体 $\mathbf{I_4}$。

2.9 3*β*-己酰氧基-乌苏烷型-12-烯-28-酸(1-苯基三氮唑-4-)甲酯 (I_5) 的合成 按照制备 $\mathbf{I_1}$ 的方法, 由母体 UA (1.10 mmol) 与己酸酐 (6.00 mmol) 反应。最终粗品经硅胶柱色谱纯化, 洗脱液 [石油醚∶乙酸乙酯 = 20∶1 (*v*/*v*)], 得到白色固体 $\mathbf{I_5}$。

2.10 3*β*-(1-苯基三氮唑-4-)乙酰氧基-乌苏烷型-12-烯-28-羧酸乙酯 (II_1) 的合成 按照制备 $\mathbf{I_1}$ 的方法, 将化合物 **5-1** (385 mg, 0.70 mmol) 加入反应, 得到的粗产物经硅胶柱层析纯化, 洗脱液 [石油醚∶乙酸乙酯 = 15∶1 (*v*/*v*)], 得到白色固体 $\mathbf{II_1}$。

2.11 3*β*-(1-苯基三氮唑-4-)乙酰氧基-乌苏烷型-12-烯-28-羧酸丙酯 (II_2) 的合成 按照制备 **4-1** 和 **5-1** 的方法, UA 与溴丙烷反应得到 **5-2** 后, 再按照制备 $\mathbf{I_1}$ 的方法, 将化合物 **5-2** (0.70 mmol) 加入反应, 得到的粗产物经硅胶柱层析纯化, 洗脱液 [石油醚∶乙酸乙酯 = 15∶1 (*v*/*v*)], 得到白色固体 $\mathbf{II_2}$。

2.12 3*β*-(1-苯基三氮唑-4-)乙酰氧基-乌苏烷型-12-烯-28-羧酸丁酯 (II_3) 的合成 按照制备 **4-1** 和 **5-1** 的方法, UA 与溴丁烷反应得到 **5-3** 后, 再按照制备 $\mathbf{I_1}$ 的方法, 将化合物 **5-3** (0.70 mmol) 加入反应, 得到的粗产物经硅胶柱层析纯化, 洗脱液 [石油醚∶乙酸乙酯 = 15∶1 (*v*/*v*)], 得到白色固体 $\mathbf{II_3}$。

2.13 3*β*-(1-苯基三氮唑-4-)乙酰氧基-乌苏烷型-12-烯-28-羧酸戊酯 (II_4) 的合成 按照制备 **4-1** 和 **5-1** 的方法, UA 与溴戊烷反应得到 **5-4** 后, 再按照制备 $\mathbf{I_1}$ 的方法, 将化合物 **5-4** (0.70 mmol) 加入反应, 得到的粗产物经硅胶柱层析纯化, 洗脱液 [石油醚∶乙酸乙酯 = 15∶1 (*v*/*v*)], 得到白色固体 $\mathbf{II_4}$。

2.14 3*β*-(1-苯基三氮唑-4-)乙酰氧基-乌苏烷型-12-烯-28-羧酸己酯 (II_5) 的合成 按照制备 **4-1** 和 **5-1** 的方法, UA 与溴己烷反应得到 **5-5** 后, 再按照制备 $\mathbf{I_1}$ 的方法, 将化合物 **5-5** (0.70 mmol) 加入反应, 得到的粗产物经硅胶柱层析纯化, 洗脱液 [石油醚∶乙酸乙酯 = 15∶1 (*v*/*v*)], 得到白色固体 $\mathbf{II_5}$。

3 生物活性试验

采用 MTT 法, 对数生长期的肿瘤细胞用胰蛋白酶消化, 然后用 RPMI-1640 培养基制备每毫升 2.5×10^4 个

单细胞悬液。将对数生长期的肿瘤细胞置于96孔培养板中, 每孔100 μL, 置于37 ℃恒温箱 (5% CO_2) 中培养24 h后, 给药组加入配置成0.1、1、10、20、50 μmol·L^{-1}的测试物, 并设置3个平行板, 加入化合物后轻摇板, 孵育72 h, 每孔加入20 μL MTT溶液 (5 mg·mL^{-1}), 培养4 h, 去除上清后加入DMSO, 使形成的紫色甲酰胺晶体溶解。测定490 nm下的光密度 (OD) 值, 使用GraphPad 5.0软件确定IC_{50}值。该实验独立重复3次。

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利益冲突: 本文所有作者声明不存在利益冲突关系。

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