Synthesis of Ursolic Acid Derivatives and Research on Their Cytotoxic Activities

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Abstract: In order to search for effective hepatic protectant and antineoplastic drugs, methods using ursolic acid as the lead compound, eighteen novel compounds were designed and synthesized by modified at the C-3 and the C-28 positions of ursolic acid (UA). The structures of the derivatives were confirmed by IR, MS, ^{1}HNMR,^{13}C NMR andelemental analysis. Effects of the derivatives on in vitro growth of 3 cell lines (HeLa, $SKOV_3$ and BGC-823) were determined by MTT method. The results show that compound 9and11 has high antineoplastic activity. So the results of this thesis will benefit the further investigating on the modification and anti-tumor activity of pentacyclic triterpenes.
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1. Introduction

Ursolic acid (UA) , a pentacyclic triterpenoid which is a member of cyclosqualenoid family, derived from berries, leaves, flowers, and fruits of medicinal plants such as *Etiobotrya japonica*, *Rosmarinus officinalis* and *Glecheoma hederaceae*[1]. Ursolic acid is really a versatile compound which possesses anti-tumor[2,3], anti-inflammatory[4,5], anti-HIV[6] and anti-angiogenic effects[7] in chick chorioallantotic membrane (CAM). Several of these effects of ursolic acid are mediated through suppression of the expression of lipoxygenase ,COX-2, MMP-9[8], and iNOS, some of which are genes regulated by NF-κB[9]. In addition, UA could act on almost all steps in the whole cancer process: initiation, promotion, progression and metastasis.

It was proved that the ester functionality at C-3 or a hydrogen donor group at C-3 and/or C-28 is necessary for the cytotoxic activity[0]. Based on above analysis, eighteen UA derivatives have been designed and synthesized. Most of the derivatives have an ester functionality at C-3. And some of them have been evaluated against three cancer call lines (HeLa, $SKOV_3$ and BGC-823).The results show that compound9and11has antineoplastic activity against HeLa,$SKOV_3$and BGC-823.

2. Experimental

2.1 General

UA (98%) was purchased from China Chengdu Scholar Bio-Tech.Co., Ltd. Unless otherwise indicated other reagents were all analytical grade, purchased from commercial suppliers and used without further purification. The melting points were determined on an electrically heated X-4 digital visual melting point apparatus and are uncorrected. IR spectra were recorded on a ThermoNicolet 470FT spectrometer. ^{1}H-NMR and^{13}C-NMR spectra were measured on Bruker ARX-300MHz spectrometer at room temperature, with TMS as the internal standard. ESI-MS were determined with Thermo-Finnigan LCQ equipment.

2.2 Methyl N-[3β-butyryloxyl-urs-12-en-28-oyl]-2-amino-3-(4`-hydroxy)-phenyl propionate (4)

To a stirred solution of UA(100mg, 0.22mmol) in THF(40mL) and a small amount of DMAP, was added n-butyric anhydride (139.20mg, 4.4mmol) at room temperature. TLC method was used to decide the end of reaction. The crude material was concentrated by vacuum distillation. The solids was added ethanol (3mL) and concentrate the solution, this was done twice. Dissolve the solid in warm methanol then the solution was added ether (6-10mL) to the solution would give a precipitate. The mixture was filtered through Buchner funnel dried at room temperature to

give raw compound 2.

The raw compound 2 was purified by silica gel chromatography with a gradient elution of petroleum ether/ ethyl acetate (6:1, v/v), to yield compound 2 (92.9mg, yield: 80.2%); m.p.183~184°C; ^{1}H NMR(300MHz, $CDCl_3$):δ 5.23 (s, 1H, H-12), 4.50 (1H, t-like, H-3), 2.31~2.26 (m, 2H, -CH_2CO), 2.19 (d, 1H, J=11.4 Hz, H-18), 1.08 (s, 3H, CH3), 0.97~0.92 (s, 9H, CH_3×3), 0.86 ~ 0.85 (s, 9H, CH_3×3), 0.78 (s, 3H, CH_3); IR (KBr): 3440, 2927, 1732, 1692, 1460, 1384, 1255, 1186cm-1; ESI-MS m/z: 525.5 (M-H)- Elemental analysis (%, found) C, 77.68 (77.52); H,10.28 (10.33).

Compound 2 (80mg, 0.1519mmol) was dissolved in CH_2Cl_2 (3mL), the oxalyl chloride was added and stirred for 24h. Concentrate the crude material by vacuum distillation, then residue was dissolved in cyclohexane (2mL) to eliminate the unreacted oxalyl chloride. This was done twice to give compound 3. Compound 3 was dissolved in CH_2Cl_2 (2mL) and alkalified to pH 9-10 with Et_3N. The mixture solution was stirred for 5 min and appropriate amino (0.6076mmol) was added at room temperature. The reaction completion was decided by TLC. The mixture was evaporated under reduced pressure to remove CH_2Cl_2, water (3mL) was added and acidified with 2M HCl to pH 3 to doposit the solid. Filtered the mixture, the filter cake was wash by water and dried to give white powder.

Compound 3(80mg ,0.1519mmol) was dissolved in CH_2Cl_2 (2mL) and alkalified to pH 9-10 with Et_3N. The mixture solution was stirred for 5 min and L-tyrosine methyl ester hydrochloride (140.77mg, 0.6076mmol) was added in at room temperature. The reaction completion was decided by TLC. The mixture was evaporated under reduced pressure to remove CH_2Cl_2, water (3mL) was added and acidified with 2M HCl to pH 3 to doposit the solid. Filtered the mixture, the filter cake was wash by water and dried to give white powder.The residue was purified by silica gel chromatography with a gradient elution of petroleum ether/acetone (3:1, v/v), to yield compound 4(43.4mg, yield: 40.6%); m.p.118-121°C; ^{1}H NMR (300MHz, $CDCl_3$):δ6.733~6.966 (m, 4H, Ph-H) ,6.426 (d, 1H, NH), 5.272 (t-like, 1H, H-12), 4.483 (m, 1H, H-3), 4.691 (m, 1H, NHCH), 3.678 (s, 3H, OCH_3), 3.042~3.075 (m, 1H, Ph- CHa) , 2.937~2.970 (m, 1H, Ph- CHb) , 2.289 (s, 3H, CH_3CO), 1.057 (s, 3H, CH_3) , 0.946 (m, 6H, CH_3×2), 0.899 (s, 3H, CH3), 0.840 (m ,9H, CH_3×3), 0.606 (s, 3H, CH_3); ESI-MS m/z: 704.3 (M+H)+. Elemental analysis (%, found) C, 76.37 (76.40); H,9.57 (9.69); N, 2.14 (2.07).

2.3 General procedure for the synthesis of N-[3β-acetoxy-urs-12-en-28-oyl]-amine compounds 5,6,7,8,9

To a stirred solution of UA(200mg, 0.44mmol) in THF(20mL) and a small amount of DMAP was added acetic anhydride (1347.1mg, 13.2mmol) at room temperature. TLC method was used to decide the end of reaction. The crude material was concentrated by vacuum distillation. The solids were dispersed in water, then acidified to pH 3-4 with 2M HCl. The mixture was filtered through Buchner funnel, washed with water to neutrality, and dried at room temperature to give raw compound 2.

The raw compound 2 was purified by silica gel chromatography with a gradient elution of petroleum ether/ ethyl acetate (10:1, v/v), to yield compound 2 (185.4mg, yield: 84.5%); m.p.285~288°C; ^{1}H NMR (300MHz, CDCl3):δ 5.23 (s, 1H, H-12), 4.50 (t, 1H, H-3), 2.20 (d-like, 1H, H-18), 2.04 (s, 3H, CH_3CO), 1.07 (s, 3H, CH_3), 0.95 (s, 6H, CH_3×2), 0.86~0.81 (m, 9H, CH_3×3), 0.76 (s, 3H, CH_3); IR (KBr): 3445, 2926, 1735,1694, 1460, 1383, 1246 cm-1. Elemental analysis (%, found) C, 77.23 (77.06); H,10.11 (11.10).

Compound 3 was reacted with oxalyl chloride following the above steps (see 3.2)

2.3.1N-[3β-acetoxy-urs-12-en-28-oyl]-3-aminopropanol acetate (5)

Compound 3 (80mg , 0.1519mmol) was reacted with 3-aminopropanol (45.63mg, 0.6076mmol) following the method of compound 4. The residue was purified by silica gel chromatography with a gradient elution of petroleum ether/acetone(3:1,v/v), to yield N-[3β-butyryloxyl-urs-12-en-28-oyl]-3-aminopropanol (37.3mg, yield: 42.0%); m.p.103-107°C; ^{1}H NMR (300MHz, $CDCl_3$):δ 6.219 (m, 1H, NH), 5.311 (m, 1H, H-12), 4.500 (t, 1H, H-3), 3.586 (m, 2H, CH_2OH) , 3.523(m, 1H, NHCHa), 3.103 (m, 1H, NHCHb), 2.277 (m, 2H, CH_2CO), 1.090 (s, 3H, CH_3) , 0.944 (s ,9H,

CH_3×3) , 0.864 (m, 9H, CH_3×3); ESI-MS m/z: 584.8 (M+H)+. Elemental analysis (%, found) C, 78.36 (78.43); H,10.80 (10.91); N, 2.29 (2.41).

To a stirred solution of N-[3β-acetoxy-urs-12-en-28-oyl]-3-aminopropanol (102.2mg, 0.1839mmol) in THF(40mL) and a small amount of DMAP, was added acetic anhydride (0.5629g, 5.516mmol) at room temperature following the general steps. The residue was purified by silica gel chromatography with a gradient elution of petroleum ether/ ethyl acetate (6:1, v/v), to yield compound 5 (76.4mg, yield: 69.5%); m.p.83-85°C; ^{1}H NMR(300MHz, $CDCl_3$): δ6.069 (br, 1H, NH), 5.327 (s, 1H, H-12), 4.492 (t-like, 1H, H-3), 4.107(m, 2H, CH_2OCOCH_3), 3.387～3.420 (m, 1H, NHCHa), 3.062～3.115 (m, 1H, NHCHb), 2.071 (s, 3H, $OCOCH_3$), 2.052 (s, 3H, CH_3COO), 1.092 (s, 3H, CH_3), 0.857 (m, 9H, CH_3×3), 0.949 (s, 6H, CH_3×2), 0.766 (s, 3H, CH_3); ESI-MS: 598.9 (M+H)+.

2.3.2N-[3β- acetoxy-urs-12-en-28-oyl]-4'-morpholinopiperidine (6)

Compound 3(100mg,0.20mmol)was reacted with 4-(Piperidin-4-yl)morpholine (102.4mg, 0.6mmol) following the general steps. The residue was purified by silica gel chromatography with a gradient elution of petroleum ether/acetone (10:1, v/v), to yield compound 6 (59.6mg, yield: 45.8%); m.p.120-122°C; ^{1}H NMR(300MHz, $CDCl_3$): δ5.218 (s, 1H, H-12), 4.499(t, 1H, H-3), 3.71 (br, 1H, NHCH), 2.047 (s, 3H, CH_3CO), 1.09 (s, 3H, CH_3), 0.96 (s, 6H, CH_3×2), 0.88～0.86 (m, 9H, CH_3×3), 0.83 (s, 3H, CH_3); ESI-MS: 651.8$(M+H)^+$.

2.3.3N-[3β- acetoxy-urs-12-en-28-oyl]-5'-methyl-2'- thiozolamine (7)

Compound 3 (80mg , 0.1604mmol) was reacted with 2-amino-5-methylthiazole (54.94mg, 0.4812mmol) following the general steps. The residue was purified by silica gel chromatography with a gradient elution of petroleum ether/ ethyl acetate (2:1, v/v), to yield compound 7 (28.8mg, yield: 30.2%); m.p.203-205°C; ^{1}H NMR(300MHz, $CDCl_3$): δ6.840 (s, 1H, NH), 6.840 (s, 1H, Ar-H), 5.228~5.505(m, 1H, H-12), 4.496 (m, 1H, H-3), 2.052 (s, 3H, CH_3CO), 1.083 (s, 3H, CH_3), 0.962 (s, 6H, CH_3×2), 0.870～0.849 (m, 12H, CH_3×4), 0.823 (s, 3H, CH_3); ESI-MS: 595.4$(M+H)^+$.

2.3.4N-[3β- acetoxy-urs-12-en-28-oyl]- 4'-methyl piperazine (8)

Compound 3 (100mg , 0.20mmol) was reacted with N-methyl piperazine (60.10mg, 0.60mmol) following the general steps. The residue was purified by silica gel chromatography with a gradient elution of petroleum ether/ acetone (3:1, v/v), to yield compound 8(51.9mg, yield: 44.7%); m.p.91-93°C; ^{1}H NMR(300MHz, $CDCl_3$): δ5.221 (s, 1H, H-12), 4.497 (t, 1H, H-3), 3.634 (brs, 3H, NCH_3), 2.155～2.399 (br, 8H, N(CH_2×4))， 2.049 (s, 3H, CH_3CO), 1.072 (s, 3H, CH_3), 0.940 (s, 6H, CH_3×2), 0.853 (m, 9H, CH_3×3), 0.745 (s, 3H, CH_3); ESI-MS: 581.8$(M+H)^+$， 582.8$(M+2H)^+$.

2.3.5 N-[3β-acetoxy-urs-12-en-28-oyl]-morpholine (9)

Compound 3 (145mg , 0.29mmol) was reacted with morpholine (69 mg ,0.88 mmol)following the general steps. The residue was purified by silica gel chromatography with a gradient elution of petroleum ether/ acetone (6:1, v/v), to yield compound 9(65 mg, 52.0%): mp 174～177℃; ^{1}H NMR(600MHz, $CDCl_3$): δ5.22 (s, 1H, H-12), 4.51 ~ 4.48 (t-like, 1H, H-3), 3.64 ~ 3.59 (m, 8H, N(CH_2CH_2)2O), 2.04 (s, 3H, CH_3CO), 1.07 (s, 3H, CH_3), 0.98 (s, 3H, CH_3), 0.94(d, 3H, J=6.2 Hz, CH_3), 0.89 (s, 3H, CH_3), 0.87(d, 3H, J=6.2 Hz, CH_3), 0.84 (s, 3H, CH_3), 0.75 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ175.1(C-28), 170.7 (CH_3COO), 138.3(C-13), 124.8(C-12) 80.6(C-3), 66.6(CH_2OCH_2), 55.0(C-5), 54.6(C-18), 48.2 (CH_2NCH_2), 47.2(C-17), 45.8(C-9), 41.8(C-14), 39.1 (C-8), 38.4(C-19) 38.4(C-20), 37.9(C-4), 37.9(C-1), 37.3(C-10), 36.6(C-22), 32.97(C-7), 30.15(C-21), 28.1 (C-23), 27.8 (C-15), 27.1(C-2), 24.9(C-16), 23.2(C-27), 23.1(C-11), 21.0 (CH_3CO), 20.9(C-30), 17.4(C-6), 17.1(C-29), 16.5 (C-26), 16.4(C-24), 15.2(C-25).IR (KBr): 3431, 2921, 1733, 1637, 1452, 1376, 1248, 1117 cm^{-1}..ESI-MS: 568.5$(M+H)^+$.

2.3.6 N-[3β-propionylox-urs-12-en-28-oyl]-morpholine (10)

3β-propionyloxy-urs-12-ene-28-oic acid was prepared from ursolic acid 1 (50 mg, 0.11 mmol) and propionic anhydride under the similar conditions as preparing Compound 2. The solid was purified by flash column chromatography [petroleum ether/ethyl acetate(6:1)] to give an amorphous solid (19.5 mg, 45.8%).

According to the same method as

compound 9, compound 10 was prepared from 3β-propionyloxy-urs-12-ene-28-oic acid (100 mg, 0.20 mmol) and morpholine (69 mg, 0.88 mmol) through 3-O- propionylursolyl chloride 7. The solid was purified by flash column chromatography [petroleum ether/ ethyl acetate(6:1)] to give 10 as an amorphous solid (36.2mg, 31.1%): mp129 ~ 131℃; ^{1}H NMR(300MHz, $CDCl_3$): δ5.22 (s, 1H, H-12), 4.50(t, 1H, J=7.9Hz, H-3), 3.64 (br, 8H, $N(CH_2CH_2)_2O$), 2.32 (q, 2H, J=5.7Hz, CH_2CO), 1.14 (d, 3H, J=7.6Hz CH_3CH_2COO), 1.08 (s, 3H, CH_3), 0.97(s, 3H, CH_3), 0.94(d, J=6.2Hz, CH_3), 0.89(s, 3H, CH_3), 0.86(d, J=6.2Hz, CH_3), 0.83(s, 3H, CH_3), 0.75(s, 3H, CH_3); ESI-MS: 582.7 (M+H)+.

2.3.7 N-[3β- butyroxy -urs-12-en-28-oyl]-morpholine (11)

Compound 3 (100 mg, 0.19 mmol) reacted with morpholine(66.2 mg, 0.76 mmol) following the general steps. The residue was purified by silica gel chromatography with a gradient elution of petroleum ether/ acetone (6:1, v/v), to yield compound 11(17.1mg, 15.1%): mp243 ~ 257℃; ^{1}H NMR(300MHz, CDCl3): δ5.22 (s, 1H, H-12), 4.50 (t-like, 1H, H-3), 3.64 (br, 8H, $N(CH_2CH_2)_2O$), 2.28(t, 2H, J=7.2Hz, CH_2CO), 1.08 (s, 3H, CH_3), 0.97(s, 3H, CH_3), 0.95(d, J=6.4Hz, CH_3), 0.91(s, 3H, CH_3), 0.89(d, J=6.4Hz, CH_3) 0.86(s, 3H, CH_3), 0.83(s, 3H, CH_3), 0.75 (s, 3H, CH_3); IR(KBr): 3436, 2965, 1731, 1637, 1458, 1384, 1184, 1119cm^{-1} ESI-MS: 596.5(M+H)+.

2.3.8N-[3β-hydroxy-urs-12-en-28-oyl]-morpholine (12*)

A mixture of 9 (40mg, 0.07mmol) and 4 N sodium hydroide (0.2 mL) in a 1:1 mixture of CH_3OH and THF(2 mL) was stirred at 40℃ for 12 h . After concentrated under reduced pressure, the residue was dispersed by water, then filtered and the filter cake was washed with water to pH 7, evaporated in vacuo to give white solid. The solid was purified by flash column chromatography [petroleum ether/ ethyl acetate(6:1)] to give compond 12* as an amorphous solid (34.8 mg, 93.3%): mp 126 ~ 129℃; 1H NMR (600MHz, $CDCl_3$): δ5.22 (s, 1H, H-12), 3.64 ~ 3.59 (m, 8H, $N(CH_2CH_2)_2O$), 3.21 ~ 3.20 (m, 1H, H-3), 1.08 (s, 3H, CH_3), 0.99 (s, 3H, CH_3), 0.95 (d, 3H, J=6.2Hz, CH_3), 0.91 (s, 3H, CH_3), 0.88 (d, 3H, J=6.3Hz, CH_3), 0.78 (s, 3H, CH_3), 0.75 (s, 3H, CH_3); ^{13}C NMR (75MHz, $CDCl_3$): δ175.4 (CONH), 138.6 (C-13), 125.8 (C-12), 79.0 (C-3), 66.8 (CH_2OCH_2); IR(KBr): 3449, 2925, 1628, 1455, 1395, 1116cm^{-1} ESI-MS: 526.4 (M+H)+.

2.3.9N-[3β-ethoxy-urs-12-en-28-oyl]-morpholine (13)

To a solution of comppund 12* (80mg, 0.15mmol) in anhydrous DMF (3 mL) were added sodium hydride(100 mg) and ethyl bromide (0.5 mL) with stirring at room temperature, and the mixture was heated at 70℃ for 3 h. After removal of DMF under reduced pressure, the residue was dispersed by water, then acidified to pH 4 ~ 5 with dilute hydrochloric acid, filtered and the filter cake was washed with water to pH 7, evaporated in vacuo to give white solid. The solid was purified by column chromatography. [petroleum ether/ ethyl acetate(6:1)] to give compound 13 as an amorphous solid (36.5 mg, 43.3%): mp 197 ~ 199℃; ^{13}C NMR (75MHz, $CDCl_3$): δ175.4(C-28), 138.6(C-13), 125.5(C-12), 86.7(C-3), 66.9(CH_2OCH_2), 65.2 (OCH_2CH_3), 55.8(C-5), 54.9 (C-18), 48.5(CH_2NCH_2), 47.6(C-17), 46.1(C-9), 42.1 (C-14), 39.5 (C-8), 38.7(C-19), 38.7(C-20), 37.0(C-4), 37.0(C-1), 34.3(C-10), 34.3(C-22), 33.1(C-7), 30.5 C-21), 29.7(C-23), 28.2(C-15), 27.1(C-2), 23.8(C-16), 23.3(C-27), 23.2(C-11), 21.2 (C-30), 18.3(C-6), 17.4 (C-29), 16.9(C-26), 16.5(CH_3CH_2O), 15.6(C-24), 15.4 (C-25). IR (KBr): 3431, 2923, 1623, 1459, 1387, 1118cm^{-1}; ESI-MS: 630.6(M+H)+.

2.3.10N-[3β-propoxy-urs-12-en-28-oyl]-morpholine (14)

Following the procedure described for compound 13, compound 12* (80mg, 0.15 mmol) and propyl bromide(0.5 mL) led to crude compound 14. The solid was purified by flash column chromato -graphy [petroleum ether/ ethyl acetate(6:1)] to give compund 14 as an amorphous solid (32.1 mg,37.8%): mp 206 ~ 210℃; ^{1}H NMR(300MHz, CDCl3): δ5.22 (s, 1H, H-12), 3.64 (br, 8H, $N(CH_2CH_2)_2O$), 3.64 (br, 1H, $CH_3CH_2CH_aO$), 3.23 ~ 3.20 (m, 1H, $CH_3CH_2CH_bO$), 2.74 ~ 2.72 (m, 1H, H-3), 1.08 (s, 3H, CH_3), 0.98(s, 3H, CH_3), 0.96(d, J=6.4Hz, CH_3), 0.90(s, 3H, CH_3), 0.87(d, J=6.4Hz, CH_3), 0.85(s, 3H, CH_3), 0.83(s, 3H, CH_3), 0.77 (s, 3H, CH_3), 0.69 (s, 3H, CH_3); IR (KBr): 3424, 2925,

1627, 1458, 1384, 1118 cm^{-1}; ESI-MS: 568.5(M+H)+.

2.3.11N-[3β-benzyloxy-urs-12-en-28-oyl]-morpholine (15)

Compound 15 an amorphous solid was prepared from compound 12*(80mg, 0.15 mmol) and benzyl bromide (0.5 mL) under the similar conditions as preparing compound 13 (yield 39.6%):mp 60 ~ 62℃; ^1H NMR(300MHz, $CDCl_3$): δ7.34 ~ 7.29 (m, 5H, Ar-H), 5.22 (s, 1H, H-12), 4.67 (d, 1H, J=11.9Hz, $PhCH_a$-O), 4.43(d, 1H, J=11.9Hz, $PhCH_b$-O), 3.64(br, 8H, $N(CH_2CH_2)_2O$), 2.93 ~ 2.91(m, 1H, H-3), 1.07 (s, 3H, CH_3), 0.99 (s, 3H, CH_3), 0.96(d, 3H, J=6.3Hz, CH_3), 0.93 (s, 3H, CH_3), 0.87(d, 3H, J=6.3Hz, CH_3), 0.84 (s, 3H, CH_3),. ESI-MS: 616.5(M+H)+.

2.3.12N-[3β-benzoyloxy-urs-12-en-28-oyl]-morpholine (16)

To a solution of 12*(80 mg, 0.15 mmol) in THF (4 mL) were added benzoyl chloride (0.05 mL) and a small amount of DMAP, and the mixture was heated at reflux for 3 h. After removal of THF under reduced pressure, the residue was added water(3 mL) and CH_2Cl_2(3 mL),then acidified to pH 8 ~ 9 with 10% aqueous K_2CO_3, standing and the layers were separated. The organic layer was washed with saturated NaCl, dried over MgSO4, and concentrated. The residue was purified by flash column chromatography [petroleum ether/ethyl acetate(8:1)] to give compound 16 as an amorphous solid (16.4 mg, 17.1%):mp 243 ~ 257℃; ^{13}C NMR(75MHz, $CDCl_3$): δ175.4 (CONH), 166.3 (PhCO), 133.5 (C-13), 132.7 (C-4`), 130.9 (C-1`), 129.5 (C-2`, 6`), 128.4, 128.3 (C-3`, 5`), 125.2 (C-12), 81.6 (C-3), 66.8 (CH_2OCH_2); IR(KBr): 3415, 2925, 1717, 1636, 1453, 1392, 1274, 1115, 712cm^{-1}. ESI-MS: 630.6(M+H)+.

2.4 General procedure for the synthesis of 3-Oxo-urs-12-en-28-oic acid ester compounds 18,19,20

To a stirred solution of UA(100mg, 0.22mmol) in DMF(4mL) and a modicum of anhydrous potassium carbonate (60mg), bromoalkane (139.20mg, 4.4mmol) was dropped slowly to this mixture at room temperature. TLC method was used to decide the end of reaction. The crude material was concentrated by vacuum distillation. After dilution with ethyl acetate (4mL×3), the mixture was washed with saturated NaCl solution (2mL). The organic phase was dried over anhydrous magnesium sulfate. Filtration and evaporation of solvent at reduced pressure gave compound17. A solution of pyridinium chlorochromate (PCC, 142mg, 0,6639mmol) in CH_2Cl_2 was added dropwise to compound 17 in CH_2Cl_2 and refluxed for 2h. Filtered it through Buchner filter by using filter paper and silica gel. The filter cake was wash by water and ethyl acetate (7mL×3). The organic phase was dried over anhydrous magnesium sulfate. Filtration and evaporation of solvent an reduced pressure gave a while solid, which was purified by silica gel chromatography.

2.4.1 3-Oxo-urs-12-en-28-oic acid propyl ester (18)

UA (100mg, 0.22mol) was reacted with bromopropane (108.23mg, 0.88mmol) following the general steps to give compound 17 (93.0mg, yield:84. 9%), ^1H NMR(300MHz, $CDCl_3$): δ 5.302 (s, 1H, H-12), 3.947 (t, 2H, $OCH_2C_2H_5$)，3.200 (brs, 1H, H-3)，2.20 (d, 1H, H-18), 1.075 (s, 6H, CH_3×2), 0.987 (s, 3H, CH_3), 0.943 (s, 3H, CH_3), 0.914 (d, 3H, CH_3), 0.864 (d, 3H, CH_3), 0.777 (s, 3H, CH_3). Compound 17 (110.2mg, 0.2213mmol) was reacted with PCC (142mg, 0.6639mmol) following the general steps. The residue was purified by silica gel chromatography with a gradient elution of petroleum ether/ ethyl acetate (24:1, v/v), to yield compound 18 (91.8mg, yield: 82.8%); m.p.105-107°C; ^1H NMR(300MHz, $CDCl_3$): δ 5.260 (s, 1H, H-12), 3.951 (t, 2H, $OCH_2C_2H_5$)，2.510 (m, 1H, H-2a), 2.400 (m, 1H, H-2b), 2.20 (d, 1H, H-18), 1.080 (s, 6H, CH_3×2), 1.038 (d, 3H, CH_3), 0.943 (s, 3H, CH_3), 0.938 (s, 3H, CH_3), 0.867 (d, 3H, CH_3), 0.802 (s, 3H, CH_3).

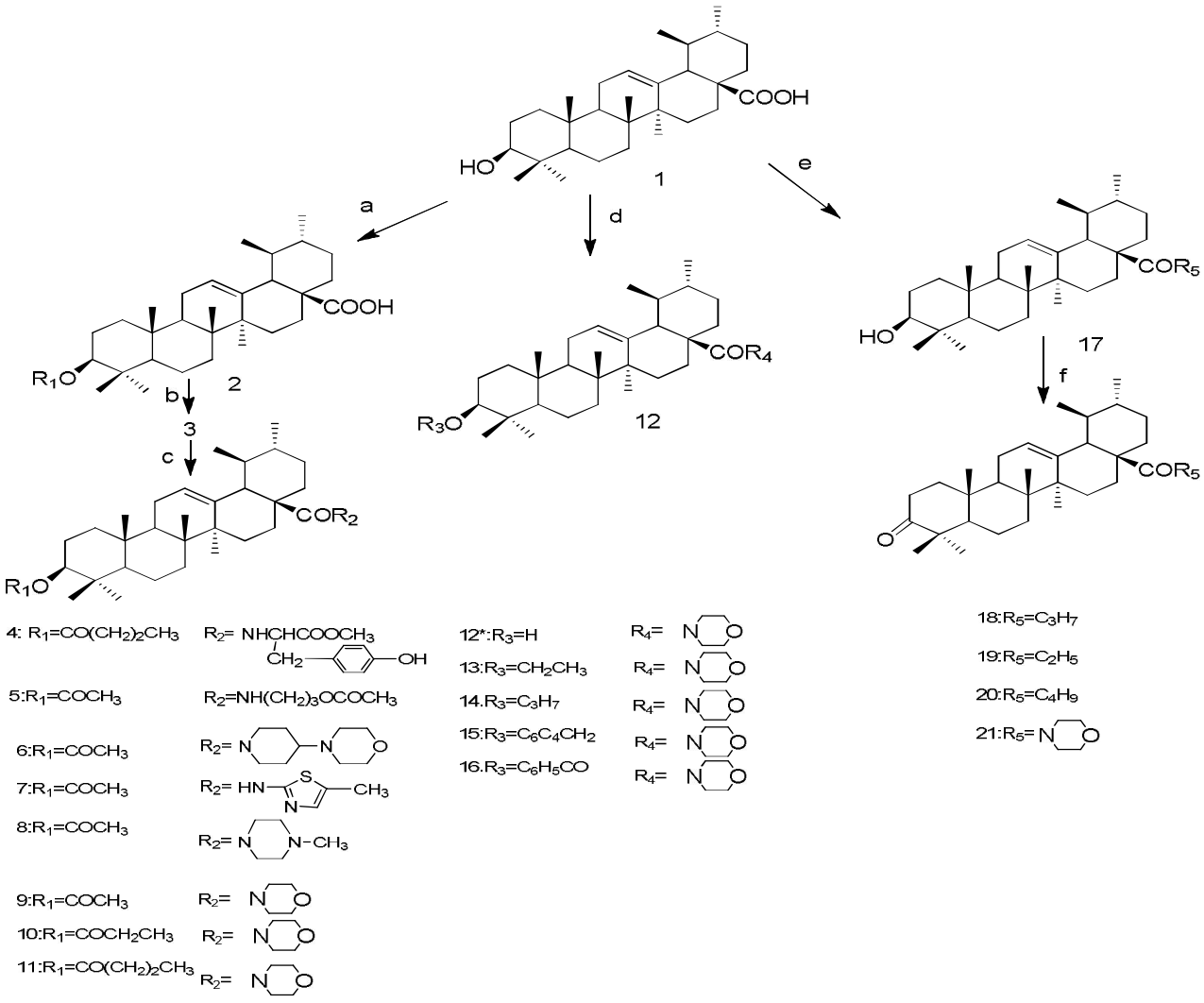
2.4.2. 3-Oxo-urs-12-en-28-oic acid ethyl ester (19)

UA (100mg, 0.22mmol)was reacted with bromoethane (95.9mg, 0.88mmol) following the general steps to give compound 17 (85.8mg, yield: 80.56%), IR (KBr): 3446, 2927, 2871，1724,1456, 1384, 1230 cm^{-1}. Compound 17 (102.9mg, 0.2126mmol) was reacted with PCC (137.5mg, 0.6378mmol) following the general steps. The residue was purified by silica gel chromatography with a gradient elution of petroleum ether/ ethyl acetate (25:1, v/v), to yield compound 19 (77.08mg, yield: 75.1%); m.p.89-89°C; IR(KBr):2975, 2924, 1705, 1454, 1384 cm^{-1}.

2.4.3 3-Oxo-urs-12-en-28-oic acid butyl ester (20)

UA (200mg, 0.44mmol) was reacted

with n-butyl bromide (241.2mg, 1.76mmol) to give compound 17 (193.6mg, yield:85.8%), IR (KBr): 3550, 2928, 1717, 1459, 1382, 1200, 1182cm^{-1}.Compound 17 (154.3mg, 0.3009mmol) was reacted with PCC (259.4mg, 1.2036mmol) following the general steps. The residue was purified by silica gel chromatography with a gradient elution of petroleum ether/ ethyl acetate (20:1, v/v), to yield compound20 (102.8mg, yield: 66.8%); m.p.139-140°C; IR(KBr): 2929, 1720, 1457, 1382, 1271 cm^{-1}.



Scheme 1. Synthesis of Ursolic acid derivatives

2.4.4 N-[3- Oxo-urs-12-en-28-oyl]- morpholine (21)

To a solution of 12*(44 mg, 0.084 mmol) in THF (2 mL) and CH_2Cl_2 (15 mL)were added celite (150 mg),PDC (99.1 mg, 0.336 mmol). The mixture was stirred at room temperature for 2h,filtered and the filter cake was washed with CH_2Cl_2 (2 ×5 mL), then evaporated in vacuo to give white solid. The solid was purified by flash column chromatography [petroleum ether/ ethyl acetate(4:1)] to give compound 21 as an amorphous solid (6.7 mg, 15.2%): mp 106～108℃; ^{1}H NMR(300MHz, CDCl3): δ5.25 (s,1H, H-12), 3.64 (br 8H, NH(CH_2CH_2)$_2$O), 1.09(s, 3H, CH_3), 1.06(s, 3H, CH_3),1.04(s, 3H, CH_3), 1.00(d, 3H, J=6.3, CH_3), 0.95 (s, 3H, CH_3), 0.87 (d, 3H, J=6.3, CH_3), 0.81 (s, 3H, CH_3); ESI-MS: 524.5 (M+H)+.

3. Results and discussion

3.1 Synthesis of UA derivatives

The paper focused on ursolic acid as a lead compound, 18 derivatives were designed and synthesized by three routes. These structure modification in the three routes were all done at the positons C-3 and C-28.

Compound 2 was synthesized through reaction of UA with n-butyric anhydride, acetic anhydride or propionic anhydride in the presence of DMAP; Afterward, oxalyl chloride was added to a solution of compound 2 in methylene dichloride to give compound 3. Then compound 3 was treated with corresponding amines in the presence of triethylamine to give compound 4-11. Ethers **13-15** prepared from **12*** with ethyl bromide, propyl bromide and benzyl bromide in presence of sodium hydride in anhydrous DMF, respectively. Similarly, compound **16** was synthesized from **12*** with benzoyl chloride by using the same method of preparing compound **2**.UA was reacted with appropriate bromoalkane or morpholine in the presence of potassium carbonate in DMF then these compounds were oxidized by PCC or PDC, to give compounds 18-21. (Scheme 1)

Reagents and conditions: (a) $(CH_3CH_2CH_2CO)_2O$, $(C_3H_7O)_2O$ or Ac_2O, DMAP, THF, r.t.; (b) CH_2Cl_2, $(COCl)_2$, r.t.; (c) Et_3N, HR_2, r.t.; (d) C_2H_5Br or C_3H_7Br or $C_6H_5CH_2Br$ or PhCOCl; (e) R_5Br, K_2CO_3, DMF; (f)PCC, CH_2Cl_2.

All the target compounds were purified by a silica gel column with petroleum ether/ethyl acetate or petroleum ether/acetone as eluents. Their structures were characterized by means of mp, IR, MS, ^{1}HNMR, ^{13}CNMR and elemental analysis.

3.2 Bioactivity

Some of antitumor activities of compounds were evaluated in vitro using the MTT method against HeLa, BGC-823 and SKOV3 cell lines with UA as the positive control. The results are summarized in Table 1.

Table 1. Inhibitory activity of ursolic acid and its derivatives on different cancer cell proliferation

Comp.	$IC_{50}(\mu mol \cdot L^{-1})$[a]		
	HeLa	SKOV3	BGC-823
1	>10	>10	>10
4	>100	>100	>100
5	>10	>10	9.20±
6	>100	>100	>100
7	>100	>100	>100
8	>10	>10	>10
9	2.16±0.26	nt[b]	>10
10	>10	>10	>10
11	>10	>10	9.7±1.01
12*	>10	nt	nt
13	>10	>10	nt
14	>10	>10	>10
15	>10	>10	cell growth
16	>10	>10	nt
18	nt	nt	nt
19	nt	nt	nt
20	nt	nt	nt
21	>10	>10	nt
VP-16[c]	2.37±0.99	5.62±2.11	5.01±2.85

[a] The agent concentration that inhibited HeLa cells growth by 50%;
[b] Not tested;
[c] Etoposide.

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